Effects of Glycyrrhetic Acid (GE) on Some Gluconeogenic Enzymes, Lipoprotein Lipase and Peroxisome Proliferator-Activated Receptors Alpha and Gamma

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Abstract: The aim of this study was to examine the role of glycyrrhetic acid (GE) as a potential compound in the amelioration of metabolic syndrome. Rats given intraperitoneal injection of GE were sacrificed after 24 hours. Blood was collected for the determination of glucose, insulin and lipid profiles; while tissues were used for 11β-HSD1, gluconeogenic enzymes activities, PPAR-α/-γ and LPL expression by RT-PCR. Intraperitoneal injection of 50mg/kg GE to normal rats significantly lowered blood glucose while insulin level and HOMA-IR showed no significant changes. H6PDH activities increased in the liver, kidney, subcutaneous and visceral adipose tissues and quadriceps femoris but decreased in the abdominal muscle. PEPCK activities were significantly reduced in the kidney and decreased in the liver but showed an increase in the subcutaneous and visceral adipose tissues. G6Pase activities were found to be reduced in both the liver and kidney. 11β-HSD1 activities increased in the liver but decreased in all other tissues. There were improvements in lipid profiles in GE-treated rats. Up-regulation of LPL activity was seen in all tissues except quadriceps femoris. PPAR-α expression was up-regulated in the liver, heart and abdominal muscle while down-regulated in the kidney and quadriceps femoris but were undetectable in the subcutaneous and visceral adipose tissues. PPAR-γ expression was up-regulated in all tissues except the kidney. GE prevented hyperglycaemia and improved lipid profiles possibly through 11β-HSD1 inhibition instead of via PPAR agonism.

Keywords: Glycyrrhetic acid, glucose-6-phosphatase (G6Pase), hexose-6-phosphate dehydrogenase (H6PDH), lipoprotein lipase, phosphoenolpyruvate kinase (PEPCK), peroxisome proliferator-activated receptors (PPAR), 11β-hydroxysteroid dehydrogenase (11β-HSD1).

INTRODUCTION

The metabolic syndrome (MetS) is a cluster of risk factors which are dyslipidaemia, hyperglycaemia, hypertension, central obesity and insulin resistance (IR) [1]. When an individual has three out of these five factors, he/she will be considered to have MetS and is at risk of getting cardiovascular disease (CVD) and type 2 diabetes mellitus (T2DM) [1]. The adaptation of obesogenic diet and sedentary lifestyle has been recognized as the major contributor to MetS which can be related to rapid urbanization and improved socioeconomic status [2]. Obesity, especially increased accumulation of fat in the visceral region, has been shown to have a strong link with dyslipidaemia, IR, hypertension, T2DM and CVD [3,4]. This can be associated with increased free fatty acids (FFA) release into the portal circulation, thus impairing hepatic metabolism followed by systemic IR and metabolic derangements [5].

Lipoprotein lipase (LPL) is an enzyme involved in lipid metabolism. It hydrolyzes the triacylglycerol (TAG) store within lipoproteins and facilitates uptake of fatty acids by the peripheral tissues [6]. Low LPL activity inhibits chylomicrons and very-low-density lipoprotein (VLDL) lipolysis. This contributes to excessive TAG level in the circulation and can lead to dyslipidaemia [7]. LPL expression is regulated by the peroxisome proliferator-activated receptor (PPAR) under different physiological conditions [8].

Glucocorticoids (GC) are important regulators of carbohydrate and lipid metabolism [9]. Alterations of GC level have been related to obesity and IR which could finally lead to MetS [10]. 11β-hydroxysteroid dehydrogenase (11β-HSD) is an enzyme that catalyzes the inter-conversion of active GC and inactive GC [10]. One of the major effects of GC is the increase in hepatic glucose production due to their opposing actions on insulin that counteracts the increased level of gluconeogenic enzymes which include phosphoenolpyruvate carboxykinase (PEPCK) and glucose-6-phosphatase (G6Pase) [11,12]. Hexose-6-phosphate dehydrogenase (H6PDH), an enzyme found in the endoplasmic reticulum lumen, is involved in the pentose phosphate pathway. H6PDH catalyzes the i.) conversion of glucose-6-phosphate (G6P) to 6-phosphogluconolactone (6PGL) and ii.) generation of reduced adenine dinucleotide phosphate (NADPH) from $NADP^+$ [13,14]. 11-βHSD1 converts inactive GC to active GC. This reaction utilizes NADPH produced from the reaction catalyzed by H6PDH, which in turn depend on the

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availability of G6P [10,15]. Overexpressions of H6PDH, PEPCK and G6pase have been shown in T2DM patients where GC were in excess [11,12].

The peroxisome proliferator-activated receptors (PPAR) is a group of ligand-activated transcription factors from the nuclear receptor superfamily that controls the expression of various genes involved in glucose and lipid metabolism e.g. PPAR-α, PPAR-β/δ and PPAR-γ [16]. PPAR-α is predominantly expressed in tissues with a high capacity for fatty acid oxidation (FAO) which include the liver, heart, kidney and skeletal muscle [17]. PPAR-γ is mainly involved in adipogenesis and storage of FFA in the adipose tissues [16]. PPAR-γ can be found abundantly in the adipose tissues and large intestine while existing at intermediate levels in the kidney, liver as well as small intestine [18].

Fibrates (a PPAR-α agonist) are a widely used class of lipid-modifying agents for dyslipidaemia while thiazolidinediones (TZDs) (a PPAR-γ agonist) are a class of insulin-sensitizing agents used in T2DM patients [19]. Combined therapy of fibrates and TZDs is often used since most patients diagnosed with T2DM also suffer from dyslipidaemia [20]. Several PPAR-α/-γ agonists with dual actions had been tested on subjects which showed significant anti-hyperglycaemic and anti-hyperlipidaemic effects. However, some of these dual PPAR-α/PPAR-γ agonists were withdrawn at the late-stage of drug development due to safety concerns, for example, in 2004, ragaglitazar was discontinued as it was found to induce anaemia and urothelial cancer [20].

Despite the effectiveness of fibrates and TZDs in the treatment of dyslipidaemia and T2DM, both classes of these drugs have been associated with unwanted side effects. Cases of rhabdomyolysis and cholesterol gall stone formation have been reported with the use of fibrates while the most common side effects of TZDs are weight gain and fluid retention [21,22]. Therefore, potential PPAR-α and PPAR-γ ligands from natural sources e.g. glycyrrhetic acid (GE) are being looked into.

GE, a triterpenoid aglycone, is an active metabolite of glycyrrhizic acid (GA) which is the primary active ingredient of the root extract of licorice plant, *Glycyrrhiza glabra* [23,24]. GA has been found to lower blood glucose in rats [6,25,26]. Both GA and GE are known inhibitors of both isoforms of 11β-HSD i.e. 11β-HSD1 and 11β-HSD2 [27,28], thus resulting in reduced GC level. Such alteration in GC action could be utilized as the therapeutic strategy for T2DM. Furthermore, triterpenoids have been found to be PPAR agonists [29,30]. GE, as a triterpenoid compound with reported inhibitory effects 200-1000 times more potent than GA on 11β-HSD1 [31] may exert anti-dyslipidaemic and anti-diabetic effects at a lower concentration.

As GE has been reported to be 200-1000 times more potent than GA [31], the roles of GE in modulating glucose and lipid metabolism were being looked into in this study. The parameters measured include:

i.) blood glucose, serum insulin and Homeostatic-Model Assessment for insulin resistance (HOMA-IR),

ii.) lipid profiles

iii.) LPL, PPAR-α and PPAR-γ expression

iv.) PEPCK, H6PDH, G6Pase activities

v.) 11β-HSD1 activities.

MATERIALS AND METHODS

Animal Sampling and Treatment

All animal procedures were approved by Monash University School of Biomedical Sciences Animal Ethics Committee (AEC Approval number MARP/2012/043). Sixteen male Sprague-Dawley rats (180-200g) were supplied by Monash University Sunway Campus Animal House. The rats were divided into two groups (8 rats per group) i.e. the control and treated groups. All rats were kept in individual polypropylene cages in a room maintained at 24°C and exposed to 12h light- 12h dark cycle. The rats from the control group were given intraperitoneal (IP) injection of 99% dimethyl sulfoxide (DMSO) while rats from the treated group were given IP injection of 50mg/kg GE in 99% DMSO. After injection, the rats from both groups were fed *ad libitum* with standard rat chow (Gold Coin, Malaysia) and drinking water.

