



Exercise increases the expression of glucose transport and lipid metabolism genes at optimum level time point 6 h post-exercise in rat skeletal muscle

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Abstract

More than 90% of diabetes cases are type 2 diabetes characterized by persistent increase in glucose (hyperglycemia), lipid, and protein metabolic disorders that may induce insulin resistance. Individuals who suffer from type 2 diabetes are partly characterized by down-regulation of glucose transport and mitochondrial lipid oxidizing genes. Nuclear respiratory factor, (NRF)-1, is a mitochondrial transcriptional factor shown to be involved in glucose transport and acts as potential therapeutic modality in the management of T2DM. In this study, we accessed NRF-1 and its target gene expression crucial in glucose transport and lipid oxidation during exercise. Five- to 6-week-old male Wistar rats were exercised to identify the time-point for an optimum increase in the levels of NRF-1 and target genes. Gastrocnemius muscles were harvested after 0, 2, 4, 6, 8, 10, 12, and 15 h post-exercise and non-exercise rats. Primers were used to amplify the region of the genes; Nrf-1, glut 4, carnitine palmitoyltransferase, peroxisome proliferator-activated receptor gamma co-activator 1, mef2a, and acetyl-CoA carboxylase-1. Relative mRNA expression was normalized to the Actin reference gene. Cpt-1, Nrf-1, mef2a, glut4, cpt2, and Pgc-1 showed 2.5, 8, 1.2, 4.1, 4.6, 3.5-folds increase respectively after 8 h post-exercise compared with control, whereas Acc-1 showed a 3.1-fold decrease in gene expression ratio after 6 h post-exercise. Nrf-1 binding to cpt-1 and mef2a increased with 3 and 3.5-folds, respectively. Nrf-1 was increased by exercise with its binding to target genes which has huge implications in ameliorating type 2 diabetes and insulin resistance.

Keywords Type 2 diabetes · NRF · Exercise · Glut4 · Lipid oxidation · Glucose transport

Introduction

Exercise is known to protect individual from metabolic syndromes, in part by increasing gene transcription and induction of several signalling pathways vital in correcting impaired

metabolic pathways related with a sedentary lifestyle. The regulation of carbohydrate and lipid metabolism is very crucial in patients with insulin resistance and type 2 diabetes. Glucose transporter protein (GLUT-4) is responsible for more than 80% of whole body glucose disposal in skeletal muscle (Shulman et al. 1990). In type 2 diabetes, insulin-mediated glucose uptake, oxidation, and storage by the skeletal muscle are critically impaired (Kelley et al. 2002). GLUT4 is an insulin-responsive glucose transporter that controls glucose uptake (Charron et al. 1999; Sparling et al. 2008). GLUT4 transports glucose from blood to the cells, thereby supporting glucose homeostasis (euglycemia). It is reported that GLUT4 levels are low in diabetes mellitus (Garvey et al. 1991; Gibbs et al. 1995; Kahn et al. 1988; Sinha et al. 1991); hence, over-expression of Glut4 transcription is seen as a means to ease type 2 diabetes (Brozinick et al. 2001; Christ-Roberts et al. 2004; Oshel et al. 2000). Moreover, Glut4 gene is regulated by the myocyte enhancing (Mef2) transcription factor that binds to its cis-elements as a hetero-dimer (Mef2a/D)

Highlights

- Nuclear respiratory factor (NRF)-1 is involved in glucose transport.
- Exercise increases NRF-1 and its binding to target gene.
- NRF-1 acts as potential therapeutic modality in the management of T2DM.
- The expression of NRF-1 gene expression is crucial in lipid oxidation.

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resulting in GLUT4 expression (Oshel et al. 2000; Thai et al. 1998). GLUT4 expression is also regulated by nuclear respiratory factor (nrf-1), a mitochondrial transcription factor, which regulates *mef2a* gene to regulate *Glut4* (Baar et al. 2003; Ramachandran et al. 2008). Thus, owing to their essential roles in blood sugar metabolism, these genes have become targets for pharmacological therapeutics in the management and treatment of type 2 diabetes.

