

## Epinephrine-mediated regulation of PDK4 mRNA in rat adipose tissue

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Submitted 18 May 2010; accepted in final form 21 August 2010

**Wan Z, Thrush AB, Legare M, Frier BC, Sutherland LN, Williams DB, Wright DC.** Epinephrine-mediated regulation of PDK4 mRNA in rat adipose tissue. *Am J Physiol Cell Physiol* 299: C1162–C1170, 2010. First published August 25, 2010; doi:10.1152/ajpcell.00188.2010.—Fatty acid reesterification in adipose tissue is dependent on the generation of glycerol 3-phosphate, and, at least in rodent adipose tissue, this appears to occur primarily through glyceroneogenesis. A key enzyme in this process is pyruvate dehydrogenase kinase 4 (PDK4). PDK4 is induced in white adipose tissue by thiazolidinediones (TZDs) and the inhibition or knockdown of PDK4 inhibits TZD-induced increases in glyceroneogenesis. Since TZDs have many unwanted side effects, we were interested in identifying alternative mechanisms that could regulate PDK4 mRNA expression in white adipose tissue. In this regard we hypothesized that exercise, fasting, and epinephrine would increase PDK4 mRNA levels in rat epididymal adipose tissue. We further postulated that the p38 mitogen-activated protein kinase (MAPK) and 5'-AMP-activated protein kinase (AMPK) signaling pathways would control PDK4 mRNA expression in cultured adipose tissue. Exercise, fasting, and in or ex vivo epinephrine treatment increased PDK4 mRNA levels. These perturbations did not increase the expression of PDK1, -2, or -3. Pyruvate dehydrogenase phosphorylation was increased after an overnight fast and 4 h after the cessation of exercise. In cultured adipose tissue, epinephrine increased p38 and AMPK signaling; however, the direct activation of AMPK by AICAR or metformin led to reductions in PDK4 mRNA levels. The p38 inhibitor SB202190 reduced epinephrine-mediated increases in p38 MAPK activation without altering hormone-sensitive lipase or AMPK phosphorylation or attenuating epinephrine-induced increases in lipolysis. Reductions in p38 MAPK signaling were associated with decreases in PDK4 mRNA expression. The inhibition of peroxisome proliferator-activated receptor- $\gamma$  (PPAR $\gamma$ ) also attenuated the induction of PDK4. Our results are the very first to demonstrate an epinephrine-mediated regulation of PDK4 mRNA levels in white adipose tissue and suggest that p38 MAPK and PPAR $\gamma$  could be involved in this pathway.

p38 mitogen-activated protein kinase; pyruvate dehydrogenase kinase 4

ADIPOSE IS A VIBRANT METABOLIC tissue secreting a wide range of hormones and metabolites that influence whole body fuel metabolism (41). The primary metabolic function of adipose tissue is to serve as a storage depot for lipids. In conditions of nutrient excess, glucose and fatty acids are stored in adipose tissue as triglycerides (TGs). When blood glucose levels become limiting, such as during fasting or prolonged exercise, fatty acids are liberated from TGs via lipolysis. While a large percentage of these fatty acids are released into the circulation, a significant amount are reesterified back to TG (49). Reesterification requires the provision of glycerol 3-phosphate (G-3-P), and, at least in rodent adipose tissue, the generation of G-3-P

occurs primarily through de novo synthesis from sources such as lactate and pyruvate, in a process termed glyceroneogenesis (31, 32). Since fluctuations in fatty acid levels have been linked to the development of systemic insulin resistance (4), an understanding of the factors that regulate glyceroneogenesis is vital in understanding the pathogenesis of type 2 diabetes.

Accumulating evidence suggests that pyruvate dehydrogenase kinase 4 (PDK4) is involved in the regulation of glyceroneogenesis in white adipose tissue (6). PDK4, along with the related enzymes PDK1, -2 and -3, phosphorylates and inhibits pyruvate dehydrogenase (PDH) complexes, leading to decreases in the formation of acetyl-CoA and a greater flux of pyruvate toward oxaloacetate and the glyceroneogenic pathway (6). Thiazolidinediones (TZDs), a commonly prescribed class of antidiabetic drugs and potent peroxisome proliferator-activated receptor- $\gamma$  (PPAR $\gamma$ ) agonists, increase PDK4 expression, lower fasting fatty acid levels, and improve insulin sensitivity in insulin-resistant Zucker *falfa* rats (6). Moreover, TZD treatment of adipocytes leads to increases in the expression of PDK4 concomitant with increased rates of glyceroneogenesis (6). PDK4 would appear to be essential for the upregulation of glyceroneogenesis by TZDs because the attenuation of PDK4 activity pharmacologically with leelamine or reducing PDK4 content by short interfering RNA both result in decreases in fatty acid reesterification (6). Given the effects of TZDs on PDK4 expression and glyceroneogenesis (6, 43) and owing to the unwanted side effects of these drugs (45), the identification of alternative approaches to induce PDK4 in white adipose tissue is warranted.

The regulation of PDK4 expression has been extensively studied in skeletal muscle and has been shown to be robustly induced by fasting (42) and exercise (23, 36, 37). The overexpression of the transcriptional coactivator PPAR $\gamma$  coactivator 1- $\alpha$  (PGC-1 $\alpha$ ) in rodent skeletal muscle (51) and C2C12 muscle cells (50) results in increases in PDK4 expression, thus lending credence to the supposition that this molecule is a key regulator of PDK4. Interestingly, we have recently made the novel discovery that exercise and epinephrine increase PGC-1 $\alpha$  mRNA expression in rat epididymal adipose tissue (47). Moreover,  $\beta$ -adrenergic agonists have been reported to increase the activity of the 5'-AMP-activated protein kinase (AMPK) (17, 25, 33) and p38 mitogen-activated protein kinase (MAPK) pathways in adipocytes (7, 30), signaling cascades that have been implicated in the regulation of PDK4 (23, 29, 39). Taken in concert, these findings suggest a role for  $\beta$ -adrenergic agonists in the regulation of PDK4 in white adipose tissue. In this context we hypothesized that fasting, exercise, and epinephrine would increase PDK4 mRNA expression in rat epididymal adipose tissue. We further surmised that epinephrine would increase p38 and AMPK signaling and that these pathways would be involved in the regulation of PDK4 in rat adipose tissue. Lastly, since PPAR $\gamma$  agonists have been impli-

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cated in regulating PDK4 mRNA expression, we postulated that this transcription factor would also be involved in the pathway through which epinephrine increases PDK4 in adipose tissue.

