

Glucose Transporter 4 Expression in White Blood Cells of Young and Old Sled Dogs



Theresa Schnurr, Arleigh Reynolds, Kriya Dunlap
Department of Chemistry and Biochemistry, University of Alaska Fairbanks



Background

Obesity has reached alarming levels in the United States. Recent statistics show that 1 out of 3 individuals are either obese or overweight!

The principle role of the hormone insulin is to mediate the redistribution of the glucose transporter-4 (Glut4) from an intracellular vesicle pool into plasma membranes of insulin-responsive tissues and thus regulating the uptake of glucose. Insulin resistance 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.

Glut4 is the only glucose transporter responsive to insulin and is thought to be found exclusively in muscle and adipose cells. But recently, Maratou et al (2007) demonstrated that there is Glut4 in white blood cells (WBC) collected from human subjects in response to insulin activation.

Dogs have been used as a proven biomedical research model for diabetes for over a century since dogs develop insulin dependent and independent forms of diabetes similar to humans. Sled dogs are incredible athletes that provide a homogenous population for studying environmental impacts such as nutrition and exercise on blood parameters.

The goal of this study was to 1) develop a protocol to measure Glut4 in white blood cells of sled dogs and 2) compare Glut4 levels in young versus old sled dogs assuming that old sled dogs are at higher risk of diabetes.

Methods

Blood samples of 4mL were collected from 11 healthy sled dogs in using the cephalic vein: 6 young, physically fit and 5 old, obese. Samples were collected in ethylenediaminetetraacetic acid (EDTA) vacutainer tubes and centrifuged. Buffy coat (containing WBC) was collected, washed in phosphate buffer saline and stimulate with 100nM of insulin in order to measure Glut4 concentration of each dog. Samples were collected in serum separating tubes before and after a meal in order to measure insulin levels.

ELISA kits were used to determine Glut4 and Insulin concentrations. The kits are 96-well plate coated in Glut4 or insulin specific antibody conjugated to horseradish peroxidase. After the addition of substrate color is generated in proportion of the amount of Glut4 or insulin in the sample. Absorbance is read with a microplate reader at 450nm: The ELISA kit used for Glucose Transporter 4 was from the manufacturer Uscn Life Science Inc., the ELISA kit used for the quantitative measurement of insulin in Porcine/Canine serum and plasma was from ALPCO Diagnostics.

Data acquisition and analysis was performed with BD Cell Quest Pro software (BD Biosciences).

The Protocol was IACUC approved.

Results

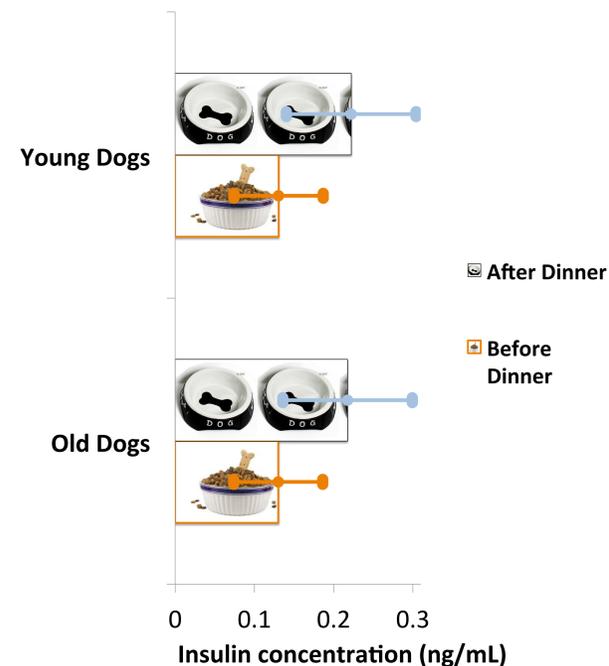


Figure 1. Insulin Concentration in old and young sled dogs, before and after dinner.

The insulin concentration of the old dogs was 0.13 (+/- 0.05) ng/mL before dinner and 0.22 (+/- 0.06) ng/mL after having dinner (Fig. 1).