The study was conducted to identify optimum time point's increase/decrease of gene expression occurring in response to exercise. Genes express at specific various times and decay at different times. The optimum time point of induction/degradation of glucose transport and lipid metabolism gene expression were specifically targeted as well as the investigation on the optimum time point increase of Nrf-1 bound *Mef2a* and *Cpt-1* promoters.

Methodology

Animal care

Five- to 6-week-old male Wistar rats were used for this study. All animal procedures were approved by the Animal ethics committee of the University of Witwatersrand (WITS University), RSA. Rats were fed standard rat chow and water ad libitum. Room temperature was maintained at 21–24 °C with a 12-h light/dark cycle. Their welfare and weight were checked daily. The rats were familiarized to handling as well as to exercise protocol prior to the experiment to ensure that the animals were not stressed during the experiment. The temperature of water for swimming was monitored and maintained at 35 °C to ensure that the rats did not suffer from hypothermia or overheating during exercise. Following each bout of exercise, rats were gently towel-dried and allowed to rest in their cages to ensure that they experienced minimal discomfort by being wet.

Experimental design

In this study, a total number of 36 rats were used. Rats were divided into 2 groups, namely, exercise and non-exercise (control). The purpose of these 2 groups was to investigate the level of gene expression in response to exercise compared with non-exercising rats. The exercise group were then subdivided into 8 different groups, in which 4 rats formed a group. Four rats also formed the control group (Group 9). Rats were exercised according to protocol reported by Smith et al. (2007) and Terada et al. (2001). The swimming protocol used to exercise the rats is stipulated by a flow diagram in Supplementary file

(Fig. 1). From days 1 to 4, rats were housed and familiarized to handling. Thereafter, from days 5 to 8, rats were familiarized to swimming protocol as follows: on day 5, rats were subjected to 2 bouts of 17 min swimming and 3 min rest. From day 9 to day 14, rats rested in their cages in order to ensure that the familiarization protocol did not affect the experiment. From days 15 to 19, the exercise group performed 5 bouts of 17 min of swimming with 3 min of rest in between bouts. The control group remained in the cages for the entire duration of the experiment. Rats in groups 1 to 8 were then anaesthetized at different time periods: 0, 2, 4, 6, 8, 10, 12, and 15 h post exercise by intraperitoneal injection of Ketamine. Gastrocnemius muscle was dissected out for analysis, snap frozen in liquid nitrogen, and stored at –80 °C freezer in cryovial until required for use.

