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Conditioning causes an increase in glucose transporter-4 levels in mononuclear cells in sled dogs

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ABSTRACT

This study was designed to investigate the effects of physical conditioning on the expression of the insulin sensitive glucose transporter-4 protein (GLUT4) on mononuclear cells and HOMA-IR levels in dogs and compared to results reported in human skeletal muscle and the skeletal muscle of rodent models. Blood was sampled from conditioned dogs ($n = 8$) and sedentary dogs ($n = 8$). The conditioned dogs were exercised four months prior the experiment and were following a uniform training protocol, whereas the sedentary dogs were not. GLUT4 expression in mononuclear cells and plasma insulin levels were measured using commercially available enzyme-linked immunosorbent assay (ELISA). Blood glucose levels were determined using blood plasma. HOMA-IR was calculated using plasma insulin and blood glucose levels using the linear approximation formula. Our results indicate that the state of conditioning had a significant effect on the GLUT4 expression at the surface of mononuclear cells. HOMA-IR was also affected by conditioning in dogs. GLUT4 levels in mononuclear cells of sled dogs were inversely correlated with the homeostasis model assessment of insulin sensitivity. This study demonstrates that conditioning increases GLUT4 levels in mononuclear cells of sled dogs as it has been previously reported in skeletal muscle. Our results support the potential of white blood cells as a proxy tissue for studying insulin signaling and may lead to development of a minimally invasive and direct marker of insulin resistance. This may be the first report of GLUT4 in mononuclear cells in response to exercise and measured with ELISA.

1. Introduction

The glucose transporter-4 (GLUT4) plays a central role in whole-body glucose homeostasis and defective GLUT4 trafficking likely represents one of the earliest defects contributing to insulin resistance in humans (Stöckli et al., 2011). Insulin resistance (IR) is characterized by an inability of cells to respond to insulin upon stimulation with glucose and presents as an important risk factor for the development of type 2 diabetes (T2D) (Bastard et al., 2006). Current methods for diagnosing IR and T2D is often done with a combination of comorbidities and a mathematical index based on fasting glucose–insulin ratios, or glucose tolerance test (Wallace et al., 2004). As prevalence of IR and T2D reach alarming rates (Seidell, 2000; Sharma and Chetty, 2005), the search for direct and reliable diagnostic methods becomes increasingly more important.

GLUT4 is found and studied predominately in muscle and adipose tissue, requiring invasive tissue biopsies (Melling et al., 2013), however as early as 1975, Schwartz et al. reported on insulin binding in monocytes (Schwartz et al., 1975). It was then discovered that the ratio between insulin binding to monocytes and lymphocytes is constant from person to person, suggesting that it is possible to estimate the insulin binding to monocytes from the binding data obtained from a mixed suspension of mononuclear leucocytes (Beck-Nielsen et al., 1977). Further, insulin receptors on monocytes have been successfully correlated with glucose intolerance and

Abbreviations: BCA, bicinchoninic acid; EDTA, ethylenediaminetetraacetic acid; ELISA, enzyme-linked immunosorbent assay; GLUT4, glucose transporter-4 protein; HbA1c, glycated hemoglobin; HOMA-IR, homeostasis model assessment of insulin resistance; IACUC, The Institute of Animal Care and Use Committee; IR, insulin resistance; RPM, revolutions per minute; RPMI, Roswell Park Memorial Institute medium; T2D, type 2 diabetes; WBC, white blood cells.

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insulin sensitivity (Beck-Nielsen and Pedersen, 1978). The glucose transporter isoform expressed by subpopulations of mononuclear cells had not been identified until 2002, at which time Korgun et al. discovered the GLUT4 isoform (Korgun et al., 2002). In 2007, Maratou et al. showed an increase in translocation of GLUT4 on the plasma membrane of mononuclear cells collected from human subjects after stimulation with insulin (Maratou et al., 2007). In a subsequent study this research group reported a negative correlation between GLUT4 expression in mononuclear cells and the homeostatic model assessment of insulin resistance (HOMA-IR) in diabetic patients (Maratou et al., 2009). While there is a growing body of research that investigates the presence of GLUT4 expression in mononuclear cells and the effects of insulin, to our knowledge no research has been performed on exercise induced GLUT4 translocation in mononuclear cells.

Regular exercise has a wide array of health promoting effects and much research is directed at better understand the molecular mechanisms underlying these benefits (Carey and Kingwell, 2009). Exercise training has been shown to mediate skeletal muscle enzymes, transcription factors, transporters and chaperones through an adaptive response to chronic training (Carey and Kingwell, 2009). Of particular interest for this study are well-documented effects of exercise on GLUT4 in skeletal muscle. The exercise-induced response occurs by recruiting more GLUT4 to the cell surface from a larger total muscle pool of GLUT4 (Reynolds et al., 2000) and the resulting increase in muscle GLUT4 protein is associated with an increased capacity for glucose transport (Rodnick et al., 1992, Goodpaster et al., 2001). Similar findings have been reported in rats (Ploug et al., 1990). Several studies have reported that the adaptive increase in the GLUT4 protein in muscle cells occurs as early as the first week of exercise training (Host et al., 1998; Ren et al., 1994).