Sample Collection

Upon completion of 24 hours treatment, the rats were subjected to overnight fast and anaesthetized by intraperitoneal injection of pentobarbital sodium (Nembutal) (150mg/kg). The blood and plasma were collected and plasma was stored at -80°C. Liver, kidney, heart, subcutaneous adipose tissue (SAT), visceral adipose tissue (VAT), abdominal muscle (AM) and quadriceps femoris (QM) were harvested and stored at -80°C until required for further analysis.

Blood and Serum Biochemical Analysis

Blood glucose was measured using Trinder's glucose oxidase reaction while serum insulin was determined using Rat/Mouse Insulin ELISA (Milipore, USA). Serum FFA, TAG and total cholesterol were determined using the Randox FA115 Non-Esterified Fatty Acids kit (Randox, UK), Randox Triglycerides Kit (Randox, UK) and Randox CH200 Cholesterol Kit (Randox, UK) respectively. HDL-Cholesterol and LDL-Cholesterol were determined using Randox CH203 HDL Precipitant (Randox, UK) and Friedewald formula [32], respectively.

RNA Extraction and cDNA Synthesis

Qiagen RNeasy Mini Kit (Qiagen, USA) and Qiagen RNeasy Lipid Tissue Mini Kit (Qiagen, USA) were used for extraction of RNA from the liver, kidney, heart, AM and QF; and SAT and VAT, respectively. The concentration and purity of RNA were determined by measuring the absorbance at 260 and 280nm. cDNA synthesis was done using the Qiagen Omniscript Reverse Transcriptase Kit (Qiagen, USA).

Real Time Reverse Transcription Polymerase Chain Reaction qRT-PCR of Lipoprotein lipase (LPL), Peroxisome proliferator-Activated Receptor-α and -γ (PPAR-α and PPAR-γ) Gene

The comparisons of LPL, PPAR-α and PPAR-γ expressions between control and GE-treated rats were performed using the Comparative Ct (ΔΔCt) Method, with BAC as reference, GE-treated group as target and control group as calibrator [33]. Agarose gel electrophoresis was performed on the amplicons obtained from qRT-PCR to ensure primer specificity. The expression of LPL, PPAR-α and PPAR-γ was determined by qRT-PCR using the probe, forward and reverse primers that are specific for *Rattus norvegicus* as listed below:

Table 1. The Probe, Forward and Reverse Primers for LPL, PPAR-α and PPAR-γ Specific for *Rattus norvegicus*

Primer/probe	Primer sequence (5' → 3')
PPAR-α forward primer	TGTGGAGATCGGCCTGGCCTT
PPAR-α reverse primer	CCGGATGGTTGCTCTGCAGGT
PPAR-α probe	(6-FAM) TGCAG-GAGGGGATTGTGCACGTGCTCA (BHQ1)
PPAR-γ forward primer	CCCTGGCAAAGCATTTGTAT
PPAR-γ reverse primer	GGTGATTTGTCTGTTGTCTTTCC
PPAR-γ LNA probe	(6-FAM) TCCTTCCCGCTGACCA (BHQ1)
BAC forward primer	GTATGGGTCAGAAGGACTCC
BAC reverse primer	GTTCAATGGGGTACTTCAGG
BAC LNA probe	(TET) CCTCTCTTGCTCTGGGC (BHQ1)
LPL forward primer	CAGCAAGGCATACAGGTG
LPL reverse primer	CGAGTCTTCAGGTACATCTTAC
LPL LNA probe	(6-FAM) TTCTCTTGGCTCTGACC (BHQ1)

Protein Determination

Tissue samples were homogenized using Heidolph DIAX 900 rotor stator homogenizer (Sigma-Aldrich, U.S.A) prior to enzyme activity determination and centrifuged to obtain the appropriate fraction. A modified Lowry's method was used to determine the protein concentration [6].

Determination of 11β-Hydroxysteroid Dehydrogenase (11β-HSD1) Activities

The 11β-hydroxysteroid dehydrogenase (11β-HSD1) activities were determined using high performance liquid chromatography (HPLC) following methods described by Chandramouli *et al.* [6]. A unit of 11β-HSD1 activity is defined as one pmole of 11-dehydrocorticosterone produced/50 mg of tissue protein used/ hour [34].

Phosphoenolpyruvate Carboxykinase (PEPCK), Hexose-6-Phosphate Dehydrogenase (H6PDH), Glucose-6-Phosphatase (G6Pase) Activities

Measurement of PEPCK activities in the liver, kidney, SAT and VAT was done as previously reported [35]. One unit of PEPCK activity was defined as one nmole of NADH oxidized/min/mg protein. H6PDH assay was performed based on the method of Banhegyi *et al.* [36]. One unit of H6PDH activity was defined as one nmole of NADPH formed/minute/mg protein. Determination of G6Pase activities in the liver and kidney were carried out following the protocol by Csala *et al.* [37]. One unit of G6Pase activity was expressed as one nmole of inorganic phosphate produced/minute/mg protein.

Statistical Analysis

The Relative Expression Software Tool (REST©) MCS Beta 2006 and Statistical Package for the Social Science (SPSS) Version 16.0 were used for the statistical analysis of the LPL, PPAR-α and PPAR-γ expressions and all the other data, respectively. Data distribution was determined using Kolmogorov-Smirnov test. Parametric data was analyzed using 2 sample t-test while non-parametric data was analysed with Mann Whitney U-test. A P-value$\leq$ 0.05 was considered statistically significant.

RESULTS

GE Treatment Led to Improved Insulin Sensitivity in Rats

The mean fasting blood glucose concentrations of the control and GE-treated groups were 8.80 ± 0.51 mmol/L and 6.82 ± 0.39 mmol/L respectively with the GE-treated group being significantly lower than the control group by 22.5% ($P<0.01$) (Fig. **1**). The mean insulin levels of the control and GE-treated groups were 0.90 ± 0.22 ng/mL and 0.95 ± 0.31 ng/mL respectively (Fig. **2**). The HOMA-IR index for the control and GE-treated groups were 0.58 ± 0.07 and 0.52 ± 0.15 respectively with the GE-treated group being 10% lower than the control group, although the results were insignificant ($P>0.05$) (Fig. **3**). There was no significant difference in the insulin level and HOMA-IR index between the control and GE-treated groups ($P>0.05$).

Hexose-6-Phosphate Dehydrogenase (H6PDH) Activities

There was no significant difference for H6PDH activities in the liver between the control (0.58 ± 0.07 units) and GE-treated groups (0.90 ± 0.18 units) ($P>0.05$) as well as the kidney between the two groups with the control group being 3.22 ± 0.44 units and the GE-treated group being 3.29 ± 0.28 units respectively ($P>0.05$). In the SAT, the mean H6PDH activities in the control and GE-treated groups were 6.04 ± 0.50 units and 7.76 ± 0.84 units respectively while in the VAT, the mean activities in control and GE-treated groups were 4.18 ± 0.61 units and 4.99 ± 0.66 units respectively ($P>0.05$) (Fig. **4**). In the abdominal muscle (AM), the mean H6PDH activities in the control and GE-treated groups were 4.79 ± 0.51 units and 4.12 ± 0.36 units respectively while in the quadriceps femoris muscle (QF), the mean H6PDH activities in the control and GE-treated groups were 5.12 ± 0.33 units and 6.15 ± 0.58 units respectively ($P>0.05$) (Fig. **4**).

