

# Potential Protective Effects of Neonatal Supplementation with Oleanolic Acid on Peroxisome Proliferator-Activated Receptor Gamma (PPAR $\gamma$ )-Ligand Dependent Regulation of Glucose Homeostasis in High Fructose-Fed Rats

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## Abstract

The effect of neonatal oral supplementation of oleanolic acid (OA) on peroxisome proliferator-activated receptor gamma (PPAR $\gamma$ )-1 on glucose homeostasis in high fructose-fed rats was investigated. Rat pups (7 days old) were randomly assigned to and randomly administered with control (Distilled water, DW), OA (60 mg/kg), metformin (MET, 500 mg/kg), high fructose solution (HFS, 20% w/v), OA + HFS, MET + HFS, and treated till postnatal day (PND) 14. The pups were weaned onto a standard diet on PND 21 up to PND 111 and terminated on PND 112. Glucose derivatives and gene expressions of PPAR $\gamma$ -1 and glucose transporter type 4 (Glut-4) in the skeletal muscles were determined by using reverse transcription-quantitative polymerase chain reaction and gas chromatography-mass spectrometry, respectively. HFS significantly lowered glucose concentration and showed the propensity to suppress the expression of PPAR $\gamma$ -1, but not significantly. OA and MET alone significantly increased PPAR $\gamma$ -1 and Glut-4 expressions. There was no significant difference between the OA and OA + HFS for PPAR $\gamma$ -1 and Glut-4 expressions, although OA expressions were always higher than that of the OA + HFS group. An elevated level of glucose-6-phosphate was observed in OA, MET, and OA + HFS groups. Ribose-5-phosphate was significantly higher in OA and MET groups than the control. Ribose-5-phosphate was also significantly high in OA-treated group compared with OA + HFS. It is concluded that the neonatal supplementation with OA could help to improve the activity of PPAR $\gamma$  in reducing the burden of metabolic diseases.

## Keywords

obesity, oleanolic acid, high fructose, peroxisome proliferator-activated receptor gamma, metabolic diseases

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Obesity, type 2 diabetes mellitus (T2DM), and other health outcomes associated with metabolic syndrome are of global concern and are influenced by the modern lifestyle of prolonged exposure to high-energy diets,<sup>1</sup> decreased physical activity,<sup>2</sup> and other environmental factors which result in epigenetic changes.<sup>3</sup> According to the World Health Organisation (WHO, 2016), obesity is described as a neglected global public health problem.<sup>4</sup> Obesity and T2DM can result from a long period of decreased glucose-induced insulin secretion and reduced cellular responses to insulin action. The failure of fat cells to respond to the antilipolytic effects of insulin leads to pathological and chronic elevations in plasma levels of free fatty acids.<sup>5,6</sup> T2DM reduces insulin sensitivity in the liver and skeletal muscles resulting in the development of insulin

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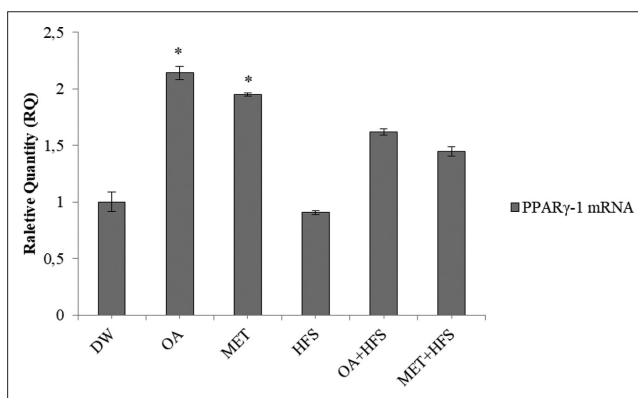
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resistance (IR) in these tissues.<sup>5,6</sup> The IR reduces glucose uptake in the skeletal muscles and increases gluconeogenesis in the liver.<sup>6</sup> Over the past decades, the management of obesity and T2DM depended on a combination of dietary modification, exercise, and administration of insulin-sensitizing pharmaceutical drugs such as metformin (MET).<sup>1</sup> However, most patients with obesity and T2DM often neglected dietary intervention and exercise but relied heavily on the administration of pharmaceutical drugs. The use of pharmaceutical drugs in the management of metabolic disorders such as T2DM is often associated with poor compliance by the patients taking the treatment regimes. These drugs are often too expensive for patients in under-served rural areas and are sometimes associated with the development of adverse side effects.<sup>1</sup> Due to the several disadvantages associated with the use of therapeutic drugs, several African communities in resource-limited areas and some urbanites rely on plant-based alternatives and complementary treatments for their primary health care.<sup>7</sup> The efficacy of the plant-based medicines is due to the presence of bioactive phytochemical compounds such as oleanolic acid (OA). OA is a pentacyclic triterpenoid that is commonly found in plants such as fruits and vegetables.<sup>8</sup> The therapeutic efficacy of OA in the management of metabolic disorders has been previously investigated and has been shown to be effective even without the introduction of complementary dietary interventions.<sup>1</sup> OA and its derivatives have been found to ameliorate the symptoms of obesity and T2DM.<sup>1,9</sup> OA also upregulated the expression of glucose transporter type 4 (GLUT-4) in skeletal muscle cells and adipocytes<sup>1</sup> but the mechanism of actions is yet to be investigated. There are a number of ligand-activated transcriptional factors that are involved in adipogenesis, glucose uptake, and glycolysis pathways and they represent important therapeutic targets for the management of obesity, T2DM, and other components of metabolic syndrome.<sup>10,11</sup> The peroxisome proliferator-activated receptor gamma (PPAR $\gamma$ ), GLUT-4, and adipokines such as leptins are the most important transcriptional factors that play such a function in adipogenesis and glucose homeostasis.<sup>10</sup>

