JPP
Journal of
Pharmacy and Pharmacology

JPP 2009, 61: 1339–1346

Received March 28, 2009
Accepted July 21, 2009
DOI 10.1211/jpp/61.10.0010
ISSN 0022-3573

Research Paper

Comparative study between the effect of the peroxisome proliferator activated receptor-α ligands fenofibrate and n-3 polyunsaturated fatty acids on activation of 5′-AMP-activated protein kinase-α1 in high-fat fed rats

Tarek M. Kamal Motawi[a], Reem M. Hashem[b], Laila A. Rashed[c] and Sabry M. Abd El-Razek[b]

[a]Department of Biochemistry, Faculty of Pharmacy, Cairo University, [b]Beni-Sueif University and [c]Faculty of Medicine, Cairo University, Egypt

Abstract

Objectives Obesity is a risk factor for type 2 diabetes mellitus. It results from an energy imbalance in which energy intake exceeds energy expenditure. The cellular fuel gauge 5′-AMP-activated protein kinase (AMPK) is a heterotrimeric protein consisting of one catalytic subunit (α) and two non-catalytic subunits (β and γ), and approximately equal levels of α1 and α2 complexes are present in the liver. AMPK regulates metabolic pathways in response to metabolic stress and in particular ATP depletion to switch on energy-producing catabolic pathways such as β-oxidation of fatty acids and switch off energy-depleting processes such as synthesis of fatty acid and cholesterol. A high-fat diet alters AMPK-α1 gene expression in the liver and skeletal muscle of rats and results in body weight gain and hyperglycaemia. The aim of this study was to investigate and compare the potential effects of peroxisome proliferator-activated receptor (PPAR)-α agonists fenofibrate and n-3 polyunsaturated fatty acids (PUFAs) in modulation of AMPK-α1 activity in liver and skeletal muscle of high-fat diet fed rats.

Methods Reverse transcription–polymerase chain reaction was used for determination of AMPK-α1 in liver and soleus muscle and both PPAR-α and CPT-1 in hepatic tissues. Serum, total cholesterol, triacylglycerol, fatty acid and fasting blood glucose were determined colorimetrically.

Key findings Both PPAR-α agonists, fenofibrate and n-3 PUFA, increased the mRNA expression of AMPK-α1 activity in liver and skeletal muscle of obese diabetic rats. Fenofibrate was superior in its activation of hepatic mRNA expression of AMPK-α 1 to exert more lipolytic effect and body weight reduction, as estimated through the decrease of triacylglycerol output and serum levels of fatty acid on the one hand and the increase in CPT-1 mRNA expression, the key enzyme in β-oxidation of fatty acid, on the other hand. n-3 PUFA activated AMPK-α1 mRNA expression in skeletal muscle much more than fenofibrate to reveal more hypoglycaemic effect.

Conclusions The PPAR-α agonists fenofibrate and n-3 PUFA could efficiently activate AMPK-α1 mRNA expression in liver and skeletal muscle to exert body weight reduction and hypoglycaemic effect, respectively.

Keywords AMPK-α1; CPT-1; diabetes mellitus; fenofibrate; PPAR-α

Introduction

Obesity is a major risk factor for developing type 2 diabetes mellitus and cardiovascular disease.[1,2] It is attributed to an energy imbalance in which energy intake exceeds energy expenditure.[3] Cellular energy homeostasis is regulated by 5′-AMP-activated protein kinase (AMPK) within individual cells and at the level of the whole body.[4]

The energy sensing/signalling AMPK is a heterotrimeric serine/threonine kinase comprised of a catalytic α and regulatory β and γ subunits. It has multiple isoforms (α1, α2, β1, β2, γ1–γ3); approximately equal levels of α1 and α2 complexes are present in the liver.[5,6] Changes in AMPK-α1, rather than α2, activity may play some role in the

Correspondence: Reem M. Hashem, Department of Biochemistry, Faculty of Pharmacy, Beni-Sueif University, 62514, Egypt.
E-mail: drreem30@yahoo.com

defective contraction-induced glucose metabolism in diabetes.[7] Only changes in α1 activity were affected in skeletal muscle of the Zucker diabetic fatty rat.[8,9]

AMPK is activated by metabolic stresses that either inhibit ATP production or accelerate ATP consumption. Once activated, AMPK phosphorylates its downstream substrates to switch on energy-producing pathways at the expense of energy-depleting processes, such as synthesis of fatty acids and cholesterol.[10,11] AMPK regulates fatty acid metabolism by stimulating fatty acid oxidation through phosphorylation of acetyl CoA carboxylase (ACC) thereby decreasing malonyl CoA levels, which disinhibits carnitine palmitoyltransferase-1(CPT-1) and increases fatty acid entry into mitochondria.[12]

Nuclear peroxisome proliferator-activated receptors (PPAR) are ligand activated metabolic transcription factors containing ligand binding and DNA binding domains, where PPAR is expressed in tissues with high-energy demands. Three PPAR isoforms have been described: α, δ (or β), and γ. PPAR-α is mainly present in liver where it activates a programme of target gene expression involved in fatty acid uptake and β-oxidation.[13]

PPAR-α is activated by natural ligands, including fish oil enriched with n-3 polyunsaturated fatty acids (PUFAs) such as docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), and by synthetic ligands, including fibrates such as fenofibrate.[14]

Recent data suggested that several drugs currently in wide usage in the treatment of obesity and diabetes may act, at least in part, by regulating AMPK activity.[15] These observations reinforced the view that modulation of AMPK activity may be an important means of regulating fuel (specifically glucose and fatty acid) usage in such tissues. In spite of having a very important role in regulation of energy metabolism, PPAR-α agonists have not been linked to the energy sensor molecule, AMPK, as a mediator of its action as yet.

