Electrical stimulation of human lower extremities enhances energy consumption, carbohydrate oxidation, and whole body glucose uptake

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activated first because of their larger axons, which in turn have much lower electrical resistance for a given externally applied electrical current (4, 34), suggesting “reversed-size principle” of motor unit recruitment by ES. It is thus reasonable to assume that ES may become a better approach to enhance the glucose transport activity in skeletal muscle, without requiring vigorous voluntary exercise, that ensures the activation of type II fibers with subsequent enhancement of postexercise glucose uptake, particularly for those individuals who are unable to exercise because of orthopedic problems or other complications. Although functional and enzymatic adaptations in response to chronic low-frequency ES of skeletal muscle has been obtained in human subjects (3, 26, 28, 36), the clinical relevance of percutaneous ES for therapeutic purposes of glucose metabolism has not yet been well established in humans.

In our laboratory’s earlier communication (14), we demonstrated in human subjects that ES significantly increased the glucose disposal rate (GDR) during euglycemic clamp. The present study was, therefore, undertaken to investigate the acute metabolic effect of ES of lower extremities compared with voluntary cycle exercise (VE) under experimental condition of identical intensity (\(V_{O_2}\)). Our hypothesis was that ES would induce greater glycogen utilization than VE at identical body glucose uptake in humans.

The present follow-up study provided further fundamental evidence for a possible therapeutic potential associated with ES-induced enhancement of energy consumption and whole body glucose uptake in humans.

METHODS

Subjects. Eight men served as subjects. Their age, height, and body mass were 24.8 ± 0.6 (SE) yr, 172.4 ± 2.8 cm, and 68.3 ± 4.7 kg, respectively. They were not taking medications, were free of metabolic, neuromuscular, cardiovascular disorders or recent illness, and were not engaging in any regular endurance and resistance training exercise program at time of study. All the subjects signed an informed consent after being fully informed about all aspects of the experimental protocol and were asked to abstain from alcoholic beverages, exercise, and caffeine for 24 h before experiments. The Ethical Committee of Kyoto University Graduate School approved the experimental protocol.

Experimental procedure. On each of two occasions after an overnight fast, the subjects came to laboratory at 8:30 AM and were instrumented with ECG electrodes and then quietly rested for at least 30 min before the beginning of the experiment. All subjects were then asked to lie in the supine position with the lower legs extended over the end of the bed. Two trials of experiment were performed on a day separated by a minimum of 1 wk.

The initial trial required subjects to complete involuntary muscle contraction by percutaneous ES of lower extremities. Two rubber stimulation surface electrodes (6.5 × 8.5 cm) were placed over the lower legs (tibialis anterior and triceps surae) and thigh (quadriceps and hamstrings) muscles. Before application of stimulation electrodes, underlying skin was prepared by shaving, sanding, and application of isopropyl alcohols. We adopted the appropriate stimulation parameter, which consisted of square-wave biphasic pulses of 0.2- ms duration at a frequency of 20 Hz with a duty cycle of 1-s stimulation/1-s pause, because our laboratory has previously reported that parameters used can induce the highest \(V_{O_2}\) with this procedures (14). Both muscle groups (lower legs and tight) were sequentially stimulated to cocontract in an isometric manner elicited from an electrical stimulator (Omron, Kyoto, Japan). Stimulator output voltage was limited to 80 V without discomfort. In the second trial, the same subjects performed voluntary supine exercise for 20 min using a cycle ergometer (model 771, Monark) that was adjusted for each subject so that a knee angle at maximal leg extension was consistent for all tests. Exercise intensity was individually adjusted according to the subject’s corresponding levels of \(V_{O_2}\) observed during percutaneous ES, and all of the subjects were requested to maintain a pedal cadence of 50 rpm for the duration of exercise by using a metronome.