## METHODS AND MATERIALS

**Materials.** SuperScript II Reverse Transcriptase, oligo(dT), and dNTP were products from Invitrogen (Burlington, ON, Canada). Taqman Gene Expression Assays for  $\beta_2$ -microglobulin,  $\beta$ -actin, PDK1, PDK2, PDK3, PDK4, pyruvate dehydrogenase phosphatase 1 (PDP1) and PDP2 were from Applied Biosystems (Foster City, CA). AICAR (5-aminoimidazole-4-carboxamide-1- $\beta$ -D-ribose nucleoside) was purchased from Toronto Research Chemicals (Toronto, ON, Canada) while rosiglitazone was obtained from Cayman (Ann Arbor, MI). Total and phosphorylation-specific antibodies for the detection of AMPK, acetyl-CoA carboxylase (ACC), p38 MAPK, MAPK-activated protein kinase 2 (MK2) and hormone-sensitive lipase (HSL) were from Cell Signaling (Danvers, MA). Phosphorylated PDH antibodies and SB202190/SB203580 were products of EMD Biosciences (San Diego, CA). An antibody against the E1  $\alpha$ -subunit of PDH was from Mitosciences (Eugene, OR). All other chemicals were purchased from Sigma (Oakville, ON, Canada).

**Treatment of rodents.** All protocols followed Canadian Council on Animal Care guidelines and were approved by the Animal Use and Welfare Committee at the University of Alberta. Male Wistar rats (Charles River, Wilmington, MA) weighing  $\sim$ 200 g were housed 2 per cage, with a 12:12-h light-dark cycle, and were provided with water and standard rat chow ad libitum. The 12-h light cycle was from 6 AM to 6 PM, and all experimental protocols were performed between 7 AM and 10 AM. Swim exercise and epinephrine injections were performed as described previously by our laboratory (47). Briefly, rats were acclimatized to the animal housing unit for 1 wk before experiments. For the exercise studies, half of the animals swam for 2 h while the remaining animals served as controls. For the epinephrine experiment, the rats were injected [intraperitoneally (ip)] with a weight adjusted bolus of epinephrine (20  $\mu$ g/100 g body wt) or an equivalent volume of sterile saline. To determine the effects of fasting on PDK4 mRNA levels in adipose tissue, food was removed at  $\sim$ 5:00 PM the evening before the experiment. Immediately following exercise, 2 h after epinephrine injections or  $\sim$ 18 h following the removal of food, the animals were anesthetized with pentobarbital sodium (5 mg/100 g body wt) and epididymal adipose tissue was dissected free of the testes, immediately weighed, and then clamp frozen in tongs cooled to the temperature of liquid nitrogen and stored at  $-80^\circ\text{C}$  until further analysis.

**Adipose tissue organ culture.** Adipose tissue organ culture is a well-characterized technique that has been used to determine changes in adipose tissue metabolism and gene expression (12). Epididymal adipose tissue was cultured as we have described in detail previously (47). To assess the effects of epinephrine (1  $\mu$ M), AICAR (1 mM), and metformin (1 mM) on the expression of PDK4 mRNA, adipose tissue was cultured for 24 h and then treated with these agents for 2 h. To determine the effects of p38 inhibition on PDK4 expression, cultures were treated with 1  $\mu$ M SB202190 or SB203580 for 30 min before and during the 2-h epinephrine treatment. These compounds are cell-permeable inhibitors of p38 MAPK that bind to the ATP pocket of the active kinase. We chose this concentration of SB202190/SB203580 since previous studies have demonstrated that higher concentrations of the pyridinyl imidazole compounds may possess some nonspecific effects (2). To assess the effects of GW9662, a PPAR $\gamma$  antagonist, on rosiglitazone and epinephrine-induced PDK4 mRNA levels, cultures were treated with 20  $\mu$ M GW9662 for 30 min before and during 2 h of epinephrine or rosiglitazone (2  $\mu$ M) treatment. For the determination of changes in the phosphorylation of AMPK, ACC, p38, MK2, and HSL, experiments were conducted as described above except that cultures were treated with epinephrine for

30 min. At the end of the experiments, adipose tissue cultures were rinsed in ice-cold sterile PBS and strained, and adipose tissue fragments were snap frozen and stored at  $-80^\circ\text{C}$  for further analysis.

**Western blot analysis.** Protein was extracted from adipose tissue, and changes in the phosphorylation status of AMPK, ACC, p38, MK2, and HSL were determined by Western blotting as described in detail by our laboratory previously (13). Briefly, adipose tissue samples were homogenized in 1.5 volumes of ice-cold cell lysis buffer supplemented with Protease Inhibitor Cocktail (Sigma) and phenylmethylsulfonyl fluoride. Homogenized samples were centrifuged for 10 min at 1,500 g at  $4^\circ\text{C}$ . The fat cake was removed, the infranatant was collected, and protein concentration was determined using the bicinchoninic acid method (44). Equal amounts of protein were separated on 7.5% gels. Proteins were wet transferred to nitrocellulose membranes at 200 mA/tank and subsequently blocked in Tris-buffered saline-0.1% Tween 20 (TBST) supplemented with 5% nonfat dry milk for 1 h at room temperature with gentle agitation. Membranes were incubated in TBST-5% nonfat dry milk supplemented with appropriate primary antibodies overnight at  $4^\circ\text{C}$  with gentle agitation. The following morning, membranes were briefly washed in TBST and then incubated in TBST-1% nonfat dry milk supplemented with horseradish peroxidase-conjugated secondary antibodies for 1 h at room temperature. Bands were visualized using ECL plus and captured using a Typhoon Imaging system (GE Health Care, Baie d'Urfe, Quebec, Canada). Imagequant software (GE Healthcare) was used to quantify relative band intensities.  $\beta$ -Actin or tubulin were used as internal controls.

