Kawano H, Tanaka H, Miyachi M	Resistance Training and Arterial Compliance: Keeping the Benefits While Minimizing the Stiffening	Journal of Hypertension	24	1753－1759	2006
Wu J, Oka J, Tabata I, Higuchi M, Toda T, Fuku N, Ezaki J, Sugiyama F, Uchiyama S, Yamada K, Ishimi Y	Effects of isoflavone and exercise on bone and lipid metabolism in postmenopausal Japanese women: One year randomized placebo-controlled trial	Journal of Bone and Mineral Research	21	780－789	2006
山本英彦，武友麻衣，田中宏暁，吉田るみ子，萱島誠，小野敦子，名取省一，橋口照人，丸山征郎	生活習慣病の予防・改善のための運動療法－ベンチステップ運動を用いた無作為化比較試験	人間ドック	21巻４号	18－23	2006

Sanada K、 Kuchiki T, Miyachi M, McGrath K, Higuchi M、 Ebashi H	Effects of age on ventilatory threshold and peak oxygen uptake normalised for regional skeletal muscle mass in Japanese men and women aged 20- 80 years	European Journal of Applied Physiology	99	475－483	2007

J Appl Physiol 96: 911–916, 2004.
First published October 31, 2003; 10.1152/japplphysiol.00664.2003.

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

Taku Hamada,[1] Tatsuya Hayashi,[2] Tetsuya Kimura,[1] Kazuwa Nakao,[2] and Toshio Moritani[1]

[1]*Laboratory of Applied Physiology, Kyoto University Graduate School of Human and Environmental Studies, Kyoto 606-8501; and* [2]*Department of Medicine and Clinical Science, Kyoto University Graduate School of Medicine, Kyoto 606-8507, Japan*

Submitted 27 June 2003; accepted in final form 27 October 2003

Hamada, Taku, Tatsuya Hayashi, Tetsuya Kimura, Kazuwa Nakao, and Toshio Moritani. Electrical stimulation of human lower extremities enhances energy consumption, carbohydrate oxidation, and whole body glucose uptake. *J Appl Physiol* 96: 911–916, 2004. First published October 31, 2003; 10.1152/japplphysiol.00664.2003.—Our laboratory has recently demonstrated that low-frequency electrical stimulation (ES) of quadriceps muscles alone significantly enhanced glucose disposal rate (GDR) during euglycemic clamp (Hamada T, Sasaki H, Hayashi T, Moritani T, and Nakao K. *J Appl Physiol* 94: 2107–2112, 2003). The present study is further follow-up to examine the acute metabolic effects of ES to lower extremities compared with voluntary cycle exercise (VE) at identical intensity. In eight male subjects lying in the supine position, both lower leg (tibialis anterior and triceps surae) and thigh (quadriceps and hamstrings) muscles were sequentially stimulated to cocontract in an isometric manner at 20 Hz with a 1-s on-off duty cycle for 20 min. Despite small elevation of oxygen uptake by 7.3 ± 0.3 ml·kg^{-1}·min^{-1} during ES, the blood lactate concentration was significantly increased by 3.2 ± 0.3 mmol/l in initial period (5 min) after the onset of the ES ($P < 0.01$), whereas VE showed no such changes at identical oxygen uptake (7.5 ± 0.3 ml·kg^{-1}·min^{-1}). ES also induced enhanced whole body carbohydrate oxidation as shown by the significantly higher respiratory gas exchange ratio than with VE ($P < 0.01$). These data indicated increased anaerobic glycolysis by ES. Furthermore, whole body glucose uptake determined by GDR during euglycemic clamp demonstrated a significant increase during and after the cessation of ES for at least 90 min ($P < 0.01$). This post-ES effect was significantly greater than that of the post-VE period ($P < 0.01$). These results suggest that ES can substantially enhance energy consumption, carbohydrate oxidation, and whole body glucose uptake at low intensity of exercise. Percutaneous ES may become a therapeutic utility to enhance glucose metabolism in humans.

