Glucose supplements increase human muscle in vitro Na\(^{+}\)-K\(^{+}\)-ATPase activity during prolonged exercise


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Glucose supplements increase human muscle in vitro Na\(^{+}\)-K\(^{+}\)-ATPase activity during prolonged exercise (1 female) with a peak aerobic power (V\(_{\text{O2peak}}\)) of 44.8 ml\(\cdot\)kg\(^{-1}\)\(\cdot\)min\(^{-1}\); mean \(\pm\) SE cycled at \(\sim\)57\% V\(_{\text{O2peak}}\) to fatigue during both NG (artificial sweeteners) and G (6.13 \(\pm\) 0.09\% glucose) in randomized order. Consumption of beverage began at 30 min and continued every 15 min until fatigue. Time to fatigue was increased (P < 0.05) in G compared with NG (137 \(\pm\) 7 vs. 115 \(\pm\) 6 min). Maximal Na\(^{+}\)-K\(^{+}\)-ATPase activity (V\(_{\text{max}}\)) as measured by the 3-O-methylfluorescein phosphatase assay (nmol/mg \(\cdot\)h \(-\)1) was not different between conditions prior to exercise (85.2 \(\pm\) 3.3 or 86.0 \(\pm\) 3.9), at 30 min (91.4 \(\pm\) 4.7 vs. 91.9 \(\pm\) 4.1) and at fatigue (92.8 \(\pm\) 4.3 vs. 100 \(\pm\) 5.0) but was higher (P < 0.05) in G at 90 min (86.7 \(\pm\) 4.2 vs. 109 \(\pm\) 4.1). Na\(^{+}\)-K\(^{+}\)-ATPase content (B\(_{\text{max}}\)) measured by the vanadate facilitated \(^{1}\text{H}\)ouabain-binding technique (pmol/g wet wt) although elevated (P < 0.05) by exercise (0\(\sim\)30, 90, and fatigue) was not different between NG and G. At 60 and 90 min of exercise, blood glucose was higher (P < 0.05) in G compared with NG. The G condition also resulted in higher (P < 0.05) serum insulin at similar time points to glucose and lower (P < 0.05) plasma epinephrine and norepinephrine at 90 min of exercise and at fatigue. These results suggest that G results in an increase in V\(_{\text{max}}\) by mechanisms that are unclear.

contractile activity; Na\(^{+}\)-K\(^{+}\)-pump; glucose; regulation

Although both \(\alpha\)- and \(\beta\)-subunits are required for catalytic activity, the \(\alpha\)-subunit contains the active nucleotide site (4). As well, the \(\alpha\)-subunit has binding sites for ATP, Na\(^{+}\), and K\(^{+}\) and for cardiac glycosides, such as digoxin (4). The \(\beta\)-subunit is believed to be involved in assembly and anchoring functions (23).

Multiple mechanisms appear to be involved in the rapid increases in catalytic activity with the onset of exercise (6). Particularly important is the increase in intrinsic activity mediated by the increase in Na\(^{+}\) inside and K\(^{+}\) outside the cell. As well, although contradictory findings exist (46), contraction is thought to result in a translocation of Na\(^{+}\)-K\(^{+}\)-pump subunits to the sarcolemma and t-tubules (35, 36, 53). As a result of these mechanisms, the requirements for Na\(^{+}\) and K\(^{+}\) transport can be successfully accomplished at least in the short term over a broad range of exercise intensities.

As the time of contractile activity increases, additional challenges result which could alter Na\(^{+}\)-K\(^{+}\)-ATPase function and consequently, membrane excitability. Both muscle glycogen and blood glucose are progressively reduced (16, 21), and since the Na\(^{+}\)-K\(^{+}\)-pump appears to prefer carbohydrate as a substrate for ATP resynthesis to meet its energy requirements (48), it is possible that in vivo catalytic activity could be compromised. Several studies have also reported decreases in the maximal activity of the Na\(^{+}\)-K\(^{+}\)-ATPase during prolonged exercise as assessed in vitro (17). The inactivation, which would also be expected to occur in vivo could be due to structural alterations in the \(\alpha\)-subunit (40) and specifically in the region of adenine nucleotide binding, as has been observed with the sarcoplasmic reticulum Ca\(^{2+}\)-ATPase (44). The agent mediating this effect could be exercise-induced increases in free radicals (47).