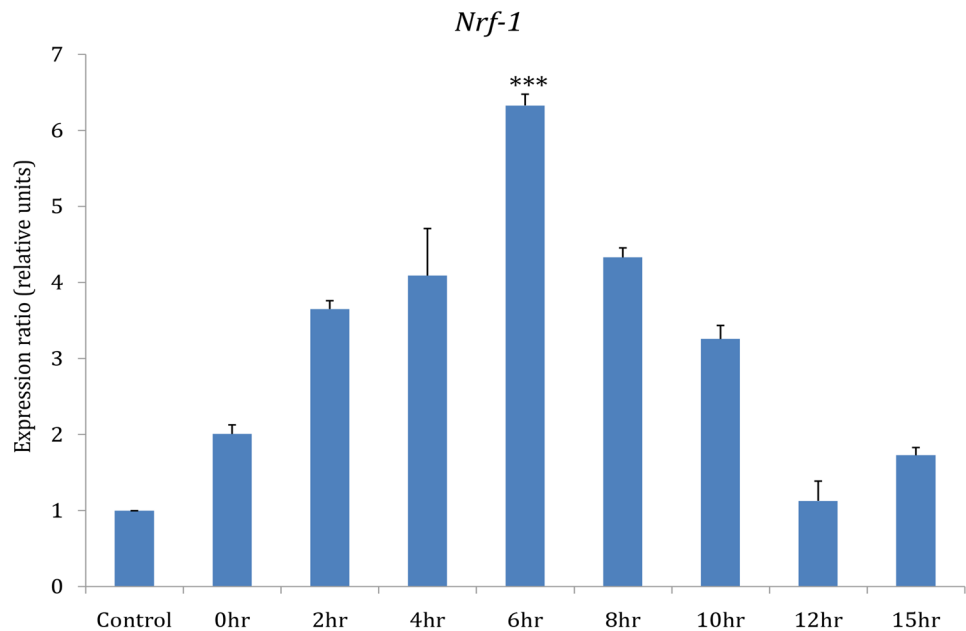
Quantitative real-time PCR

Total RNA was isolated and purified from ~100 mg frozen Gastrocnemius muscle using QIAzol lysis reagent (QIAGEN Sciences, USA) and RNA clean and Concentrator-25 (Inqaba Biotech, SA). Double stranded cDNA was synthesized from ~3 mg of total RNA using Superscript Reverse Transcriptase III (Invitrogen, USA). Quantitative real-time PCR were set up using SensiMix SYBER Noe ROX one-step kit (Bioline, UK) and were cycled according to the Sensi Mix kit instructions in Rotor Gene e3000 (QIAGEN Sciences, USA) qPCR machine. Real-time PCR was performed in triplicate using Rotor gene-3000 Thermo cycler PCR machine; Sensi Mix SYBER green PCR reagent and primers (Integrated DNA technologies, US) were used to amplify the genes. The primers are Nrf-1 forward 5'-TTA CTCTGCTGTGGCTGATGG-3 reverse 5'-CCTCTGATG CTTGCGTCTCT-3'; Mef2a forward: 5'-CCG CCT CAG AAC TTC TCA ATG-3' reverse 5'-TTG GAG AGG CCC TTG AGT TTA C-3'. Amplification occurred in a three-step cycle, denaturation at 95 °C for 5 s, annealing at 62 °C for 10 s and extension at 72 °C for 15 s. Relative mRNA expression was normalized to Actin reference gene (Actin forward primer- 5'-GAC GAG GCC CAG AGC AAG AGA 3' reverse primer 5' GGG TGT TGA AGG TCT CAA ACA 3'). Expression ratio was calculated according to relative standard method.

Chromatin immunoprecipitation assay

NRF-1 is known to bind to MEF2A and CPT-1 to regulate both glucose metabolism and lipid oxidation, respectively. To assess if exercise had any effect on NRF-1 binding activities to these promoters, we performed CHIP assay. This was

Fig. 1 Exercise increases *Nrf-1* gene expression to optimum level at time point 6 h post exercise. Gastrocnemius muscles were extracted 0, 2, 4, 6, 8, 10, 12, and 15 h post exercise. The *Nrf-1* gene expression of 6 h post exercise showed ~6.3 fold increase compared with the control. The *P* value exercise (6 h) vs control $P < 0.001$ (***)



used to identify the optimum time point of the increase of NRF-1 bound Mef2a and Cpt-1 promoter1 promoter regions after exercise compared with the control group (Table 1).

Approximately 100 mg of Gastrocnemius muscle from each group (Groups 1–9) was grounded with 10 mL of 1% formaldehyde in a phosphate buffered saline (PBS: pH 7.4) to cross-link protein to DNA. Cross-linking was stopped by adding 500 μ L of 2.5 M glycine, which was incubated for 5 min, centrifuged for 5 min at 13,000 rpm, and washed 3 times using 5 mL chilled PBS. Supernatant was then removed and pellet sonicated with 500 μ L of Lysis buffer (50 mM Tris HCl pH 8.0, 10 mM EDTA, 10% SDS, 10 mM Na₄P₂O₇, 20 mM NaF, 4 mM Na₃VO₄, 1 \times RCPI) on ice. The resultant chromatin was sheared 8 times for 15 s (1 min rest in between the cycles) at a maximum intensity of 33% on ice to achieve size ~300 to 1000 bp. The sonicated sample was then centrifuged at 13,000 rpm for 10 min, and the supernatant was transferred to a new tube. One hundred microlitres of the resultant supernatant containing chromatin was diluted with 900 μ L of ChIP dilution buffer (0.01% SDS, 1% Triton-X100, 1.2 mM EDTA, 16.7 mM Tris-HCl (pH 8) and 167 mM NaCl). Thirty microlitres of sample from each group was kept as an input sample (IN) and stored at -80°C

to cross-link together with the immune-precipitation (IP) sample. The remaining 970 μ L sample from each group was used for IP.