Glucose-6-Phosphatase (G6Pase) Activities in the Liver and Kidney

The mean hepatic G6Pase activities in the control and GE-treated rats were 140.45 ± 5.33 units and 137.24 ± 7.77

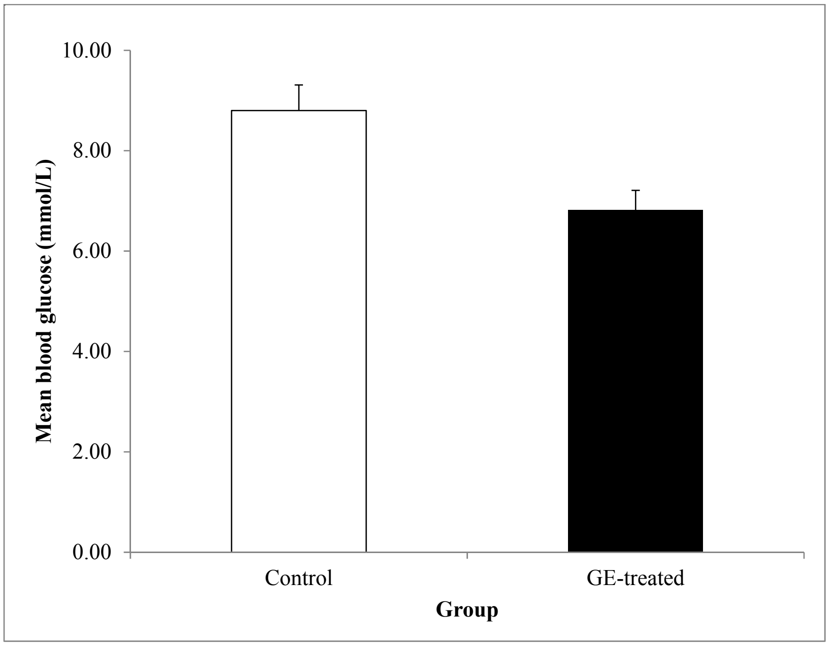

Fig. (1). Mean blood glucose concentration (mmol/L) of control and GE-treated groups (** indicates P<0.01 between groups).

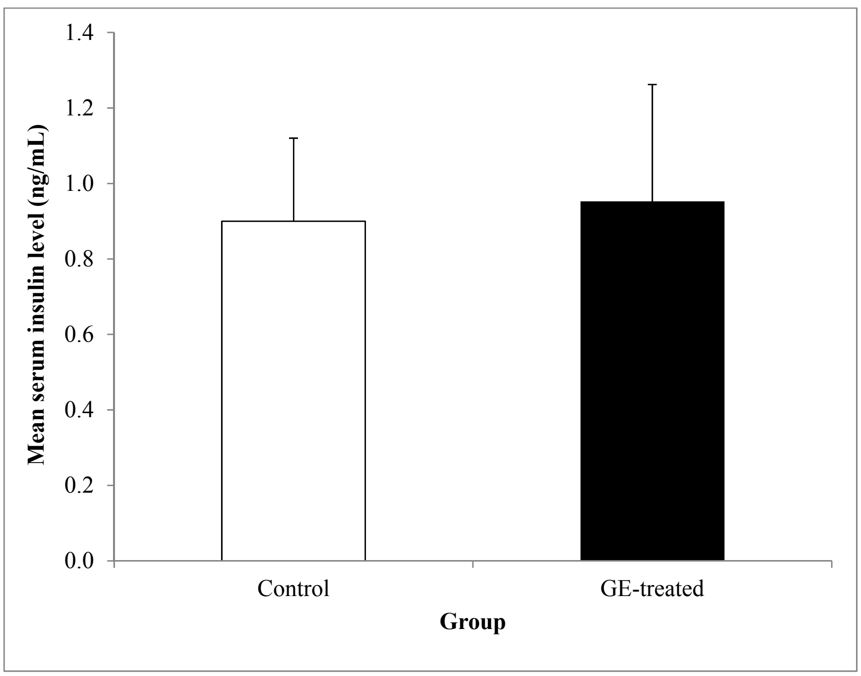

Fig. (2). Mean serum insulin (ng/mL) of the control and GE-treated groups (P>0.05).

units respectively (P>0.05) while the mean renal G6Pase activities in the control and GE-treated rats were 75.80 ± 4.61 units and 70.05 ± 2.86 units respectively (P>0.05) (Fig. **5**).

Phosphoenolpyruvate Carboxykinase (PEPCK) Activities in the Liver, Kidney, Subcutaneous and Visceral Adipose Tissues

The mean hepatic PEPCK activities in the control and GE-treated groups were 3.04 ± 0.45 units and 2.35 ± 0.44 units respectively (P>0.05) while the mean renal activities in the control group were 5.69 ± 0.67 units and the GE-treated group were 3.66 ± 0.28 units. A significant reduction of 55.71% was seen in the GE-treated group (P<0.05). In the SAT, no significant difference (P>0.05) was seen between the control (6.88 ± 1.05 units) and the GE-treated groups (7.23 ± 1.25 units) while in the VAT, similar observation was seen for the control (10.79 ± 1.75 units) and the GE-treated (11.68 ± 1.27 units) groups (Fig. **6**).

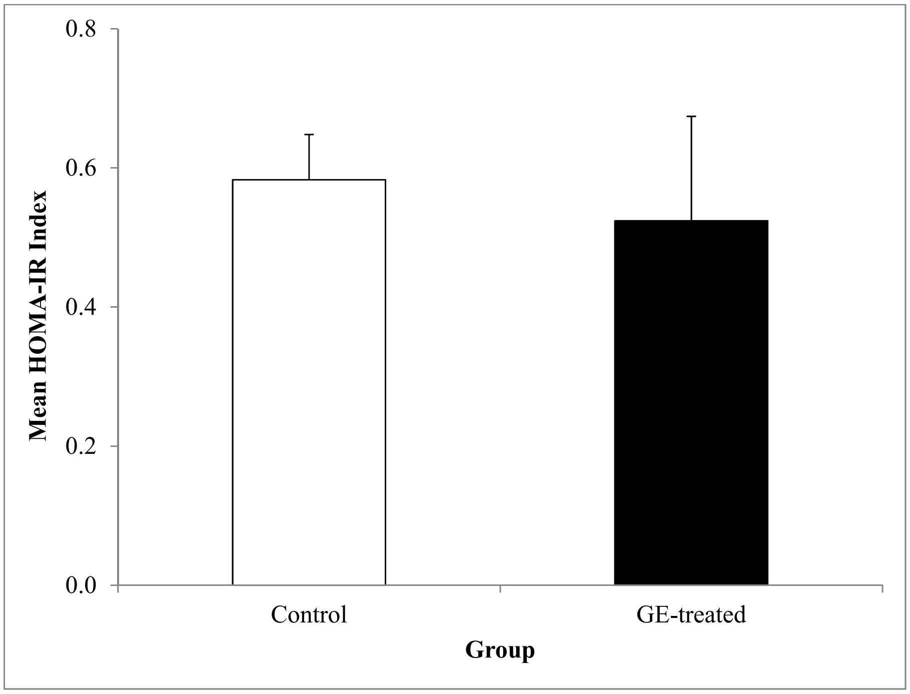

Fig. (3). Mean HOMA-IR index of the control and GE-treated groups (P>0.05).

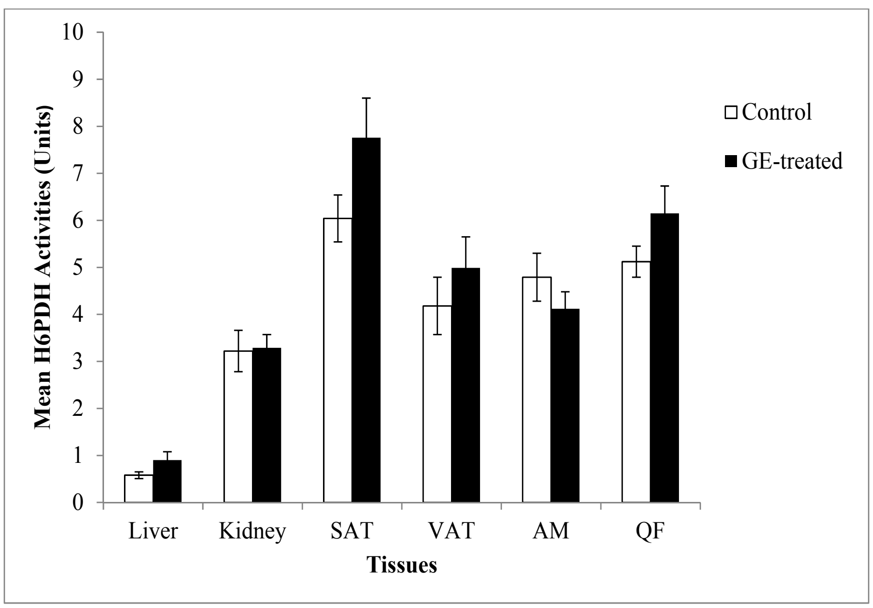

Fig. (4). Mean hexose-6-phosphate dehydrogenase (H6PDH) activities in the control and GE-treated rats (P>0.05). [AM, abdominal muscle; QF, quadriceps femoris; SAT, subcutaneous adipose tissue; VAT, visceral adipose tissue].