Prolonged submaximal exercise in general also results in extensive changes in blood hormonal concentrations, which could impact on the catalytic activities of the Na\(^{+}\)-K\(^{+}\)-pump. Pronounced increases in both plasma epinephrine (Epi) and norepinephrine (Norepi) occur (15), which increase in vivo the intrinsic activity of the Na\(^{+}\)-K\(^{+}\)-ATPase by cAMP-mediated phosphorylation processes (6). In contrast, prolonged exercise results not only in a reduction of blood glucose but in serum insulin as well (15). Increases in insulin are thought to increase in vivo Na\(^{+}\)-K\(^{+}\)-ATPase activity, possibly by increases in intrinsic activity and translocation of the Na\(^{+}\)-K\(^{+}\)-ATPase subunits to the plasma membrane (as reviewed in Ref. 6). However, not all studies support the translocation function of insulin (46).

Artificially induced increases in blood glucose do not appear to result in increases in Na\(^{+}\)-K\(^{+}\)-ATPase activity (5). How-
ever, there is indirect evidence that a different effect of elevated blood glucose could occur during repetitive stimulation. Karelis et al. (37) have reported that infusion of glucose into the blood of anesthetized rats during repetitive stimulation of the plantaris muscle resulted in an attenuation of the disturbances in the properties of the muscle mass action potential, namely the amplitude, area, and duration. The properties of the muscle action potential are believed to provide a measure of membrane excitability and, consequently, an indirect index of the adequacy of $Na^+-K^+-ATPase$ function (6). These effects appear not to be due to the accompanying changes in serum insulin that occur (38). Although the blood catecholamines were not measured, an elevated blood glucose would be expected to attenuate the increases in these hormones accompanying repetitive activity (for a review see Ref. 15) and consequently reduce the $Na^+-K^+-ATPase$ activity. Under these conditions, it would appear that the increases in $Na^+-K^+-ATPase$ activity would occur independently of the blood hormonal changes.

The purpose of this study was to investigate the effects of prolonged cycle exercise and prolonged cycle exercise plus glucose supplementation on the maximal activity of the $Na^+-K^+-ATPase$ in working skeletal muscle. We have hypothesized that prolonged exercise would result in a progressive reduction in maximal $Na^+-K^+-ATPase$ activity in whole homogenates, while prolonged exercise with glucose would reverse or prevent any decline. These changes would occur in the absence of any changes in $Na^+-K^+-ATPase$ content.

**METHODS**

Fifteen untrained volunteers (14 males and 1 female) with a mean age of $20.1 \pm 0.31 \text{ yr (mean \pm SE)}$ participated in the study. Peak aerobic power ($V_{O_2peak}$), measured during progressive cycle exercise to fatigue (30) was $3.48 \pm 0.20 \text{l/min and } 44.8 \pm 1.9 \text{ ml/kg\text{-min.}^{-1}}$. None of the volunteers were engaged in regular physical activity either at the time of the study or for at least 3 mo preceding the study. All participants were healthy and free from any medications as determined by a questionnaire. As a condition of entry into the study, each subject was required to read and sign an Information Consent Form which detailed all experimental procedures and associated risks involved and which had been approved by the Office of Research at the University of Waterloo.

**Experimental Design**

The experimental design involved two conditions, the order of which was randomly assigned with participants blinded as to condition and with each condition separated by at least 4 wk (Fig. 1). In both conditions, the volunteers were required to cycle at the same absolute power output, representing $\sim 57\% V_{O_2peak}$ until fatigue. We have previously shown that this exercise protocol results in a reduction in maximal $Na^+-K^+-ATPase$ activity (51). One condition, the control condition, designated as no glucose (NG), involved ingestion of a placebo beverage consisting of artificial sweetener (Sugar Twin; Alberto-Culver Canada, Toronto, ON, Canada) consisting of 7.5% solution of water, sodium cyclamate, benzoic acid, and methyl paraben, beginning at 30 min of exercise and at 15 min intervals thereafter, until fatigue. The second condition, the experimental condition, designated as the glucose condition (G), involved ingestion of a $6.13 \pm 0.09\%$ glucose beverage following the same schedule as used for NG. No other supplements, either beverage or solid food, was provided during the exercise. The glucose supplement schedule and drink concentration were patterned from previous studies that have demonstrated increases in cycle time to fatigue (as cited in Ref. 7). The total glucose ingested during the exercise amounted to $1.23 \pm 0.11 \text{ g/kg body mass}$. The total volume of fluid ingested in G amounted to $1,564 \pm 142 \text{ ml, involving } 6.7 \pm 0.5 \text{ drinks with a volume of } 241 \pm 16 \text{ ml/drink}$. Comparable amounts for the NG condition were a total $1,262 \pm 137 \text{ ml, with } 5.3 \pm 0.5 \text{ drinks and } 243 \pm 17 \text{ ml/drink}$. The total volume of fluid ingested in G was greater in NG as a result of the longer exercise duration.