**Lipolysis assays.** Adipose tissue was cultured as described above. On the morning of the experiment, media were removed and replaced with fresh M199 supplemented with 3% BSA. Epinephrine, SB202190, or the PDK inhibitor dichloroacetate (DCA) (2 mM) was added as described above. Media were collected at the end of the 2-h epinephrine treatment, and adipose tissue fragments were weighed and frozen in liquid nitrogen. Glycerol release into the media was determined using a Free Glycerol Kit (Sigma, St. Louis, MO). The coefficient of variation for these assays in our laboratory is  $<10\%$ .

**Real-time RT-PCR.** RNA was isolated from epididymal adipose tissue, cDNA was synthesized, and PCR analysis was performed as we have described in detail previously (13, 47). Relative differences in gene expression between groups were determined using the  $2^{-\Delta\Delta\text{CT}}$  method (28). The amplification efficiencies of the gene of interest and the housekeeping gene were equivalent, and there was no effect of the experimental manipulation on the expression of housekeeping genes.

**Statistical analysis.** Data are presented as means  $\pm$  SE. Comparisons between control and exercised rats were made using a one-way ANOVA followed by a post hoc comparison using Fishers least significant difference (LSD) test. Comparisons between fed and fasted rats, vehicle and epinephrine, AICAR- or metformin-treated cultures were made using a Student's *t*-test. The effects of SB202190 on cell signaling and lipolysis were analyzed using a  $2 \times 2$  ANOVA with LSD post hoc comparisons. The effects of SB202190, SB203580, and GW9662 on PDK4 mRNA expression were analyzed using a one-way ANOVA on ranks followed by Dunn's test. Statistical significance was set at  $P < 0.05$ .

## RESULTS

**Exercise, fasting, and epinephrine increase PDK4 mRNA expression in epididymal adipose tissue.** PDK-4 mRNA expression was increased approximately sixfold immediately following 2 h of swim exercise (Fig. 1) and remained elevated 2 h ( $3.83 \pm 0.32$  fold increase versus control;  $P < 0.05$ ) and 4 h ( $3.34 \pm 0.70$  fold increase versus control;  $P < 0.05$ ) following exercise cessation. Similarly, an overnight fast led to an approximately threefold increase in PDK-4 mRNA levels. PDK4 mRNA levels were increased in epididymal adipose