Respiratory gas measurement. Our methods for measuring respiratory gas exchange parameters online have been fully described in our laboratory’s previous studies (14, 27). Briefly, gas measurement was continuously performed for a total period of 35 min, including before (5 min), during (20 min), and after (10 min) exercise periods, with respiratory gas exchange ratio (RER) and \(V_{O_2}\) being calculated online every 15 s. Subjects breathed through a low-resistance valve, and expired gas was sampled in synchrony with the breath cycle from a mixing chamber. Analog signals of fractional concentrations of oxygen and carbon dioxide and flow rate from AE 280 analyzer (Minato Medical Science, Tokyo, Japan) were continuously digitized at a sampling rate of 50 Hz by a 13-bit analog-to-digital converter. Simultaneously, heart rate (HR) was recorded from a bipolar lead (CM5) ECG. Blood lactate measurement was performed every 5 min and was measured by the lactate oxidase method with an automated analyzer (Lactate Pro, Arklay, Kyoto, Japan).

Glucose uptake measurement. We measured glucose uptake in whole body by the hyperinsulinemic-euglycemic clamp, according to the method of DeFronzo et al. (7), with the aid of a blood glucose monitoring and glucose-insulin infusion system (Artificial pancreas model STG22, Nikkiso, Tokyo, Japan) (14). After an overnight fast, subjects arrived at a clinical research room in Kyoto University Hospital and were kept in the supine position with both knees extended. A polyethylene catheter was placed into an antecubital vein in the right side and connected to the STG22 for continuous monitoring of blood glucose with glucose oxidase method, and the hand, forearm, elbow and brachial regions were kept warm by disposable warmers to provide an arterialized venous blood source. A second catheter was inserted into the left antecubital vein for continuous infusion of insulin and glucose. After 15–20 min, baseline blood samples were drawn for the determination of fasting glucose and insulin concentrations. Insulin (Humulin R, Eli Lilly, Indianapolis, IN) was continuously infused at a rate of 1.12 mU/kg·min−1 throughout the experimental period before priming insulin infusion (0–1 min, 3.56 mU/kg·min−1; 1–2 min, 3.17 mU/kg·min−1; 2–3 min, 2.82 mU/kg·min−1; 3–4 min, 2.52 mU/kg·min−1; 4–5 min, 2.24 mU/kg·min−1; 5–6 min, 1.99 mU/kg·min−1; 6–7 min, 1.77 mU/kg·min−1; 7–8 min, 1.58 mU/kg·min−1; 8–9 min, 1.41 mU/kg·min−1; 9–10 min, 1.25 mU/kg·min−1) followed by constant insulin infusion at 1.12 mU/kg·min−1. Priming of glucose infusion with the use of a 20% glucose solution was also performed (4–10 min, 2.0 mg·kg−1·min−1; 10–15 min, 2.5 mg·kg−1·min−1; 15–16 min, 4.0 mg·kg−1·min−1), and thereafter, baseline plasma glucose level was maintained by adjusting the glucose infusion rate. At 100 min, at least [ES, 104 ± 4 (SE) min; VE, 107 ± 3 min], after the start of insulin infusion, ES or VE trial was performed for 20 min as described above. GDR was determined as the average value of every 5 min throughout the experiment period, and its values are expressed as milligrams per kilogram per minute. Blood samples for insulin measurements were obtained at the beginning and the end of ES and every 30 min during the poststimulation period of 90 min and were determined by blood enzyme immunoassay (Eiken Chemical, Tokyo, Japan), and GDR data were collected in only seven subjects because of analytic difficulty with blood sample for insulin in one subject. During the entire clamp procedures, subjects were in a prone
bars have been omitted for clarity. Mean \( \dot{V}O_2 \) change analysis in electrical stimulation (ES) of lower extremities

\[ \text{Fi} \]

The probability level for statistical significance when the significance level of V\( \dot{O}_2 \) was nearly identical before, during, and after ES (open bars) and VE (solid bars). Ex, exercise (ES and VE); Pre, preexercise; Post, postexercise. **Significantly different from preexercise, \( P < 0.01 \).