Measurement of respiratory gas exchange was performed prior to the beginning of exercise and periodically throughout the submaximal cycle protocol and used for determinations of $V_{O_2}$ (31). Blood samples were also collected at frequent intervals from the dorsal vein of a prewarmed hand and used for the measurement of glucose, the catecholamines, Epi and Noradp, and insulin. Muscle tissue was extracted from the vastus lateralis of both legs using the needle biopsy technique (2) with suction to increase tissue yield. A total of four biopsies were performed during a given condition, two on each leg. Further details on site preparation, location, and procedure were as are previously published by our group (20). The tissue obtained, which was stored at $-80^\circ \text{ C prior to analyses, was used for measurements of glycerogen, energy metabolites, and } Na^+-K^+-ATPase \text{ properties. The schedule for the respiratory gas, blood, and muscle sampling is described in Fig. 1. Exercise was performed using an electronically braked cycle ergometer (Quinton 870) calibrated on a regular basis. For each participant, } V_{O_2peak} \text{ was measured at least 1 wk prior to the submaximal cycling protocol. Participants were requested to refrain from exercise outside the study during the period of experimentation. Due to restrictions in the number of biopsies that can reasonably be performed on a given individual in a given condition, it was necessary to make some adjustments to the tissue sampling schedule. This was necessary so that the time-dependent effects of exercise between conditions could be examined. A total of eight separate sites were sampled per individual, four per condition. For the participants randomly assigned to the NG condition first (n = 9), tissue samples were obtained just prior to the beginning of exercise (0 min) and at 30 and 90 min of exercise and at fatigue (NG). For these participants who performed the G condition last (n = 6), tissue samples were extracted at 30 and 90 min and at fatigue (NG) and again at fatigue (G). For these volunteers, to keep the number of biopsies at four, no resting samples were collected. In those participants, we used the resting samples from the NG condition for analyses. For the participants who were randomly assigned to the G condition as the first condition, the same sampling occurred at 0, 30, and 90 min of exercise and at fatigue for this condition. The same sampling schedule was followed for the NG condition in these volunteers, namely at 0, 30, and 90 min of exercise.
exercise and at fatigue (NG). We have shown in previous experiments using similar experimental designs, namely two prolonged exercise conditions separated by at least 4 wk, the resting samples for all chemical constituents examined, are not altered (10). Other studies have also reported no differences in resting metabolic contents between NG and G conditions (45, 57, 59). We also compared the resting samples for the subgroup in the present study in which we obtained resting samples for each condition. We found no differences in metabolite content and in Na\(^+\)-K\(^+\)-ATPase activity and Na\(^+\)-K\(^+\)-ATPase content.

The prolonged cycle exercise was performed during the morning hours ~60 min after the subjects had reported to the laboratory. For each condition, the exercise test was performed at approximately the same time of day. All subjects were instructed to refrain from any other supplements, including coffee, for at least 12 h before reporting to the laboratory. During each exercise test, only the glucose supplements (G) or placebo (NG) were provided. Testing was conducted at a controlled room temperature (24°C) and at a relative humidity of between 40 and 50%.

