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Exercise Increases Glucose Transporter-4 Levels on Peripheral Blood Mononuclear Cells

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Abstract

PURPOSE—Glucose transporter 4 (GLUT4) plays a key role in the pathophysiology of type 2 diabetes. GLUT4 is upregulated in response to exercise, enhancing cellular glucose transport in skeletal muscle tissue. This mechanism appears to remain intact in individuals with insulin resistance. Details of the mechanism are poorly understood and are challenging to study due to the invasive nature of muscle biopsy. Peripheral blood mononuclear cells (PBMC) have documented insulin-sensitive GLUT4 activity and may serve as a proxy tissue for studying skeletal muscle GLUT4. The purpose of this study was to investigate whether GLUT4 in PBMC is affected by conditioning.

METHOD—We recruited sixteen student athletes from the cross-country running and skiing teams and fifteen sedentary students matched for age and sex from the University of Alaska Fairbanks. PBMC were collected with mononuclear cell separation tubes. GLUT4 concentrations were measured using a commercially available enzyme linked immunosorbent assay. Additionally, correlations between PBMC GLUT4 and common indicators of insulin resistance were examined.

RESULTS—Results indicate significantly higher PBMC GLUT4 levels in conditioned athletes than in their sedentary counterparts, similar to what has been documented in myocytes. Females were observed to have higher PBMC GLUT4 levels than males. Correlations were not detected between PBMC GLUT4 and hemoglobin A1c (HbA1c), glucose, insulin, HOMA-IR, BMI, or body fat.

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CONCLUSION—This study provides evidence to support exploration of PBMC as a proxy tissue for studying GLUT4 response to exercise or other non-insulin factors.

Keywords

exercise; insulin signaling; PBMC; GLUT4; college athlete

INTRODUCTION

Glucose transporter-4 (GLUT4) resides in intracellular pools in membranes of small vesicles and is translocated to the cell membrane in response to insulin (1). Impaired GLUT4 trafficking is likely one of the first events involved in glucose metabolism disorders (2). GLUT4 increases in skeletal muscle in humans and animals as an adaptive response to exercise, independent of insulin and has thus made exercise an important treatment option for glucose metabolic disorders (3–11). It is commonly believed the increase in GLUT4 levels is due to increased translocation from different intracellular pools rather than increased transcription (12, 13) and that regular physical conditioning elevates basal GLUT4 protein levels (10, 14, 15). However, acute exercise is also known to increase GLUT4 in skeletal muscle (9, 10, 13) resulting in as much as a 50% increase within 16 hours post exercise (16). Most importantly, while individuals with insulin resistance (IR) or type 2 diabetes have diminished GLUT4 activity in response to insulin, their response to exercise appears to be uninhibited (6, 13) (17). This is an important physiological phenomenon, as it provides a potential alternate pathway to improve blood glucose control in patients with IR.

An obstacle to studying GLUT4 is that it is predominantly found in muscle and adipose tissue. The invasive nature and expense of tissue biopsy needed to study in vivo GLUT4 has resulted in limited human studies and small sample sizes. In the past decade, it was discovered that peripheral blood mononuclear cells (PBMC) have insulin-sensitive GLUT4 activity and could potentially serve as a proxy tissue (18, 19). Maratou et al reported an increase in PMBC GLUT4 in response to insulin (18) and found a negative correlation with the homeostatic model assessment of insulin resistance (HOMA-IR) in diabetic patients (19). The research to date on GLUT4 activity in mononuclear cells suggests these cells respond to insulin in a similar manner to muscle tissue (18–20). What has not been explored is whether GLUT4 activity in PMBC also responds to exercise in the same way that it does in muscle.

Our lab recently reported for the first time higher GLUT4 on mononuclear cells of conditioned versus sedentary sled dogs (21). Similar to Maratou et al (19), we also reported an inverse relationship with PBMC GLUT4 and HOMA-IR. In a follow up study we reported a 50% increase in PBMC GLUT4 with a single bout of exercise in sled dogs (22), akin to what is reported in skeletal muscle of humans and rodents (7, 16, 23, 24). In this study we extend these finding in humans for the first time, which further supports the hypothesis that conditioning increases GLUT4 concentration in PBMC. Using University of Alaska Fairbanks NCAA cross-country skiing and running student athletes compared to matched sedentary students, we reported significantly higher PBMC GLUT4 concentrations in student athletes after three months of uniform conditioning. These findings add to the

growing body of evidence that supports PBMC as a proxy tissue for studying GLUT4 in vivo.