The roles of the PPAR-α agonists, and in particular n-3 PUFA, are not fully understood. Contradictory data was reported considering the role of n-3 PUFA on activation of hepatic AMPK, while their effects on AMPK in skeletal muscle is still obscure.[16,17] Also, fenofibrate prevents and reduces body weight gain and adiposity in diet-induced obese rats, but its role on activation of AMPK has not been studied.[18]

The aim of this study was to throw some light on the potential role of PPAR-α agonists, either natural such as fish oil (n-3 PUFA) or synthetic such as fenofibrate, on activation of the AMPK-α1 subunit in obesity and its complication, type 2 diabetes mellitus lessening in high-fat diet fed rats.

Materials and Methods

Experimental animals

Forty male Wistar rats (150 ± 20 g) were supplied by the Egyptian Organization for Biological Products and Vaccines. Rats were subjected to controlled conditions of temperature (25 ± 2°C) and illumination (12-h light/dark), and allowed free access to normal rat chow diet and water. This protocol was approved by the Animal Care and Use Committee of the Biochemistry Department, Faculty of Pharmacy, Beni-Sueif University.

Experimental design

One week after acclimatization, rats were randomly divided into two main groups. Normal control rats (n = 10) were placed on a control chow diet, which was freely available. The chow consisted of 59% carbohydrate (32% maize starch and 27% sucrose), 31.5% protein (casein) and 9.5% fat (lard). Thirty rats were fed a high-fat diet and to induce obesity food was freely available. The high-fat diet contained 65% carbohydrate, 19% protein and 16% as fat.[19] After 16 weeks, the obese rats were divided into three groups of n = 10 for each and underwent a treatment for six weeks. The first group was administered fenofibrate 100 mg/kg per day orally (Eva pharma, Pyramids, Giza, Egypt). The second group was supplemented with 10% v/w n-3 PUFA as fish oil, which contained eicosapentaenoic acid (C20:5 n-3, EPA) and docosahexaenoic acid (C22:6 n-3, DHA) (Technopharma, New Borg El Arab City, Alexandria, Egypt). The third group was kept as control obese rats. At the end of the 22nd week of the experiment, the rats from each group were anaesthetized with urethane (1.3 mg/kg) and blood samples were collected through retro-orbital bleeding. Serum was separated and samples stored at −20°C for further analysis. The rats were then killed by decapitation and the liver and the soleus muscles were harvested for mRNA determination.

Biochemical methods

Blood total cholesterol, triacylglycerol, and fasting blood glucose were determined enzymatically using commercially available kits (Spinreact, Gerona, Spain). The serum free fatty acids were converted to chloroform-soluble copper salts and measured colormetrically.[20]

Semi-quantitative determination of polymerase chain reaction products

mRNA determination

Muscle and liver tissue samples (approximately 30 mg) were homogenized, and then centrifuged at 15 000 rev/min. The supernatant was examined for detection of gene expression and β-actin was used as an internal control.

RNA extraction

Total RNA was extracted from tissue homogenate using an RNA extraction kit supplied by Promega (Madison, WI, USA), according to the manufacturer's instructions. The extracted RNA was quantitated spectrophotometrically at 260 nm.

Reverse transcription–polymerase chain reaction experiments

Reverse transcription–polymerase chain reaction (RT-PCR) was performed using the extracted RNA for detection of the expression of AMPK-α1, PPAR-α and CPT-1 genes.

The extracted RNA was reverse transcribed into cDNA using an RT-PCR kit (Stratagene, USA). Random primers

Table 1 Primer sequences from the experiment

	Primer sequence	Annealing temperature	Product size
AMPK-α1	Forward 5′-TCAGGCACCCTCATATAATC-3′ reverse 5′-TGACAATAGTCCACACCAGA-3′ R1	65°C	210 bp
CPT-1	Forward: 5′-TATGTGAGGATGCTGCTT-3′ Reverse: 5′-CTCGGAGAGCTAAGCTTG-3′ R2	60°C	629 bp
PPAR-α	Forward: 5′-GGCTCGGAGGGCTCTGTCATC-3′ Reverse: 5′-ACATGCACTGGCAGCAGTGGA-3′ R3	60°C	654 bp

(3 μl) were added to 10 μl RNA which was denatured for 5 min at 65°C in the thermal cycler and cooled to 4°C. The cDNA master mix (buffer, 10 mM dNTPs, 40 U/μl RNase inhibitor, 50 U/μl MMLV-RT enzyme) was added and incubated for 1 h at 37°C, followed by heating to 95°C for 10 min. This was finally cooled at 4°C and then PCR was performed in a total volume of 50 μl. The PCR mixture contained 100 mM Tris HCl (pH 8.8), 500 mM KCl, 1.5 mM $MgCl_2$, primers, 10 mM dNTPs and 5 U Taq polymerase. The reaction mixture was then subjected to 35 cycles of 95°C for 1 min, 55°C for 1 min and 72°C for 2 min. After the last cycle, a final extension at 72°C for 10 min was carried out.

The primer sequences are shown in Table 1.

Agarose gel electrophoresis

All the PCR products of the three genes were electrophoresed on 2% agarose gel, stained with ethidium bromide and visualized using a UV transilluminator.

