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The effect of acute exercise on GLUT4 levels in peripheral blood mononuclear cells of sled dogs

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ABSTRACT

Using sled dogs as exercise model, our objectives of this study were to (1) assess the effects of one acute bout of high-intensity exercise on surface GLUT4 concentrations on easily accessible peripheral blood mononuclear cells (PBMC) and (2) compare our findings with published research on exercise induced GLUT4 in skeletal muscle. During the exercise bout, dogs ran 5 miles at approximately 90% of VO_2 max. PMBC were collected before exercise (baseline), immediately after exercise and after 24 h recovery. GLUT4 was measured via ELISA. Acute exercise resulted in a significant increase on surface GLUT4 content on PBMC. GLUT4 was increased significantly immediately after exercise ($\sim$50%; $p < 0.05$) and reduced slightly by 24 h post-exercise as compared to baseline ($\sim$22%; $p > 0.05$). An effect of acute exercise on GLUT4 levels translocated to the cell membrane was observed, with GLUT4 levels not yet returned to baseline after 24 h post-exercise. In conclusion, the present investigation demonstrated that acute high-intensity exercise increased GLUT4 content at the surface of PBMC of sled dogs as it has been reported in skeletal muscle in other species. Our findings underline the potential use of peripheral blood mononuclear cell GLUT4 protein content as minimally invasive proxy to investigate relationships between insulin sensitivity, insulin resistance, GLUT4 expression and glucose metabolism.

1. Introduction

Exercise training improves insulin sensitivity [1–4] and is a cornerstone treatment for insulin resistance and diabetes [5]. A fundamental adaptation to exercise training is the increase in glucose transporter 4 (GLUT4) protein in skeletal muscle [6]. GLUT4 is one of 13 facilitative glucose transport proteins. Both exercise and insulin cause GLUT4 to translocate from intracellular storage vesicles to the plasma membrane allowing the entry of glucose into the cell that can then be used as substrate during exercise or during recovery from exercise to replenish glycogen stores [6]. While GLUT4 protein is expressed most abundantly in adipose tissue and cardiac and skeletal muscle, there is a growing body of research investigating the effect of GLUT4 expression on the plasma membrane of peripheral blood mononuclear cells (PBMC) and the effects of insulin under resting and stimulated conditions both in vivo and in vitro [7]. However, more studies are needed to characterize the relationship of other physiological or laboratory stimuli on GLUT4 translocation in PBMC. Dogs have been a traditional model of health research, and, in Arctic Medicine, sled dogs have been used to study the immune system, nutrition and exercise endurance in extreme environments [8,9].

Until 20 years ago, there was a common belief that the adaptation of skeletal muscle to exercise is a slow process and GLUT4 protein levels increase after several weeks of regular exercise in rodents [10–13] and humans [2,14–16] but soon researchers started to hypothesize that a rapid (within hours) increase in GLUT4 is necessary to help organisms deal with environmental changes that are responsible for an adaptive response [17]. Ren et al. demonstrated through an experiment in which rats that were exercised by swimming, had a 50% increase in GLUT4 protein expression in epitrochlearis muscle 16 h after the exercise session and was the first to show that a rapid increase in GLUT4 expression is an early adaptive response of muscle to exercise [17]. Shortly after, Gulve et al. were the first that reported an increase in GLUT4 protein in human skeletal muscle after 7–10 days of cycle ergometer exercise [18]. Greiwe et al. measured skeletal muscle GLUT4 protein content before, 8 h, and 22 h after a single bout of exercise and were the first to report that a single bout of exercise increases human skeletal muscle GLUT4 protein

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content [19] and this was subsequently reaffirmed by Green et al., who found that GLUT4 protein content in skeletal muscle was rapidly up regulated after just one 6 min session of heavy intermittent exercise [20]. Taken together, these studies contribute to the body of evidence that show an effect of exercise as a stimulus for upregulating skeletal muscle GLUT4 protein content occurring soon after acute exercise in rodent and human models.