There are three types of PPAR isoforms, which are PPAR $\gamma$ , PPAR $\alpha$ , and PPAR $\beta/\delta$ .<sup>6</sup> PPAR $\gamma$  is highly expressed in white and brown adipose tissues and sometimes in skeletal and cardiac muscles.<sup>6,10</sup> In the adipose tissue, PPAR $\gamma$  plays a role in the long-term regulation of lipid metabolism, particularly adipogenesis and in skeletal muscles where it is important in glucose homeostasis.<sup>5,12,13</sup> The PPAR isoforms are usually activated by the presence of free fatty acids and fatty acid-derived eicosanoids.<sup>6,14,15</sup> These groups of receptors are important in mediating the pleiotropic effects of different environmental factors, food contaminants, and drugs.<sup>15</sup> PPARs influence the expression of genes by binding to specific PPAR elements (PPAREs).<sup>14</sup> PPAR $\gamma$  has both natural and synthetic ligands.<sup>5</sup> Natural ligands of PPAR $\gamma$  include oleate, linoleate, eicosapentaenoic and arachidonic acids.<sup>5</sup> Synthetic ligands for PPAR $\gamma$  are of particular interest in treating obesity and T2DM, as they have the ability to restore insulin sensitivity.<sup>6</sup>

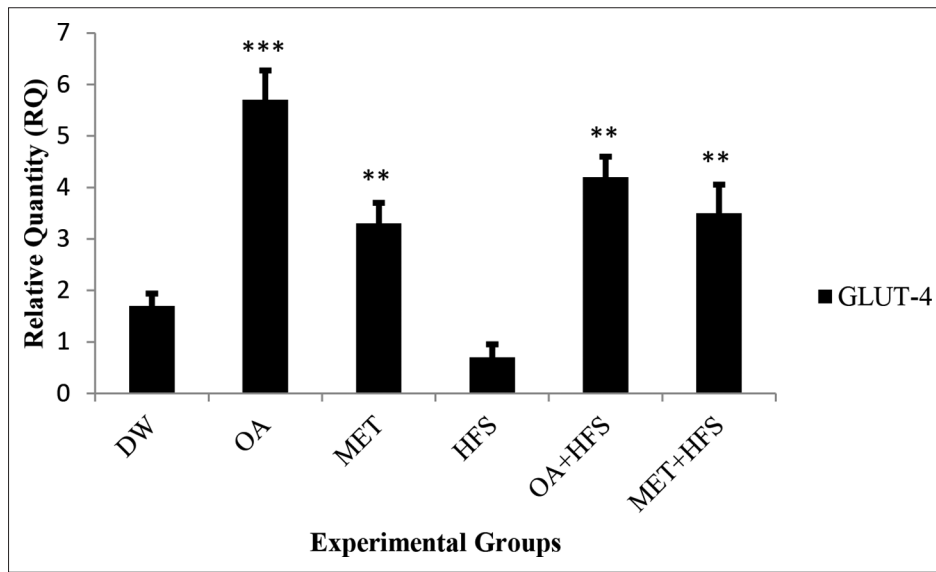


**Figure 1.** The expression of PPAR $\gamma$ -1 messenger ribonucleic acid gene in the skeletal muscles of adult female rats, where each treatment group was compared with the control group; OA vs DW, \* $P < 0.05$  and MET versus DW, \* $P < 0.05$ . DW, control; HFS, high fructose diet; MET, metformin; OA, oleanolic acid; PPAR $\gamma$ , peroxisome proliferator-activated receptor gamma.