Statistical analysis. All data are expressed as means \( \pm \) SE. A one-factor (time) repeated-measures ANOVA was used to test whether a single bout of ES and VE increased respiratory gas exchange, blood lactate concentration, and GDR from baseline values. A two-factor ANOVA (between, ES and VE condition; within, time) was used for comparison of gas parameters, blood lactate concentration, and GDR. Tukey’s post hoc test was used to determine the significant difference when the significant interaction was found. The probability level for statistical significance was set at \( P < 0.05 \).

RESULTS

Figure 1 is a time course of the changes in heart rate (HR; A) and whole body oxygen uptake (\( \dot{V}O_2 \); B). \( \dot{V}O_2 \) was continuously determined by respiratory gas exchange analysis in electrical stimulation (ES) of lower extremities (C) and voluntary cycle exercise (VE, •). Values are means \( \pm \) SE for 8 subjects. Error bars have been omitted for clarity. Mean \( \dot{V}O_2 \) (C) was nearly identical before, during, and after ES (open bars) and VE (solid bars). Ex, exercise (ES and VE); Pre, preexercise; Post, postexercise. **Significantly different from preexercise, \( P < 0.01 \).

Figure 2 is a time course of the change in blood lactate concentration and RER. As we expected, lactate significantly increased at initial period (5 min) after the onset of the stimulation period (lactate, pre 1.2 \( \pm \) 0.1 vs. ES 3.2 \( \pm \) 0.3 mmol/l; \( P < 0.01 \)), whereas no such drastic changes were observed during VE, despite the identical \( \dot{V}O_2 \) (lactate, pre 1.2 vs. VE 1.4 mmol/l; not significant). Similarly, it was found that RER rose sharply and significantly at 5 min after the onset of ES far greater than during VE (RER, pre 0.80 \( \pm \) 0.02 vs. ES 0.99 \( \pm \) 0.03, \( P < 0.01 \); pre 0.79 \( \pm \) 0.02 vs. VE 0.83 \( \pm \) 0.03, \( P < 0.01 \)).

Figure 3 indicates changes in whole body glucose uptake determined by GDR in euglycemic clamp. Steady-state clamp concentration of plasma glucose was quite satisfactory in both ES and VE conditions. The coefficient of variation was found to be 2.5% for ES and 2.6% for VE. Serum insulin concentration throughout both clamp experiments was constant within the range of physiological hyperinsulinemia that was sufficient to suppress endogenous glucose production (30) (Table 1). GDR was significantly increased in response to ES and VE (\( P < 0.01 \)). However, there was a significant requirement for glucose during the post-ES period (20–50 min, 3.9 \( \pm \) 0.4

posição com ambas as mãos mantidas ao redor da tabela e instruído não a mover e contrair os braços.

A análise estatística. Todos os dados são expressos como médias \( \pm \) SE. Uma análise de variância (ANOVA) a um fator (tempo) repetido-medidas foi usada para testar se uma única dose de ES e VE aumentou os parâmetros de troca de gases respiratórios, concentração de lactato sanguíneo, e GDR a partir de valores de base. Uma análise de variância a dois fatores (entre, ES e VE condição; dentro, tempo) foi usada para comparação de gas parâmetros, concentração de lactato sanguíneo, e GDR. Teste post-hoc de Tukey foi usado para determinar a diferença significativa quando a interação significativa foi encontrada. O nível de probabilidade para significância estatística foi definido como \( P < 0.05 \).

RESULTADOS

A Figura 1 mostra um tempo curso das mudanças de \( \dot{V}O_2 \) e HR durante o período pré-ES e VE (5 min), durante ES e VE (20 min), e durante-ES e VE período (10 min). HR e \( \dot{V}O_2 \) foram rapidamente aumentados com o início de ES para extremidades, mantendo-se de forma constante durante o ES, e então retornado ao nível de estímulo imediatamente após o término de ES. \( \dot{V}O_2 \) médio durante ES foi significativamente aumentado de 3.6 \( \pm \) 0.1 para 7.3 \( \pm \) 0.3 ml·kg\(^{-1}\)·min\(^{-1}\) (\( P < 0.01 \)).