Similarly, it has been shown that GLUT4 protein content remains elevated for about 24 h after the last exercise bout [19,21,22], there is a rapid decline in skeletal muscle GLUT4 protein content with detraining [23,24] and the adaptive increase in GLUT4 is lost within 40 h [24]. Taken together, these findings suggest that the GLUT4 protein has a short half-life in the range of 8–10 h [24]; at what time point after exercise GLUT4 protein content is at its peak has yet to be characterized.

It still remains unclear whether exercise and muscle contraction, which stimulates GLUT4 translocation locally, would also have an effect on the expression or translocation of GLUT4 in circulating mononuclear. In a previous study we have shown that four months of long term conditioning significantly increased GLUT4 content in mononuclear cells of sled dogs [25]. The present study was undertaken to address the question of whether the observed increase in skeletal muscle GLUT4 protein after one acute bout of exercise, measured by highly invasive biopsies, can also be measured in peripheral mononuclear cells, assessed via minimally-invasive blood sampling, to further examine the potential use of this technique as reliable and feasible technique in a clinical setting. To answer this question we measured mononuclear cell surface GLUT4 protein content before, immediately after and 24 h after one acute bout of exercise in sled dogs.

2. Materials and methods

2.1. Animals and diet

Sled dogs in Salcha, Alaska (Latitude 65°N, 147°W) were used as test subjects. The protocol of this study was approved by the Institute of Animal Care and Use Committee (IACUC) at the University of Alaska Fairbanks (#02-14). The dogs that were used were typical racing sled dogs. Eight sled dogs ($n=8$), conditioned for 6 months, were sampled after completion of their racing season at the end of March. The dogs used in this experiment were sprint racing sled dogs that exercised on average 4–5 times per week. Their training season began in September, starting with 3 miles at approximately 15 miles per hour (~80% VO_2 max); by the end of the 6 months, when sampling occurred, the dogs were training and racing between 8 and 15 miles at speeds between 18 and 21 mph (~90% VO_2 max). Dogs were balanced for age and ability, and sex (all dogs were sexually intact). Age ranged from 2 to 7 years (4 ± 2 years). All dogs were of similar lineage. During the study, the dogs had access, at all times, to his or her house to which they were tethered on 2 m chains. Dogs were fed the same high performance diet and were allowed ad libidium access to water. Each dog was fed to maintain its ideal body condition score of 3–4 [26]. The temperature on the day of the exercise session was −15 °C (March 27th, 2013).

2.2. Experimental protocol

The dogs were brought to the research facility at 9:00 AM after an overnight fast. Dogs were kept at rest for three days before the test. Blood samples were obtained before (PRE), immediately after (POST), and 24 h after exercise (24 h POST). Dogs were run in front of a dog sled. A distance of 5 miles at around 90% VO_2 max was chosen as an acute exercise bout in order to physically exert the animals while staying well within their capabilities. The exercise bout lasted about 17 min and the dogs averaged 18 mph during the 5 miles. Blood was collected into EDTA tubes (6 mL, for measurement of insulin and glucose concentrations) and BD Separation tubes (6 mL, for measurement of GLUT4 levels) using the cephalic vein. All tubes were stored upright at room temperature until centrifugation. Whole blood was spun within 2 h of blood collection at room temperature at 3600 rpm for 15 min. Immediately after, aliquots of plasma collected from EDTA tubes were frozen at −80 °C and stored for later insulin and glucose analysis. BD mononuclear separation tubes were used to collect the buffy coat (mononuclear interphase layer containing white blood cells). Mononuclear cells were washed a total of three times and resuspended in 4 mL of RPMI w/5% calf serum. Aliquots of the resuspended samples were used for GLUT4 ELISA analysis and adjusted for protein content using BCA Protein assay. Both assays were run on the same day of the blood draws (within 6 h of blood collection).