Gel documentation

The PCR products were semiquantitated using the gel documentation system (Bio Doc Analyzer) supplied by Biometra, Germany. Relative expression of each studied gene (R) was calculated using the following formula: R = Densitometric Units of each studied gene/Densitometric Units of β-actin.

Statistical analysis

All the data are expressed as mean ± SD. Statistical analyses were performed using SPSS software (SPSS Science, Chicago, IL, USA). Analysis of variance with post-hoc LSD test was performed for comparison between the different groups. Pearson correlation was used to study any association between variables. P values < 0.05 were considered statistically significant.

Results

Determination of body weight gain and metabolic parameters

Feeding the rats with a high-fat diet for 16 weeks induced obesity, as demonstrated by significant ($P < 0.001$) body weight gain as compared with normal rats. Supplementation of obese rats with PPAR-α agonists fenofibrate and n-3 PUFAs caused significant weight loss at $P < 0.001$. Fenofibrate caused a greater decrease in weight gain than n-3 PUFAs, but this difference in action was not statistically significant. Body weight gain was more negatively correlated with hepatic AMPK-α1 (r, –0.377, $P < 0.01$) than the isoform in skeletal muscle (r, –0.324, $P < 0.05$). This statistical data clearly linked the reduction in body weight gain to the activation of hepatic AMPK-α1 rather than the muscular isoform. Significant hyperglycaemia was recorded for obese rats when they were compared with the normal rats (167.2 ± 13.2 vs 92.1 ± 7.6, $P < 0.001$), while treatment with fenofibrate or n-3 PUFAs significantly decreased the serum glucose level when compared with obese rats (126.4 ± 10.8 and 104.5 ± 9.66 vs 167.2 ± 13.2, $P < 0.001$, respectively). A more hypoglycaemic effect was exerted by n-3 PUFAs as compared with fenofibrate (104.5 ± 9.66 vs 126.4 ± 10.8, $P < 0.05$, respectively). The hypoglycaemic effect was more closely related to stimulation of AMPK-α1 in the skeletal muscle than the hepatic isoform (r, –0.810 and –0.769, respectively) at a P value less than 0.001. Resultant obesity was accompanied with a significant increment in energy consuming metabolic pathways, whereas high-fat fed rats recorded significant increases in cholesterol (21.5%) and triacylglycerol (130%) ($P < 0.001$) when compared with normal control rats. A significant decrease in the concentration of serum total cholesterol (19.3 and 19.7%, $P < 0.001$) as well as triacylglycerol output (52.3 and 57.17%, $P < 0.001$) as a result of fenofibrate or n-3 PUFA treatment of obese rats, respectively, is shown in Table 2.

Table 2 Effect of fenofibrate and fish oil on body weight gain and metabolic parameters in high-fat diet fed rats

	Normal control	High-fat fed group	Fenofibrate-treated group	Fish oil-treated group
Weight gain (g)	240.5 ± 35.8	315 ± 34.5$^{\#}$	226.7 ± 27.8*	241.6 ± 34.4*
Glucose (mg/dl)	92.1 ± 7.6	167.2 ± 13.2$^{\#}$	126.4 ± 10.8*	104.5 ± 9.6*†
Total cholesterol (mg/dl)	122.8 ± 10.6	149.3 ± 14.3$^{\#}$	120.4 ± 9.2*	119.8 ± 11.1*
Triacylglycerol (mg/dl)	82.3 ± 18.2	188.7 ± 19.4$^{\#}$	86.0 ± 14.4*	81.1 ± 11.5*

Values are expressed as mean ± SD (n = 10). $^{\#}P < 0.001$ compared with normal control group; *$P < 0.001$ compared with high-fat fed group; $^{\dagger}P < 0.001$ compared with fenofibrate-treated group.

Determination of AMPK-α1 in liver

There was a significant 2.7-fold decrease in gene expression of hepatic AMPK-α1 in the high-fat diet fed rats in comparison with normal rats at P value less than 0.001. However, supplementation with PPAR-α agonists fenofibrate and n-3 PUFA significantly increased ($P < 0.001$) mRNA expression of AMPK-α1 in liver by approximately 2- and 1.7-fold, respectively, in comparison with the high-fat fed rat group. Fenofibrate was significantly ($P < 0.05$) more efficient than n-3 PUFA (Figure 1).

Determination of AMPK-α1 in muscle

Figure 2 illustrates the significant ($P < 0.001$) decrease in AMPK-α1 mRNA levels in soleus muscle in the high-fat fed rats, up to 46.73% in comparison with normal control rats. Meanwhile, fenofibrate and n-3 PUFA significantly ($P < 0.001$) increased the mRNA level of AMPK-α1, up to 29 and 38%, respectively, as compared with obese rats. More significant activation of AMPK-α1 gene expression in the skeletal soleus muscle was achieved by n-3 PUFAs as compared with fenofibrate at $P < 0.05$.

Determination of PPA-α in liver

Feeding the rats with the high-fat diet significantly ($P < 0.001$) altered PPAR-α mRNA level in its prominent site of expression, the liver, up to 3.1-fold compared with the normal control group. The synthetic ligand fenofibrate and the natural ligand fish oil, enriched with n-3 PUFAs, induced a significant increase in gene expression of PPAR-α, up to 2- and 1.7-fold, respectively, and in comparison with the high-fat fed group. PPAR-α correlated significantly with hepatic AMPK-α1 and muscle AMPK-α1 (r, 0.793 and 0.751, respectively, $P < 0.001$). It was noteworthy that both of the PPAR-α agonists exerted a significant ($P < 0.001$) and synchronized parallel increase in activation of hepatic AMPK-α1 and PPAR-α. Consequently, fenofibrate was significantly ($P < 0.05$) more efficient than n-3 PUFAs (Figure 3).