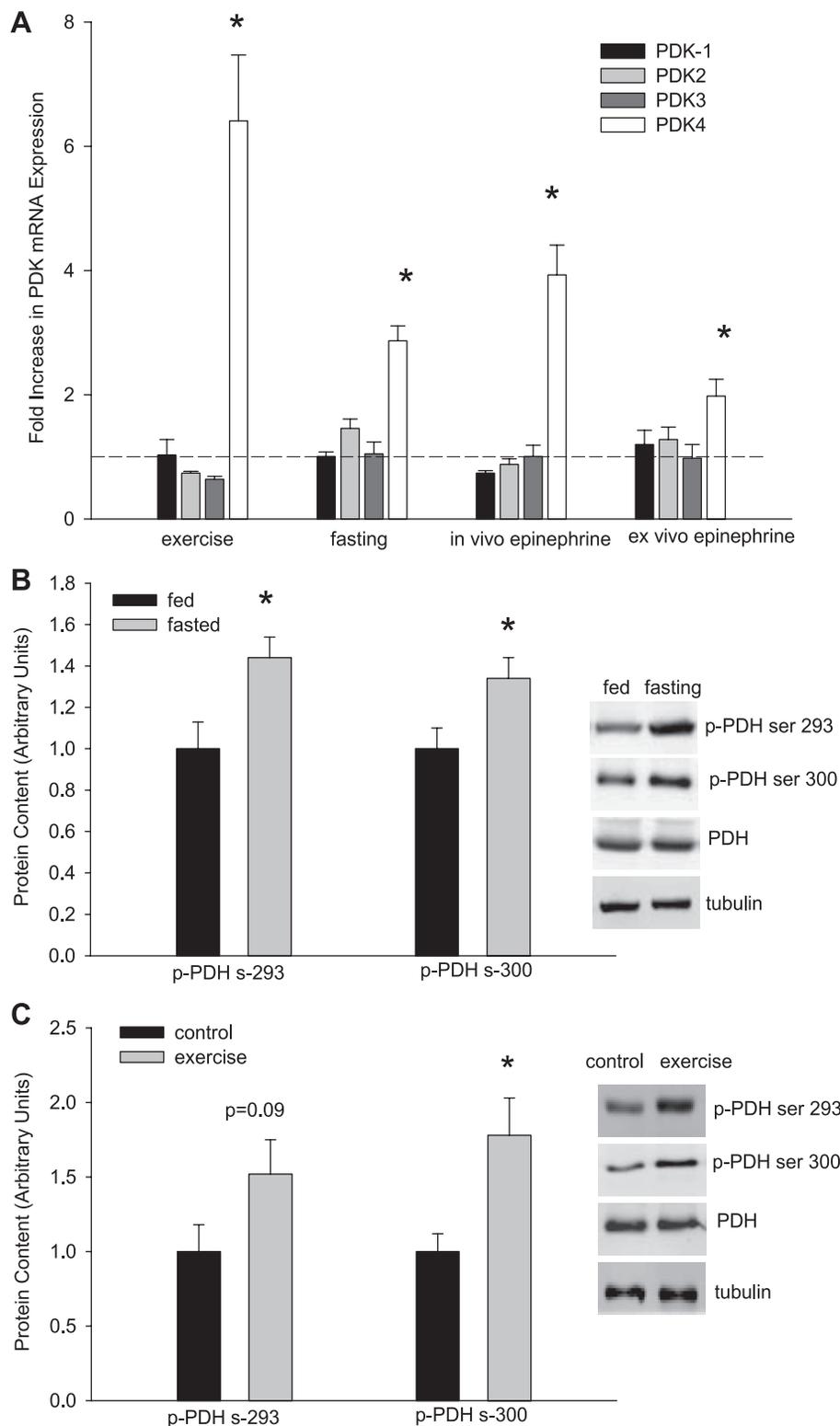


Fig. 1. *A*: exercise, fasting, and epinephrine increase the mRNA expression of pyruvate dehydrogenase kinase 4 (PDK4), but not PDK1, PDK2, or PDK3 in rat epididymal adipose tissue. *B* and *C*: increases in PDK4 mRNA levels are associated with increases in the phosphorylation of pyruvate dehydrogenase (p-PDH) on serine 293 and 300 after an overnight fast (*B*) and 4 h following exercise cessation (*C*). Data are presented as means  $\pm$  SE for 5–11 animals or tissue cultures per group. The mRNA data were normalized to  $\beta$ -actin and are expressed as fold differences compared with sedentary controls, fed controls, saline-injected animals, or vehicle-treated tissue cultures. Representative Western blot images are given to the right of the quantified data in *B* and *C*. \* $P < 0.05$  vs. control.

tissue 2 h following a bolus injection of epinephrine (20  $\mu$ g/100 g body wt) and were also increased in cultured epididymal adipose tissue following a 2-h epinephrine (1  $\mu$ M epinephrine) treatment. Exercise, fasting, and epinephrine did not increase mRNA levels of PDK1, -2, or -3 (Fig. 1). Similarly, the mRNA expression of PDP1 and 2, enzymes that dephosphorylate PDH complexes, were not altered by any of our

experimental manipulations (data not shown). Increases in PDK4 mRNA levels after an overnight fast or 4 h following exercise cessation were associated with increases in the phosphorylation of PDH on serine 293 and serine 300, PDK4 phosphorylation sites. Confirming the importance of PDK4 in regulating fatty acid release (6), we found that DCA, a PDK inhibitor (40), increased the ratio ( $\mu$ mol/g tissue fatty acid to

$\mu\text{mol/g}$  tissue glycerol) of fatty acid to glycerol release in cultured adipose tissue ( $1.76 \pm 0.10$  control,  $2.67 \pm 0.45$  DCA  $P = 0.05$ ).

**Epinephrine increases AMPK and P38 MAPK signaling in cultured epididymal adipose tissue.** As illustrated in Fig. 2, epinephrine treatment ( $1 \mu\text{M}$ , 30 min) led to increases in the phosphorylation of AMPK and its downstream substrate ACC (18). Similarly, epinephrine also increased the activity of the p38 MAPK signaling pathway as evidenced by increases in the phosphorylation of p38 and MK2. p38 MAPK phosphorylates MK2 on threonine 222, and thus MK2 phosphorylation can be used as a marker of p38 MAPK activity (3).

**AMPK agonists do not increase PDK4 mRNA expression in rat epididymal adipose tissue.** As illustrated in Fig. 3, AICAR ( $1 \text{ mM}$ , 1 h) and metformin ( $1 \text{ mM}$ , 1 h) both increased AMPK and ACC phosphorylation while having no effect on p38 MAPK phosphorylation. These compounds decreased PDK4 mRNA expression (Fig. 2C).

**SB202190 does not attenuate AMPK or PKA signaling in epididymal adipose tissue cultures.** Treating cultured epididymal adipose tissue with  $1 \mu\text{M}$  SB202190 for 30 min before and during epinephrine treatment led to reductions in MK2 phosphorylation (Fig. 4A). SB202190 did not inhibit the phosphorylation of HSL on serine 563 and 660 (Fig. 4B), which are protein kinase A (PKA) phosphorylation sites (1). Similarly, AMPK phosphorylation was not inhibited by SB202190 (Fig. 4C). Epinephrine-induced increases in lipolysis, as determined by glycerol release into the culture media, were not reduced by prior treatment with SB202190 (Fig. 4D).

**SB202190 attenuates epinephrine-induced increases in PDK4 mRNA.** Epinephrine-induced increases in PDK4 mRNA were blunted by SB202190 (Fig. 5). Similarly, the related compound SB203580 ( $1 \mu\text{M}$ ) also blocked the effects of epinephrine on PDK4 mRNA levels. In the absence of epinephrine these compounds had no effect on PDK4 mRNA (data not shown).

**Epinephrine-mediated increases in PDK4 are inhibited by a PPAR $\gamma$  antagonist.** The PPAR $\gamma$  agonist rosiglitazone ( $2 \mu\text{M}$ ) increased PDK4 mRNA levels in cultured adipose tissue, and

this effect was abrogated by GW9662 ( $20 \mu\text{M}$ ), a PPAR $\gamma$  antagonist. These reductions did not appear to be secondary to decreases in  $\beta$ -adrenergic signaling because GW9662 did not effect epinephrine-mediated increases in the phosphorylation of p38 MAPK, AMPK, or HSL (Fig. 5).