Pre-clearing of endogenous antibodies from the IP sample was done by adding 30 μ L of 50% agarose beads/Salmon sperm (Santa Cruz Biotechnology, US), followed by incubation at 4°C on a rotating flat-form using VWR tube rotator for 2 h. The sample was then centrifuged to pellet beads at 2000 rpm for 2 min at 4°C , and the supernatant was transferred to a new tube. Immunoprecipitation was allowed by incubating samples with 5 μ L of monoclonal anti-NRF-1 (Sigma) at 4°C (cold room) for 48 h, rotating gently. After incubation, immunocomplexes were precipitated with 40 μ L of 50% slurry agarose/salmon sperm for 6 h in a rotating flat form at 4°C . Beads were pelleted by centrifuge at 5000 rpm for 1 min at 4°C , and pellets from the IP sample were sequentially washed with different buffers and centrifuged at 2000 rpm: (1) low salt wash buffer, 1 mL 1×5 min at 4°C (0.1% SDS, 1% Triton-X100, 2 mM EDTA, 20 mM Tris-HCl (pH 8.0), 150 mM NaCl); (2) high salt buffer, 1 mL 1×5 min at 4°C (0.1% SDS, 1% Triton-X100, 2 mM EDTA, 20 mM Tris-HCl (pH 8.0), 500 mM NaCl); (3) lithium chloride wash buffer, 1 mL 1×5 min at 4°C (0.25 M LiCl, 1 mM EDTA, 10 mM Tris-HCl (pH 8.0)); and (4) Tris EDTA buffer, 1 mL 2×5 min at room temperature (RT) (1 mM EDTA, 10 mM Tris-HCl). After washing, beads were suspended in a fresh Elution buffer (1% SDS, 1 M NaHCO₃).

Elution was done with 150 μ L elution buffer for 15 min at RT and repeated twice to make a 300- μ L final volume. The above-mentioned IN sample was used from this point

Table 1 ChIP assay primer sequence

Gene	Primer sequence
Mef2a Forward	5' CCT TCC TGT GCC GGG TGA TCT 3'
Mef2a Reverse	5' CTA TTT TTA GGA GTC AGG CCC GG 3'
Cpt-1 Forward	5' TCG GGA CAC ACC CTT GGT GC 3'
Cpt-1 Reverse	5' CCG AGA CGA AGG GTA CCG GCT 3'

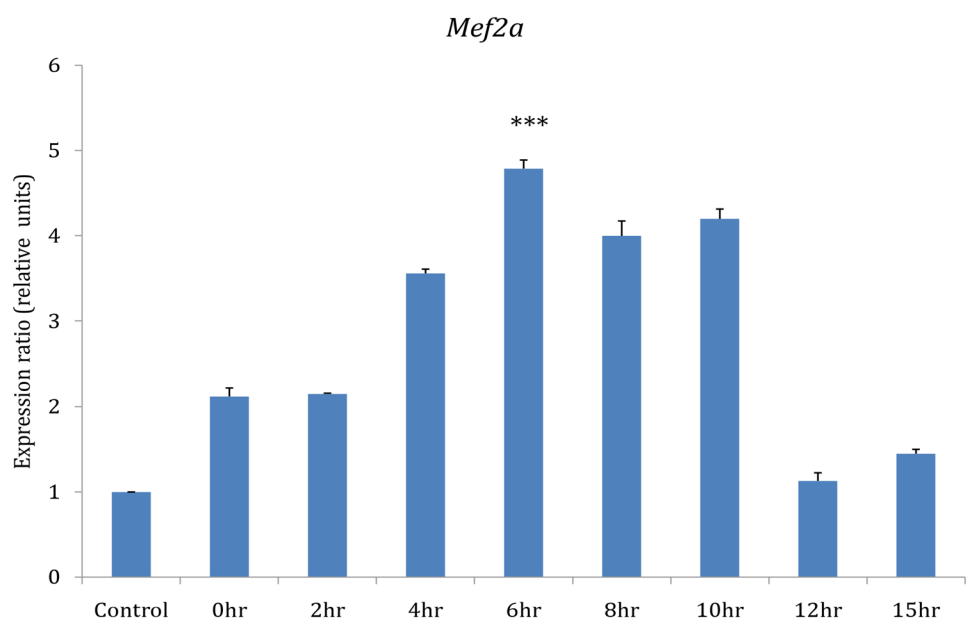
onwards. Ninety microlitres of elution buffer and 7.5 μL of 5 M NaCl (final concentration: 300 nM) were added to the 30 μL of IN sample mentioned above. IP and IN samples were then reverse cross-linked with 5 M NaCl (Final: 300 nM) at 65 °C overnight. This was followed by an addition of 0.5 M EDTA, 1 M Tris- HCl (pH 6.5) and proteinase K (stock: 10 $\mu\text{g}/\mu\text{L}$) and incubation at 45 °C for 1 h. This was to destroy the protein in the sample.