2.3. Biochemical analysis

The protocol for the assessment of GLUT4 protein concentrations translocated to the surface of mononuclear cells has been described elsewhere [25]. We controlled for the measurement of GLUT4 translocated to the cell membrane, and not total GLUT4, in comparing sonicated to unsonicated samples. We could detect the same trend as we have seen in an earlier study, namely that sonicated samples had 3 fold higher GLUT4 levels compared to unsonicated samples (data not shown) [27]. Protein content was determined using the BCA Protein Assay (Pierce, Thermo Scientific, United States). Insulin levels were measured using an ELISA (Porcine/Canine; ALPCO, Salem NH), following the protocol by the manufacturer. All absorbance readings were done using Synergy HT multi-mode microplate reader (BioTek, United States). Plasma Glucose analysis was performed by the North Pole Veterinary Clinic (North Pole, AK) using the in-house diagnostic Catalyst® Chemistry Analyzer (IDEXX, United States). All samples were assayed in duplicates as an internal control.

2.4. Statistical analysis of data

Samples were analyzed using GraphPad Prism statistical software (version 5.0). Data were analyzed using one-way ANOVA with Tukey post hoc analysis. All results are expressed as means ± SD. Differences were considered significant at $P \leq 0.05$.

3. Results

The effects of one acute bout of exercise on surface GLUT4 protein content in mononuclear cells are summarized in Fig. 1. Surface GLUT4 protein levels increased 50% immediately after exercise (5414 ± 631 ng/g protein vs. 3617 ± 948 ng/g protein; $P < 0.05$) and remained slightly, but non-significantly, elevated 24 h after the exercise bout relative to pre exercise (4421 ± 722 ng/g protein vs. 3617 ± 948 ng/g protein; $P > 0.05$).

The effects of exercise on plasma insulin and glucose levels are summarized in Fig. 2. Plasma insulin levels did not significantly change after exercise (3.2 ± 0.66 μU/mL (POST) and 3.2 ± 0.36 μU/mL (24 h POST) vs. 3.8 ± 0.66 μU/mL (PRE); $P > 0.05$). Blood glucose levels increased significantly immediately after exercise (156 ± 58 mg/dL vs. 92 ± 4.9 mg/dL; $P < 0.05$), but were back to baseline by 24 h POST (96 ± 9.0 mg/dL vs. 92 ± 4.9 mg/dL; $P > 0.05$). The drop in blood glucose levels between POST and 24 h POST was statistically significant ($P < 0.05$).

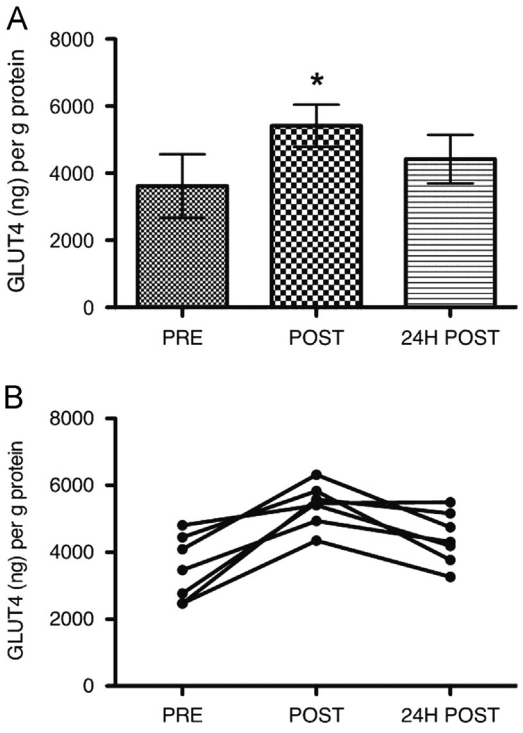

Fig. 1. Mononuclear cell surface GLUT4 levels in exercising sled dogs. Values are means ± SD. (a) GLUT4 protein levels in mononuclear cells of sled dogs before (PRE), immediately after (POST) and 24 h after (24 h POST) exercise at 90–95% VO_2 max. *Significantly different from PRE exercise ($p < 0.05$). (b) GLUT4 protein levels are shown for each dog individually (dots connected by lines).