exercise; glucose transport; euglycemic clamp; insulin sensitivity; oxygen uptake

IT HAS BEEN RECOMMENDED THAT, for the greatest improvement in glycemic control and insulin sensitivity, exercise intensity needs to be set at 50–80% of maximal aerobic capacity in Type 2 diabetes (1). An acute bout of physical exercise increases glucose disposal into the contracting muscles, leading to clinically significant decreases in blood glucose concentrations. This is due partly to the translocation of GLUT4 glucose transporters to the cell surface via insulin-independent mechanism (11, 23). In addition, after a single exercise session at intensity of 60–85% maximal oxygen uptake (V_{O_2}), substantial glucose utilization continues to occur at a significantly elevated level in the previously exercised muscles, primarily functioning to restore muscle glycogen concentrations (8, 25). This postexercise effect has been well characterized by a substantial increase in insulin-stimulated whole body glucose uptake (insulin sensitivity), as demonstrated by the hyperinsulimic-euglycemic clamp (8, 25). These clinically important effects of insulin-independent and -dependent stimulation by exercise have widely been accepted as a useful modality to prevent and treat Type 2 diabetes.

However, attention has not been given to those individuals who are restricted from voluntary physical activity and in a bedridden state because of chronic illness or other forms of disability. It is quite difficult to require the recommended exercise intensity for those individuals. Lipman et al. (22) have shown that a chronic lack of physical activity is associated with reduced peripheral glucose uptake due to insulin resistance. More recently, Mikines et al. (24) and Stuart et al. (35) have demonstrated that physical inactivity caused by bed rest for as little as 7 days is associated with a substantial reduction in insulin sensitivity in inactive skeletal muscle without changing the effect of insulin on hepatic glucose production. In addition, prolonged physical inactivity has been shown to decrease the oxygen transport capacity of skeletal muscle (32). Thus it is important to develop a new way of enhancing energy and glucose metabolism in individuals who are unable to exercise.

Electrical stimulation (ES) produces skeletal muscle contractions as results of the percutaneous stimulation of peripheral nerves. Clinically, the use of ES has been shown to potentially improve or compensate for disadvantages in disabled or chronic patients with physical inactivity. In fact, ES of skeletal muscles might not only improve cardiovascular function for tetra- or paraplegic individuals but may also increase the strength and endurance of their paralyzed muscles during daily activity, such as wheelchair locomotion or body transfer (5, 18). Furthermore, there is substantial evidence that the anaerobic metabolism in the glycolytic pathway with the formation of lactate and hydrogen ions and through the degradation of phosphocreatine (PCr) is more pronounced in ES than in voluntary exercise when the exercise protocols were performed at identical low intensity (17, 21, 33, 37). In addition, previous studies in rat have shown that glucose transport activity can be higher in type II than type I fibers when ES is employed (19, 31). Unlike the orderly recruitment of motor units during low-intensity voluntary exercise in which type I slow-twitch fibers are utilized first (10), during ES, large and fatigable fast-twitch motor units with glycolytic fibers are

Address for reprint requests and other correspondence: T. Moritani, Laboratory of Applied Physiology, Graduate School of Human and Environmental Studies, Kyoto University, Kyoto 606-8501, Japan (E-mail: moritani@virgo.jinkan.kyoto-u.ac.jp).

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C Section 1734 solely to indicate this fact.

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 ($\dot{V}O_2$). Our hypothesis was that ES would induce greater glycogen utilization than VE at identical $\dot{V}O_2$. We have employed a new way of giving involuntary exercise induced by percutaneous electrical muscle stimulation. This was performed by means of rhythmic muscle contractions of lower leg and thigh muscles of both lower extremities. 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 $\dot{V}O_2$ 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 $\dot{V}O_2$ 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 $\dot{V}O_2$ 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 hyperinsulimic-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^{-1}·min^{-1} throughout the experimental period after priming insulin infusion (0–1 min, 3.56 mU·kg^{-1}·min^{-1}; 1–2 min, 3.17 mU·kg^{-1}·min^{-1}; 2–3 min, 2.82 mU·kg^{-1}·min^{-1}; 3–4 min, 2.52 mU·kg^{-1}·min^{-1}; 4–5 min, 2.24 mU·kg^{-1}·min^{-1}; 5–6 min, 1.99 mU·kg^{-1}·min^{-1}; 6–7 min, 1.77 mU·kg^{-1}·min^{-1}; 7–8 min, 1.58 mU·kg^{-1}·min^{-1}; 8–9 min, 1.41 mU·kg^{-1}·min^{-1}; 9–10 min, 1.25 mU·kg^{-1}·min^{-1}) followed by constant insulin infusion at 1.12 mU·kg^{-1}·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