To assess the amount of NRF-1 bound to Mef2a, a 315-bp fragment Mef2a gene was PCR amplified from IN and IP samples. Quantitative real-time PCR also used to determine the amount of NRF-1 bound Mef2a and Cpt-1. Real-time PCR was performed in triplicate using Rotorgene-3000 thermo cycler PCR machine, Sensimix syber green PCR reagents (Bioline, UK), and primers were used to amplify regions of Mef2a. In brief, 2 μL of DNA were added to the following reagents: 10 μL of Sensi fast, 1 μL of 10 μM forward primer, 1 μL of 10 μM reverse primer, and made up to 20 μL with milliQ water. Amplification occurred in a 3-step cycle: denaturation at 95 °C for 5 s, annealing at 55 °C for 10 s, and extension at 72 °C for 15 s. Density of DNA was assessed by running 2% agarose gel electrophoresis.

Statistical analysis

Results are presented as means \pm SD. Statistical analysis was performed by one-way ANOVA followed by the Tukey's post hoc test. The level of significance was accepted at $P < 0.05$. All statistical analyses were performed using GraphPad InStat 3 software. *** indicates $P < 0.001$, ** indicates $P < 0.01$, and * indicates $P < 0.05$.

Fig. 2 Exercise increase *Mef2a* gene expression to optimum level at time point 6 h post exercise. Gastrocnemius muscles were extracted 0, 2, 4, 6, 8, 10, 12, and 15 h post exercise. At 6 h post exercise, the *Mef2a* expression showed ~4.6 fold increase compared with the control group. The P value exercise (6 h) vs control $P < 0.001$ (***)



Results and discussion

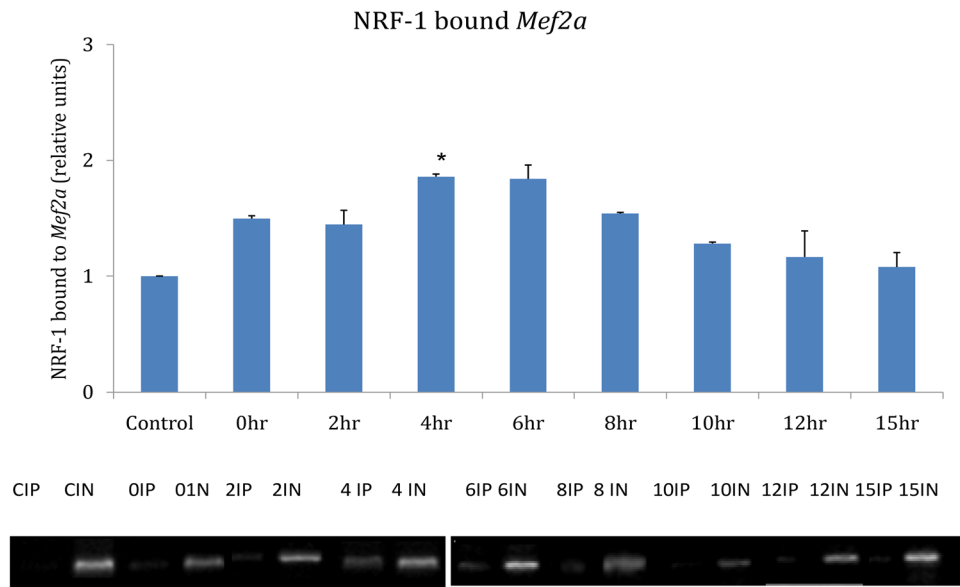
Exercise induces expression of glucose transport and lipid metabolising genes in rat skeletal muscle