## DISCUSSION

Adipose tissue is an active metabolic organ that is intimately involved in the regulation of systemic carbohydrate and lipid metabolism (41). Accumulating evidence suggests that the modulation of white adipose tissue metabolism is a beneficial approach in the treatment of type 2 diabetes. In this regard it has been demonstrated that TZDs increase fatty acid reesterification and limit fatty acid release from white adipocytes (6). Cadoual and colleagues (6) have reported that the inhibition or knockdown of PDK4 attenuates the effects of TZDs on glyceroneogenesis, thus demonstrating the essential role of this enzyme in fatty acid recycling. Given the key role of PDK4 in the regulation of white adipose tissue metabolism, we sought to explore the regulation of this kinase by physiological stimuli such as exercise, fasting, and epinephrine. We have made the novel discovery that exercise leads to rapid increases in PDK4 mRNA levels that persist for upward of 4 h following exercise cessation. These findings are similar in magnitude and time course to the reported effects of exercise on PDK4 mRNA levels in skeletal muscle (23, 36, 37) and further demonstrate (47) the plasticity of adipose tissue in response to exercise. In addition to exercise, and consistent with one previous report (27), we found that PDK4 mRNA levels were also increased by fasting. In contrast to PDK4, PDK1, -2, and -3 were not responsive to these perturbations. These findings are congruent with a previous study that demonstrated negligible changes in PDK2 levels in skeletal muscle following fasting (35). In addition to increasing PDK4 mRNA levels we found that the phosphorylation of PDH on serine 293 and 300 were increased after an overnight fast or 4 h following exercise cessation. Since PDK4 phosphorylates these residues (40), these findings suggest an increase in PDK4 activity.

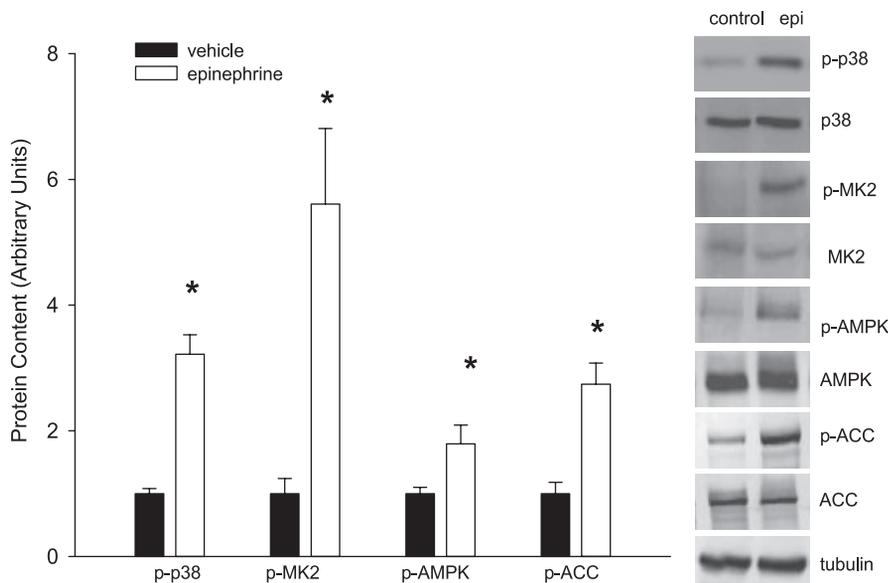


Fig. 2. Ex vivo epinephrine (Epi) treatment (30 min,  $1 \mu\text{M}$ ) increases the phosphorylation of p38, MAPK-activated protein kinase 2 (MK2), 5'-AMP-activated protein kinase (AMPK), and acetyl-CoA carboxylase (ACC) in cultured rat epididymal adipose tissue. Data are presented as means  $\pm$  SE for 5–8 tissue cultures per group. Representative Western blot images are given to the right of the quantified data. \* $P < 0.05$  vs. control.