METHODS

IRB and consent

The study protocol was approved by the Institutional Review Board of the University of Alaska Fairbanks (#492213-4) and acknowledged by the Institutional Review Board of the University of Alaska Anchorage. Written consent was obtained prior to beginning data collection.

Study population

All participants were students at the University of Alaska Fairbanks (UAF), between 18 and 25 years of age, non-pregnant, and non-diabetic. The sample consisted of two groups matched for age and sex: conditioned athletes ($n = 16$; 8 males, 8 females age 20.1 ± 2.0) and sedentary students ($n = 15$; 8 males, 7 females age 21.5 ± 2.1). Conditioned participants were recruited through the UAF cross-country skiing and cross-country running teams. This group consisted of endurance athletes who had been training with the UAF team for ten to twenty hours per week for three months prior to sample collection. Sedentary students did not participate in regular moderate physical activity (defined to participants as “physical activity that takes moderate effort and makes you breathe somewhat harder than normal”) for more than 20 minutes per session, per week for three months prior to sample collection. A prescreening questionnaire was administered. Students who participated in “any exercise more than once per week for the past 2 months” were not selected.

Assessment of physical activity

Participants completed the International Physical Activity Questionnaire (IPAQ) Short Form with instructions from a researcher. The IPAQ has been assessed for validity and reliability in multiple populations (25). The data in the questionnaires were analyzed and cleaned according to the IPAQ Guidelines for Data Processing and Analysis. MET-minute/week scores were calculated and participants were classified categorically according to the protocol as low, moderate, or high physical activity levels.

Demographics and health information

Participants completed a health history questionnaire, which included demographic information such as age, sex, and ethnicity. Demographic data indicates that there were no significant differences in age or sex between the groups. Participants were primarily white.

Anthropometric Data

A Registered Nurse at UAF's Center for Native Health Research (CANHR) performed anthropometric measurements. Height was measured to the nearest 1/8 inch. Weight in pounds and percent body fat were measured with a TANITA TBF-300A (Tanita Corporation of America Inc., Arlington Hills, Illinois). Waist circumference was measured with the

Gulick II 150 cm anthropometric tape. Two measurements to the nearest 0.2 cm were obtained and the average was utilized in the analysis.

Diet

Dietary data was collected using the ASA24 Automated Self-Administered 24-hour Recall system from the National Cancer Institute. The ASA24 has acceptable performance in measuring true intake (26). Participants were instructed on the automated system following informed consent. They were asked to record one typical weekend day and one typical weekday of food intake. Dietary data was reviewed and cleaned according to ASA24 recommendations.

Blood collection

Participants were instructed to fast for 12 hours prior to the blood draw with nothing consumed except water. A Registered nurse obtained blood by venipuncture into one 8-ml BD separation tube, one 4-ml EDTA tube, and one 2-ml heparinized tube. Samples not immediately used were refrigerated or centrifuged within two hours of sample collection. Plasma from heparinized tubes was frozen at -80°C for future measurement of insulin.

Biochemical analysis

Blood lipids, glucose, and HbA1c were measured immediately following each blood draw utilizing blood from the heparinized tube at UAF's CANHR clinic. The Cholestech LDX system measured total cholesterol, LDL, HDL, triglycerides, and glucose; the Bayer DCA 2000+ Analyzer measured HbA1c. The same registered nurse that obtained anthropometric data and sample blood, is in charge of maintaining the Cholestech LDX and Bayer DCA 2000+; this includes running control tests and keeping records of the results all tests and maintenance.