Determination of CPT-1 in liver

Figure 4 illustrates the significant ($P < 0.001$) decrease (up to 6%) in gene expression of CPT-1 in high-fat fed rats as

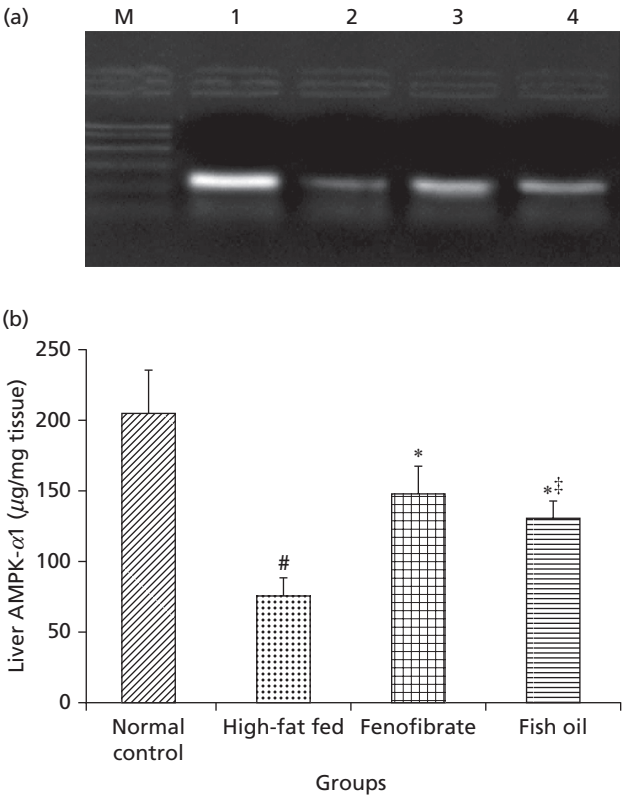

Figure 1 Determination of AMPK-α1 in the liver. (a) Agarose gel electrophoresis showing gene expression of AMPK-α1 in liver by RT-PCR. Lane M, PCR marker at 100 pb; lane 1, normal control; lane 2, high-fat fed group; lane 3, high-fat fed rats treated with fenofibrate; lane 4, high-fat fed rats treated with fish oil. (b) Effect of fenofibrate and fish oil on concentration of PCR products of gene expression of AMPK-α1 in liver in high-fat fed rats. Values are expressed as mean ± SD, $n = 10$, $^{\#}P < 0.001$ compared with normal control group, $^{*}P < 0.001$ compared with high-fat fed rat group, $^{\ddagger}P < 0.05$ compared with fenofibrate-treated group.

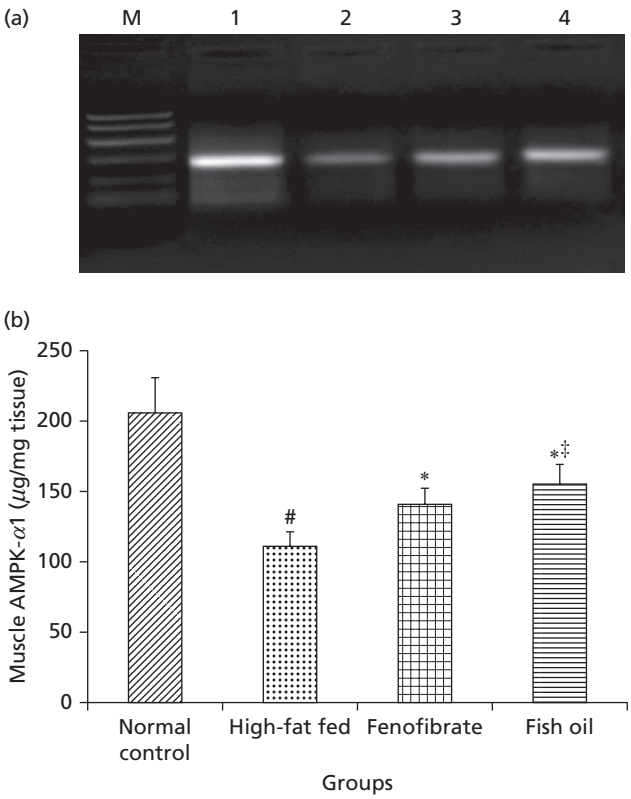

Figure 2 Determination of AMPK-α1 in muscle. (a) Agarose gel electrophoresis showing gene expression of AMPK-α1 in muscle by RT-PCR. Lane M, PCR marker at 100 pb; lane 1, normal control; lane 2, high-fat fed group; lane 3, high-fat fed rats treated with fenofibrate; lane 4, high-fat fed rats treated with fish oil. (b) Effect of fenofibrate and fish oil on concentration of PCR products of gene expression of AMPK-α1 in muscle in high-fat fed rats. Values are expressed as mean ± SD, $n = 10$, $^{\#}P < 0.001$ compared with normal control group, $^{*}P < 0.001$ compared with high-fat fed group, $^{\ddagger}P < 0.05$ compared with fenofibrate-treated group.