Administration of GE to Rats did not Improve 11β-HSD1 Activities in all Tissues Except the Liver

In the liver, the control and GE-treated groups had mean 11β-HSD1 activities of 261.54 ± 34.14 units and 166.26 ± 25.78 units, respectively with a significant reduction of 36.43% in the GE-treated group (P<0.05). However, the reduction of renal PEPCK was not significant in the GE-treated group (P>0.05). Renal 11β-HSD1 activities were 44.93 ± 3.83 units and 35.75 ± 2.91 units, respectively. In the SAT, the control and GE-treated groups had mean 11β-HSD1 activities of 27.50 ± 3.76 units and 24.27 ± 2.60 units, respectively. No significant difference was seen between the two groups (P>0.05). No significant difference (P>0.05) was observed between the control (24.03 ± 2.14 units) and the GE-treated group (23.42 ± 3.97 units) in the VAT. The activities in SAT were observed to be 16.25 ± 4.47 units for the control and 13.01 ± 3.51 units for the GE-treated group while in the QF, the values were 10.43 ± 3.82 units and 9.48 ± 1.71 units respectively. No significant difference was seen between the two groups (P>0.05) (Fig. **7**).

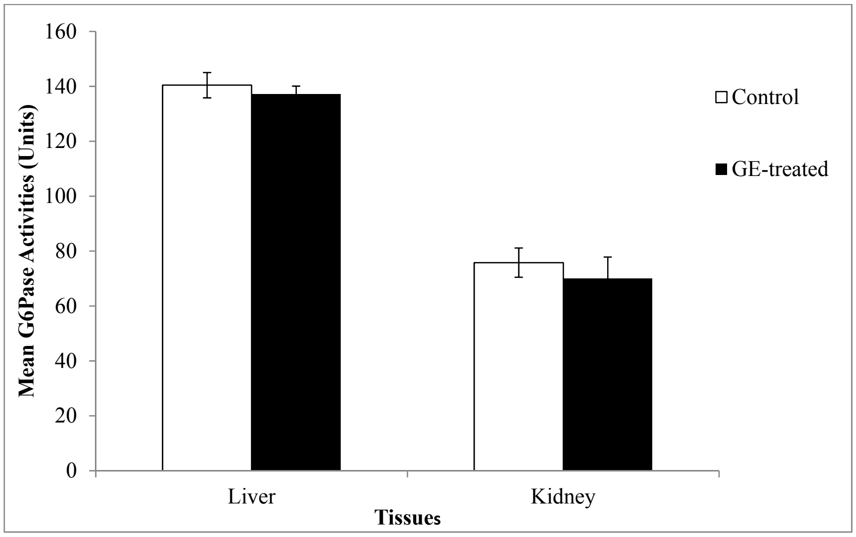

Fig. (5). Mean glucose-6-phosphatase (G6Pase) activities in the control and GE-treated rats (P>0.05).

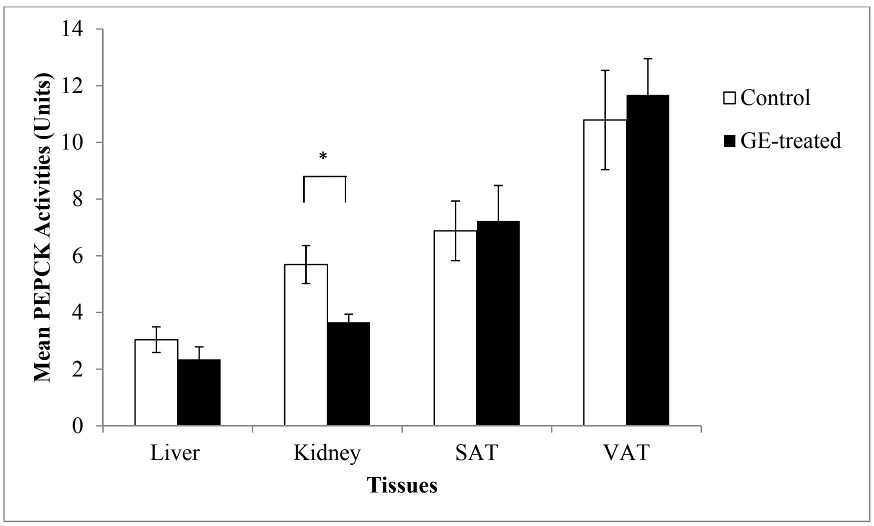

Fig. (6). Mean phosphoenolpyruvate carboxykinase (PEPCK) activities in the control and GE-treated rats (* indicates P<0.05). [SAT, subcutaneous adipose tissue; VAT, visceral adipose tissue].

Positive Shift in Lipid Profile in GE-Treated Rats

Consistent improvements in all the lipid parameters were observed in the treated group relative to the control group. A comparison of the lipid parameters and FFA between control and GE-treated groups is shown in (Fig. **8**). The mean serum TAG concentration of control and GE-treated groups were 0.51 ± 0.05 mmol/L and 0.29 ± 0.02 mmol/L respectively, representing a significant 43% decrease in the GE-treated group (P<0.01). The mean serum FFA concentration of control and GE-treated groups were 0.38 ± 0.06 mmol/L and 0.27 ± 0.04 mmol/L respectively. Although insignificant, the GE-treated group showed a 29% decrease (P>0.05). As for the mean total cholesterol concentration, the value in the GE-treated group was significantly lower than the control group with the control group being 2.11 ± 0.12 mmol/L while the GE-treated group was 1.47 ± 0.10 mmol/L (P<0.01). The mean HDL cholesterol of the control group was 0.56 ± 0.04 mmol/L and that of the GE-treated group was 0.66 ± 0.04 mmol/L respectively with a non-significant increase of 18% in the GE-treated group (P>0.05). However, the mean LDL cholesterol of the control and GE-treated groups showed a significant difference with the value of the control group being 1.29 ± 0.12 mmol/L and GE-treated group being 0.95 ± 0.09 mmol/L indicating a significant decrease of 27% in the GE-treated group (P<0.05). Overall, intraperitoneal administration of 50mg/kg of GE to rats showed significant improvement in lipid profiles with a significant decrease in TAG, total cholesterol and LDL-cholesterol and a non-significant decrease in FFA and an increase in HDL.

Increased Lipoprotein Lipase (LPL) Expression in all Tissues With GE Treatment

It was observed that LPL expression was insignificantly up-regulated in all tissues except QF in the GE-treated rats. The LPL expression was up-regulated by 1.63 ± 0.66 fold in the liver and 1.32 ± 1.18 in the kidney. The up-regulation

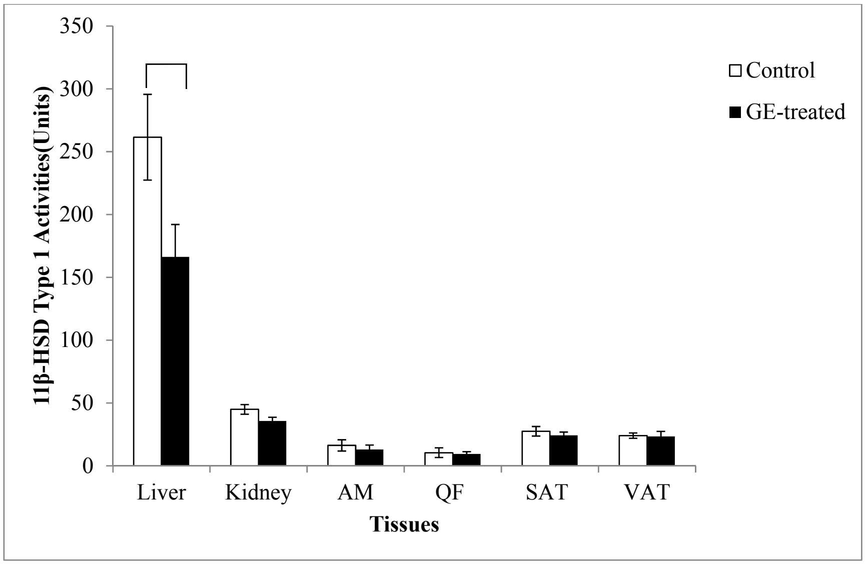

Fig. (7). Activities of 11β-HSD type 1 in all tissues from both the control and GE-treated groups (* indicates P<0.05). [AM, abdominal muscle; QF, quadriceps femoris; SAT, subcutaneous adipose tissue; VAT, visceral adipose tissue].