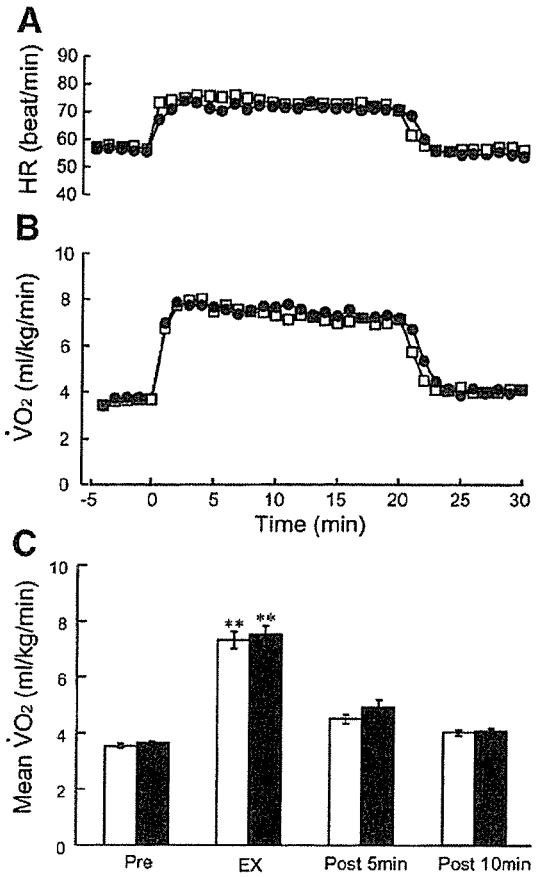

Fig. 1. Time course of changes in heart rate (HR; *A*) and whole body oxygen uptake ($\dot{V}O_2$; *B*). $\dot{V}O_2$ was continuously determined by respiratory gas exchange analysis in electrical stimulation (ES) of lower extremities (□) and voluntary cycle exercise (VE, ●). Values are means ± SE for 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, $P < 0.01$.

position with both hands kept flat on the table and instructed not to move and contract upper arms.

Statistical analysis. All data are expressed as means ± SE. A one-factor (time) repeated-measures ANOVA was used to test whether a single bout of ES and VE increased respiratory gas parameters, 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 $\dot{V}O_2$ and HR throughout the pre-ES and VE (5 min), during ES and VE (20 min), and post-ES and VE period (10 min). HR and $\dot{V}O_2$ were rapidly increased with the onset of ES to lower extremities, maintained fairly constant throughout the ES, and then returned to the prestimulation level immediately after the cessation of ES. Mean $\dot{V}O_2$ during ES was significantly increased from 3.6 ± 0.1 to 7.3 ± 0.3 ml·kg^{-1}·min^{-1} ($P < 0.01$). On a separate day, when the same subjects performed VE using a supine cycle ergometer for 20 min, exercise intensity (work rate) was individually adjusted, and thereby $\dot{V}O_2$ was increased to the same level as observed during ES (7.5 ± 0.3 ml·kg^{-1}·min^{-1}, not significant vs. ES; Fig. 1).