In skeletal muscle, PPAR $\gamma$  regulates the genes that are involved in uptake and storage of fatty acid, development of inflammation, and glucose homeostasis.<sup>6</sup>

Due to its fundamental role in the improvement of obesity, T2DM, and other metabolic disorders, several pharmacological studies using murine models have been conducted on PPARs.<sup>16</sup> It is believed that the activation of PPAR $\gamma$  increases the expression of the insulin-dependent glucose transporters (GLUT-4 and GLUT-1), which then increases glucose uptake into liver and skeletal muscles, resulting in reduced plasma glucose levels.<sup>5,6</sup> This makes PPAR $\gamma$  one of the major key receptors in the pharmacological treatment of T2DM.<sup>15</sup> Furthermore, skeletal muscle is regarded as the most quantitatively important tissue for insulin-dependent glucose utilization.<sup>5</sup> The neonatal period is considered as an important period of developmental plasticity during which dietary interventions tend to program long-lasting epigenetic changes that affect the health outcomes of individuals.<sup>3,17</sup> As such, the aim of this study was to assess the potential of neonatal supplementation of OA on PPAR $\gamma$ -1 expression to control selected key elements involved in glucose homeostasis of fructose-fed rats.

Administration of OA and MET alone in the rats both increased the expression of PPAR $\gamma$ -1 significantly by 2-fold (Figure 1;  $P < 0.05$ ). High fructose solution (HFS) showed a tendency to suppress the expression of PPAR $\gamma$ -1 but was not significantly different from the control (Figure 1). However, a combination of OA and HFS (OA + HFS) treatment increased the expression of PPAR $\gamma$ -1 by approximately 1-fold compared with that of the control (DW) and HFS groups (Figure 1). When OA was compared with OA + HFS, there was no significant difference, although OA was always higher than OA + HFS in PPAR $\gamma$ -1 expression.

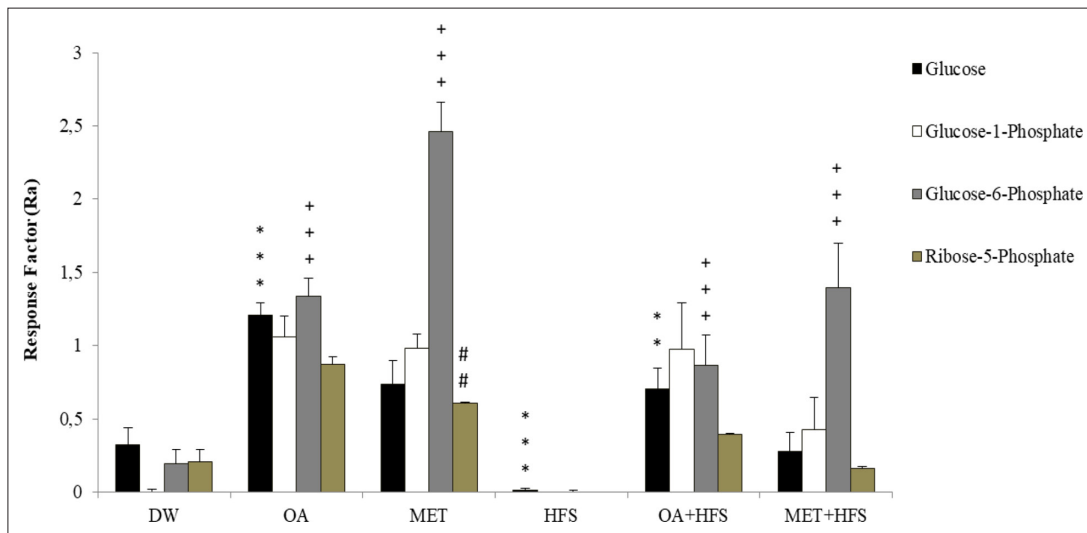


**Figure 2.** The expression of *GLUT-4* gene in the skeletal muscles of adult female rats, where each treatment group was compared with the control group; OA vs DW, \*\*\* $P < 0.001$ ; MET vs DW, \*\* $P < 0.01$ ; OA + HFS vs DW, \*\* $P < 0.01$ ; and MET + HFS vs DW, \*\* $P < 0.01$ . DW, control; GLUT4, glucose transporter type 4; HFS, high fructose diet; MET, metformin; OA, oleanolic acid.

The expression of GLUT-4 in the experimental groups that were administered with OA ( $P < 0.001$ ), MET ( $P < 0.01$ ), OA + HFS ( $P < 0.01$ ), and MET + HFS ( $P < 0.01$ ) groups were significantly higher than the control (DW) group (Figure 2). The GLUT-4 expression in the HFS group was lower than that of the control but not significant ( $P > 0.05$ ). However, it was significantly ( $P < 0.001$ ) lower than the other groups (Figure 2). There was no significant difference between OA and OA +

HFS groups. The HFS group had suppressed expression of *GLUT-4* gene. OA administration increased the expression of *GLUT-4* when compared with metformin in this experiment (Figure 2).