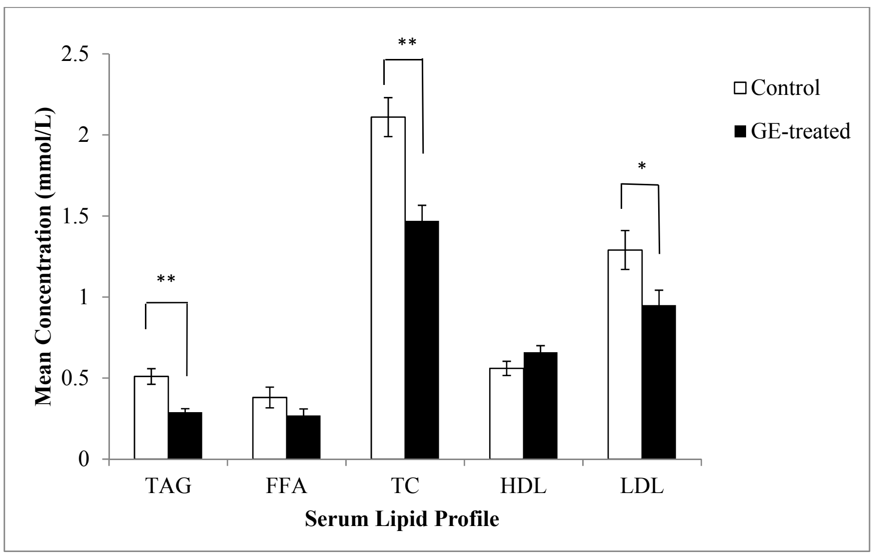

Fig. (8). Mean concentration of serum triglycerides (TAG), free fatty acids (FFA), total cholesterol (TC), high-density lipoprotein (HDL) cholesterol and low-density lipoprotein (LDL) cholesterol in rats from the control and GE-treated groups (** indicates P<0.01; * indicates P<0.05).

trend was also observed in the heart (1.14 ± 0.04 fold) and the AM (1.21 ± 0.75 fold). In adipose tissues, i.e. SAT and VAT, LPL expression was up-regulated by 1.19 ± 0.47 fold and 2.67 ± 0.52 fold respectively. On the other hand, LPL expression was hardly detectable in the QF (0.01 ± 0.16 fold). However, all the observations were statistically insignificant (P>0.05) (Fig. **9**).

PPAR-α and PPAR-γ Expression in the GE-Treated Rats

The PPAR-α expression was insignificantly up-regulated in the liver (1.02 ± 0.14 fold), heart (1.17 ± 0.41 fold) and AM (1.16 ± 0.30 fold) (P>0.05). However, non-significant down-regulation was observed in the kidney (-1.31 ± 0.29 fold) and QF (-1.36 ± 0.34 fold) (P>0.05). The PPAR-α expression was not detectable in both the SAT and VAT of all rats (Fig. **10**). The PPAR-γ expression was insignificantly up-regulated in the liver (1.12 ± 0.18 fold), heart (1.12 ± 0.53 fold), AM (1.45 ± 0.80 fold), QF (1.02 ± 0.47 fold), SAT (1.23 ± 0.58 fold) and VAT (1.29 ± 0.95 fold) (P>0.05). However, non-significant down-regulation was observed in the kidney (-1.21 ± 0.41 fold) (P>0.05) (Fig. **11**).

DISCUSSION

The GE-treated rats had significantly lower blood glucose level than the control group. Previous studies conducted by Chia *et al.* [38] with 50mg/kg GA showed the same anti-hyperglycaemic effect on rats. GE, the pharmacologically active compound of GA, has been reported to be 200-1000

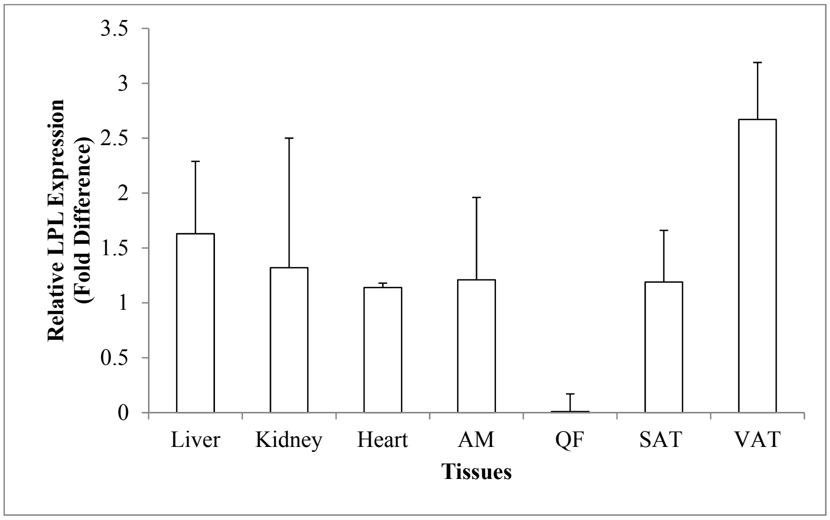

Fig. (9). Fold difference of LPL expression in different tissues with BAC as the endogenous reference, tissues from GE-treated group as target and tissues from control group as calibrator. LPL was up-regulated in all tissues except QF (P>0.05). [AM, abdominal muscle; QF, quadriceps femoris; SAT, subcutaneous adipose tissue; VAT, visceral adipose tissue].

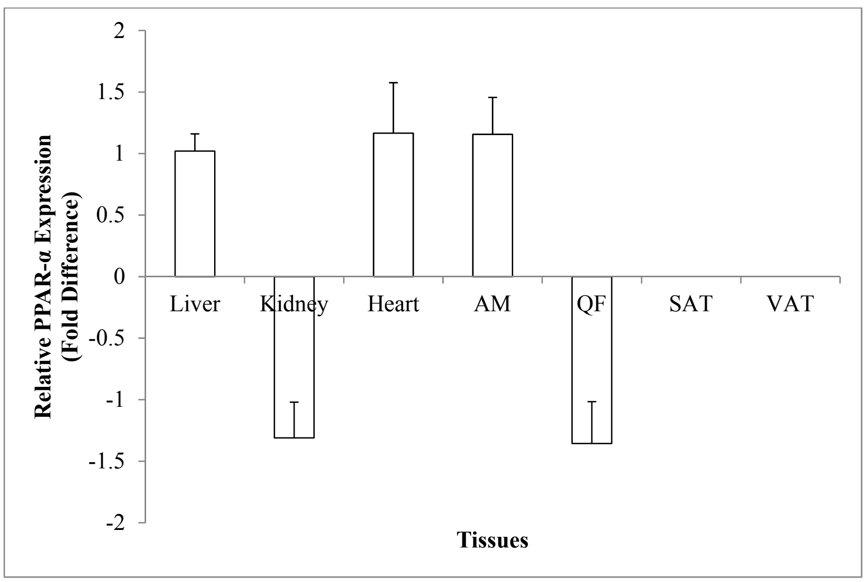

Fig. (10). Fold difference of PPAR-α expression in different tissues with BAC as the reference, GE-treated group as target and control group as calibrator (P>0.05). [AM, abdominal muscle; QF, quadriceps femoris; SAT, subcutaneous adipose tissue; VAT, visceral adipose tissue].

times more potent than GA [31] and it is postulated to improve glucose and lipid metabolism at a lower concentration than GA. The lowering of blood glucose level can be related to the antiglucocorticoid effect of GE. GC, as an antagonist of insulin, (i) inhibits insulin secretion from the pancreatic β-cells of Langerhans; (ii) inhibits peripheral glucose uptake by preventing translocation of glucose transporter to the plasma membrane and (iii) promotes gluconeogenesis in the liver, skeletal muscles and adipose tissues [39]. Inhibition of 11β-HSD1 decreases concentration of active GC that antagonizes insulin activities and inhibits the events that contribute to elevated blood glucose level [27]. It was also found that inhibition of 11β-HSD1 promotes hepatic glucose uptake by increasing translocation of glucose-transporter 2 (GLUT-2) to the membrane of hepatocytes hence resulting in the lowering of blood glucose level [39].