**Analytical Techniques**

Na\(^+\)-K\(^+\)-ATPase properties. The maximal activity (V\(_{\text{max}}\)) of the Na\(^+\)-K\(^+\)-ATPase was assessed using the K\(^+\)-stimulated 3-O-methylflourescein phosphatease (3-O-MFPase) assay (28) as modified by Fraser and McKenna (14) and by our laboratory (1). This assay is based on the K\(^+\)-dependent phosphatase activity using 3-O-methylflourescein phosphate (3-O-MFP) as the fluorogenic substrate. This substrate substitutes for the aspartylphosphate intermediate of the enzyme (39). In effect, the activity of the Na\(^+\)-K\(^+\)-ATPase to represent the terminal site is ATP hydrolysis (28). In effect, the correction of TCr is based on the stability of TCr between conditions (NG vs. G). No differences were determined after the addition of 10 mM KCl and 160 mM MgCl\(_2\), 1.25 EDTA, 1.25 EGTA, 5 Na\(_2\)SO\(_4\), and 100 Tris (pH 7.4). Maximal activity was determined after the addition of 10 mM KCl and 160 mM 3-O-MFP (14). The slope of the reaction was followed using fluorescence spectrophotometry (excitation wavelength = 475 nm; emission wavelength = 515 nm; slit width = 5 nm). The maximal activity was determined by calculating the difference in slopes obtained with and without KCl (ΔF/min). The specificity of the reaction was demonstrated using the specific inhibitor ouabain, which completely inhibited 3-O-MFPase activity (H. Green, unpublished observations). Homogenate protein content was determined spectrophotometrically by using the Lowry method as modified by Schacterle and Pollock (54). All measurements of 3-O-MFPase activity and protein were performed in triplicate.

It should be noted that the value for V\(_{\text{max}}\) both within and between laboratories can show appreciable variability. In large part, the variability is due to the nonspecific activity that is thought to be due to spontaneous hydrolysis and unspecified Mg\(^2+\)-activated phosphatase activity of the homogenate (27). Given the variability inherent in the measurement of V\(_{\text{max}}\), it was important to analyze all tissue for both conditions in a given individual in a given analytical session. This we have done in this study.

Maximal Na\(^+\)-K\(^+\)-ATPase content (β\(_{\text{max}}\)) was assessed using the [\(^3\)H]ouabain binding procedure as described previously (39) and as employed in our laboratory (11). The measurement of β\(_{\text{max}}\) is based on the maximal binding of the [\(^3\)H]ouabain to the α-subunit of the enzyme (39). For these measurements, two individual tissue samples weighing between 2 and 8 mg were prewashed twice for 10-min periods in a Tris-sucrose buffer containing (in mM) 10 Tris·HCl, 3 MgSO\(_4\), 1 Tris vanadate, 250 sucrose, and 1 NaVO\(_3\) at 0°C. Maximal binding was accomplished by incubating the samples in the Tris-sucrose buffer with a saturating concentration of [\(^3\)H]ouabain (1 μM, at a specific activity of 0.9 μCi/ml) for 180 min at 37°C. To remove unbound ouabain, the samples were washed three times for 15 min in an ice-cold buffer. After being washed, the samples were blotted, weighed, placed in 1.5-ml Eppendorf tubes, soaked in 1 ml 5% TCA for 16 h at room temperature. Then 0.5 ml of the above sample was counted for [\(^3\)H]ouabain binding corrected for the loss of specifically bound [\(^3\)H]ouabain during the washout and expressed as picomoles per gram wet weight, as previously described (39).

**Blood glucose and hormones.** Following extraction of the arterialized blood sample from the prewarmed dorsal hand vein, the blood was separated into 3 aliquots and prepared for the measurement of different constituents. For blood glucose, the blood was placed in a prechilled tube containing 0.6 M perchloric acid and neutralized in 1.25 mM KHCO\(_3\), centrifuged, and the supernatant analyzed for glucose fluorometrically (43). Serum insulin was measured using serum obtained after clotting and centrifugation with standard radioimmunoassay methods. Plasma Epi and Norepi were performed using HPLC techniques as modified by our laboratory (18). For the measurement of the catecholamines, whole blood was collected in a tube containing ethylene glycol-bis(β-aminoethyl ether)-N,N,N',N'-tetraacetic acid and glutathione as antioxidant. The blood was then centrifuged at 2,000 g at 2°C, and the plasma was removed and stored at ~80°C prior to analyses.