Administration of HFS to the rats significantly decreased glucose diffusion into skeletal muscles (Figure 3;  $P < 0.001$ ), while treatment with OA significantly improved the effects (Figure 3;  $P < 0.01$ ). Neonatal OA ( $P < 0.001$ ) and MET ( $P <$



**Figure 3.** Monosaccharide levels in the skeletal muscle samples of the experiment. Response factors of all monosaccharides were calculated in R statistics. Student's *t*-test results for glucose: OA vs control (DW), \*\*\* $P < 0.001$ ; MET vs DW, \*\* $P < 0.01$ ; HFS vs DW, \*\*\* $P < 0.001$ ; and OA + HFS, \*\* $P < 0.01$ . Glucose-6-phosphate: OA vs DW, +++ $P < 0.001$ ; MET vs DW, +++ $P < 0.001$ ; OA + HFS vs DW +++ $P < 0.001$ ; and MET + HFS vs DW, +++ $P < 0.001$ . Ribose-5-phosphate: OA vs DW, #### $P < 0.001$  and MET vs DW, ### $P < 0.01$ . DW, control; HFS, high fructose diet; MET, metformin; OA, oleanolic acid.

0.01) administration significantly increased glucose diffusion into the skeletal muscles when they were administered alone (Figure 3). Glucose-6-phosphate was detected at the lowest level of 0.002 response factor (Ra) in the HFS group (Figure 3). However, glucose-6-phosphate detection was significantly higher in OA, MET, OA + HFS, and MET + HFS treatment groups when compared with the control group (Figure 3;  $P < 0.001$ ). Neonatal MET treatment increased levels of glucose-6-phosphate by 2 times more than that of OA (Figure 3). Although glucose-6-phosphate was not detected in significant amounts in the HFS group, it was detected in significantly higher amounts in the OA + HFS group in comparison with the control group (Figure 3;  $P < 0.001$ ). Glucose-1-phosphate was only detected in the control (0.979 Ra), HFS (1.061 Ra), and OA + HFS (0.983 Ra) groups (Figure 3). In all the treatment groups, the level of glucose-1-phosphate was not significantly different when compared with the control (Figure 3;  $P > 0.05$ ). The level of ribose-5-phosphate was significantly higher in both the OA ( $P < 0.001$ ) and MET ( $P < 0.01$ ) groups (Figure 3). Ribose-5-phosphate was not detected in the HFS group (Figure 3). The treatment of high fructose-fed rats with OA elevated the levels of ribose-5-phosphate when compared with the control, but not significantly (Figure 3). Ribose-5-phosphate levels in the OA group were significantly lower than that of the OA + HFS group (Figure 3).

Our findings showed that glucose trafficking into the skeletal muscles was increased in the rats fed with OA and MET when compared with the HFS group.<sup>1</sup> It was also showed that OA restored the regulation of glucose homeostasis with or without dietary intervention in male rats. Neonatal administration of OA increased *Glut-4* gene expression and glucose levels in the skeletal muscles.<sup>10</sup> It was also reported that elevated expression of *GLUT-4* is associated with lower blood glucose and enhanced glucose transport and utility. However, the neonatal intake of HFS suppressed *GLUT-4* gene expression which resulted in significantly lower glucose levels within the skeletal muscles. This suggests that high glucose levels will be in circulation and could result in insulin resistance and therefore, T2DM. Conditional depletion of *GLUT-4* in skeletal muscles results in the development of insulin resistance and an approximately corresponding increase in the incidence of T2DM in animal models.<sup>18</sup> Since *GLUT-4* plays a fundamental role in the removal of glucose from circulation and is a key regulator of whole-body glucose homeostasis,<sup>19,20</sup> its expression is important in the development of risk factors associated with metabolic dysfunction and the management of such risk factors. Most of the *GLUT-4* protein is found intracellularly in the basal state, it can then be translocated rapidly to the plasma membrane and transverse tubules.<sup>19</sup> This increases the presence of cell surface glucose transporters, which, in turn, increases the rate of glucose uptake into the skeletal muscle.<sup>19</sup> The translocation of *GLUT-4* glucose transporters depends on insulin stimulation or exercise.<sup>18,19</sup> Insulin has been shown to increase glucose transport activity in rats muscle by 7-fold but only by 2-fold in human muscle.<sup>21</sup>