The improved serum lipid parameters in GE-treated rats can also be related to GE-mediated inhibition of 11β-HSD1. The reduced serum FFA may be related to the increased expression of plasma membrane fatty acid binding protein (FABP) that facilitates uptake of FFA into cells following inhibition of 11β-HSD1, thus lowering circulating FFA level [40]. The significant reduction in serum TAG may be related to reduced VLDL secretion following inhibition of 11β-HSD1. The rate of VLDL secretion depends on the amount of lipid available for VLDL assembly [41]. Adipose tissues which act as a reservoir for unlimited TAG storage have

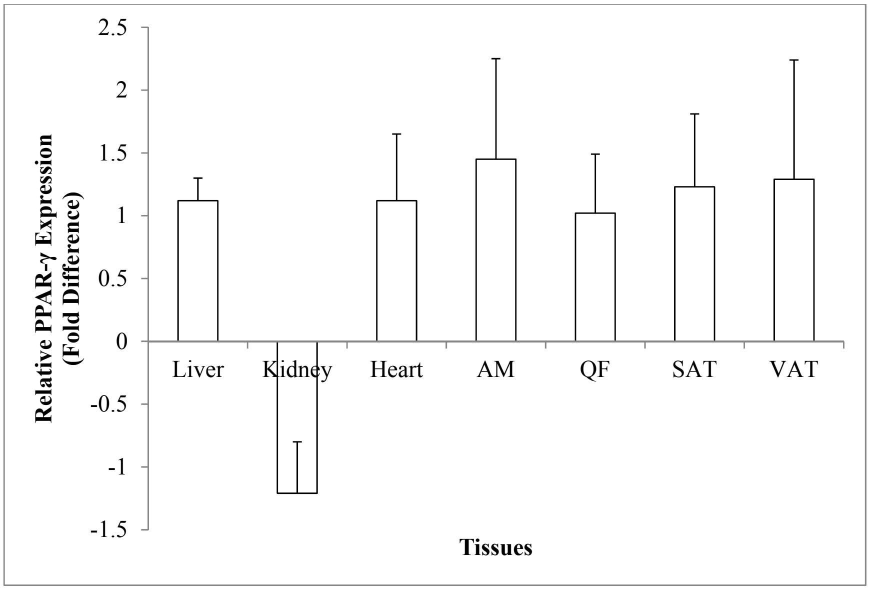

Fig. (11). Fold difference of PPAR-γ expression in different tissues with BAC as the reference, GE-treated group as target and control group as calibrator (P>0.05). [AM, abdominal muscle; QF, quadriceps femoris; SAT, subcutaneous adipose tissue; VAT, visceral adipose tissue].

been the primary target of intense investigations in obesity and MetS [42]. Nevertheless, increased evidence has shown that other tissues, particularly the liver, are able to store significant amount of fat, mostly as TAG in obesity and T2DM [43]. Hence, FAO plays a crucial role in energy homeostasis where maintenance of a high FAO rate is expected to aid in the reduction of fat accumulation despite excess energy consumption [44]. The ability of the tissues to up-regulate the FAO enzyme system in response to the increased fat uptake becomes critical to prevent the development of MetS [42,45]. It was found that the rate of lipid catabolism increases following increased expression of enzymes involved in fatty acid catabolism (e.g. carnithine palmitoyltransferase-I) following 11β-HSD1 inhibition [41]. Since GC is known to promote lipogenesis and VLDL secretion, the increased channeling of lipid to the oxidative pathway reduce the amount of lipid available for VLDL production, hence leading to reduced TAG level [41]. The increased HDL level may be related to the increase in synthesis of apo-AI, the main component of HDL-cholesterol. Morton *et al.* [41] found a significant elevated apo-AI level in 11β-HSD1 knockout mice. Followed by reduced hepatic TAG production and increased FFA oxidation as induced by 11β-HSD1 inhibition, there is reduced generation of small, dense LDL from TAG-rich VLDL by hepatic lipase [40]. This contributes to the significant reduction in serum LDL-cholesterol.

Some gluconeogenic enzymes like H6PDH, PEPCK and G6Pase were shown to be affected by the GE. Most tissues showed slight elevation in H6PDH activities. However, the increase was found to be non-significant which could be related to the increased availability of G6P. GE, as an insulinotropic agent, may increase the substrates i.e. G6P for H6PDH via induction of glucose transport, thereby enhancing the H6PDH activities. Chandramouli *et al.* [6] demonstrated that GA administration at 100mg/kg in rats showed parallel reduction in 11β-HSD1 and H6PDH activities while Walker and Andrew [46] commented that the relationship between 11β-HSD1 activity and the physiological variations in H6PDH remains elusive.

PEPCK is the key regulatory enzyme involved in gluconeogenesis (in the liver and kidney) and glyceroneogenesis (in the adipose tissues) [27]. The liver and kidney of GE-treated rats displayed decreased PEPCK activities (only significant in the latter tissue) which could be related to the GE-mediated reduction in 11β-HSD1. This leads to decreased production of active GC that mediate PEPCK gene transcription, thereby decreasing PEPCK activities and gluconeogenesis rate. The decreased PEPCK activities in the liver and kidney could also be related to the slight increase in insulin as insulin has been related to inhibition of PEPCK transcription via the activation of PI3K pathway [47]. In addition, PPAR-γ activation was also found to down-regulate PEPCK gene expression in the liver and kidney, thereby decreasing gluconeogenesis. GE, as a potential PPAR-γ agonist, would lead to down-regulation of PEPCK gene transcription and hence lower PEPCK activities in the GE-treated rats. Similarly, the transcription of G6Pase is subjected to control by GC [48]. Elevated circulating FFA is also known to exert allosteric simulation on G6Pase through the PPAR-mediated pathway, resulting in increased G6Pase expression and gluconeogenesis [49]. The non-significant decrease in G6pase activities could hence be related to the lowering effect of GE in both the activating compounds of G6pase- FFA and GC.

In the present study, PPAR-γ expression was detected in both SAT and VAT, suggesting their importance in both adipogenesis and adipocyte differentiation. The PPAR-α expression in both SAT and VAT was too low to be detected, which corresponded to the low FAO activity in the adipose tissues [50]. This is because the adipose tissues act as the primary energy storage site instead of energy utilization. The non-significant down-regulation of PPAR-α expression in quadriceps femoris may be due to fiber-type-selective transcriptional response of PPAR-α agonists. De Souza *et al.* [51] showed that administration of PPAR-α agonists, fenofibrates and Wy-14,643 to rats selectively induced PPAR-α activation in the type 1 (slow twitch) but not type 2 (fast twitch) skeletal muscle fibers. Abdominal muscle contains higher composition of type 1 muscle fibers as compared to quadriceps femoris (QF) which is mainly composed of type 2 muscle fibers [52]. Hence, as a potential PPAR-α agonist, GE may selectively induce PPAR-α transcriptional activity in the AM instead of QF.