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 ± 0.1 vs. ES 3.2 ± 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 ± 0.02 vs. ES 0.99 ± 0.03, $P < 0.01$; pre 0.79 ± 0.02 vs. VE 0.83 ± 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 ± 0.4

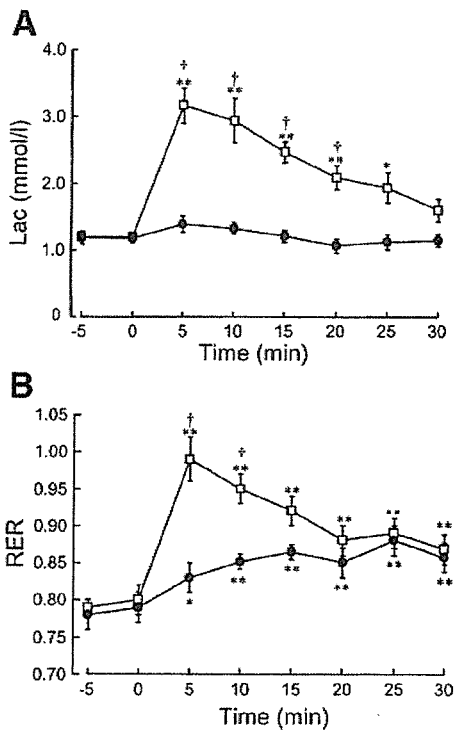

Fig. 2. Comparison of time course of changes in blood lactate concentration (Lac; *A*) and respiratory gas exchange ratio (RER; *B*). Data are indicated in a time point of every 5 min before, during, and after ES (open bars) and VE (solid bars). Values are means + SE for 8 subjects. **Significantly different from preexercise (0 min), $P < 0.01$. *Significantly different from preexercise (0 min), $P < 0.05$. †Significantly different from VE, $P < 0.01$.

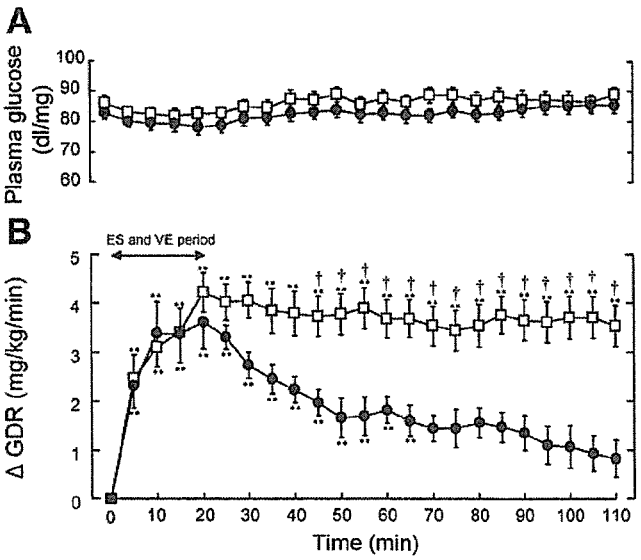

Fig. 3. Mean time course of changes in plasma glucose (*A*) and glucose disposal rate (GDR; *B*) during steady-state euglycemic clamp in both ES (open bars) and VE (solid bars) conditions. Change in GDR (ΔGDR) data determined every 5 min are indicated as increasing time point from preexercise period (ES and VE). Values are means ± SE for 7 subjects. **Significantly different from preexercise (0 min), $P < 0.01$. †Significantly different from VE. $P < 0.01$.

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 $\dot{V}O_2$ 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 $\dot{V}O_2$ 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*

	Preinfusion	Pre-Ex (−30–0 min)	Ex (0–20 min)	Post-Ex (20–50 min)	Post-Ex (50–80 min)	Post-Ex (80–110 min)
ES						
Plasma glucose, mg/dl	86.6 ± 2.4	85.0 ± 1.9	82.5 ± 1.5	84.9 ± 2.4	86.4 ± 2.3	86.8 ± 2.7
Serum insulin, μU/ml	6.4 ± 1.0	88.3 ± 5.0	91.2 ± 4.6	89.4 ± 4.1	92.4 ± 6.8	91.8 ± 5.9
GDR, mg·kg^{-1}·min^{-1}		8.8 ± 0.6	12.1 ± 0.8*	12.7 ± 0.8*	12.5 ± 0.9*	12.5 ± 0.9*
VE						
Plasma glucose, mg/dl	84.9 ± 1.0	82.1 ± 1.0	81.1 ± 0.7	81.8 ± 1.3	82.6 ± 1.1	84.8 ± 1.6
Serum insulin, μU/ml	5.3 ± 0.8	83.1 ± 5.3	81.7 ± 4.0	82.7 ± 4.5	82.8 ± 5.7	81.4 ± 4.4
GDR, mg·kg^{-1}·min^{-1}		8.7 ± 0.7	11.9 ± 0.9*	11.3 ± 0.8*	10.4 ± 0.7†	9.9 ± 0.7