A Figura 2 é um tempo curso da mudança na concentração de lactato sanguíneo (Lac; A) e troca de gases respiratórios (RER; B). Os dados são indicados para um ponto de tempo de cada 5 min anterior, durante, e após ES (barras abertas) e VE (barras soltas). \( \pm \) SE para 8 sub支. **Significativamente diferente do pré-exercício, \( P < 0.01 \).

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mg·kg⁻¹·min⁻¹; 50–80 min, 3.7 ± 0.5 mg·kg⁻¹·min⁻¹; 80–110 min, 3.7 ± 0.5 mg·kg⁻¹·min⁻¹ above pre-ES). Thus the stimulatory effect of ES on GDR persisted not only during but also after the stimulation. In contrast to the recovery period after ES, GDR decreased rapidly during the recovery period after VE (20–50 min, 2.6 ± 0.4 mg·kg⁻¹·min⁻¹; 50–80 min, 1.7 ± 0.3 mg·kg⁻¹·min⁻¹; 80–110 min, 1.2 ± 0.3 mg·kg⁻¹·min⁻¹ above pre-VE). The difference in GDR during the recovery periods was statistically significant (P < 0.01).

**DISCUSSION**

The significant finding of this study was that a single bout of ES to lower limb muscles induced significantly greater carbohydrate utilization than VE when the same subjects were compared at the identical intensity and duration of exercise. It is well known that, unlike during voluntary contraction, the large motoneurons innervating fast-twitch fibers are the first ones to be activated, owing to their large nerve axons with low-input resistance against external stimulation current (4).

The present findings seem to support this notion. In fact, we found a larger concomitant increase in blood lactate concentration and RER in response to ES compared with VE, and this appears to reflect increased carbohydrate utilization. It has been shown in humans that there is a greater reliance on anaerobic glycolysis for energy production together with the degradation of PCr and the formation of lactate during electrically elicited muscle contractions. Greenhaff et al. (13) have found that the rates of glycolysis in contracted muscles during ES of human quadriceps muscles were twofold higher in type II fibers than type I fibers. Furthermore, the most recent evidence has shown that, for identical low-intensity level (10% maximal voluntary contraction), ES to quadriceps muscle induced a faster decline of intracellular pH together with the degradation of PCr after the onset of ES, whereas no such changes resulted from voluntary contraction (37). Our finding and these earlier observations seem to suggest that differential metabolic response to VE and ES could be primarily due to a large activation of glycolytic type II fibers by ES. One may consider the possibility that our findings of quite high RER together with considerable blood lactate concentration might have been a result of involvement of small amounts of muscle during ES, leading to much smaller overall blood flow than during VE. Therefore, lactate transport to other tissues would likely be less in the ES trial, resulting in higher blood lactate as well as RER values. Although we are not able to discard such a possibility, the nearly identical VO₂ during ES and VE trials suggests the similar blood supply to the working muscles in addition to a potentially higher venous blood pumping action by simultaneous and quite rhythmic contractions by ES. The higher RER values during ES could also result from hyperventilation. However, it is unlikely that such a hyperventilation continues for more than 10 min. The nearly identical time course changes in lactate and RER during constant VO₂ also seem to refute such a possibility.

In the present study, the most novel aspect of the present finding was that the acute stimulatory effect of ES to lower extremities on whole body glucose uptake persisted not only during but also for at least 90 min after ES under physiological hyperinsulinemia that was sufficient to suppress endogenous glucose production. This further supports our laboratory’s previous findings on possible beneficial effects of ES to quadriceps alone (14), and we consider the present results as more encouraging.