CONCLUSION

The current study has shown that GE administration at dosage of 50mg/kg for 24 hours could lower blood glucose level significantly. GE also modulates serum lipid towards the beneficial side as shown by the improvement in each lipid parameter which includes TAG, FFA, total cholesterol, HDL-cholesterol and LDL-cholesterol following inhibition of 11β-HSD1. Furthermore, selective induction of PEPCK activities took place in parallel with the lowering of blood glucose level upon GE administration. This suggests that GE could be a potential compound in ameliorating hyperglycaemia and dyslipidaemia mediated through its antiglucocorticoid effect instead of via PPAR activation. However, even though GE has shown to be 200-1000 times more potent than GA [31,34], the potency of GE at 50mg/kg was not shown in the present study as it did not exert much significant effect on most of the parameters studied compared to the earlier work with GA [9,35,36].

CONFLICT OF INTEREST

The author(s) confirm that this article content has no conflicts of interest.

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REFERENCES

[1] Alberti, K.G.M.M.; Eckel, R.H.; Zimmet, P.Z.; Cleeman, J.L.; Donato, K.A.; Fruchart, J., Pharm, D.; James, W.P.T.; Loria, C.M.; Smith, S.C.Jr. Harmonizing the metabolic syndrome. A joint interim statement of the International Diabetes Federation Task Force on Epidemiology and Prevention; National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International Atherosclerosis Society; and International Association of the Study of Obesity. *Circulation*, **2009**, *120*, 1640-1645.

[2] Wan Nazaimoon, W.M.; al-Safi Ismail, A.; Md Khir, A.S.; Ismail, I.K.; Musa. K.I.; Abdul Kadir, K.; Kamarudin, N.A.; Yaacob, N.A.; Mustafa, N.; Ali, O.; Md Isa, S.H.; Wan Bebakar, W.M. Prevalence of metabolic syndrome and its risk factors in adult Malaysians: Results of a nationwide survey. *Diabetes. Res. Clin. Pr.*, **2012**, *96*, 91-97.

[3] Carr, M.C.; Brunzell, J.D. Abdominal obesity and dyslipidaemia in the metabolic syndrome: Importance of type 2 diabetes and familial combined hyperlipidaemia in coronary artery disease risk. *J. Clin. Endocr. Metab.*, **2004**, *89*(6), 2602-2607.

[4] Klein, S.; Allison, D.B.; Heymsfield, S.B.; Kelley, D.E.; Leibel, R.L.; Nonas, C.; Klein, R. Waist Circumference and Cardiometabolic Risk: A Consensus Statement from Shaping America's Health: Association for Weight Management and Obesity Prevention; NAASO, The Obesity Society; the American Society for Nutrition; and the American Diabetes Association. *Obesity*, **2007**, *15*(5), 1061-1067.

[5] Grundy, S.M.; Cleeman, J.I.; Daniels, S.R.; Donato, K.A.; Eckel, R.H.; Franklin, B.A.; Gordon, D.J.; Krauss, R.M.; Savage, P.J.; Smith Jr., S.C.; Spertus, J.A.; Costa, F. Diagnosis and management of the metabolic syndrome. *Circulation*, **2005**, *112*, 2735-2752.

[6] Chandramouli, C.; Yong, S.T.; Lam, Y.L.; Ton, S.H.; Khalid, B.A.K. Glycyrrhizic Acid Improves Lipid and Glucose Metabolism in High-Sucrose-Fed Rats. *Endocrinol. Metab.*, **2011**, *1*(3), 125-141.

[7] Preiss-Land, K.; Zimmermann, R.; Hammerle, G.; Zechner, R. Lipoprotein lipase: the regulation of tissue specific expression and its role in lipid and: energy metabolism. *Curr. Opin. Lipidol.*, **2002**, *13*(5), 471-481.

[8] Lann, D.; LeRoith, D. Insulin resistance as the underlying cause for the metabolic syndrome. *Med. Clin. N. Am.*, **2007**, *91*, 1063-1077.

[9] Aguilera, A.A.; Diaz, G.H.; Barcelata, M.L.; Guerrero, O.A.; Ros, R.M. Effects of fish oil on hypertension, plasma lipids, and tumor necrosis factor-alpha in rats with sucrose-induced metabolic syndrome. *J. Nutr. Biochem.*, **2004**, *15*(6), 350-357.

[10] London, E.; Castonguay, T.W. Diet and the role of 11 betahydroxysteroid dehydrogenase 1 on obesity. *J. Nutr. Biochem.*, **2009**, *20*(7), 485-493.

[11] Reynolds, R.M.; Walker, B.R.; Syddall, H.E.; Andrew, R.; Wood, P.J.; Whorwood, C.B.; Phillips, D.I. Altered control of cortisol secretion in adult men with low birth weight and cardiovascular risk factors. *J. Clin. Endocrinol. Metab.*, **2001**, *86*(1), 245-250.

[12] Wang, M. The role of glucocorticoid action in the pathophysiology of the Metabolic Syndrome. *Nutr. Metab(Lond).*, **2005**, *2*(1), 3.

[13] Hewitt, K.N.; Walker, E.A.; Stewart, P.M. Minireview: hexose-6-phosphate dehydrogenase and redox control of 11{beta}-hydroxysteroid dehydrogenase type 1 activity. *Endocrinology*, **2005**, *146*(6), 2539-2543.

[14] Lavery, G.G.; Hauton, D.; Hewitt, K.N.; Brice, S.M.; Sherlock, M.; Walker, E.A.; Stewart, P.M. Hypoglycemia with enhanced hepatic glycogen synthesis in recombinant mice lacking hexose-6-phosphate dehydrogenase. *Endocrinology*, **2007**, *148*(12), 6100-6106.

[15] Nashev, L.G.; Chandsawangbhuwana, C.; Balazs, Z.; Atanasov, A.G.; Dick, B.; Frey, F.J.; Baker, M.E.; Odermatt, A. Hexose-6-phosphate dehydrogenase modulates 11beta-hydroxysteroid dehydrogenase type 1-dependent metabolism of 7-keto- and 7beta-hydroxy-neurosteroids. *PLoS One*, **2007**, *2*(6), e561.

[16] Kota, B.P.; Huang, T.H.; Roufogalis, B.D. An overview on biological mechanisms of PPARs. *Pharmacol. Res.*, **2005**, *51*, 85-94.

[17] Berger, J.; Moller, D.E. The mechanisms of action of PPARs. *Annu. Rev. Med.*, **2002**, *53*, 409-435.

[18] Desvergne, B.; Wahli, W. Peroxisome proliferator-activated receptors: nuclear control of metabolism. *Endocr. Rev.*, **1999**, *20*, 649-88.

[19] Mandard, S.; Muller, M.; Kersten, S. Peroxisome proliferator-activated receptor α target genes. *Cell. Mol. Life. Sci.*, **2004**, *61*, 393-416.

[20] Lalloyer, F.; Staels, B. Fibrates, Glitazones, and Peroxisome Proliferator Activated Receptors. *Arterioscl. Throm. Vasc.*, **2010**, *30*, 894-899.

[21] Rang, H.P.; Dale, Maureen, M.; Ritter, J.M.; Flower, R.J. *Rang and Dale's Pharmacology*, 6th ed.; Churchill Livingstone: Philadelphia, **2007**.

[22] Post, S.M.; Duez, H.; Gervois, P.P.; Staels, B.; Kuipers, F.; Princen, H.M.G. Fibrates suppress bile acid synthesis via peroxisome proliferator-activated receptor-α-mediated downregulation of cholesterol 7α-hydroxylase and sterol 27-hydroxylase expression. *Arterioscl. Throm. Vasc.*, **2001**, *21*, 1840-1845.

[23] Wang, Z.Y.; Athar, M.; Bickers, D.R. *Herbs, Botanicals & Teas*; Technomic Publishing Co. Inc: Pennsylvania, **2000**.

[24] Isbrucker, R.A.; Burdock, G.A. Risk and safety assessment on the consumption of licorice root (*Glycyrrhiza* sp.), its extract and powder as a food ingredient, with emphasis on the pharmacology and toxicology of glycyrrhizin. *Regul. Toxicol. Pharm.*, **2006**, *46*, 167-192.

[25] Lim. W.Y.A; Chia, Y.Y.; Liong, S.Y.; Ton, S.H.; Khalid, A.B.K; Sharifah, N.A.S.H. Lipoprotein lipase expression, serum lipid and tissue lipid deposition in orally-administered glycyrrhizic acid-treated rats. *Lipids. Health. Dis.*, **2009**, *8*, 31-40.