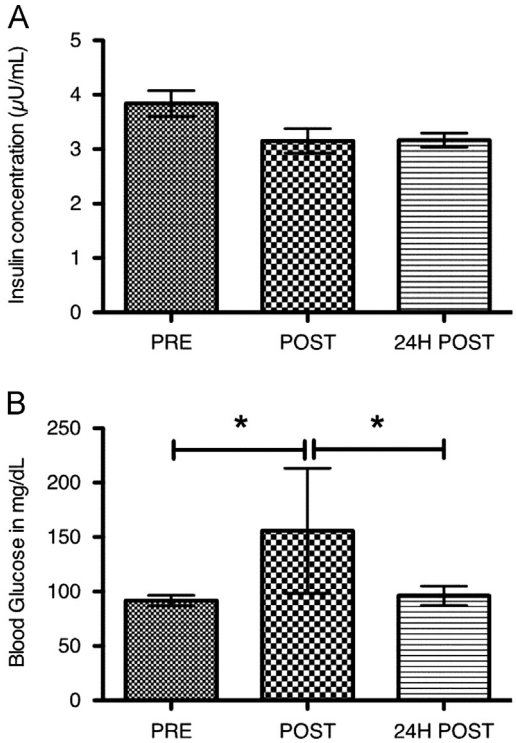

Fig. 2. Insulin and Glucose concentrations in exercising sled dogs. Values are means ± SD. (a) Plasma insulin levels in sled dogs before (PRE), immediately after (POST) and 24 h after (24 h POST) exercise at 90–95% VO_2 max. Statistically significant differences were not detected in plasma insulin levels at rest, after exercise or after 24 h recovery ($p > 0.05$). (b) Blood glucose levels in sled dogs before (PRE), immediately after (POST) and 24 h after (24 h POST) exercise at 90–95% VO_2 max. *Significant difference between PRE vs. POST ($p < 0.05$) and POST vs. 24 h POST ($p < 0.05$) blood glucose levels.

4. Discussion

Prior to this investigation it was unknown whether exercise and muscle contraction, which stimulates GLUT4 translocation locally (skeletal muscle), would have any effect on circulating mononuclear cell surface GLUT4 levels. Our study demonstrated, for the first time, that a single high intensity bout of exercise, increases surface GLUT4 levels in mononuclear cells of sled dogs and agrees with observations made in skeletal muscle of humans and rodents [17,19,21,22,28]. We have previously examined whether long-term conditioning had an adaptation effect on mononuclear cell surface GLUT4 content and found that four months of endurance training increased GLUT4 protein levels expressed at the surface in mononuclear cells of sled dogs [25]. These results are consistent with observations made in rodents and humans skeletal muscle [2,3,10–12,15,29].

Another important finding in the present study was the 50% increase in surface GLUT4 protein levels immediately after exercise (Fig. 1). A similar increase in magnitude has been described in a previous study that reported a 60% increase in human skeletal muscle GLUT4 protein expression 3 h after exercise [22]. Similarly, studies in rats have observed rapid (1.5–24 h) upregulation (~50–100%) of GLUT4 protein levels after acute exercise [17,21,30]. The rapid increases in GLUT4 immediately after exercise and slight decrease 24 h post exercise suggest that the half-time of this protein in vivo in mononuclear cells using the dog model is short, similar to results obtained in muscle [18]. These adaptive responses to exercise may contribute to enhanced insulin action in the post-exercise or recovery period [22].