NRF-1 is a transcriptional factor that regulates mitochondrial biogenesis and GLUT4 expression in parallel. This study assessed the optimum time point of *Nrf-1* gene expression in response to exercise. As shown in Fig. 1, *Nrf-1* gene expression of the exercise group showed significant increase compared with the control group. *Nrf-1* gene expression increased to the optimum level at (~6.3 fold) time point at 6 h post exercise. This result indicates that the optimum time point of *Nrf-1* gene expression is 6 h post exercise. As such, in intervention studies 6 h was used as the optimal time for its expression.

Furthermore, *Pgc-1* gene expression in response to exercise was investigated. PGC-1 is a transcriptional factor known to co-activate NRF-1 to induce mitochondrial biogenesis and glucose transport genes. As shown in Supplementary Fig. 2, after exercise *Pgc-1* gene expression showed significant increase when compared with the control group. *Pgc-1* expression increased to the optimum level (~4.0 fold) at time point 4 h post exercise compared with other time points. This result indicates that the optimum level of *Pgc-1* expression is at 4 h post exercise.

Glut4 expression was also analysed in response to exercise using qPCR. As shown in Supplementary Fig. 3, *Glut4* gene expression increased after exercise as compared with the control group. After 8 h of exercise, *Glut4* gene expression showed higher levels, ~threefold increase, compared with the control. This result indicates that *Glut4* gene expression increased to an optimum level at time point 15 h post exercise.

Fig. 3 Exercise increases NRF-1 bound *Mef2a* to optimum level at time point 4 h post exercise. Gastrocnemius muscles were extracted from rats 0, 2, 4, 6, 8, 10, 12, and 15 h post exercise. The NRF-1 binding to *Mef2a* gene increases after exercise and it showed an optimum increase 4 h post exercise. The *P* value, exercise (4 h) vs control $P < 0.05$ (*)



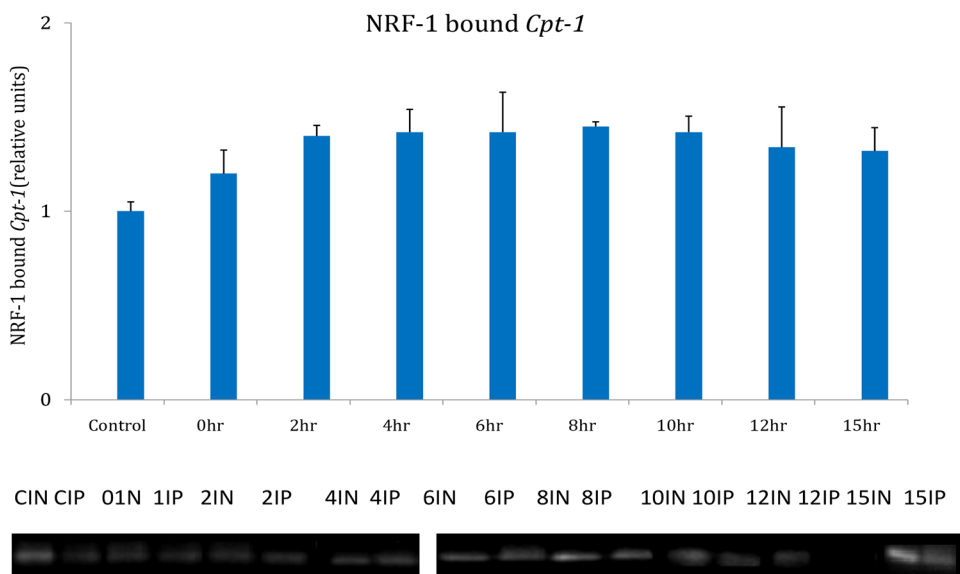
In addition, the optimum time point of *Mef2a* gene expression in response to exercise was investigated. Results shown in Fig. 2 indicate that gene expression of *Mef2a* increases after exercise as compared with the control group. After 6 h of exercise, *Mef2a* gene expression showed higher levels, ~4.2-fold increase compared with the control. This result indicates that the optimum time point of *Mef2a* gene expression is 6 h post exercise.