Table 1. Plasma glucose, serum insulin, and glucose disposal rate during euglycemic clamp

<table>
<thead>
<tr>
<th></th>
<th>Preinfusion</th>
<th>Pre-Ex (−30–0 min)</th>
<th>Ex (0–20 min)</th>
<th>Post-Ex (20–50 min)</th>
<th>Post-Ex (50–80 min)</th>
<th>Post-Ex (80–110 min)</th>
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<tr>
<td><strong>ES</strong></td>
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<tr>
<td>Plasma glucose, mg/dl</td>
<td>86.6±2.4</td>
<td>85.0±1.9</td>
<td>82.5±1.5</td>
<td>84.9±2.4</td>
<td>86.4±2.3</td>
<td>86.8±2.7</td>
</tr>
<tr>
<td>Serum insulin, μU/ml</td>
<td>6.4±1.0</td>
<td>6.8±5.0</td>
<td>91.2±4.6</td>
<td>89.4±4.1</td>
<td>92.4±6.8</td>
<td>91.8±5.9</td>
</tr>
<tr>
<td>GDR, mg·kg⁻¹·min⁻¹</td>
<td>8.8±0.6</td>
<td>12.1±0.8*</td>
<td>12.7±0.8*</td>
<td>12.5±0.9*</td>
<td>12.5±0.9*</td>
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<td><strong>VE</strong></td>
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<tr>
<td>Plasma glucose, mg/dl</td>
<td>84.9±1.0</td>
<td>81.2±1.0</td>
<td>81.1±0.7</td>
<td>81.8±1.3</td>
<td>82.6±1.1</td>
<td>84.8±1.6</td>
</tr>
<tr>
<td>Serum insulin, μU/ml</td>
<td>5.3±0.8</td>
<td>8.3±5.3</td>
<td>81.7±4.0</td>
<td>82.7±4.5</td>
<td>82.8±5.7</td>
<td>81.4±4.4</td>
</tr>
<tr>
<td>GDR, mg·kg⁻¹·min⁻¹</td>
<td>8.7±0.7</td>
<td>11.9±0.9*</td>
<td>11.3±0.8*</td>
<td>10.4±0.7*</td>
<td></td>
<td>9.9±0.7</td>
</tr>
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</table>

Values are means ± SE for 7 subjects. Plasma glucose and glucose disposal rate (GDR) were continuously determined, and mean values of the indicated time period are shown. Serum insulin was determined at the end of the indicated time period. ES, electrical stimulation; VE, voluntary cycle exercise; −30–0 min, preexercise (Ex) period (ES and VE); 0–20 min, during Ex period; 20–50, 50–80, and 80–110 min, post-Ex period. *Significantly different from pre-Ex, P < 0.01. †Significantly different from pre-Ex, P < 0.05.
One effect, evident during exercise and for a relatively short period after exercise, is an insulin-independent simulation of glucose uptake. The second effect, which becomes evident while the acute effect of exercise on glucose uptake disappears, consists of a large increase in the insulin-dependent stimulation (insulin sensitivity). Although increased glycogenolysis was not necessarily associated with glucose disposal (9, 15), there is substantial evidence to suggest that, for glycogen repletion after a single exercise session, both insulin-independent and insulin-dependent glucose uptake may play a role. In fact, Price et al. (29) showed in humans that postexercise glycogen repletion occurred in an insulin-independent manner for ~1 h after exercise, and thereafter insulin-dependent glycogen repletion became significant. In line with this, Wallberg-Henriksson et al. (38) have shown in isolated rat skeletal muscle that the activity of insulin-independent glucose uptake is maximally enhanced immediately after exercise and then gradually wears off but that ~34% of the initial activity is still present over 180 min. Because our data are limited to 90 min post-ES, it appears that acute and persistent enhancement of GDR during and after ES is likely due, at least in large part, to the insulin-independent-mediated effect and that the insulin-dependent effect may come into play, particularly during the latter part of the post-ES period. In addition, it has been recently suggested that 5′-AMP-activated protein kinase (AMPK) may have a regulatory role in contraction-stimulated (insulin-independent) glucose transport in skeletal muscle (16). AMPK is stimulated by various glycogen-depleting stimuli, including contraction, with a close correlation to glucose transport activity in rat skeletal muscle (15). In fact, contraction-induced activation of AMPK and GLUT4 translocation and glucose uptake is impaired in glycogen-supercompensated muscles of exercised rats (20). With regard to insulin sensitivity, carbohydrate deprivation after exercise results in delayed glycogen restoration and prolonged increase in insulin sensitivity in rat skeletal muscle (2), and, furthermore, insulin-stimulated GLUT4 translocation and glucose uptake are impaired in supercompensated muscles of exercised rats (20). It thus seems that exercise-induced increase in carbohydrate depletion may have provided the stimulus for increased glucose uptake in the postexercise period. Muscle fuel and energy state in contracted muscles may play an important role in regulating the acute and persistent effect of contraction on glucose transport and may partly explain different persistent duration of increased glucose uptake.