While total GLUT4 concentrations are valuable, cell surface GLUT4 concentration are of more interest because this indicates that a stimuli has resulted in the translocation of GLUT4 from intracellular vesicles. Our laboratory previously developed a method that results in minimal cell damage using sled dog PBMC. If the cells remain intact, then the GLUT4 measured represents surface GLUT4. In developing this method, we validated surface GLUT4 via two different methods; 1) we found the same significant increase in PBMC GLUT4 after exercise when analyzed by flow cytometry as we did with this technique (unpublished) and 2) rupturing the cells with sonication resulted in a 3-fold higher increase in GLUT4 concentration (21, 22, 27). Using BD separation tubes results in a diffuse layer of mononuclear cells that can be carefully collected and gently washed. The BD separation tubes were centrifuged at 3600 RPM for 15 minutes at room temperature. The mononuclear cell layer was then collected and transferred to a 15-mL conical vial. Each sample was gently suspended in 15 mL RPMI 1640 with 5% calf serum. Tubes were centrifuged for 15 minutes at 1500 RPM. Samples were re-suspended in RPMI solution two more times, followed by centrifugation for total of three washes. The final sample was re-suspended with the RPMI solution to a final volume of 8 mL, from which the sample for the GLUT4 protein analysis was drawn. GLUT4 was measured within two hours of collection. Protein concentrations for each sample was determine using Pierce BCA Protein Assay (Fisher

Scientific). Statistical analysis was completed on both ng GLUT4/g of protein concentrations and GLUT4 concentrations without adjusting for protein content. Both resulted in significant differences, therefore we chose to report on GLUT4 concentrations without adjustment for protein (Figure 2)(21, 22).

The GLUT4 protein and plasma insulin were measured using commercially available ELISA kits. Each test was run in duplicate. The Glucose Transporter 4 (GLUT4) kit (USCN Life Sciences, Inc., United States) and Insulin ELISA (ALPCO Immunoassays, United States) were used according to manufacturers' instructions; absorbance was read at 450 nm for both tests. Absorbance readings were collected using a Synergy HT multi-mode microplate reader (BioTek, United States).

The homeostasis model assessment of insulin resistance (HOMA-IR) has been widely used as a surrogate for assessing insulin resistance, however universal cut-off values have not been defined. HOMA-IR was calculated with the equation (fasting plasma insulin * fasting plasma glucose)/22.5 (28–30).

Statistical analysis

Sample size was determined from an identical protocol published by our laboratory in sled dogs (21), in which eight dogs in each group had significant findings. We doubled the sample size in each group. SPSS statistical analysis software (version 21) and R (version 3.3.3) were used to analyze the data. SPSS was used to conduct Student's independent sample t-tests to assess differences between the conditioned and sedentary groups related to demographic, anthropometric, biochemical, and dietary data. Differences were considered significance at $\alpha = 0.05$. The SPSS box-plot was used to assess outliers, the Shapiro-Wilk test to assess normal distribution of the data, and Levene's test to assess homogeneity of variances prior to conducting the t-test. Mann-Whitney U tests to compare mean ranks were conducted on all data that were not normally distributed; this included serum insulin, HOMA-IR, triglycerides, physical activity, servings of fruit and vegetable, and grams of fiber intake per calorie. The R package "stats" was used to conduct a Levene's test to ensure homogeneity of variances, followed by a Wilcoxon Signed Rank test to assess GLUT4 concentration differences between the groups. Spearman's rank correlation coefficient on the combined groups was used to investigate associations between GLUT4 and glucose, HbA1c, insulin, HOMA-IR, BMI, and percent body fat.

RESULTS

Anthropometric Data

Of all anthropometric measurements (Table 1), a significant difference was only detected between the groups for Body Mass Index ($p=0.050$). However, when analyzing by sex (data not reported), this difference was only observed between conditioned males and sedentary males; conditioned males having a higher BMI than sedentary males. Interestingly, no difference was detected in females. Percent body fat was not different between groups, but again when analyzing by sex, we observed that sedentary males had significantly higher percent body fat compared to the conditioned males ($p = 0.004$). All of the participants in

the conditioned group had a BMI in a healthy range (18.6–25.0). In the sedentary group, ten participants had a healthy BMI, three were classified as overweight (BMI = 25-29.9), and two were classified as obese (BMI >30). Percent body fat and waist circumference was not significant ($p = 0.10$ and 0.06 , respectively).