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 $\dot{V}O_2$ 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 physiological 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 glyconeogenolysis after ES. It is unlikely that significant epinephrine spill out would have occurred during a low-intensity exercises, 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 $\dot{V}O_2$ 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.

ACKNOWLEDGMENTS

We thank the subjects for participation in this study. We are grateful to Omron (Kyoto, Japan) for providing electrical stimulation equipment.

GRANTS

This work was in part supported by the Japanese Ministry of Education, Science, Sports and Culture Grant-In-Aid for Scientific Research (B) 15300231 (to T. Moritani) and by the discretional expense of the Kyoto University's president.

REFERENCES

1. **American College of Sports Medicine and American Diabetes Association.** Diabetes mellitus and exercise. *Med Sci Sports Exerc* 29: i–vi, 1997.

2. **Cartee GD, Young DA, Sleeper MD, Zierath J, Wallberg-Henriksson H, and Holloszy JO.** Prolonged increase in insulin-stimulated glucose transport in muscle after exercise. *Am J Physiol Endocrinol Metab* 256: E494–E499, 1989.
3. **Chilibeck PD, Bell G, Jeon J, Weiss CB, Murdoch G, MacLean I, Ryan E, and Burnham R.** Functional electrical stimulation exercise increases GLUT-1 and GLUT-4 in paralyzed skeletal muscle. *Metabolism* 48: 1409–1413, 1999.
4. **Clamann HP, Gillies JD, Skinner B, and Henneman E.** Quantitative measure of output motoneuron pool during monosynaptic reflexes. *J Neurophysiol* 37: 1328–1337, 1974.
5. **Davis GM, Servedio FJ, Glaser RM, Gupta SC, and Suryaprasad AG.** Cardiovascular responses to arm cranking and FNS-induced leg exercise in paraplegics. *J Appl Physiol* 69: 671–677, 1990.
6. **DeFronzo RA, Ferrannini E, Sato Y, Felig P, and Wahren J.** Synergistic interaction between exercise and insulin on peripheral glucose uptake. *J Clin Invest* 68: 1468–1474, 1981.
7. **DeFronzo RA, Tobin JD, and Andres R.** Glucose clamp technique: a method for quantifying insulin secretion and resistance. *Am J Physiol Endocrinol Metab Gastrointest Physiol* 237: E214–E223, 1979.
8. **Devlin JT, Hirshman M, Horton ED, and Horton ES.** Enhanced peripheral and splanchnic insulin sensitivity in NIDDM men after single bout of exercise. *Diabetes* 36: 434–439, 1987.
9. **Fisher JS, Gao J, Han DH, Holloszy JO, and Nolte LA.** Activation of AMP kinase enhances sensitivity of muscle glucose transport to insulin. *Am J Physiol Endocrinol Metab* 282: E18–E23, 2002.
10. **Gollnick PD, Karlsson J, Piehl K, and Saltin B.** Selective glycogen depletion in skeletal muscle fibers of man following sustained contractions. *J Physiol* 241: 59–67, 1974.
11. **Goodyear LJ, King PA, Hirshman MF, Thompson CM, Horton ED, and Horton ES.** Contractile activity increases plasma membrane glucose transporters in absence of insulin. *Am J Physiol Endocrinol Metab* 258: E667–E672, 1990.
12. **Greenhaff PL, Ren JM, Soderlund K, and Hultman E.** Energy metabolism in single human muscle fibers during contraction without and with epinephrine infusion. *Am J Physiol Endocrinol Metab* 260: E713–E718, 1991.