[26] Chia, Y.Y.; Ton, S.H.; Khalid, B.A.K. Effects of glycyrrhizic acid on peroxisome proliferator-activated receptor gamma (PPARγ),

lipoprotein lipase (LPL) serum lipid and HOMA-IR in rats. *PPAR Res.*, **2010**, *2010*, 530265.

[27] Alberts, P.; Engblom, L.; Edling, N.; Forsgren, M.; Klingstrom, G.; Larsson, C.; Ronquist-Nii, Y.; Ohman, B.; Abrahmsen, L. Selective inhibition of 11β-hydrosteroid dehydrogenase type 1 decreases blood glucose concentrations in hyperglycaemic mice. *Diabetologia*, **2002**, *45*, 1528-1532.

[28] Wamil, M.; Seckl, J.R. Inhibition of 11β-hydroxysteroid dehydrogenase type 1 as a promising therapeutic target. *Drug. Discov. Today*, **2007**, *12*(13-14), 504-520.

[29] Wang, Y.; Porter, W.W.; Suh, N.; Honda, T.; Gribble, G.W.; Leesnitzer, L.M.; Plunkett, K.D.; Mangelsdorf, D.J.; Wilson, T.M.; Sporn, M.B. Triterpenoid, 2-cyano-3,12-dioxooleana-1,9-dien-28-oic acid (CDDO), is a ligand for the peroxisome proliferator-activated receptor gamma. *Mol Endocr*, **2000**, *14*(10), 1550-1556.

[30] Sato, M.; Nunoura, Y.; Yajima, Y.; Kawashima, S.; Tanaka, K. Dehydrotrametenolic acid induces preadipocyte differentiation and sensitizes animal models of noninsulin-dependent diabetes mellitus to insulin. *Biol. Pharm. Bull.*, **2002**, *25*(1), 81-86.

[31] Ploeger, B.A.; Meulenbelt, J.; DeJongh, J. Physiologically based pharmacokinetic modelling of glycyrrhizic acid, a compound subject to presystemic metabolism. *Toxicol. Appl. Pharm.*, **2001**, *162*, 177-188.

[32] Friedewald, W.T.; Levy, R.I.; Fredrickson, D.S. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin. Chem.*, **1972**, *18*(6), 499-502.

[33] Plaffl, M.W.; Horgan, G.W.; Dempfle, L. Relative expression software tool (REST©) for group-wise comparison and statistical analysis of relative expression results in real-time PCR. *Nucleic. Acids. Res.*, **2002**, *30*(9), 1-10.

[34] Ng, Y.; Chandramouli, C.; Ton, S.H.; Khalid, B.A.K.; Haque, F.; Tamotharan, M.S. Modulation of glucose and lipid metabolism in adrenalectomised rats given glycyrrhizic acid. *J. Mol. Pathophysiol.*, **2012**, *1*(1), 3-20.

[35] Petrescu, I.; Bojan, O.; Saied, M.; Barzu, O.; Schmidt, F.; Kuhnle, H.F. Determination of phosphoenolpyruvate carboxykinase activity with deoxyguanosine 5'-diphosphate as nucleotide substrate. *Anal. Biochem.*, **1979**, *96*(2), 279-281.

[36] Banhegyi, G.; Benedetti, A.; Fulceri, R.; Senesi, S. Cooperativity between 11beta-hydroxysteroid dehydrogenase type 1 and hexose-6-phosphate dehydrogenase in the lumen of the endoplasmic reticulum. *J. Biol. Chem.*, **2004**, *279*(26), 27017-27021.

[37] Csala, M.; Margittai, E.; Senesi, S.; Gamberucci, A.; Banhegyi, G.; Mandl, J.; Benedetti, A. Inhibition of hepatic glucose 6-phosphatase system by green tea flavanol epigallocatechin gallate. *FEBS Lett.*, **2007**, *581*(8), 1693-1698.

[38] Chia, Y.Y.; Ton, S.H.; Khalid, B.A.K. Effects of glycyrrhizic acid on 11β-hydroxysteroid dehydrogenase (11βHSD1 &2) activities and HOMA-IR in rats at different treatment periods. *Exp. Clin. Endocr. Diab.*, **2010**, *118*(9), 617-624.

[39] Vegiopoulos, A.; Herzig, S. Glucocorticois, metabolism and metabolic diseases. *Mol. Cell. Endocrinol.*, **2007**, *275*(1-2), 43-61.

[40] Berthiaume, M.; Laplante, M.; Festuccia, W.T.; Cianflone, K.; Turcotte, L.P.; Joanisse, D.R.; Olivecrona, G.; Thieringer, R.; Deshaies, Y. 11β-HSD1 inhibition improves triglyceridemia through reduced liver VLDL secretion and partitions lipids toward oxidative tissues. *Am. Endocrinol. Metab.*, **2007**, *293*, E1045-1052.

[41] Morton, N.M.; Holmes, M.C.; Fievet, C.; Staels, B.; Tailleux, A.; Mullins, J.J.; Seckl, J.R. Improved lipid and lipoprotein profile, hepatic insulin sensitivity, and glucose tolerance in 11beta-hydroxysteroid dehydrogenase type 1 null mice. *J. Biol. Chem.*, **2001**, *276*(44), 41293-41300.

[42] Spiegelman, B.M.; Flier, J.S. Obesity and regulation of energy balance. *Cell*, **2001**, *104*, 531-543.

[43] Marchesini, G.; Bugianesi, E.; Forlani, G.; Cerrelli, F.; Lenzi, M.; Manini, R.; Natale, S.; Vanni, E.; Villanova, N.; Melchionda, N.; Rizzetto, M. Nonalcoholic fatty liver, steatohepatitis, and the metabolic syndrome. *Hepatology*, **2003**, *37*, 917-923.

[44] Abu-Elheiga, L.; Matzuk, M.M.; Ab-Hashema, K.A.H.; Wakil, S.J. Continuous fatty acid oxidation and reduced fat storage in mice lacking acetyl-CoA carboxylase 2. *Science*, **2001**, *291*, 2613-2616.

[45] Reddy, J.K.; Hashimoto, T. Peroxisomal β-oxidation and peroxisomal proliferator-activated receptor: An adaptive metabolic system. *Annu. Rev. Nutr.*, **2001**, 21, 193-230.

[46] Walker, B.R.; Andrew, R. Tissue production of cortisol by 11beta-hydroxysteroid dehydrogenase type 1 and metabolic disease. *Ann. NY. Acad. Sci.*, **2006**, *1083*, 165-184.

[47] Barthel, A.; Schmoll, D. Novel concepts in insulin regulation of hepatic gluconeogenesis. *Am. J. Physiol-Endoc. M.*, **2003**, *285*, E685-E692.

[48] Streeper, R.S.; Svitek, C.A.; Goldman, J.K.; O'Brien, R.M. Differential role of hepatocyte nuclear factor-1 in the regulation of glucose-6-phosphatase catalytic subunit gene transcription by cAMP in liver-and kidney-derived cell lines. *J. Biol. Chem.*, **2000**, *275*, 12108-12118.

[49] Lam, T.K.; Carpentier, A.; Lewis, G.F.; van de Werve, G.; Fantus, I.G.; Giacca, A. Mechanisms of the free fatty acid induced increase in hepatic glucose production. *Am J. Physiol. Endocrinol. Metab.*, **2003**, *284*(5), E863-873.

[50] Wang, T.; Zang, Y.; Ling, W.; Corkey, B.E.; Guo, W. Metabolic Partitioning of endogenous fatty acid in adipocytes. *Obes. Res.*, **2003**, *11*(7), 880-887.

[51] De Souza, A.T.; Cornwell, P.D.; Dai, X.; Caguyong, M.J.; Ulrich RG. Agonists of the peroxisome-proliferator-activated receptor alpha induce a fiber-type-selective transcriptional response in rat skeletal muscle. *Toxicol. Sci.*, **2006**, *92*(2), 578-586.

[52] Haggmark, T.; Thorstensson, A. Fibre types in human abdominal muscles. *Acta Physiol. Scand.*, **1979**, *107*(4), 319-325.

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