We did not observe a significant change in plasma insulin levels immediately after exercise (Fig. 2A) which indicates that the recruitment of GLUT4 protein to the plasma membrane of mononuclear cells may be independent of insulin levels but instead was stimulated by exercise as an alternative, physiological stimulus. The same findings have been made in studies using rat skeletal muscle, concluding that insulin is may not be necessary or that there is an alternative pathway that also causes an increase in GLUT4 at the plasma membrane in contracting skeletal muscle [31–33]. Our findings in mononuclear cells of sled dogs are the first evidence that there might be an insulin-independent pathway that leads to the stimulation of mononuclear glucose GLUT4 translocation by exercise.

The potential use of GLUT4 expression in mononuclear cells as low-invasive model to investigate the relationships between insulin signaling, GLUT4 expression and insulin action in vivo is surely not a new idea. Results of several studies provide evidence that mononuclear GLUT4 translocation occurs in response to acute insulin exposure, and may be sensitive to the relative state of insulin resistance of the individual [7]. However, we are aware that further research needs to be undertaken to further validate GLUT4 translocation in mononuclear cells before applications of this method could find its way into a clinical setting as a marker for glucose homeostasis. Now that we have demonstrated the effect of mononuclear GLUT4 translocation by two physiological stimuli, acute exercise and in a prior study long-term conditioning [25], it is necessary to take further steps and set up studies examining adaptations to long-term interventions of exercise, diet, weight loss and pharmacological nature. Additionally it is necessary for the research community to compare GLUT4 expression in mononuclear cells and other tissues such as skeletal muscle and adipose tissue, simultaneously, in order to establish the clinical meaningfulness of this technique. If the technique of measuring GLUT4 protein levels on the plasma membrane via easily, minimally-invasive accessible peripheral blood cells as compared to taking standard muscle biopsies proves to be a reliable index of insulin sensitivity and glucose transport at the

whole body level it would markedly facilitate investigations in this field [7].

Maratou et al. had found an increased translocation of GLUT1, GLUT4 and GLUT3 isoforms on the plasma membrane after insulin activation of white blood cells suggesting an increase in white blood cell responsiveness to insulin as part of the immune response [34]. This could also be an explanation for the biological function of an increase in GLUT4 protein in white blood cells after exercise stimulation. Further research will be needed to establish GLUT4 as a marker of glucose metabolism in identifying whether surface GLUT1 and GLUT3 proteins are increased after exercise activation.

Sled dogs appear to be an excellent comparative model for insulin signaling in regard to exercise since the energy needs of sled dogs, exercising in arctic climate, is increased by 3–8 fold as compared to human elite athletes and therefore amplify any effect of exercise on blood parameters [35]. There are several lines of evidence that show that the sled dog model is a good example of the growing "one health movement" in combining disciplines of biomedical and environmental research in itself [36–38]. Based on the results of our study we propose that the sled dog model should be considered when designing experiments to evaluate the impact of nutritional supplements, e.g. through highly intensive, minimally invasive exercise experiments over one day, before human trials.

5. Conclusions

In conclusion, a single high intensity exercise bout at ~90% VO_2 max, increased GLUT4 protein translocation to the cell membrane in PBMC of sled dogs as it has been reported in skeletal muscle in other species. Our findings underline the potential use of peripheral blood mononuclear cells GLUT4 protein content as minimally invasive proxy to investigate relationships between insulin sensitivity, insulin resistance, GLUT4 expression and glucose metabolism. The next step we will take are to perform similar experiments using human athletes through which we hope to generate results that contribute to developing the mononuclear GLUT4 method so that its application in a clinical setting can be further validated.

Author contributions

S. TM wrote manuscript, contributed to study design, data collection and analysis, protocol development. R. AJ contributed to study design, reviewed/edited manuscript. K. AM contributed with data collection and data analysis. D. LK contributed to discussion, reviewed/edited manuscript. D. KL data collection and analysis, contributed to study design, protocol development, reviewed/edited manuscript.

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Appendix A. Transparency document

Transparency document associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.bbrep.2015.05.002.

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