**Muscle biochemistry.** Measurements were made for phosphocreatine (PCr), creatine (Cr), inosine monophosphate (IMP), and glycogen (Glyc). For the measurement of Cr, PCr, and Glyc, fluorometric procedures were employed following extraction from freeze-dried tissue according to procedures previously published (19, 22). The content of IMP was determined on the same homogenates by using reversed-phase HPLC procedures as described by Ingebretson et al. (34) and modified by our group (20). This measurement is recognized as a more sensitive indicator of ATP level (26). Total Glyc was measured as glucosyl units following hydrolysis by HCl.

The average of the total creatine concentration (TCr) for each individual was used to correct the raw values. This procedure helps to adjust content for the contaminating effects of blood and connective tissue. It should be acknowledged that the correction of TCr is based on the stability of TCr between conditions (NG vs. G). No differences (P > 0.05) were found between the NG and G conditions for TCr.

**Statistical procedures.** The data were analyzed using both one- and two-way ANOVA procedures for repeated measures with exercise time and experimental condition (NG, G) serving as independent variables. One-way ANOVA was used to examine the changes within an experimental condition, while two-way ANOVA was used to examine the responses between conditions at matched time points. Where significant differences were found, the Newman-Keuls technique was employed to determine which means was significant. Significance was set at the 0.05 level (P < 0.05).

**RESULTS**

**Steady-state \(V_{\text{O}}2\text{peak}\).** To determine whether exercise and experimental condition resulted in changes in aerobic energy expenditure, we measured \(V_{\text{O}}2\) responses (Table 1). For the NG condition, \(V_{\text{O}}2\) was increased from rest at 15 min of exercise and remained stable until 30 min of exercise before increasing further at 90 and 115 min of exercise. A similar pattern was
observed for the G condition with no further increases observed with the additional exercise time to 137 min. No differences were observed between conditions in VO2 at matched-time points. At 15 min of exercise, relative exercise intensity amounted to 57.2 ± 1.9 and 57.8 ± 1.2% VO2peak for the NG and G conditions, respectively.

The total time to fatigue was increased by 19% (P < 0.01) with the G condition (137 ± 7 min) compared with the NG condition (115 ± 6 min).

**Muscle metabolism.** No differences were observed in IMP content between conditions at different exercise time points (Fig. 2). However, IMP increased with the onset of exercise for both NG and G conditions and then remained stable until fatigue, regardless of condition. Total muscle glycogen declined progressively with time of exercise regardless of condition (Fig. 3). Reductions in total glycogen with exercise amounted to 76 and 73% for NG and G, respectively. The rates of decline in total glycogen were not different between conditions.

**Blood substrates and hormones.** Blood glucose levels declined with exercise in the NG but not the G condition (Fig. 4). For the NG condition, blood glucose was lower at 90 min than at all previous time points. No further reductions were observed at fatigue for this condition. At 60, 90, and 115 ± 6 min of exercise, blood glucose was higher in G compared with the NG condition.

Serum insulin concentration was also observed to be altered by exercise and glucose supplementation (Fig. 5). In the NG condition, lower levels of serum insulin were found at 30 min of exercise compared with 0 and 15 min. At 60, 90, and 115 min, further reductions in serum insulin were observed compared with 30 min. Following initiation of the oral glucose supplements at 30 min of exercise, serum insulin initially increased at 60 min of exercise compared with 30 min. At 115 and 137 min of exercise, serum insulin regressed from the value observed at 60 min of exercise. At no point in the exercise was serum insulin below rest values in the G condition. At 60, 90, and 115 min of exercise, serum insulin was higher in G compared with NG. The glucose supplements also altered the plasma Epi and Norepi responses to exercise (Fig. 6). In the case of Epi, increases in NG were initially observed at 90 min of exercise, followed by further increases at 115 min of exercise. For the G condition, initial increases in Epi were not observed until 115 min of exercise. No further increases were noted at fatigue in this condition. The Epi concentration was lower in G compared with NG at both 90 and 115 min of exercise. For Norepi, a lower concentration was also found at 90 and 115 min of exercise in G compared with NG. For this hormone, initial increases in concentration were observed at 15 min of exercise, regardless of condition. This was followed by further progressive increases until fatigue in NG. Essentially the same pattern was observed for Norepi in G condition except that the rate of increases was blunted.
**Na⁺-K⁺-ATPase properties.** No changes in $V_{\text{max}}$ were observed with exercise for the NG condition (Fig. 7). In contrast, $V_{\text{max}}$ was elevated at 90 min, and at fatigue compared with rest in the G condition. In G, $V_{\text{max}}$ was also higher at 90 min compared with 0 and 30 min, while at fatigue $V_{\text{max}}$ was higher than at 0 min, as well as being lower than at 90 min. At 90 min of exercise, $V_{\text{max}}$ was higher ($P < 0.05$) in G compared with NG. No effect of glucose was observed for $V_{\text{max}}$. However, $\beta_{\text{max}}$ was observed to increase ($P = 0.01$) with exercise, such that higher values were observed at 30 and 90 min of exercise and at fatigue compared with rest. In a secondary analysis, we have examined the responses for the subgroup in which we had measurements for both NG and G conditions at the same time point, namely 115 ± 6 min, which represented fatigue for NG.

