humans, or both, but an increasing trend has been observed nonetheless (Catchpole *et al.*, 2005). Dogs a proven model for biochemical research (Dunlap *et al.*, 2006; Greeley *et al.*, 2001; Milgram *et al.*, 2002), can be an innovative model to link activity and nutrition to the physiological and immune effects seen in metabolic syndrome and related disorders.

For the circumpolar north, racing sled dogs are excellent models for studying health effects related to exercise, nutrition and diabetes (Felsburg, 2002; Kararli, 2006). Nutritional intervention and exercise has shown to improve insulin sensitivity and increase GLUT4 expression (Carey and Kingwell, 2009; Ruel and Couillard, 2007). The main purpose of this study was to validate quantifiable amounts of GLUT4 in white blood cells of dogs using a simple commercially available ELISA and furthermore compare GLUT4 levels in young versus old sled dogs. Additionally, we examined the blood insulin response to a meal. While our preliminary results have shown the ability to detect GLUT4 in white blood cells of sled dogs, this study focuses on **1) comparison of GLUT4 levels in conditioned versus non-conditioned sled dogs, pre and post exercise.** Additionally we compare **2) blood glucose and plasma insulin levels pre and post exercise.**

II. MATERIALS AND METHODS

A. Animals and diet

Sled dogs, raised in Salcha, Alaska (Latitude 65°N, 147°W) were used as test subjects. The Institute of Animal Use and Care Committee at the University of Alaska Fairbanks approved this study (#02-14). The dogs that were used were typical racing sled dogs. The conditioned dogs (COND, n =8) were from the Piledriver Kennel in Salcha, the non-conditioned dogs (n =8) were from the Dunlap Kennel in

Salcha. There were eight conditioned dogs (COND) and eight non-conditioned dogs (NON-COND). The conditioned dogs (COND) were exercised daily for the three months prior the experiment and were following training protocols in preparation for various mushing championships. The non-conditioned dogs (NON-COND) were not exercised for the three months prior the experiment but were at the on-set of their training season. Groups were balanced for age and ability, but not for sex. Values did not significantly change for age [COND, range 1.5 to 6 years (3 $\pm$ 2 years); NON-COND, range 1.5 to 6 years (3 $\pm$ 2 years)]. All dogs were sexually intact. Housing arrangements consisted of 2 meter 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) and were allowed *ad libidium* access to water. Each dog was fed to maintain its ideal body condition score of 3 (Laflamme, 1997). The temperature range on the day of the experiment was 4°C-7°C (September 5*th*, 2012).

B. Conditioning and exercise

The COND group was exercised for three months prior the experiment, the NON-COND group was not. Sled dogs are often run 2-5 miles in an untrained state at the on-set of the training season. A distance of 3.5 miles was chosen to physical exert the animals while staying within the capabilities of the animals. Both groups were run in two teams of 8 dogs in front of an All Train Vehicle (ATV) for consistency. The COND dogs ran 2.5 miles at VO_2 max of about 80-85%, averaging a speed of 21 km/h before increasing the speed at the last mile to an VO_2 of about 90-95% and an average speed of 26 km/h. NON-COND dogs ran the first 2.5 miles at the same percentage of VO_2 max as the COND dogs (80-85%), but averaging a slightly lower speed of 18 km/h due to the fact that dogs had not been exercising for the past three

months. For consistency, the NON-COND dogs also increased their speed to achieve VO_2 max levels of 90-95%, averaging about 23 km/h.