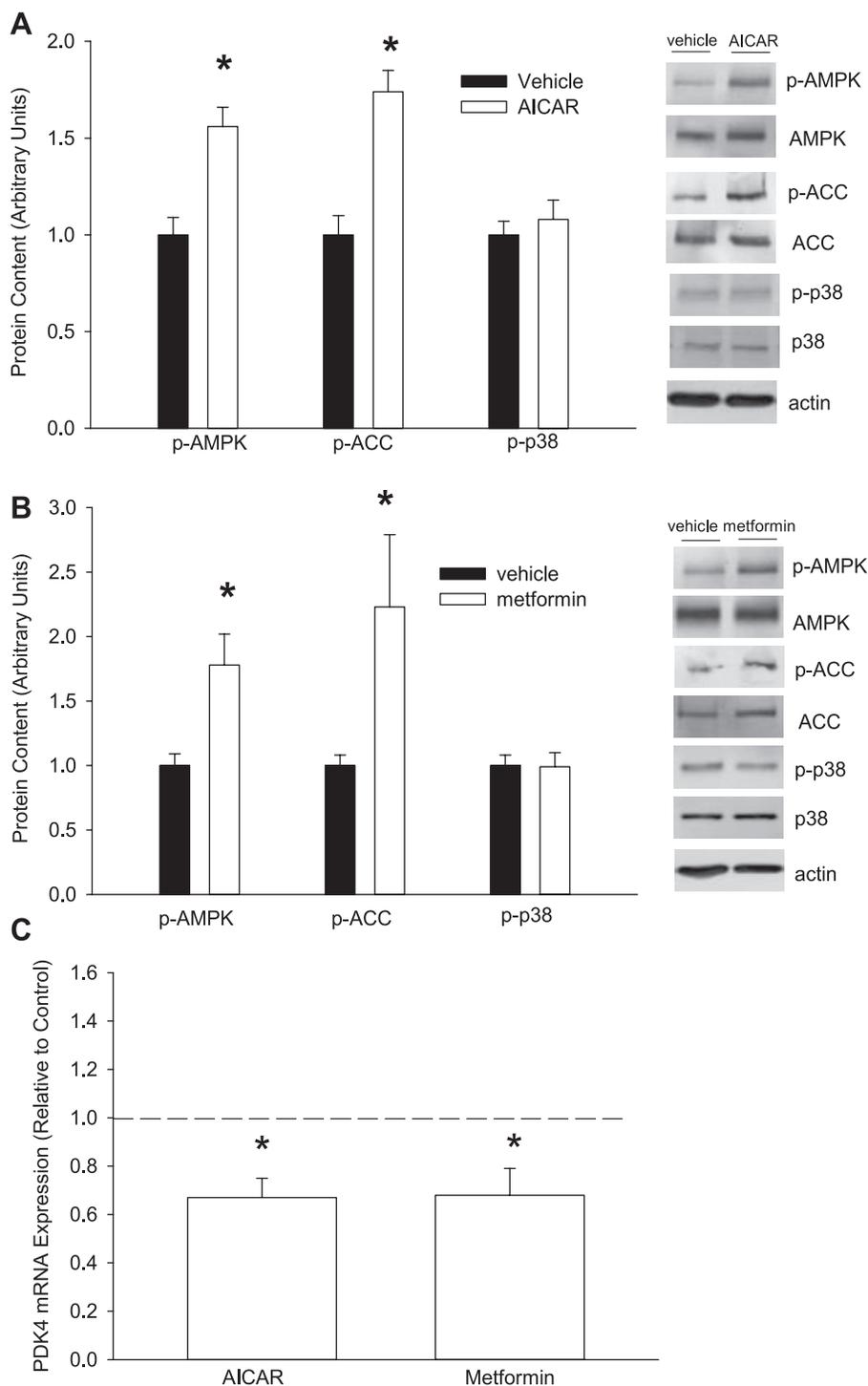


Fig. 3. The AMPK agonists AICAR (1 mM; *A*) and metformin (1 mM; *B*) increase AMPK signaling (1 h treatment) in cultured epididymal adipose tissue but do not increase the expression of PDK4 mRNA levels (2 h treatment; *C*). Data are presented as means  $\pm$  SE for 5–11 samples per group. RT-PCR data were normalized to  $\beta$ -actin. Representative Western blot images are given to the right of the quantified Western blot data. \* $P < 0.05$  vs. control.

Swim exercise (19) and fasting (Frier BC and Wright DC, unpublished observations) increase circulating epinephrine levels. To determine whether epinephrine could be a signal that initiates increases in PDK4 mRNA levels, we examined PDK4 in adipose tissue harvested from rats 2 h following epinephrine injections or in adipose tissue cultures treated with epinephrine. Similar to what has previously been reported in mouse skeletal muscle following treatment with the  $\beta_2$ -adrenergic agonist formoterol (34), we found that epinephrine increased PDK4 mRNA levels both in vivo and ex vivo.

In isolated adipocytes,  $\beta$ -adrenergic agonists increase the activity of AMPK (15, 17, 24). Since AMPK has been postulated to regulate PDK4 mRNA expression in skeletal muscle (23, 29), we reasoned that this kinase may serve a similar role in white adipose tissue. Consistent with this line of thinking, we found that ex vivo epinephrine treatment increased the phosphorylation of AMPK and ACC concomitant with increases in PDK4 mRNA expression. To determine whether the direct activation of AMPK can increase PDK4 mRNA levels, we treated adipose tissue cultures with AICAR or metformin.