We have found that $V_{\text{max}}$ (111 ± 3.3 vs. 89.7 ± 5.4 nmol·mg⁻¹·h⁻¹) but not $\beta_{\text{max}}$ (350 ± 14 vs. 347 ± 12 pmol/g wt) was higher in G compared with NG.

**DISCUSSION**

**Summary of Results**

The most significant finding produced from this study was that $V_{\text{max}}$ was increased with glucose supplementation. This increase in $V_{\text{max}}$ was observed at 90 min of exercise, the first sampling point studied following the initiation of glucose supplementation at 30 min of exercise. Contrary to our expectations, exercise, regardless of duration, did not result in a decrease in $V_{\text{max}}$. A second and intriguing observation was the increase in $\beta_{\text{max}}$ that occurred early in exercise, regardless of condition. Interestingly, glucose supplementation was without effect in modifying the change in $\beta_{\text{max}}$ that was observed. The changes in $V_{\text{max}}$ with G occurred in the face of a blunting of both the normal exercise-induced decrease in serum insulin and increase in plasma catecholamine concentrations. The uncoupled response between $V_{\text{max}}$ and $\beta_{\text{max}}$ with regard to both
The increase in \( V_{\text{max}} \) that we have observed with glucose supplementation, since a blunting of the exercise-induced increase in both Epi and Norepi were observed. The reduction in these hormones is a well-known effect of strategies, such as oral glucose supplementation designed to stabilize or increase blood glucose during prolonged exercise (for a review, see Ref. 15).

A second potential mechanism that could be involved in the increase in \( V_{\text{max}} \) observed with the glucose condition is the translocation of functionally active enzyme from intracellular storage sites to the plasma membrane (5, 32, 41). This is a well-documented phenomenon in rat muscle in response to increases in serum insulin. However, this does not seem to be a viable mechanism to explain the increase in \( V_{\text{max}} \) in this study, since the primary effect of G was to prevent the normal exercise-induced depression in serum insulin. Moreover, for insulin to be involved, given that our measurements were performed on whole muscle homogenates and not isolated plasma membrane fractions, the subunits translocated would have to undergo transformation to functional enzyme only when inserted into the plasma membrane. Increases in insulin are known to induce translocation of the \( \alpha \)-subunit, which promotes an increase in \( V_{\text{max}} \) in isolated plasma membrane fractions but not whole homogenates in rat muscle (5). The increase in \( V_{\text{max}} \) has been ascribed to increases in the number of functional enzymes and not to increases in catalytic activity of single enzymes (5). In fact, at least in the rat soleus, a muscle of predominant slow-twitch or Type I composition (9), no change in \( V_{\text{max}} \) occur at a given enzyme content as a result of insulin-mediated phosphorylation of the \( \alpha \)-subunits via protein kinase C (PKC) and tyrosine kinase-dependent mechanisms (5). It should be noted that the conditions under which these effects were observed occurred in conjunction with high insulin levels. In our exercise protocol, the normal response of serum insulin to prolonged exercise is to decrease (15) as we have documented in the current investigation. The fact that we have shown increases in whole homogenates in \( V_{\text{max}} \) in G compared with NG in conjunction with maintained serum insulin levels while others have not (5), is inconsistent with a role for insulin in our results. It is possible that a net translocation of active enzyme to the plasma membrane may have occurred with G compared with NG, resulting in an increase in \( V_{\text{max}} \) in enriched plasma membrane fractions but this would not elevate \( V_{\text{max}} \) in whole homogenates.