Thereafter, gene expressions of *Cpt-1* and *Acc-1* were also assessed in response to exercise. CPT-1 is an enzyme that encoded the *cpt-1* gene that controls the rate-limiting step in lipid oxidation. The result shown in

Supplementary Fig. 4 indicates that *Cpt-1* gene expression showed significant increase after 2, 4, 6, 8, 10, 12, and 15 h post exercise compared with the control group. *Cpt-1* gene expression shows an optimum level at time point 4 h post exercise.

In addition, *Acc-1* gene expression decreased after 0, 2, and 4 post exercise compared with the control (Supplementary Fig. 5). After 4 h post exercise, *Acc-1* gene expression shows optimum decrease ~2.4 fold, decrease compared with the control. This result indicates that the optimum time point of *Acc-1* gene expression is 4 h post exercise.

Fig. 4 Exercise increased NRF-1 bound *Cpt-1* in rat skeletal muscle, but not statistically significant



Exercise increases amount of NRF-1 bound Mef2a 4 h post exercise in rat skeletal muscle

NRF-1 is a transcriptional factor that regulates mitochondrial biogenesis and Glut4 expression indirectly via Mef2a. NRF-1 regulates Glut4 expression by regulating Mef2a gene expression because MEF2, which has binding sites on Glut4 promoter (Michael 2001). As such, we focussed on NRF-1 binding to its target genes, *Mef2a* and also *Cpt-1*. NRF-1 binding *Mef2a* promoter was assessed for how NRF-1 regulates glucose transport and its binding to *Cpt-1* gene on how NRF-1 regulate fatty acid metabolism. ChIP assay was used to determine NRF-1 bound *Mef2a* promoter at different time intervals. The results shown in Fig. 3 indicate that exercise increases the amount of NRF-1 bound *mef2a* compared with the control group. Exercise increases the binding of NRF-1 to *Mef2a* at an optimum time point 4 h post exercise, and it shows approximately 1.8-fold increases compared the control. This indicates that the optimum time point of NRF-1 bound *Mef2a* is 4 h post exercise.

Furthermore, the amount of NRF-1 bound *Cpt-1* were analysed in response to exercise. As shown in Fig. 4, NRF-1 bound *Cpt-1* was changed but not statistically significant change after exercise compared with the control group.

Conclusion

This study primarily focused on determining the optimum time point of gene expression in response to exercise. Genes are expressed at specific times and degrade very rapidly. Therefore, this study conducted to find out the optimum expression time point of genes that are responsible for glucose transport and lipid oxidation in response to exercise. This study indicates that glucose transport genes such as *Nrf-1*, *Pgc-1*, *Mef2a*, and *Glut4* show an optimum level at time points 6, 4, 6, and 8 h post exercise, respectively. After 6 h of exercise, expression of glucose transport genes shows significant increases compared with the control. Lipid metabolism genes such as *Cpt-1* show an optimum increase of gene expression 6 h post exercise, and *Acc-1* shows an optimum decrease 8 h post exercise. These results indicate that most genes are highly expressed after 6 h of exercise. Furthermore, this study showed that the optimum time point increases in the level of NRF-1 bound *Mef2a* and *Cpt-1* was 4 h and 8 h post exercise, respectively. In conclusion, the optimum time point of most glucose transport and lipid metabolism genes is 6 h post exercise.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s00580-022-03318-4>.

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Data availability The data analysed during the current study are available from the corresponding author on request.

Declarations

Ethical approval All animal procedures were approved by the Animal ethics committee of the University of Witwatersrand (WITS University), RSA.

Conflict of interest The authors declare no competing interests.