In the current study, OA treatment increased skeletal muscle *GLUT-4* gene expression probably through insulin stimulation by activating the *PPAR $\gamma$ -1* transcriptional factor. *GLUT-4* gene transcription is regulated by metabolites, hormones, ligands, and transcriptional factors.<sup>22</sup> *PPAR $\gamma$*  also acts as a regulator of *GLUT-4* gene transcription.<sup>23</sup> In the presence of a ligand, *PPAR $\gamma$*  is activated and forms a heterodimer with a retinoid X receptor (RXR).<sup>6,11,15</sup> The *PPAR $\gamma$* -RXR dimers then bind to deoxyribonucleic acid (DNA)-specific sequences known as PPREs resulting in stimulation of transcription of target genes, in this case, *Glut-4*.<sup>6,11,15</sup> The ligands that bind and form a heterodimer with *PPAR $\gamma$*  control cellular glucose homeostasis and lipid metabolism.<sup>15</sup> Our findings showed that neonatal administration of OA significantly increases the activity of *PPAR $\gamma$ -1* in skeletal muscles in adult rats, whereas a high fructose diet suppresses the activity of *PPAR $\gamma$ -1*. As a result, it is most likely that OA acted as a ligand in activating the *PPAR $\gamma$ -1* transcriptional factor which led to increased *GLUT-4* expression and promotes glucose transport into the skeletal muscle.<sup>5</sup> Thiazolidinediones decreased hyperglycemia in adipose tissues through their capacity to activate *PPAR $\gamma$* . However, a cascade of signal transduction such as the PI3K and insulin receptor substrate (IRS) are needed for transmitting signals to *Glut-4* transporter so that it can be translocated for utilization in skeletal muscle.<sup>22,23</sup> PI3K influences the IRS through tyrosine phosphorylation that is required for insulin transmission signals.<sup>22,23</sup>

The derivatives of glucose, such as glucose-1-phosphate, glucose-6-phosphate, and ribose-5-phosphate are formed during glucose breakdown. As soon as glucose has been transported across the sarcolemma of the skeletal muscle, it is then phosphorylated to glucose-6-phosphate in a reaction catalyzed by hexokinase II (HKII).<sup>24</sup> This is the first step in the metabolism of glucose either for glycolysis, glycogenolysis, or nucleotide biosynthesis.<sup>24</sup> Glucose phosphorylation is a rate-determining step and a potential limitation to glucose uptake and utilization.<sup>24</sup> Administration of OA with a high fructose diet in the neonatal period significantly increased the levels of glucose-6-phosphate within the rats' skeletal muscles, suggesting that the dietary intervention with OA increases glucose breakdown. However, the high fructose diet did not promote the breakdown of glucose into glucose-6-phosphate as there was none detected (within the limitations of our equipment and methodology) from the skeletal muscles. An increase in glucose uptake influences a decrease in intracellular glucose concentration, as the HKII inhibition is relieved by a lower glucose-6-phosphate concentration.<sup>24</sup> In this study, the high fructose diet slightly increased the levels of glucose-1-phosphate in the skeletal muscles. However, the dietary inclusion of OA in rats on a high fructose diet decreased this concentration in the rat skeletal muscle. This suggests that there was some degree of breakdown of glucose-6-phosphate into glucose-1-phosphate for energy storage in the HFS group. Increased *GLUT-4* gene expression together with increased glucose uptake into the muscles contributes to increased

glucose-1-phosphate and therefore glycogen levels in rat's muscles.<sup>24</sup>

Our findings showed an increase in *GLUT4* gene expression associated with high concentrations of glucose-6-phosphate and glucose uptake and not glucose-1-phosphate. This indicates that PPAR $\gamma$ -1 activation by OA increases *GLUT4* expression for glucose uptake and oxidation of fuel in muscles, in turn improving insulin sensitivity.<sup>24,25</sup> However,<sup>25</sup> Armoni et al outlined that PPAR isoforms have a ligand-dependent binding domain and a ligand-independent activation domain. The PPAR $\gamma$  repressed *GLUT4* expression through the ligand-independent binding domain, which was ameliorated by the use of thiazolidinediones to recover insulin sensitivity.<sup>25,26</sup> Ribose-5-phosphate is essential for nucleotide biosynthesis in skeletal muscles.<sup>24</sup> Ribose-5-phosphate was also found to be high in rats treated with OA and was not detected in the high fructose diet-fed group.

This study shows that PPAR $\gamma$ -1 which is central to the regulation of glucose homeostasis is a target for the mechanism of action by which OA can be employed as a strong activator of the PPAR isoform and, in turn, could help to restore insulin sensitivity. It is suggested that OA should be explored further as a natural ligand of PPARs for the management of obesity, insulin resistance, and T2DM.