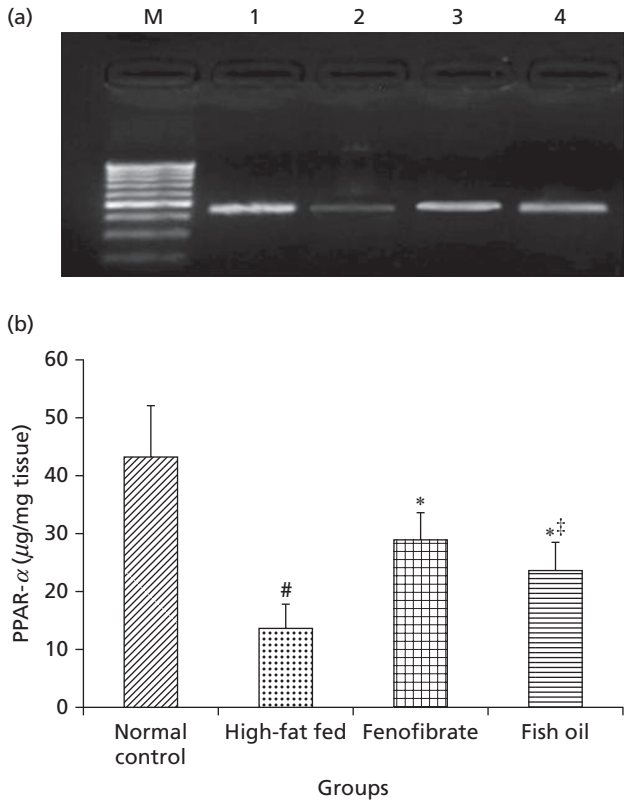

Figure 3 Determination of PPAR-α in liver. (a) Agarose gel electrophoresis showing gene expression of PPAR-α by RT-PCR. Lane M, PCR marker at 100 pb; lane 1, normal control; lane 2, high-fat fed group; lane 3, high-fat fed rats treated with fenofibrate; lane 4, high-fat fed rats treated with fish oil. (b) Effect of fenofibrate and fish oil on concentration of PCR products of gene expression of PPAR-α in high-fat fed rats. Values are expressed as mean ± SD, $n = 10$, $^{\#}P < 0.001$ compared with normal control group, $^{*}P < 0.001$ compared with high-fat fed group, $^{\ddagger}P < 0.05$ compared with fenofibrate-treated group.

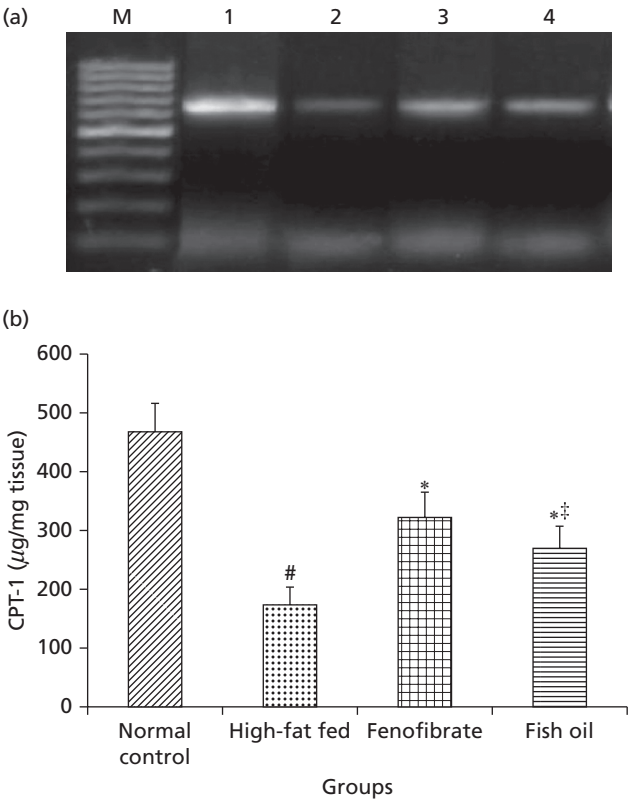

Figure 4 Determination of CPT-1 in liver. (a) Agarose gel electrophoresis showing gene expression of CPT-1 by RT-PCR. Lane M, PCR marker at 100 pb; lane 1, normal control; lane 2, high-fat fed group; lane 3, high-fat fed rats treated with fenofibrate; lane 4, high-fat fed rats treated with fish oil. (b) Effect of fenofibrate and fish oil on concentration of PCR products of gene expression of CPT-1 in high-fat fed rats. Values are expressed as mean ± SD, $n = 10$, $^{\#}P < 0.001$ compared with normal control group, $^{*}P < 0.001$ compared with high-fat fed group, $^{\ddagger}P < 0.05$ compared with fenofibrate-treated group.

compared with normal rats. Fenofibrate and n-3 PUFAs significantly induced the gene expression of CPT-1 by up to 87.4 and 56%, at $P < 0.001$, respectively, as compared with the high-fat fed group. The key enzyme of β-oxidation, CPT-1, was significantly correlated with AMPK-α1 in liver and skeletal muscle (r, 0.876 and 0.833, respectively, $P < 0.001$). Obese rats administered fenofibrate showed a significant increase in the level of CPT-1 mRNA compared with n-3 PUFA-treated rats at $P < 0.05$.