13. **Greenhaff PL, Soderlund K, Ren JM, and Hultman E.** Energy metabolism in single human muscle fibers during intermittent contraction with occluded circulation. *J Physiol* 460: 443–453, 1993.
14. **Hamada T, Sasaki H, Hayashi T, Moritani T, and Nakao K.** Enhancement of whole body glucose uptake during and after human skeletal muscle low-frequency electrical stimulation. *J Appl Physiol* 94: 2107–2112, 2003.
15. **Hayashi T, Hirshman MF, Fujii N, Habinowski SA, Witters LA, and Goodyear LJ.** Metabolic stress and altered glucose transport: activation of AMP-activated protein kinase as a unifying coupling mechanism. *Diabetes* 49: 527–531, 2000.
16. **Hayashi T, Hirshman MF, Kurth EJ, Winder WW, and Goodyear LJ.** Evidence for 5′ AMP-activated protein kinase mediation of the effect of muscle contraction on glucose transport. *Diabetes* 47: 1369–1373, 1998.
17. **Hultman E and Spriet LL.** Skeletal muscle metabolism, contraction force and glycogen utilization during prolonged electrical stimulation in humans. *J Physiol* 374: 493–501, 1986.
18. **Jacobs PL, Klose KJ, Guest R, Needham-Shropshire B, Bronton JG, and Green BA.** Relationships of oxygen uptake, heart rate, and ratings of perceived exertion in persons with paraplegia during functional neuromuscular stimulation assisted ambulation. *Spinal Cord* 35: 292–298, 1997.
19. **Johannsson E, Jensen J, Gundersen K, Dahl HA, and Bonen A.** Effect of electrical stimulation patterns on glucose transport in rat muscles. *Am J Physiol Regul Integr Comp Physiol* 271: R426–R431, 1996.
20. **Kawanaka K, Han DH, Nolte LA, Hansen PA, Nakatani A, and Holloszy JO.** Decreased insulin-stimulated GLUT-4 translocation in glycogen-supercompensated muscles of exercised rats. *Am J Physiol Endocrinol Metab* 276: E907–E912, 1999.
21. **Kim CK, Bangsbo J, Strange S, Karpakka J, and Saltin B.** Metabolic response and muscle glycogen depletion pattern during prolonged electrically induced dynamic exercise in man. *Scand J Rehabil Med* 27: 51–58, 1995.
22. **Lipman RL, Schunre JJ, Bradley EM, and Lecocq FR.** Impairment of peripheral glucose utilization in normal subjects by prolonged bed rest. *J Lab Clin Med* 76: 221–230, 1970.
23. **Lund S, Holman GD, Schmitz O, and Pedersen O.** Contraction stimulates translocation of glucose transporter GLUT4 in skeletal muscle through a mechanism distinct from that of insulin. *Proc Natl Acad Sci USA* 92: 5817–5821, 1995.
24. **Mikines KJ, Richter EA, Dela F, and Galbo H.** Seven days of bed rest decrease insulin action on glucose uptake in leg and whole body. *J Appl Physiol* 70: 1245–1254, 1991.
25. **Mikines KJ, Sonne B, Farrell PA, Tronier B, and Galbo H.** Effect of physical exercise on sensitivity and responsiveness to insulin in humans. *Am J Physiol Endocrinol Metab* 254: E248–E259, 1988.
26. **Mohr T, Dela F, Handberg A, Biering-Sorensen F, Galbo H, and Kjaer M.** Insulin action and long-term electrically induced training in individuals with spinal cord injuries. *Med Sci Sports Exerc* 33: 1247–1252, 2001.
27. **Moritani T, Takaishi T, and Matsumoto T.** Determination of maximal power output at neuromuscular fatigue threshold. *J Appl Physiol* 74: 1729–1734, 1993.