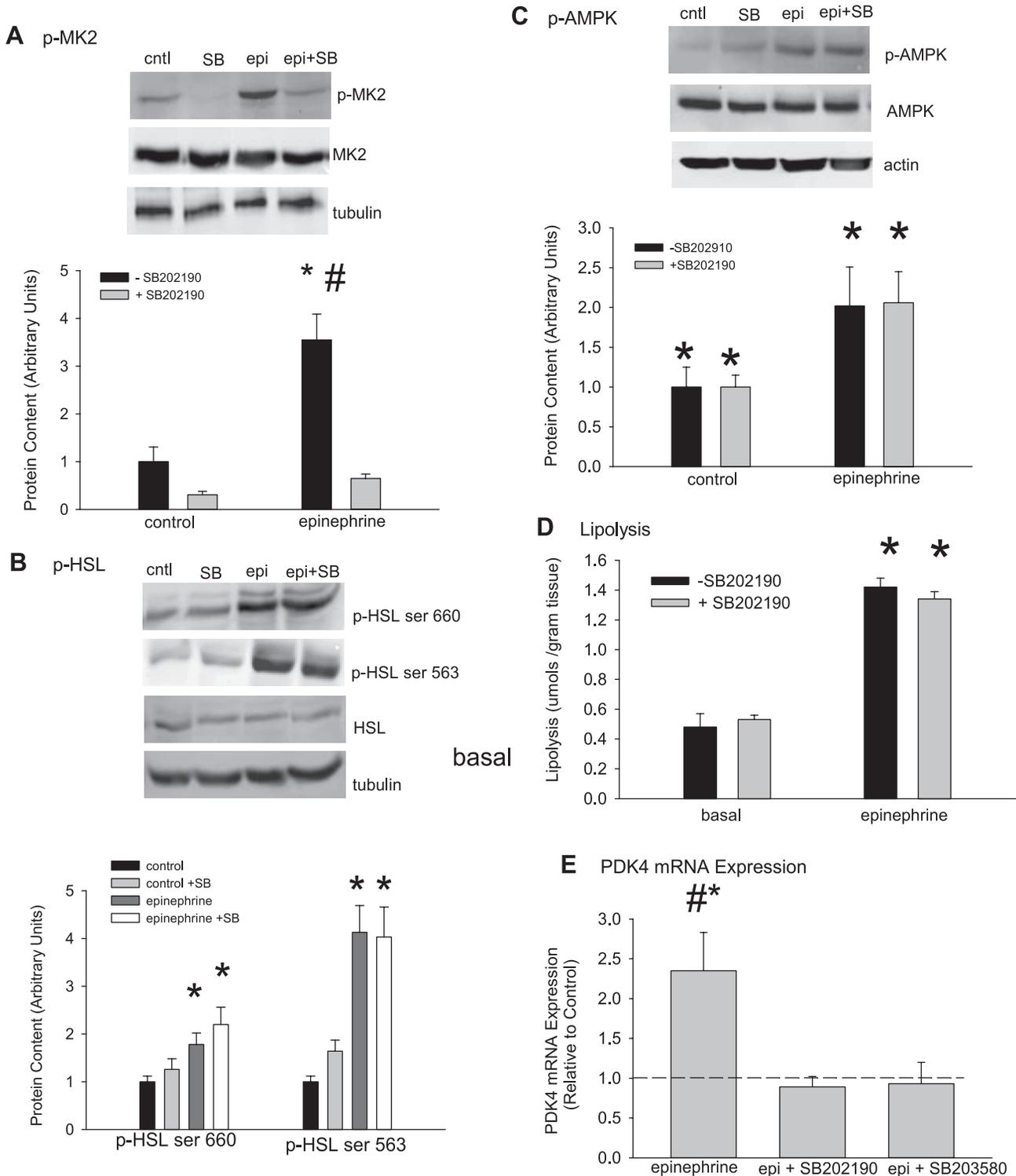


Fig. 4. A–D: SB202190 (SB) inhibits epinephrine-induced increases in MK2 phosphorylation (A) but not hormone-sensitive lipase (HSL) phosphorylation (B) (serine 563 and 660), AMPK phosphorylation (C), or lipolysis (D). Cntl, control. E: epinephrine-mediated increases in PDK4 mRNA expression are abrogated by SB202190 and SB203580. Data are presented as means  $\pm$  SE for 5–15 tissue cultures per group. The RT-PCR data were normalized to  $\beta$ -actin and are expressed as fold differences compared with controls. Representative Western blot images are given above the quantified data. \* $P < 0.05$  vs. vehicle-treated control, # $P < 0.05$  vs. SB202190-treated epinephrine condition.

AICAR is an adenosine analog that is converted to ZMP (5-aminoimidazole-4-carboxamide-1- $\beta$ -D-ribofuranosyl monophosphate) and mimics the effects of AMP on AMPK activation (14) while metformin activates AMPK independent of

changes in the AMP-to-ATP ratio (14). Despite increasing AMPK and ACC phosphorylation, we demonstrate that these mechanistically distinct AMPK agonists did not increase, and in fact reduced, PDK4 mRNA expression in cultured epididy-

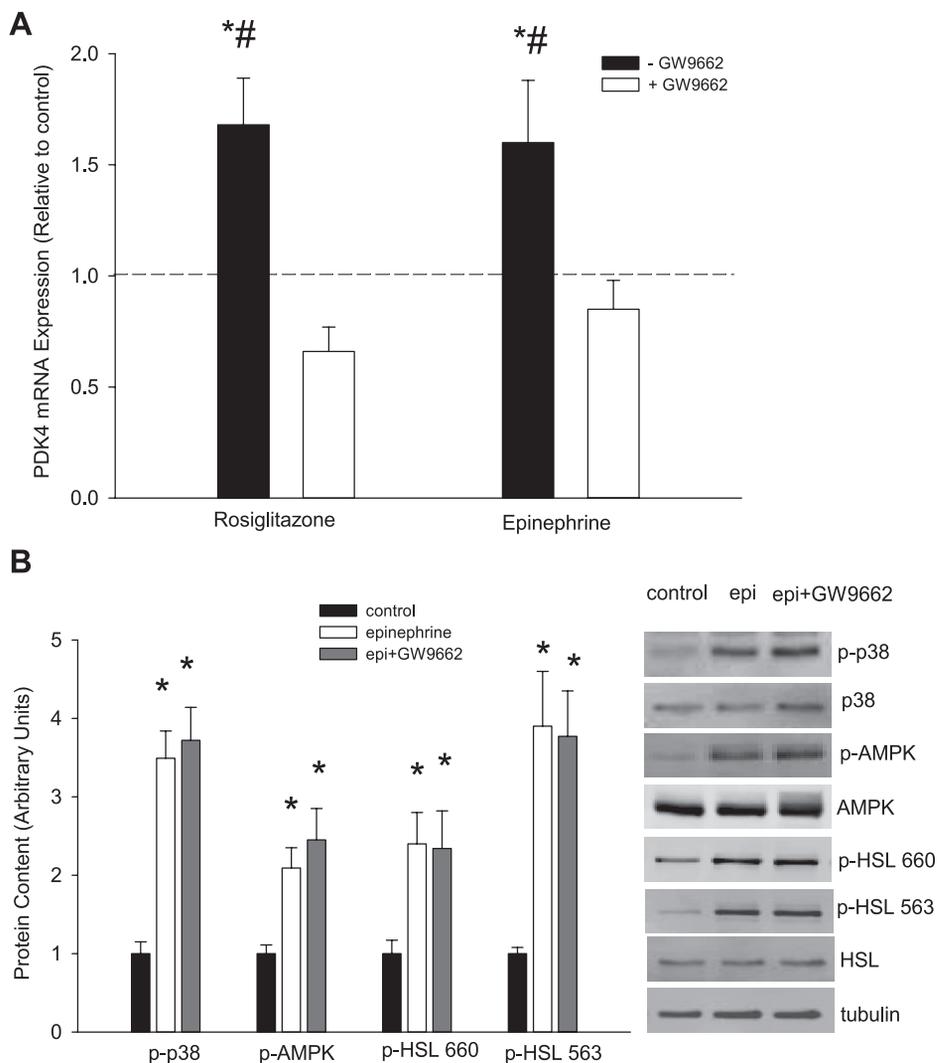


Fig. 5. GW9662 (20  $\mu$ M), a peroxisome proliferator-activated receptor- $\gamma$  (PPAR $\gamma$ ) antagonist, inhibits rosiglitazone (2  $\mu$ M) and epinephrine (1  $\mu$ M)-mediated increases in PDK4 mRNA levels (A) while having no effect on epinephrine-mediated increases in the phosphorylation of p38, AMPK, or HSL (B). Data are presented as means  $\pm$  SE for 6–8 cultures per group. The RT-PCR data were normalized to  $\beta$ -actin and are expressed as fold differences compared with control. Representative Western blot images are given to the right of the quantified data.  $*P < 0.05$  vs. control,  $\#P < 0.05$  vs. epinephrine + GW9662.