C. Blood sampling

All dogs were bled before the exercise (PRE) and immediately upon the completion of exercise (POST). Blood was withdrawn into EDTA tubes (6 mL, for measuring plasma insulin and plasma glucose levels) and BD Separation tubes (6 mL, for measuring GLUT4 levels) using the cephalic vein. Tubes were stored upright at room temperature until centrifugation and centrifuged within 2 hours of blood collection. Blood samples were centrifuged at 3600 RPM for 15 minutes at room temperature. 1 mL of the plasma sample (from EDTA tubes) was used for determination of blood glucose levels (performed by the North Pole Veterinary Hospital) and 1 mL of the plasma sample was transferred into freezer vials and flash frozen in liquid nitrogen and stored at $-80°$C for later insulin analysis. The buffy coat (mononuclear interphase layer containing white blood cells) was collected from the blood samples in the BD Separation tubes: Half of the blood plasma was aspirated without disturbing the cell layer. Next, the cell layer was collected with a Pasteur Pipette and transferred into 15 mL conical vials. The blood sample was resuspended in 3 mL RPMI w/ 5% Calf Serum and centrifuged for 15 minutes at 1500 RCF. WBC were washed one more time using the previous settings for centrifugation before resuspending in 4 mL RPMI w/ 5% Calf Serum. 1 mL of the resuspended sample was used for GLUT4-ELISA analysis and BCA Protein assay. Both assays were run on the same day of the blood draw (within 6 hours past blood collection).

D. Biochemical analysis

Plasma Glucose levels analysis was performed by the North Pole Veterinary Clinic (North Pole, AK) using in house laboratory equipment.

The concentrations of GLUT4 in white blood cells were measured with a commercially available ELISA (USCN Life Science Inc., United States). The USCN Life Science GLUT4 ELISA kit is a sandwich enzyme immunoassay for the *in vitro* quantitative measurement of GLUT4 in canine tissue homogenates and other biological fluids. The micro filter plate in the kit was pre-coated with a monoclonal antibody specific to GLUT4. GLUT4 concentrations of the samples were then determined by further extrapolation from a standard curve developed from known concentrations of GLUT4. After appropriate sample and standard dilution, the procedure supplied with the assay was followed. GLUT4 concentrations were determined by comparing the optical density (read spectrophotometric with a microplate reader at 450 nm) of each sample to the standard curve. Furthermore, a BD Protein Assay was used for protein adjustment in regard with GLUT4.

The plasma concentrations of insulin (Porcine/Canine; ALPCO, Salem NH) were measured with commercially available ELISA. The ALPCO Insulin ELISA is also a sandwich type immunoassay. Monoclonal antibodies specific for insulin are immobilized to the 96-well microplate as the solid phase. Sample concentration was determined by extrapolation from a standard curve. Again, the procedure supplied with the assay was followed. Optical Density was measured with a microplate reader at 450 nm and reference wavelength at 620 nm.

E. Statistics

Data was normally distributed. For each variable, an ANOVA was used to search for significant differences within or between treatment groups. Significant differences within or between treatment groups were further characterized, using a 2 sample t-test. Significant differences were established with P values less than or equal to 0.05.

III. RESULTS

A. GLUT4 levels in mononuclear cells

GLUT4 concentrations in mononuclear cells of conditioned dogs was 7.90 ($\pm$ 2.27 ng/g protein) and in non-conditioned dogs was 5.20 ($\pm$ 2.47 ng/g protein). The state of conditioning had a significant effect in the concentration of GLUT4 between the populations of conditioned and non-conditioned dogs. The dogs that were conditioned for three months prior to the experiment had a significant higher GLUT4 expression as compared to the population of dog that did not get conditioned during the three months prior to the experiment. Data for pre and post exercise samples were combined for both of the populations to determine an overall result, Figure 1, ($p < 0.05$, n =32). GLUT4 levels decreased in both populations after the acute bout of exercise (Pre and Post exercise), which ran for 3.5 miles at 80-95% VO_2 max, Figure 2. The GLUT4 concentration in the conditioned dogs decreased from 8.16 ($\pm$ 2.96 ng/g protein) to 7.64 ($\pm$ 0.91 ng/g protein) due to the acute exercise bout whereas the GLUT4 concentration in the non-conditioned dogs decreased from a significantly lower baseline value of 5.70 ($\pm$ 2.97 ng/g protein) to 4.70 ($\pm$ 1.79 ng/g protein), Figure 2. One bout of exercise did not have a significant