Determination of serum free fatty acid level

A significant increase of 110% in fatty acid synthesis, one of the energy consuming pathways, was estimated in the high-fat diet fed rats as compared with normal control rats at $P < 0.001$. Nevertheless, fenofibrate and n-3 PUFAs demonstrated significant ($P < 0.001$) decreases by up to 53.6 and 46.3%, respectively, in comparison with obese control rats. Furthermore, the potential effect in decreasing the serum free fatty acid level was attributed to fenofibrate as compared with n-3 PUFAs at $P < 0.05$ (Figure 5). Moreover free fatty acid showed a higher significant negative correlation with hepatic AMPK-α1 as compared with muscle AMPK-α1 (r, –0.755 and –0.657, respectively, $P < 0.001$).

Discussion

High fat feeding altered the AMPK-α1 signalling pathway in muscle and liver. Nevertheless, further altering of AMPK activity in diet-induced obesity could have therapeutic effects because AMPK activators have been shown to cause weight loss in some obese rodent models. Those data suggested that AMPK may be a key player in the development of new treatments for obesity and type 2 diabetes mellitus.[15,21]

PPAR-α is expressed predominantly in liver and, to a lesser extent, in heart, skeletal muscle, and kidney, where it appears to play a crucial role in intracellular lipid metabolism.[22,23] The activation of PPAR-α upregulates the expressions of several catabolic enzymes that are involved in mitochondrial and peroxisomal β-oxidation. PPAR-α agonists fundamentally regulate β-oxidation of fatty acids, and the n-3 PUFAs and fenofibrate are well known activators of PPAR-α.[24,25]

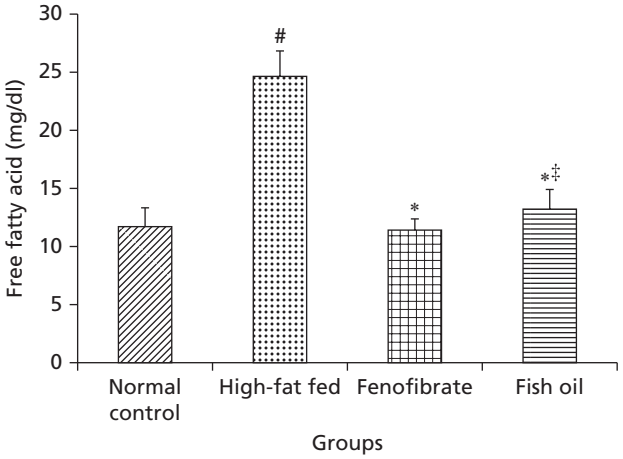

Figure 5 Effect of fenofibrate and fish oil on serum free fatty acid in high-fat fed rats Values are expressed as mean ± SD, $n = 10$. $^{\#}P < 0.001$ vs normal control group, $^{*}P < 0.001$ vs high-fat fed group, $^{\ddagger}P < 0.05$ vs fenofibrate-treated group.

Since Suchankova *et al*.[17] demonstrated that AMPK was the mediator of action of PUFAs and because PPAR-α agonists participated with AMPK in possessing the same properties, as 'fuel partitioners' via their unique ability to partition fatty acid away from lipid synthesis towards fatty acid oxidation, it led us to suppose that fenofibrate may also regulate AMPK as a mediator of its action. Our study demonstrated significant correlation between mRNA expression of PPAR-α and AMPK-α1 in liver. Recent studies showed that PUFAs and fenofibrate could regulate CPT-1 mRNA expression.[26,27] Accordingly, supplementation of obese rats with the PPAR-α agonists induced significant weight reduction in response to increased hepatic CPT-1 mRNA expression; these mechanisms are thought to be mediated through stimulation of hepatic AMPK, since a parallel increase was observed between stimulation of both mRNA expression of PPAR-α and AMPK-α1 in liver. In corroboration with our results, Suchankova *et al*.[17] reported that the natural ligand of PPAR-α receptors, fish oil, enriched with n-3 PUFA, enhanced hepatic AMPK activity *in vivo*, and implicated AMPK as a component of the nutrient-sensing mechanism through which n-3 PUFA influenced the regulation of hepatic lipid metabolism and gene expression. Others have documented that constitutive expression of hepatic AMPK-α1 completely abrogated the effects of glucose to increase the levels of mRNAs encoding lipogenic enzymes, and estimated that hepatic activation of AMPK would appear as an efficient silencer of lipogenic gene expression.[6] Moreover, recent evidence documented that chronic activation of AMPK-α1 in liver led to decreased adiposity in mice; indeed, both of the PPAR-α agonists recorded hypolipidaemic effect through decreased triacylglycerol output and marked weight reduction.[28] However, fenofibrate ingestion induced more activation of hepatic AMPK-α1 and PPAR-α than fish oil to cause more weight reduction, but the reduction was not significant. Fenofibrate was superior in switching on ATP consuming pathways, whereas enhanced free fatty acid oxidation through increased CPT-1 mRNA expression and decreased serum levels of free fatty acid were observed.