mal adipose tissue. In adipocytes the activation of AMPK results in the phosphorylation of HSL (5, 9, 16), leading to decreases in HSL activity and reductions in lipolysis (8, 11, 46). Since rates of fatty acid reesterification change in proportion to lipolysis (5, 48), it is not entirely surprising that AMPK agonists would downregulate the expression of PDK4 given reductions in lipolysis, and presumably by extension, decreases in the absolute rate of fatty acid reesterification. Regardless of the mechanisms involved, these findings clearly demonstrate tissue-specific differences in the mechanisms regulating PDK4.

In addition to AMPK, catecholamines have also been shown to activate p38 MAPK signaling in adipocytes (7, 30). Since this pathway has been implicated in modulating the expression of PDK4 in cardiomyocytes (39), we postulated that p38 MAPK would regulate PDK4 in white adipose tissue. Consistent with this hypothesis, we found that the p38 MAPK inhibitor SB202190 attenuated the epinephrine-induced increases in MK2 phosphorylation and PDK4 mRNA expression. While the pyridinyl imidazole compounds are potent inhibitors of p38 MAPK (10), these agents have also been shown to possess nonspecific effects (2, 21). In this light we thought it vital to determine whether SB202190 impaired epinephrine

signaling, independent of reductions in p38 MAPK activity. Under the conditions utilized, we found that SB202190 did not attenuate epinephrine-induced increases in the phosphorylation of HSL on serine 660 and 563, which are specific PKA phosphorylation sites (1), nor did the compound inhibit epinephrine-induced increases in lipolysis or AMPK phosphorylation. Collectively, these results strongly suggest that inhibition of p38 MAPK, and not a general abrogation of  $\beta$ -adrenergic signaling, mediates the effects of SB202190 on epinephrine-induced increases in PDK4 mRNA levels in white adipose tissue.

In addition to providing novel information regarding the processes that regulate PDK4 mRNA expression in white adipose tissue, our findings also lend insight into the relationship between AMPK and p38 MAPK signaling in this tissue. It has been suggested by some (26), but not all (20), that AMPK may lie upstream of p38 MAPK. Conversely, it has also been demonstrated that SB202190, at levels 10-fold higher than used in the current study, attenuates AMPK activity in heart muscle (22). Contrary to these studies, we did not find an interaction between p38 and AMPK signaling in cultured white adipose tissue. For instance, AICAR and metformin did not increase the phosphorylation of p38 MAPK, while the inhibi-

tion of p38 MAPK signaling was not associated with reductions in AMPK phosphorylation. Collectively, these results imply that epinephrine increases the activation of AMPK and p38 MAPK through parallel, not serial, pathways in white adipose tissue.

Previous work has demonstrated that rosiglitazone causes a rapid increase in PDK4 mRNA levels in rodent adipose tissue (6). Since the time course and the magnitude of change in PDK4 mRNA with rosiglitazone are similar to what we see following epinephrine treatment, we reasoned that PPAR $\gamma$  may be involved in the pathway through which epinephrine induces PDK4 in white adipose tissue. Consistent with this hypothesis, and in line with work from Caudoual and associates (6), we found that rosiglitazone, a potent PPAR $\gamma$  agonist, increased the expression of PDK4. Conversely, GW9662, a PPAR $\gamma$  antagonist, prevented rosiglitazone and epinephrine-mediated increases in PDK4. The reduction in epinephrine-induced PDK4 was not secondary to attenuated  $\beta$ -adrenergic signaling because epinephrine-induced increases in p38, AMPK, and HSL phosphorylation were unaffected by GW9662. In combination with the p38 inhibition experiments, these findings provide evidence suggesting the involvement of p38 MAPK and PPAR $\gamma$  in regulating epinephrine-mediated increases in PDK4. What is not clear at this juncture is whether there is a direct link between p38 activation, PPAR $\gamma$ , and increases in PDK4, or whether both p38 MAPK and PPAR $\gamma$  are required for the induction of PDK4 by epinephrine, but do so through distinct pathways. While speculative, the activation of p38 MAPK may activate PGC-1 $\alpha$  (38), which in turn may bind to and coactivate PPAR $\gamma$ , thus leading to the induction of PDK4 in white adipose tissue. Clearly, this is a question that needs to be explored in greater detail in the future.

In summary, we have made the novel observation that exercise, fasting, and epinephrine increase the mRNA expression of PDK4 in rat epididymal adipose tissue. We have further demonstrated a disconnect between the activation of AMPK and the induction of PDK4 mRNA and provide data suggesting that p38 MAPK and PPAR $\gamma$  are involved in the regulation of PDK4 mRNA levels in adipose tissue. These findings provide novel information regarding the biochemical processes regulating PDK4 and importantly highlight alternative, nonpharmaceutical-based approaches that can be used to induce PDK4 mRNA expression.

#### ACKNOWLEDGMENTS

D. C. Wright is an Alberta Heritage Foundation for Medical Research (AHFMR) Scholar, Canadian Diabetes Association Scholar, and Canadian Institutes of Health Research (CIHR) New Investigator. L. N. Sutherland was supported by a studentship from AHFMR and a graduate scholarship from the Natural Sciences and Engineering Research Council of Canada. M. Legare was a recipient of a summer studentship from the Natural Sciences and Engineering Research Council of Canada. D. B. Williams was a recipient of a studentship from the Alberta Diabetes Institute.

#### GRANTS

This research was supported by an Operating Grant from CIHR (to D. C. Wright).

#### DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

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