The insulin concentration of the young dogs was 0.13 (+/- 0.07) ng/mL before dinner and 0.22 (+/- 0.10) ng/mL after having dinner. (Fig. 1).

There is a significant difference in insulin concentration after the meal in both populations (Fig. 1, $p < 0.05$).

Figure 1 highlights that there was an increased insulin concentration in sled dogs after having dinner. The insulin spike was seen in old dogs and young dogs but there is no significant difference in the insulin concentration between old dogs and young dogs (Fig. 1, $p > 0.05$).

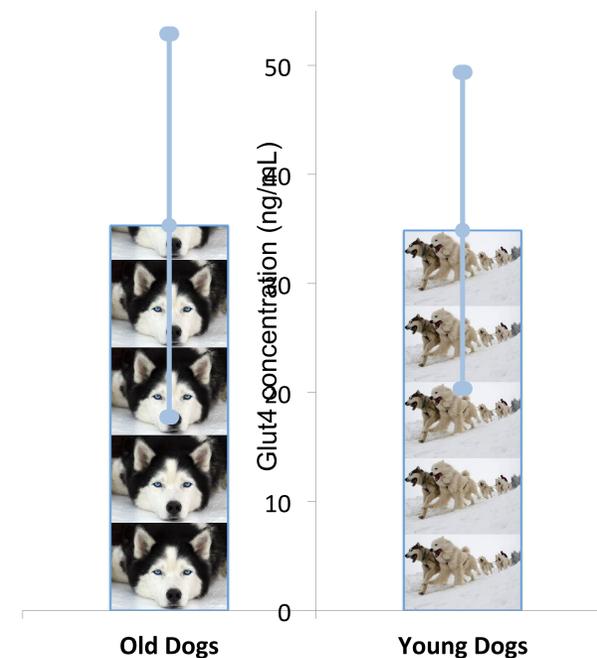


Figure 2. Glut4- concentration in old and young sled dogs.

Figure 2 accentuates that we were able to find glucose transporter-4 in white blood cells of sled dogs.

The Glut4- concentration of old dogs was 35.31 (+/- 17.57 ng/mL). The Glut4- concentration of young dogs was 34.85 (+/- 14.51 ng/mL) (Fig. 2).

In comparing the concentration of glucose transporter-4 between the populations of old and young dogs shows that there is no significant difference among the populations. (Fig. 2, $p > 0.05$).



Conclusions

Mean insulin levels increased from 0.13 to 0.22 ng/mL for the old sled dogs when having dinner, the mean insulin levels for the young dogs increased from 0.13 to 0.22 ng/mL. The change in measured insulin concentration pre and post meal was significant, indicating that the insulin level spiked after the meal. There was no significant difference in insulin levels when comparing old versus young sled dogs.

We were able to show that white blood cells have quantifiable glucose transporter-4 levels with no significant difference in Glut4 concentration between old and young sled dogs.

Finding Glut4 in white blood cells might open up non-invasive avenues for studying the underlying molecular mechanisms associated with insulin resistance in more complex, dynamic and physiological systems compared to standard in vivo studies that require invasive muscle or adipose tissue.

This study focuses on sled dogs, incredible athletes, that provide a homogenous population for studying environmental impacts such as nutrition and exercise on blood parameters. Since the results of the study indicate that we might have found a reliable and non-invasive method in using white blood cells for studying cellular mechanisms involved in insulin resistance, a long term objective would refine and employ this protocol on a human at-risk population. Additionally, nutritional therapies could be assessed and their means of action.

Due to the relatively small sample size of sled dogs in our study the question remains if it might be possible to reduce the standard error and to detect a difference in insulin levels and/or Glut4 concentrations between old, obese individuals being at high risk of diabetes and young, fit individuals being at low risk of diabetes.

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References

Maratou, E., G. Dimitriadis, et al. (2007). "Glucose transporter expression on the plasma membrane of resting and activated white blood cells." *Eur J Clin Invest* 37(4): 282-90.