Repetitive muscle contraction is also known to increase translocation of functional enzyme to the plasma membrane (53), with potential increases in \( V_{\text{max}} \). There is evidence that contractile activity increases both the translocation of the \( \alpha_1 \)-, \( \alpha_2 \)-, and \( \beta_1 \)-isoforms (35, 36).

Differences in energy expenditure between conditions could explain the different \( V_{\text{max}} \) responses. However, this does not appear important since the absolute power output between the NG and G conditions was similar, as well as the aerobic-based energy expenditure. Since the amount of glycogen loss and the change in IMP (used as a more sensitive measure of ATP) with exercise were also unaffected by glucose supplementation, there is no reason to suspect that fatigued-mediated differences in fiber-type recruitment occurred. This could be important because Type I and Type II fibers, given the different \( \alpha \)- and \( \beta \)-isoform composition (11), may
respond differently to intrinsic regulation of the catalytic behavior of the Na\(^{+}\)-K\(^{+}\)-ATPase (4, 24).

Yet to be considered is the potential role of blood glucose in increasing \(V_{\text{max}}\). In this study, the reduction in blood glucose observed during prolonged exercise without glucose was prevented with the glucose supplement. Artificial and unphysiological elevations in blood glucose have been shown to decrease \(V_{\text{max}}\) in rat soleus muscle (5) secondary to PKC-dependent phosphorylation of \(\alpha\)-subunits (5). High glucose in isolation is also without effect on Na\(^{+}\)-K\(^{+}\)-ATPase translocation (5). These results argue against a role for glucose in increasing \(V_{\text{max}}\). In spite of a maintained blood glucose level with G, the increase in \(V_{\text{max}}\) was transient, with part of the response lost with continued exercise. The dissociation between \(V_{\text{max}}\) and blood glucose adds further support that blood glucose is not directly involved in the increase in \(V_{\text{max}}\).

Increases in the amount of Na\(^{+}\)-K\(^{+}\)-ATPase protein expressed as changes in subunit isoform composition, in response to the contractile activity remain as a possibility to account for the increase in \(V_{\text{max}}\). Small increases in \(\alpha_{1}\)-isoforms have been reported in homogenates of rat soleus muscle subjected to 90 min of stimulation (53). The fact that this increase would have to occur in the 60 min period following the beginning of the oral glucose supplement and the muscle sampling and the reversal of the increase in \(V_{\text{max}}\) at fatigue, makes the possibility remote.

An additional possibility to explain the increase in \(V_{\text{max}}\) in response to glucose supplements is via energy-mediated processes, resulting in improved ATP availability to the Na\(^{+}\)-K\(^{+}\)-ATPase. It is known that Na\(^{+}\)-K\(^{+}\)-ATPase function is dependent on glycolytically derived ATP (48). As a consequence, glucose acting as a substrate could conceivably increase ATP production in the subsarcolemmal region. Although, this could occur in vivo, even though no differences in ATP concentrations was shown between conditions, it would not be expected to translate to our in vitro measurements. In the in vitro measurement of \(V_{\text{max}}\), 3-O-MFP in saturating concentrations is used as the substrate.

An unexpected finding was the lack of an effect of prolonged exercise in reducing \(V_{\text{max}}\). Our group (12, 51, 52) as well as others (13, 42, 49) have reported decreases in \(V_{\text{max}}\) in humans using a variety of exercise protocols spanning a range of exercise intensities. Although yet to be confirmed, the decrease in \(V_{\text{max}}\) is believed to occur as a result of structural changes in the region of the nucleotide binding site of the enzyme as a result of oxidation and/or nitrosylation (40). The catalytic activity of the enzyme is measured in vitro under supposedly optimal conditions. Accordingly, the changes in \(V_{\text{max}}\) observed with exercise would represent the net effect of inhibitory and excitatory factors. Unlike most previous studies, which resulted in a reduction in \(V_{\text{max}}\), this study was conducted at a lower exercise intensity. Increases in reactive oxygen species are believed to be intensity-dependent, and consequently the inhibiting effects may not have been as pronounced in the current protocol (56). However, other factors may be involved since we have reported reductions in \(V_{\text{max}}\) in a previous study (51) using prolonged cycling that was conducted at a lower percentage of \(V_{\text{O2peak}}\) than used in the current study. We have previously observed that selected individuals demonstrate a persistent increase in \(V_{\text{max}}\) with prolonged submaximal cycling (H. Green, unpublished). Such was the case in this study.