Sled dogs are elite athletes whose energy expenditure and physical endurance provide for an excellent model for studying the effects of exercise on insulin signaling and glucose uptake (Hinchcliff et al., 1997a). Historically, dogs have played a critical role in our understanding and treatment of diabetes, and scientists have used dogs as a biochemical research model for studying human metabolic disorders for over a century (Catchpole et al., 2005; Serisier et al., 2008). Though there are species-specific pathologies associated with diabetes, dogs develop insulin dependent and independent forms of diabetes, and gestational diabetes akin to humans (Catchpole et al., 2005; Bergman et al., 2006; Johnson, 2008). While the prevalence of canine diabetes remains lower than humans, an increasing trend in dogs has been observed (Catchpole et al., 2005). Furthermore, the most widely used clinical and epidemiological tool for assessing insulin sensitivity, the homeostatic model assessment, HOMA-IR (Wallace et al., 2004) has also been established in the dog (Serisier et al., 2008).

We report, for the first time, that GLUT4 levels in mononuclear cells are elevated in response to conditioning. Furthermore, GLUT4 in mononuclear cells in sled dogs displayed a negative correlation with HOMA-IR. These results provide additional support in the growing body of evidence that mononuclear cells are a promising proxy tissue for assessing insulin sensitivity.

2. Methods

2.1. Animals and diet

Sled dogs, raised in Salcha, Alaska (Latitude 65° N, 147° W) were used as test subjects. The Institute of Animal Care and Use Committee (IACUC) at the University of Alaska Fairbanks approved the protocol of this study (#02-14). The dogs used were typical racing sled dogs from similar lineage; eight conditioned dogs ($n = 8$) and eight sedentary dogs ($n = 8$) were sampled. The exercise program for the conditioned sled dogs over the 4 months prior the blood draw (May–September) consisted of varying modalities, durations, and intensities. The goal of the training program is to slowly increase miles and speed. Since this was during the summer, the training program was primarily for maintenance, which consists mostly of hooking sled dogs pulling in harness in front of an All Terrain Vehicle (ATV) and maintaining an approximate speed of between 15 and 18 mph for 3–8 miles. This is interspersed with long duration (2–3 h), low intensity exercise (approximately 7 mph), that involves an exercise wheel. An exercise wheel is a bi-directional motorized wheel that is commonly used for exercising horses and has been adapted for dogs. The animals are similarly fastened to the wheel by chains that hang down off of large extended arms to their collars and a fast walk is maintained without resistance. The sedentary dogs were not conditioned for the four months prior to the experiment but were at the onset of their training season when sampling occurred. Groups were balanced for ability and age [conditioned, range 1.5–6 years (3 ± 2 years); sedentary, range 1.5–6 years (3 ± 2 years)]. The sedentary group had slightly more males ($n = 6$), while the conditioned group had slightly more females ($n = 6$). We compared GLUT4 concentrations between males and females in both groups to ensure that sex was not a confounding issue (t-test, $p > 0.05$). All dogs were sexually intact. Housing arrangements consisted of 2-m chains on which the dogs were tethered for the duration of the study. Each dog had access to his or her own house. Dogs in both groups were fed the same diet (Purina Pro Plan Performance) and were allowed ad libitum access to water. Each dog was maintained at an ideal body condition score of 3 (Laflamme, 1997).

2.2. Blood sampling

The temperature range on the day of the experiment was 4–7 °C (September 5th, 2012). All samples were collected after an overnight fast between 9 and 10 am. 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. Aliquots of plasma collected in EDTA tubes were immediately frozen at −80 °C for later insulin and glucose analysis. The buffy coat (mononuclear interphase layer containing white blood cells) was collected using BD mononuclear separation tubes, resuspended in 3 mL of RPMI w/5% calf serum and centrifuged for 15 min at 1500 RCF. Mononuclear cells were washed a total of three times and resuspended in 4 mL of RPMI w/5% calf serum. Aliquots of the resuspended sample were used for GLUT4 enzyme-linked immunosorbent assay (ELISA) analysis and adjusted for protein content using BCA Protein assay.

2.3. Biochemical analysis

GLUT4 levels at the surface of mononuclear cells were assessed using a commercially available ELISA (USCN Life Science Inc., United States) according to manufacturer's instruction and absorbance was read at 450 nm. To ensure GLUT4 levels were indicative of surface amounts, levels were compared with samples that were sonicated. Sonicated samples had 2–3 fold higher GLUT4 levels compared with unsonicated samples (Schnurr et al., 2013). A BCA Protein Assay (Pierce, Theroms Scientific, United States) was used for protein adjustment in regard with GLUT4 and absorbance was read at 562 nm. Insulin levels were measured using an ELISA (Porcine/Canine; ALPCO, Salem NH), following the protocol by the manufacturer and taking absorbance readings at 450 nm. All