Biochemical Data

There were no significant differences between the groups for glucose, HbA1c, insulin, or HOMA-IR. Insulin had a p value of 0.14 and HOMA-IR had a p value of 0.15 . Total cholesterol, LDL, HDL, and triglycerides also were not different between groups. HDL and triglycerides both had a p value of 0.10 (Table 1).

Diet

Dietary factors related to blood glucose regulation were analyzed (Table 2). There was no difference in macronutrient distribution between the groups. The conditioned group had significantly higher total caloric intake ($p=0.004$), sugar intake ($p=0.002$), fiber intake ($p<0.0001$), and fruit and vegetable intake ($p=0.005$) than the sedentary group. When sugar, fiber, and fruit and vegetable intake were normalized to individual caloric intake, there was found to be no difference in mean grams of sugar per calorie between the groups ($p=0.461$). The conditioned group had a higher mean rank of gram of fiber per calorie intake (19.97) than the sedentary group (11.77), $U = 56.500$, $z = -2.551$, $p=0.011$. The conditioned group also had a higher mean rank of servings of fruits and vegetables per calorie intake (19.25) than the sedentary group (12.3), $U=68.000$, $z = -2.055$, $p=0.041$. No correlations were found between GLUT4 and calories, sugar, fiber, or fruit and vegetable intake.

Physical Activity

Physical activity between groups was significant ($p<0.0005$) (Figure 1). Despite a pre-screening of participants for physical activity levels, the International Physical Activity Questionnaire (IPAQ)-Short Form indicated that two of the participants in the sedentary group did in fact participate in at least one bout of moderate or high physical activity. However, their Metabolic Equivalent of Task (MET)-minutes per week were well below any of the student athletes. MET-minutes per week were calculated from the IPAQ-short form and participants were categorized as having low, moderate, or high activity levels. The distributions of MET-minutes per week and the categorical ranking of physical activity levels were compared between groups. There were statistically significant differences for both methods of analyzing the IPAQ data (MET-minutes and categorical ranking of physical activity). For MET-minutes per week, $U=7.00$, $p<0.0005$; for categorical physical activity levels, $U=17.5$, $p<0.0005$, using an exact sampling distribution for each.

Glucose Transporter-4 concentrations

The conditioned group had significantly higher GLUT4 on PBMC than the sedentary group ($p=0.035$) (Figure 2). The conditioned group had a mean of $0.6433 \text{ ng/mL} \pm 0.2871$ compared to the sedentary group ($n=13$ due to sample loss) mean of $0.3641 \text{ ng/mL} \pm 0.2752$. Females, grouped together regardless of physical activity, had a higher mean GLUT4 than males ($0.6671 \pm 0.2465 \text{ ng/mL}$ vs. $0.3972 \pm 0.3112 \text{ ng/mL}$) in the combined groups

($p=0.009$) (Figure 3). This was also the case with the sedentary students (0.5537 ± 0.2367 ng/mL vs. $0.2456 \pm .2368$ ng/mL; $p=0.041$). In the conditioned group, GLUT4 levels in females were not significantly higher than males (0.7379 ± 0.2392 ng/mL vs. 0.5488 ± 0.3145 ng/mL; $p=0.130$). There were no correlations between GLUT4 and glucose, HbA1c, fasting insulin, HOMA-IR, BMI, and percent body fat.

Discussion

GLUT4 increases on the cell surface of muscle tissue in response to acute and chronic exercise (4, 5, 7, 10, 13). Recently, we reported for the first time an increase in GLUT4 on PBMC in response to acute (22) and chronic exercise (21) in sled dogs. To our knowledge, no previous human studies have examined GLUT4 in PBMC in response to exercise or conditioning. It has, however, been well documented that GLUT4 increases on the membrane of monocytes (18, 20, 31, 32) and lymphocytes (19, 33) in response to insulin. We hypothesized that exercise would also induce an elevation in GLUT4 concentration in PBMC of humans. Our data from a population of young, healthy individuals indicates that those who participate in regular athletic training have a higher GLUT4 concentration on PBMC compared to their sedentary counterparts (Figure 2).