References

- Baar K, Song Z, Semenkovich CF, Jones TE, Han D-H, Nolte LA, Holloszy JO (2003) Skeletal muscle overexpression of nuclear respiratory factor 1 increases glucose transport capacity. *FASEB J* 17(12):1666–1673
- Brozinick JT, McCoid SC, Reynolds TH, Nardone NA, Hargrove DM, Stevenson RW, Gibbs EM (2001) GLUT4 overexpression in db/db mice dose-dependently ameliorates diabetes but is not a lifelong cure. *Diabetes* 50(3):593–600
- Charron MJ, Katz EB, Olson AL (1999) GLUT4 gene regulation and manipulation. *J Biol Chem* 274(6):3253–3256
- Christ-Roberts CY, Pratipanawatr T, Pratipanawatr W, Berria R, Belfort R, Kashyap S, Mandarino LJ (2004) Exercise training increases glycogen synthase activity and GLUT4 expression but not insulin signaling in overweight nondiabetic and type 2 diabetic subjects. *Metabolism* 53(9):1233–1242
- Garvey W, Maianu L, Huecksteadt T, Birnbaum M, Molina J, Ciaraldi T (1991) Pretranslational suppression of a glucose transporter protein causes insulin resistance in adipocytes from patients with non-insulin-dependent diabetes mellitus and obesity. *J Clin Investig* 87(3):1072–1081
- Gibbs EM, Stock JL, McCoid SC, Stukenbrok HA, Pessin JE, Stevenson RW, McNeish JD (1995) Glycemic improvement in diabetic db/db mice by overexpression of the human insulin-regulatable glucose transporter (GLUT4). *J Clin Investig* 95(4):1512–1518
- Kahn BB, Simpson IA, Cushman SW (1988) Divergent mechanisms for the insulin resistant and hyperresponsive glucose transport in adipose cells from fasted and refed rats. Alterations in both glucose transporter number and intrinsic activity. *J Clin Investig* 82(2):691–699.
- Kelley DE, He J, Menshikova EV, Ritov VB (2002) Dysfunction of mitochondria in human skeletal muscle in type 2 diabetes. *Diabetes* 51(10):2944–2950
- Michael L, Wu Z, Cheatham RB, Puigserver P, Adelman G, Lehman JJ, Kelly DP, Spiegelman BM (2001) Restoration of insulin-sensitive glucose transporter (GLUT4) gene expression in muscle cells by the transcriptional coactivator PGC-1. *Proc Natl Acad Sci USA* 98:3820–3825
- Oshel KM, Knight JB, Cao KT, Thai MV, Olson AL (2000) Identification of a 30-base pair regulatory element and novel DNA binding protein that regulates the human GLUT4 promoter in transgenic mice. *J Biol Chem* 275(31):23666–23673
- Ramachandran B, Yu G, Gulick T (2008) Nuclear respiratory factor 1 controls myocyte enhancer factor 2A transcription to provide a mechanism for coordinate expression of respiratory chain subunits. *J Biol Chem* 283(18):11935–11946
- Shulman GI, Rothman DL, Jue T, Stein P, DeFronzo RA, Shulman RG (1990) 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* 322(4):223–228
- Sinha MK, Raineri-Maldonado C, Buchanan C, Pories WJ, Carter-Su C, Pilch PF, Caro JF (1991) Adipose tissue glucose transporters in NIDDM: decreased levels of muscle/fat isoform. *Diabetes* 40(4):472–477
- Smith JA, Collins M, Grobler LA, Magee CJ, Ojuka EO (2007) Exercise and CaMK activation both increase the binding of MEF2A to the Glut4 promoter in skeletal muscle in vivo. *Am J Physiol Endocrinol Metab* 292(2):E413–E420
- Sparling DP, Griesel BA, Weems J, Olson AL (2008) GLUT4 enhancer factor (GEF) interacts with MEF2A and HDAC5 to regulate the GLUT4 promoter in adipocytes. *J Biol Chem* 283(12):7429–7437
- Terada S, Yokozeki T, Kawanaka K, Ogawa K, Higuchi M, Ezaki O, Tabata I (2001) Effects of high-intensity swimming training on GLUT-4 and glucose transport activity in rat skeletal muscle. *J Appl Physiol* 90(6):2019–2024
- Thai MV, Guruswamy S, Cao KT, Pessin JE, Olson AL (1998) Myocyte enhancer factor 2 (MEF2)-binding site is required for GLUT4 gene expression in transgenic mice: regulation of MEF2 DNA binding activity in insulin-deficient diabetes. *J Biol Chem* 273(23):14285–14292
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