## Experimental

### Materials and Chemicals

**Experimental design.** Thirty-six (36) female Sprague Dawley pups were put into 6 groups. These pups were housed in the Central Animal Services of the University of the Witwatersrand. The ethical clearance for the use of animals in this study was granted by the Animal Research Ethics Committee of the University of the Witwatersrand (Animal Research Ethics Reference Number: 2014/47/D). Suckling pups (7 days old) were randomly divided into the following treatment groups: DW, OA (60 mg/kg), MET (500 mg/kg), HFS (25% w/v), OA + HFS, and MET + HFS until postnatal day (PND) 14. No experimental treatments were administered between PND15 and PND20. Rats were then weaned at PND21 and fed standard rat chow and drinking water ad libitum until day PND 111. Results for growth parameters which were measured daily and rats weight have already been published.<sup>9</sup> On PND112, the rats were euthanized via an intraperitoneal injection of 200 mg/kg body mass of sodium pentobarbital (Eutha-naze; Bayer Corporation, Johannesburg, South Africa). Triceps muscle samples were dissected out and snap-frozen in liquid nitrogen and then stored at  $-80^{\circ}\text{C}$  until molecular analyses were performed.

### RNA Extraction

The samples that were used for the ribonucleic acid (RNA) extraction were randomly selected from the experimental groups. Muscle tissue samples were crushed in a mortar with a

pestle in liquid nitrogen. The tissue (200 mg) was then transferred into a 1.5 mL tubes and dissolved in 1 mL Trizol. The tissue was homogenized and incubated on ice for 5 minutes and then centrifuged at  $12,000\times g$  at  $4^{\circ}\text{C}$  for 15 minutes. Trizol was discarded from the sample homogenate and chloroform (200  $\mu\text{L}$ ) was added before incubation on ice for 15 minutes. Thereafter, the sample was centrifuged at  $12,000\times g$  for 15 minutes. The aqueous phase was then transferred into a 2 mL tube and isopropanol (0.5 mL) added to precipitate the RNA. The sample was incubated on ice for 10 minutes and then centrifuged for 10 minutes at  $12,000\times g$  at  $4^{\circ}\text{C}$ . The supernatant was decanted and the extracted RNA pellet was air-dried and then dissolved in RNase free water (20  $\mu\text{L}$ ). RNA concentration and ratio were determined using a nanodrop machine (manufacturer details). RNA integrity was verified with 2% gel electrophoresis.

### cDNA Synthesis

Extracted RNA samples were used to synthesize DNA with a Superscript VILO complementary DNA (cDNA) synthesis kit (iScript<sup>TM</sup> cDNA synthesis kit, Bio-Rad Laboratories, Inc., CA, USA). The RNA concentration required for the synthesis of cDNA as determined by the nanodrop varies per sample. For every single reaction, the master mix was prepared by mixing the following reagents:  $5\times$  VILO reaction mix (4  $\mu\text{L}$ ),  $10\times$  Superscript enzyme mix (2  $\mu\text{L}$ ), sample RNA (up to 2.5  $\mu\text{g}$ ), and diethyl pyrocarbonate-treated water (up to 20  $\mu\text{L}$ ). The master mix was then incubated at  $25^{\circ}\text{C}$  for 10 minutes. Incubation was repeated at  $42^{\circ}\text{C}$  for an hour. The reaction was terminated by incubating at  $85^{\circ}\text{C}$  for 5 minutes.

### Real-Time Quantitative Polymerase Chain Reaction

Real-time quantitative Polymerase Chain Reaction (RT-qPCR) was used to analyze the expression of the genes for PPAR $\gamma$ -1 and *GLUT4*. Three replicates per sample from each experimental group were analyzed. The cDNA (1  $\mu\text{L}$ ) samples were diluted with 19  $\mu\text{L}$  of RNase free water up to 20  $\mu\text{L}$ . Using the diluted cDNA samples, 1:20 ratio of cDNA to RNase free water was pipetted into 3 new qPCR tubes. The forward and reverse primers for each gene of interest and the reference gene master mix were prepared separately. The primer stocks were diluted following the manufacturer's instructions. The master mix (7  $\mu\text{L}$ ) of each gene was pipetted into the qPCR sample tubes. Thereafter, 3  $\mu\text{L}$  of cDNA was added into the qPCR sample tube in order to achieve a final volume of 10  $\mu\text{L}$ . The samples were loaded into the RT-qPCR machine (model number, 4376592, Carlifornia, USA; ThermoFisher Scientific Inc.) for analysis. The RT-qPCR was set to run according to the manufacturer's instructions. A standard cycling mode was used. The primer stocks were diluted according to the manufacturer's instructions. Briefly, from the master mix of each gene, 7  $\mu\text{L}$  was pipetted into the qPCR sample tubes. Thereafter, 3  $\mu\text{L}$  of cDNA was added into the qPCR sample tube in order to

achieve a final volume of 10  $\mu\text{L}$ . The samples were loaded into the RT-qPCR machine for analysis in a specific order of choice. The RT-qPCR was set to run according to the manufacturer's instructions. A standard cycling mode was used.