Although the majority of volunteers displayed a reduction in \(V_{\text{max}}\) with the exercise protocol, the atypical response exhibited by others, did not result in significant reductions with exercise. In those individuals displaying the increase in \(V_{\text{max}}\) with exercise, it would appear that excitatory factors dominated over inhibitory factors.

Interpretation of Changes in \(\beta_{\text{max}}\)

An additional intriguing observation was the increase that occurred in \(\beta_{\text{max}}\) as assessed by the \[^{3}\text{H}\]ouabain binding technique (39). Typically, this procedure has been used to measure Na\(^{+}\)-K\(^{+}\)-ATPase content as indicated by the increase in \(\alpha\)-subunit abundance (39). At present, there is uncertainty both with regard to the state of the enzyme that facilitates ouabain binding (25) and the location of the \(\alpha\)-subunits that are accessible to ouabain binding (46). There is evidence that the binding affinity for ouabain is based on the enzyme being in the active state or phosphorylated state (6). Previous studies have also reported increases in \(\beta_{\text{max}}\) in rat muscle with exercise (53) and insulin (60). Since increases in \(V_{\text{max}}\) in whole homogenates were observed with G, it might be expected that increases in \[^{3}\text{H}\]ouabain binding should have been observed with glucose supplementation similar to what has been observed with \(V_{\text{max}}\). However, this did not occur. It has also been claimed that, given the fact that \[^{3}\text{H}\]ouabain binding is based on whole fiber preparations, only the plasma membrane content is being assessed (46). The failure to find an increase in \[^{3}\text{H}\]ouabain binding in rat muscle following electrically induced contractions has been used to argue against the translocation hypothesis (46). This would mean that enzyme translocation did not occur to the plasma membrane as a result of contractile activity. However, we have challenged these findings (53) by using both crude homogenates and isolated fractions of the plasma membrane and intracellular endosomal fraction prepared following prolonged electrical stimulation to rat soleus muscle.

The dissociation that we have observed between \(\beta_{\text{max}}\) and \(V_{\text{max}}\) in response to insulin could be explained by the different properties assessed or the measurement protocols employed. It is possible depending on the concentration of ouabain that the measurement reflects not \(\beta_{\text{max}}\) but the rate of ouabain binding (60). While \(\beta_{\text{max}}\) is a measure of the phosphorylated Na\(^{+}\)-K\(^{+}\)-ATPase content (39), \(V_{\text{max}}\) as assessed by the 3-O-MFPase assay is a measure of phosphatase activity as measured by the dephosphorylation of 3-O-MFP (29). The effect of glucose would appear to increase the overall hydrolytic activity of the enzyme.

It is important to emphasize that the measurement of \(V_{\text{max}}\) was based on the protein content of the tissue, while the measurement of \(\beta_{\text{max}}\) was based on the wet weight of the tissue. Exercise is known to increase the water content of tissue by 10 to 15% (55) and consequently could dilute the \(\beta_{\text{max}}\). As a consequence, the increase in \(\beta_{\text{max}}\) that we have shown with our exercise protocol may be even more pronounced.

In summary, we have found that oral glucose supplements administered during prolonged exercise results in a transient increase in maximal Na\(^{+}\)-K\(^{+}\)-ATPase activity in contracting human vastus lateralis. Given the approximately equal Type I and Type II fiber distribution in the vastus lateralis (50), it is not clear whether the effect was fiber-type specific. The mech-
anisms underlying the increase in maximal Na\(^+\)-K\(^+\)-ATPase activity would be expected to associate with the hormonal effects of the altered glucose. In this respect, insulin-mediated translocation of active enzyme to the plasma membrane or increases in intrinsic Na\(^+\)-K\(^+\)-ATPase activity by the catecholamines do not remain as inviting hypotheses. It is possible that the glucose-induced increase in Na\(^+\)-K\(^+\)-ATPase activity could have beneficial effects on protecting membrane excitability depending on the demands of the contractile activity.

**GRANT**

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**REFERENCES**