High-fat diet supplementation resulted in hyperglycaemia due to elevated plasma free fatty acid levels and decreased intracellular free fatty acid oxidation, as revealed by increased serum levels of free fatty acid and decreased expression of CPT-1 mRNA.[29] McGarry[30] documented that accumulation of free fatty acid in insulin target organs led to insulin resistance and type 2 diabetes mellitus. Actually our laboratory recorded in a previous study that feeding rats with a high-fat diet for 16 weeks caused type 2 diabetes mellitus and insulin resistance.[31] Desvergne and Wahli[14] reported that acute elevation of free fatty acid moderately stimulated insulin release, and chronic exposure to free fatty acid impaired insulin secretion through stimulation of β-oxidation.[32] PPAR-α agonists might decrease tissue lipid content, thus preventing lipid accumulation and toxicity.[33] Both PUFA and fenofibrate recorded significant hypoglycaemic effects but n-3 PUFA was more potent than fenofibrate in stimulation of AMPK-α1 in skeletal muscle to reflect a more efficient hypoglycaemic effect. Skeletal muscle is a principal site of glucose and free fatty acid use, and is one of the primary tissues responsible for insulin resistance in obesity and type 2 diabetes mellitus. Activation of skeletal muscle AMPK now offers a new approach to combating insulin resistance by promoting glucose uptake and reducing lipid synthesis and ATP consuming pathways.

Barnes *et al*.[8] demonstrated that the obesity related type 2 diabetes mellitus in the Zucker fatty rat was associated with an isoform-specific impairment in AMPK-α1 activation in skeletal muscle in response to contraction.[9] Uptake of glucose into skeletal muscle accounts for >70% of glucose disposal, so this process is of paramount importance for normal glucose homoeostasis.[34,35] Moreover, glucose uptake into skeletal muscle is markedly inhibited in subjects with type 2 diabetes mellitus *in vivo* and in skeletal muscle isolated from patients with diabetes.[36,37] Glucose uptake is stimulated both by insulin, largely through the recruitment to the plasma membrane of the glucose transporter Glut4, and during muscle contraction by an insulin-independent mechanism.[38–40] AMPK may play an important role in Glut4 translocation to the cell surface, at least in the response to exercise.[41,42] Furthermore, the action of a 'hepatoportal vein glucose sensor', which appears to mediate an insulin-independent uptake of glucose into muscle following increases in the portal glucose concentration after a meal, requires the presence of AMPK in muscle.[43,44]

Conclusions

PPAR-α agonists, either natural as fish oil or synthetic as fenofibrate, could efficiently activate AMPK-α1 mRNA expression in skeletal muscle and liver. Fish oil was superior to fenofibrate in its induction of AMPK-α1 mRNA in skeletal muscle to reveal its hypoglycaemic effect, however fenofibrate showed a marked effect on weight reduction. Fenofibrate activated hepatic AMPK-α1 more than fish oil.

Declarations

Conflict of interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

Funding

This research/review received no specific grant from any funding agency in the public, commercial or not-for-profit sectors.