Increases in GLUT4 levels in myocytes have been reported after one bout of exercise (5, 22, 24, 34), but the time in which GLUT4 levels peak is unknown. Literature suggests that the peak GLUT4 levels is likely somewhere between 3 to 16 hours post-exercise (22, 24, 34–36), though some research suggest that it remains elevated for up to 22 hours (5). Host et al. found that GLUT4 protein has a relatively short half-life, between 8-12 hours, and that any adaptive response to exercise was lost within 40 hours (36). We previously reported an increase in GLUT4 levels in PBMC of sled dogs post-exercise, but it was no longer significant 24 hours after the completion of exercise. While the higher GLUT4 in our athletes may have been residual from the acute nature of the previous day's heavy training, it is more likely a result of conditioning since sampling occurred at least 24 hours post-exercise.

GLUT4 response to exercise in myocytes varies in different muscle fiber types (4, 37). GLUT4 in slow-twitch fibers may be more responsive to exercise than GLUT4 in fast-twitch fibers in both humans and rats (4, 11). The athletes in this study were cross-country skiers and runners, which are sports that rely heavily on slow-twitch fibers. This result, again, supports the similar characteristics in GLUT4 activity in PBMC compared to myocytes.

Studies examining the change in GLUT4 in myocytes in response to exercise interventions have reported elevations of 26-51% (4–6, 10, 13). A study comparing GLUT4 in myocytes of athletes and untrained controls found 93% higher GLUT4 protein per muscle protein in the athletes (14). In this study, GLUT4 on PBMC was 59% higher in athletes than sedentary participants. Increases in GLUT4 on PBMC in response to insulin have been reported in the range of 24-54% (18, 20, 31, 32); our data suggests that conditioning may have a greater impact on GLUT4 concentration in PBMC than insulin.

An important unanticipated finding was higher GLUT4 levels in females compared to males (Figure 3), when both combining student athletes with sedentary students and within the sedentary group. Interestingly, female participants in this study also exhibited significantly higher plasma 25-OH vitamin D levels than their male counterparts, despite male participants reporting higher dietary and supplemental vitamin D intake (38). It was hypothesized that body fat percent may have contributed to this finding given that vitamin D is fat soluble and is stored, in part, in adipose tissue. Percent body fat and body condition score differed significantly between male athletes and male sedentary students, but this was not observed when solely looking at female participants. Recently vitamin D deficiency and insufficiency have been linked to the development of IR and type 2 diabetes (39). While no participants in this study were diabetic or at risk of developing IR, it is worthwhile to note that females had higher vitamin D, higher body fat percent, and higher PBMC GLUT4. This relationship warrants further investigation.

A negative correlation between BMI and plasma membrane GLUT4 in both myocytes (7) and monocytes (40) has been reported. The majority of our participants were considered to have a “healthy” body weight and so it is not surprising that we did not detect a relationship between GLUT4 and BMI or percent body fat. The increase in GLUT4 seen in PBMC as a result of training, similar to what is reported in muscle, without the predisposition of glucose metabolic disorders, adds to both the body of evidence supporting exercise as a therapeutic intervention strategy for IR and the emerging hypothesis that PBMC could serve as important proxy tissue both for early detection and monitoring progress of treatments.

A considerable challenge in diabetes prevention and treatment is the lack of early identification of those at risk. Early identification of individuals at risk for type 2 diabetes or IR may allow an exercise or lifestyle intervention to be implemented prior to disease progression. Currently, routine diagnostic tools only detect physiological abnormalities once they result in inadequate glucose transport (41). Inexpensive, direct, reliable identification of early physiological changes that lead to type 2 diabetes, such as changes in GLUT4, would be beneficial for early intervention. Bernat-Karpi ska, et al. found that changes in GLUT4 in lymphocytes might precede the ability to detect insulin resistance through currently available diagnostic tools (41). In sled dogs we observed an inverse relationship with PBMC GLUT4 and HOMA-IR (21), a relationship that was not mimicked in this human subject study. This is likely due to the fact that HOMA-IR is a measure of insulin resistance and beta cell function, while this study focused on the effects of exercise. Perhaps a relationship would be observed with a larger sample size and/or a population that is at a higher risk for insulin resistance. In a small, healthy cohort of people, Maratou, et al. also did not observe any relationship between HOMA-IR and PMBC GLUT4 (18), but in diabetic patients they found a negative correlation (19).