It is interesting to note that the post-ES increase in GDR was as high as that observed by bicycle exercise at 40% of maximal V̇O₂ for 30 min under similar hyperinsulinemia (~77 μU/ml) (6). DeFronzo et al. (6) have demonstrated that exercise and insulin actually act synergistically on glucose uptake in whole body under physiologic hyperinsulinemia. The synergism could be attributed to the fact that exercise enhances blood flow, which increases glucose supply to contracting muscles, thereby reinforcing the effect of infused insulin. It has been shown that ES can increase lower limb blood flows, leading to enhanced stroke volume and cardiac output by activation of venous muscle pump when ES was used to induce rhythmic muscle contractions of calf and thigh in able-bodied and paraplegic patients (5). It thus appears that acute increase in GDR during ES and VE condition could be due to a better perfusion of the peripheral tissue within contracted muscles.

However, the results obtained from physiological hyperinsulinemia must be interpreted in light of the fact that exercise (or almost every other condition in which the insulin clamp has been applied) is not normally characterized by hyperinsulinemia and euglycemia. Indeed, DeFronzo et al. (6) have shown that the effect of combined insulin and exercise on the peripheral glucose uptake is much greater than of either one alone and is more likely to have an impact throughout exercise and postexercise period. If the effects of exercise and insulin were purely additive, then the acute stimulatory effect of ES alone on glucose uptake in the whole body would have been a smaller magnitude than that with combined insulin.

Additionally, it has been shown that increase in epinephrine may partly enhance carbohydrate utilization in humans. Because epinephrine enhances the rate of muscle glycogenolysis as well during short-term ES (12), it might have resulted in an enhanced glycogenolysis after ES. It is not likely that significant epinephrine spill out would have occurred during a low-intensity exercise, such as the ones employed in the present study (i.e., 10% maximal voluntary contraction of ES). We could, however, only speculate such a possibility in the absence of no epinephrine data.

Recent therapeutic studies have shown that ES-assisted training can increase muscle GLUT4 content and improve insulin sensitivity in patients with spinal cord injury (3, 26). It has also been reported in humans that chronic low-frequency ES to leg muscles increased V̇O₂ at anaerobic threshold as an improvement in muscle function due to its enhanced oxidative capacity (28). In addition, chronic low-frequency ES can induce improvement in the aerobic-oxidative metabolism of skeletal muscle (36). Those previous therapeutic findings, together with the present study, seem to suggest that ES may have a great potential for medical applications, e.g., counteracting the effects of disuse, decreased oxygen transport, and reduced peripheral glucose uptake due to insulin resistance in immobilized or bedridden patients, without requiring vigorous voluntary exercise.

In summary, we have demonstrated that a single bout of ES to lower extremities can significantly enhance energy consumption, carbohydrate oxidation, and whole body glucose uptake at low-intensity exercise. This could be partly due to a larger contribution of type II fibers in ES compared with in VE at identical intensity. Enhanced carbohydrate oxidation by ES may partly influence the post-ES effect on enhanced GDR. Thus percutaneous ES may become an important part of therapy in enhancing energy and glucose metabolism for those individuals who are unable to exercise because of orthopedic problems or other complications.

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