References

1. Friedman JM. Obesity in the new millennium. *Nature* 2000; 404: 632–634.
2. Kopelman PG. Obesity as a medical problem. *Nature* 2000; 404: 635–643.
3. Hellström PM *et al*. Peripheral and central signals in the control of eating in normal, obese and binge-eating human subjects. *Br J Nutr* 2004; 92(Suppl. 1): S47–S57.
4. Carling D. The role of the AMP-activated protein kinase in the regulation of energy homeostasis. *Novartis Found Symp* 2007; 286: 72–81; discussion 81–85, 162–163, 196–203.
5. Stapleton D *et al*. AMP-activated protein kinase isoenzyme family: subunit structure and chromosomal location. *FEBS Lett* 1997; 409: 452–456.
6. Woods A *et al*. Characterization of the role of AMP-activated protein kinase in the regulation of glucose-activated gene expression using constitutively active and dominant negative forms of the kinase. *Mol Cell Biol* 2000; 20: 6704–6711.
7. Lev S *et al*. Protein tyrosine kinase PYK2 involved in Ca2+-induced regulation of ion channel and MAP kinase functions. *Nature (London)* 1995; 376: 737–745.
8. Barnes BR *et al*. Isoform-specific regulation of 5′ AMP-activated protein kinase in skeletal muscle from obese Zucker (fa/fa) rats in response to contraction. *Diabetes* 2002; 51: 2703–2708.
9. Rutter GA *et al*. Roles of 5′-AMP-activated protein kinase (AMPK) in mammalian glucose homoeostasis. *Biochem J* 2003; 375: 1–16.
10. Thomson DM *et al*. AMP-activated protein kinase phosphorylates transcription factors of the CREB family. *J Appl Physiol* 2008; 104: 429–438.
11. Hardie DG *et al*. AMP-activated protein kinase–development of the energy sensor concept. *J Physiol* 2006; 574(Pt 1): 7–15.
12. Saha AK, Ruderman NB. Malonyl-CoA and AMP-activated protein kinase: an expanding partnership. *Mol Cell Biochem* 2003; 253: 65–70.
13. Cignarella A *et al*. Hypolipidemic therapy for the metabolic syndrome. *Pharmacol Res* 2006; 53: 492–500.
14. Desvergne B, Wahli W. Peroxisome proliferator-activated receptors: nuclear control of metabolism. *Endocr Rev* 1999; 20: 649–688.
15. Misra P. AMP activated protein kinase: a next generation target for total metabolic control. *Expert Opin Ther Targets* 2008; 12: 91–100.
16. Dobrzyn A *et al*. Polyunsaturated fatty acids do not activate AMP-activated protein kinase in mouse tissues. *Biochem Biophys Res Commun* 2005; 332: 892–896.
17. Suchankova G *et al*. Dietary polyunsaturated fatty acids enhance hepatic AMP-activated protein kinase activity in rats. *Biochem Biophys Res Commun* 2005; 326: 851–858.
18. Mancini FP *et al*. Fenofibrate prevents and reduces body weight gain and adiposity in diet-induced obese rats. *FEBS Lett* 2001; 491: 154–158.
19. Naderali EK *et al*. Fenofibrate lowers adiposity and corrects metabolic abnormalities, but only partially restores endothelial function in dietary obese rats. *Atherosclerosis* 2004; 177: 307–312.
20. Duncombe WG. The colorimetric micro-determination of long-chain fatty acids. *Biochem J* 1963; 88: 7–10.
21. Santomauro Júnior AC *et al*. Metformin and AMPK: an old drug and a new enzyme in the context of metabolic syndrome. *Arq Bras Endocrinol Metabol* 2008; 52: 120–125.
22. Braissant O *et al*. Differential expression of peroxisome proliferator-activated receptors (PPARs): tissue distribution of PPAR-alpha, -beta, and -gamma in the adult rat. *Endocrinology* 1996; 137: 354–366.
23. Willson TM *et al*. The PPARs: from orphan receptors to drug discovery. *J Med Chem* 2000; 43: 527–550.
24. Cho MC *et al*. Peroxisome proliferator-activated receptor (PPAR) modulators and metabolic disorders. *PPAR Res* 2008; 2008: 679137.
25. Yu S *et al*. Peroxisome proliferator-activated receptors, fatty acid oxidation, steatohepatitis and hepatocarcinogenesis. *Curr Mol Med* 2003; 3: 561–572.
26. Morash AJ *et al*. Effects of dietary fatty acid composition on the regulation of carnitine palmitoyltransferase (CPT) I in rainbow trout (*Oncorhynchus mykiss*). *Comp Biochem Physiol B Biochem Mol Biol* 2009; 152: 85–93.
27. Bai XP *et al*. Effects of fenofibrate on gene expression of carnitine palmitoyltransferase 1 in liver and skeletal muscle and its influence on insulin sensitivity. *Zhonghua Yi Xue Za Zhi* 2008; 88: 268–270.
28. Yang J *et al*. Chronic activation of AMP-activated protein kinase-alpha1 in liver leads to decreased adiposity in mice. *Biochem Biophys Res Commun* 2008; 370: 248–253.
29. Lawrence VJ, Kopelman PG. Medical consequences of obesity. *Clin Dermatol* 2004; 22: 296–302.
30. McGarry JD. Banting lecture 2001: dysregulation of fatty acid metabolism in the etiology of type 2 diabetes. *Diabetes* 2002; 51: 7–18.
31. Hashem RM *et al*. Interleukin-10 to tumor necrosis factor-alpha ratio is a predictive biomarker in nonalcoholic fatty liver disease: interleukin-10 to tumor necrosis factor-alpha ratio in steato-hepatitis. *Eur J Gastroenterol Hepatol* 2008; 10: 995–1001.
32. Zhou YP, Grill V. Long term exposure to fatty acids and ketones inhibits B-cell functions in human pancreatic islets of Langerhans. *J Clin Endocrinol Metab* 1995; 80: 1584–1590.
33. Blaschke F *et al*. Obesity, peroxisome proliferator-activated receptor, and atherosclerosis in type 2 diabetes. *Arterioscler Thromb Vasc Biol* 2006; 26: 28–40.
34. DeFronzo RA *et al*. Pathogenesis of NIDDM. A balanced overview. *Diabetes Care* 1992; 15: 318–368.
35. Shulman GI *et al*. Quantitation of muscle glycogen synthesis in normal subjects and subjects with non-insulin-dependent diabetes by 13C nuclear magnetic resonance spectroscopy. *N Engl J Med* 1990; 322: 223–228.
36. DeFronzo RA *et al*. Effects of insulin on peripheral and splanchnic glucose metabolism in noninsulin-dependent (type II) diabetes mellitus. *J Clin Invest* 1985; 76: 149–155.
37. Dohm GL *et al*. An in vitro human muscle preparation suitable for metabolic studies. Decreased insulin stimulation of glucose transport in muscle from morbidly obese and diabetic subjects. *J Clin Invest* 1988; 82: 486–494.
38. Oatey PB *et al*. GLUT4 vesicle dynamics in living 3T3 L1 adipocytes visualized with green-fluorescent protein. *Biochem J* 1997; 327: 637–642.

39. Ryder JW *et al*. Intracellular mechanisms underlying increases in glucose uptake in response to insulin or exercise in skeletal muscle. *Acta Physiol Scand* 2001; 171: 249–257.
40. Goodyear LJ, Kahn BB. Exercise, glucose transport, and insulin sensitivity. *Annu Rev Med* 1998; 49: 235–261.
41. Koistinen HA *et al*. 5-Amino-imidazole carboxamide riboside increases glucose transport and cell-surface GLUT4 content in skeletal muscle from subjects with type 2 diabetes. *Diabetes* 2003; 52: 1066–1072.
42. Kurth-Kraczek EJ *et al*. 5′ AMP-activated protein kinase activation causes GLUT4 translocation in skeletal muscle. *Diabetes* 1999; 48: 1667–1671.
43. Moore MC, Cherrington AD. The nerves, the liver, and the route of feeding: an integrated response to nutrient delivery. *Nutrition* 1996; 12: 282–284.
44. Burcelin R *et al*. GLUT4, AMP kinase, but not the insulin receptor, are required for hepatoportal glucose sensor-stimulated muscle glucose utilization. *J Clin Invest* 2003; 111: 1555–1562.