### Glucose Measurement by Gas Chromatography–Mass Spectrometry

Glucose and glucose derivatives measurements from the skeletal muscle tissue samples from the study were performed according to a method described by Folch et al.<sup>27</sup> Briefly, muscle tissue samples were placed on ice and cut into small pieces and 50 mg was weighed and placed into microcentrifuge tubes. An internal standard (3-phenylbutyric acid) was added to the weighed muscle tissue together with a mixture of solvents (methanol, water, and chloroform). Steel beads were then added to the sample mixture, and the samples were placed on a vibrator for homogenization. The steel beads were subsequently removed from the sample and the mixture transferred into a new clean tube. Following homogenization, the IS was added to the sample. The mixture was then vortexed for 60 seconds using a Vortex-2 Genie machine (Vortex-Genie 2, Scientific Industries, Inc. Bohemia, NY, USA). Thereafter, the sample mixture was centrifuged for 10 minutes at  $2000\times g$  at  $4^\circ\text{C}$  resulting in phase separation, with the clear polar layer at the bottom and the milky apolar layer at the top of the muscle pellet. The polar and apolar samples were used for analysis, wherein each sample (725  $\mu\text{L}$ ) of the polar sample was used and 400  $\mu\text{L}$  of the apolar sample was used and transferred into Agilent vials. Five replicates were used for each sample. The samples were then dried under nitrogen. An oximation solution was then prepared by dissolving 200 mg of methoxyamine into 10 mL of pyridine. The oximation solution (50  $\mu\text{L}$ ) was added to each sample and vortexed for 60 seconds. The samples were incubated at room temperature for 1 hour. Bistrifluoroacetamide (50  $\mu\text{L}$ ), which contained 1% of trimethylsilyl chloride was added and then incubated for 1 hour at  $40^\circ\text{C}$ . An external standard (methyl tricosanoic acid) was then prepared using 50  $\mu\text{L}$  of 30  $\mu\text{g}/\text{mL}$  eicosane in hexane and added into the Agilent vials inserts of each sample before running the gas chromatography–mass spectrometry analysis.

The Ra of each lipid was analyzed and calculated using the formula:

$$\text{Ra} = \text{Aa}/\text{ma}$$

where ma is the mass of the lipid analyte of the free fatty acid, *a* and Aa is the corresponding peak area of the monosaccharide *a*, and Ra is the response factor or the peak area ratio of the monosaccharide *a*.

### Statistical Analysis

Data were expressed as mean  $\pm$  standard deviation. One way analysis of variance was used to determine differences of the means from different treatment regimens. All statistical

analyses were performed in R version 3.4.0 statistical software (R Development Core Team, Auckland, New Zealand).


### Declaration of Conflicting Interests


The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.


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
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### References

- Gamede M, Mabuza L, Ngubane P, Khathi A. The effects of plant-derived oleanolic acid on selected parameters of glucose homeostasis in a diet-induced Pre-Diabetic rat model. *Molecules*. 2018;23(4):794. doi:10.3390/molecules23040794
- Sikaris KA. The clinical biochemistry of obesity. *Clin Biochem Rev*. 2004;25(3):165-181.
- Steyn K, Fourie J, Temple N. *Chronic Diseases of Lifestyle in South Africa: 1995 - 2005*. South African Medical Research Council; 2016. <http://www.mrc.ac.za/reports/chronic-diseases-lifestyle-south-africa-1995-2005>
- Hossain MA, Amin A, Paul A, et al. Recognizing obesity in adult hospitalized patients: a retrospective cohort study assessing rates of documentation and prevalence of obesity. *J Clin Med*. 2018;7(8):E203. doi:10.3390/jcm7080203
- Ferré P. The biology of peroxisome proliferator-activated receptors: relationship with lipid metabolism and insulin sensitivity. *Diabetes*. 2004;53(suppl 1):S43-S50. doi:10.2337/diabetes.53.2007.S43
- Staels B, Fruchart J-C. Therapeutic roles of peroxisome proliferator-activated receptor agonists. *Diabetes*. 2005;54(8):2460-2470. doi:10.2337/diabetes.54.8.2460
- Gurib-Fakim A. Medicinal plants: source of new bio-molecules and standardised herbal drugs. *Regulatory Rapporteur-TOpra*. 2006;3:2-4.
- Ayeleso TB, Matumba MG, Mukwevho E. Oleanolic acid and its derivatives: biological activities and therapeutic potential in chronic diseases. *Molecules*. 2017;22(11):1915. doi:10.3390/molecules22111915
- Molepo M, Ayeleso A, Nyakudya T, Erlwanger K, Mukwevho E. A Study on Neonatal Intake of Oleanolic Acid and Metformin in Rats (*Rattus norvegicus*) with Metabolic Dysfunction:

- Implications on Lipid Metabolism and Glucose Transport. *Molecules*. 2018;23(10):2528. doi:10.3390/molecules23102528
10. Taher M, Mohamed Amiroudine MZA, Tengku Zakaria T, et al.  $\alpha$ -Mangostin Improves Glucose Uptake and Inhibits Adipocytes Differentiation in 3T3-L1 Cells via PPAR $\gamma$ , GLUT4, and Leptin Expressions. *Evid Based Complement Alternat Med*. 2015;2015:740238. doi:10.1155/2015/740238
  11. Stienstra R, Duval C, Müller M, Kersten S. PPARs, obesity, and inflammation. *PPAR Res*. 2007;2007:1-. doi:10.1155/2007/95974
  12. Vidal-Puig A, Jimenez-Liñan M, Lowell BB, et al. Regulation of PPAR gamma gene expression by nutrition and obesity in rodents. *J Clin Invest*. 1996;97(11):2553-2561. doi:10.1172/JCI118703
  13. Vidal-Puig AJ, Considine RV, Jimenez-Liñan M, et al. Peroxisome proliferator-activated receptor gene expression in human tissues. Effects of obesity, weight loss, and regulation by insulin and glucocorticoids. *J Clin Invest*. 1997;99(10):2416-2422. doi:10.1172/JCI119424
  14. Moller DE, Berger JP. Role of PPARs in the regulation of obesity-related insulin sensitivity and inflammation. *Int J Obes Relat Metab Disord*. 2003;27(Suppl 3):S17-S21. doi:10.1038/sj.ijo.0802494
  15. Wang N, Kong R, Luo H, Xu X, Lu J. Peroxisome proliferator-activated receptors associated with nonalcoholic fatty liver disease. *PPAR Res*. 2017;2017(16):1-8. doi:10.1155/2017/6561701
  16. Yoon M. Ppar $\alpha$  in obesity: sex difference and estrogen involvement. *PPAR Res*. 2010;2010. 17 08 2010. doi:10.1155/2010/584296
  17. Kawada T, Hagihara K, Iwai K. Effects of capsaicin on lipid metabolism in rats fed a high fat diet. *J Nutr*. 1986;116(7):1272-1278. doi:10.1093/jn/116.7.1272
  18. Huang S, Czech MP. The GLUT4 glucose transporter. *Cell Metab*. 2007;5(4):237-252. doi:10.1016/j.cmet.2007.03.006
  19. Klip A, Volchuk A, He L, Tsakiridis T. The glucose transporters of skeletal muscle. *Semin Cell Dev Biol*. 1996;7(2):229-237. doi:10.1006/scdb.1996.0031
  20. Marín-Juez R, Díaz M, Morata J, Planas JV. Mechanisms regulating GLUT4 transcription in skeletal muscle cells are highly conserved across vertebrates. *PLoS One*. 2013;8(11):e80628.21. doi:10.1371/journal.pone.0080628
  21. Gould GW, Holman GD. The glucose transporter family: structure, function and tissue-specific expression. *Biochem J*. 1993;295(Pt 2):329-341.
  22. Im S-S, Kwon S-K, Kim T-H, Kim H-I, Ahn Y-H. Regulation of glucose transporter type 4 isoform gene expression in muscle and adipocytes. *IUBMB Life*. 2007;59(3):134-145. doi:10.1080/15216540701313788
  23. Karnieli E, Armoni M. Transcriptional regulation of the insulin-responsive glucose transporter GLUT4 gene: from physiology to pathology. *Am J Physiol Endocrinol Metab*. 2008;295(1):E38-E45. doi:10.1152/ajpendo.90306.2008
  24. Richter EA, Hargreaves M. Exercise, GLUT4, and skeletal muscle glucose uptake. *Physiol Rev*. 2013;93(3):993-1017. doi:10.1152/physrev.00038.2012
  25. Armoni M, Kritiz N, Harel C, et al. Peroxisome proliferator-activated receptor-gamma represses GLUT4 promoter activity in primary adipocytes, and rosiglitazone alleviates this effect. *J Biol Chem*. 2003;278(33):30614-30623. doi:10.1074/jbc.M304654200
  26. Leonardini A, Laviola L, Perrini S, Natalicchio A, Giorgino F. Cross-Talk between PPAR $\gamma$  and insulin signaling and modulation of insulin sensitivity. *PPAR Res*. 2009;2009:1-12. doi:10.1155/2009/818945
  27. Folch J, Lees M, Stanley GHS. A simple method for the isolation and purification of total lipides from animal tissues. *J Biol Chem*. 1957;226(1):497-509.