This study supports expanding investigations of PBMC as proxy tissue for GLUT4 activity. Our data demonstrate similar results in PBMC GLUT4 as is reported in myocyte GLUT4 for conditioned vs. sedentary individuals. To further validate PBMC GLUT4 as a useful proxy tissue, it would be beneficial to sample muscle biopsy and PBMC from the same subjects at the same time before and after an exercise intervention. Furthermore, it would be helpful to include glucose tolerance tests and subjects with insulin resistance or type 2 diabetes. More

research is also needed to address the timing of alterations in PBMC GLUT4 expression in response to exercise such as the decay time of the signal transduction network after exercise ceases.

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FIGURE 1. Physical Activity in Conditioned and Sedentary College Students

Means and standard deviation of Metabolic Equivalent of Task (MET)-minutes/week in Division II NCAA student cross-country skiing and running athletes (conditioned) were significantly higher than sedentary students ($p=0.035$) matched for age and sex. MET-minutes per week were obtained from data International Physical Activity Questionnaire (IPAQ) Short Form. The data in the questionnaires were analyzed and cleaned according to the IPAQ Guidelines for Data Processing and Analysis.

FIGURE 2. PBMC GLUT4 Protein Concentrations in Conditioned and Sedentary College Students

Means and standard deviations of PBMC GLUT4 in ng/mL. PBMC GLUT4 concentrations in Division II NCAA student cross-country skiing and running athletes (conditioned) were significantly higher than sedentary students ($p=0.035$) matched for age and sex.

FIGURE 3. PBMC GLUT4 Protein Concentrations in Male and Female College Students
Means and standard deviations of PBMC GLUT4 in ng/mL. Female college students between the ages of 18 and 25 had significantly higher ($p=0.009$) PBMC GLUT4 concentrations than male students regardless of physical activity.

TABLE 1**Anthropometric and Biometric Data**

Parameter	Conditioned Mean	Sedentary Mean	p-value
Body Mass Index (kg/m ²)	21.7 (1.8)	24.8 (5.3)	0.05
Body Fat (%)	17.2 (8.0)	22.4 (8.9)	0.10
Waist Circumference (cm)	72.9 (5.8)	80.7 (13.9)	0.06
Total Cholesterol (mg/dl)	167.4 (25.3)	168.0 (24.2)	0.95
LDL (mg/dl)	95.5 (32.8)	95.3 (16.9)	0.99
HDL (mg/dl)	68.0 (10.2)	59.5 (17.4)	0.10
	Mean Rank[†]	Mean Rank	
Triglycerides (mg/dl)*	13.38	18.80	0.10
Fasting serum insulin (μIU/ml)	13.66	18.50	0.14
HOMA-IR	13.69	18.47	0.15

Data are reported as means (standard deviations) unless otherwise noted. Difference were considered significant with $p < 0.05$

[†]Serum insulin, HOMA-IR, and triglycerides had non-normal distributions therefore the Mann-Whitney U test was conducted. For both measures, mean ranks were compared. For insulin $U = 157.5$, $z = 0.483$; for HOMA-IR $U = 157.00$, $z = 1.436$; for triglycerides $U = 162.000$, $z = 1.675$.

TABLE 2**Dietary Data**

Parameter	Conditioned Mean	Sedentary Mean	p-value
Fruit and Vegetable Intake (servings/day)	3.8 (2.2)	1.9 (1.1)	0.005 *
Caloric Intake (kcal/day)	3022 (951)	2041 (756)	0.004 *
Calories from Carbohydrate (%)	51.7 (5.1)	47.5 (6.8)	0.060
Calories from Fat (%)	34.5 (3.9)	35.6 (5.2)	0.488
Calories from Protein (%)	15.4 (2.1)	14.3 (2.7)	0.223
Sugar Intake (g/day)	171.5 (63.2)	104.7 (46.0)	0.002 *
Fiber Intake (g/day)	29.0 (11.2)	14.2 (7.5)	< 0.0001 *

Dietary data as assessed by ASA24 program. Data are reported as means (standard deviations).

* significant at p = 0.05