28. **Nuhr M, Crevenna R, Gohlsch B, Bittner C, Fialka-Moser V, Quittan M, and Pette D.** Functional and biochemical properties of chronically stimulated human skeletal muscle. *Eur J Appl Physiol* 89: 202–208, 2003.
29. **Price TB, Rothman DL, Taylor R, Avison MJ, Shulman GI, and Shulman RG.** Human muscle glycogen resynthesis after exercise: insulin-dependent and-independent phases. *J Appl Physiol* 76: 104–111, 1994.
30. **Rizza RA, Mandarino LJ, and Gerich JE.** Dose-response characteristics for effects of insulin on production and utilization of glucose in man. *Am J Physiol Endocrinol Metab* 240: E630–E639, 1981.
31. **Roy D, Johannsson E, Bonen A, and Marette A.** Electrical stimulation induces fiber type-specific translocation of GLUT-4 to T tubules in skeletal muscle. *Am J Physiol Endocrinol Metab* 273: E688–E694, 1997.
32. **Saltin B, Blomqvist G, Mitchell JH, Johnson RL Jr, Wildenthal K, and Chapman CB.** Response to exercise after bed rest and after training. *Circulation* 38, *Suppl* 7: 1–78, 1968.
33. **Saltin B, Strange S, Bangsbo J, Kim CK, Duvoisin M, Hargens A, and Gollnick PD.** Central and peripheral cardiovascular response to electrically induced and voluntary leg exercise. In: *Proceedings from the Fourth European Symposium on Life Science Research in Space.* Noordwijk, The Netherlands: European Space Agency, 1990, p. 591–595. (SP-307)
34. **Sinacore DR, Delitto A, King DS, and Rose SJ.** Type II fiber activation with electrical stimulation: a preliminary report. *Phys Ther* 70: 416–422, 1990.
35. **Stuart CA, Shangraw RE, Prince MJ, Peters EJ, and Wolfe RR.** Bed-rest-induced insulin resistance occurs primarily in muscle. *Metabolism* 37: 802–806, 1988.
36. **Theriault R, Theriault G, and Simoneau JA.** Human skeletal muscle adaptation in response to chronic low-frequency electrical stimulation. *J Appl Physiol* 77: 1885–1889, 1994.
37. **Vanderthommen M, Duteil S, Wary C, Raynaud JS, Leroy-Willig A, Crielaard JM, and Carlier PG.** A comparison of voluntary and electrically induced contractions by interleaved ^{1}H- and ^{31}P-NMRS in humans. *J Appl Physiol* 94: 1012–1024, 2003.
38. **Wallberg-Henriksson H, Constable SH, Young DA, and Holloszy JO.** Glucose transport into rat skeletal muscle: interaction between exercise and insulin. *J Appl Physiol* 65: 909–913, 1988.

J Bone Miner Metab (2004) 22:26–31
DOI 10.1007/s00774-003-0443-5

Journal of
Bone and
Mineral
Metabolism

Effects of treadmill exercise on bone mass, bone metabolism, and calciotropic hormones in young growing rats

JUN IWAMOTO[1], CHISATO SHIMAMURA[2,3], TSUYOSHI TAKEDA[1], HITOSHI ABE[3], SHOICHI ICHIMURA[4], YOSHIHIRO SATO[5], and YOSHIAKI TOYAMA[2]

[1] Department of Sports Medicine, Keio University School of Medicine, 35 Shinanomachi, Shinjuku-ku, Tokyo 160-8582, Japan
[2] Department of Orthopaedic Surgery, Keio University School of Medicine, Tokyo, Japan
[3] Department of Orthopaedic Surgery, Kitasato Institute Hospital, Tokyo, Japan
[4] Department of Orthopaedic Surgery, Kyorin University School of Medicine, Tokyo, Japan
[5] Department of Rehabilitation Medicine, Hirosaki University School of Medicine, Hirosaki, Japan

Abstract The aim of the present study was to examine the effects of exercise on bone mass, bone metabolism, and calciotropic hormones in young growing rats. Twenty 6-week-old female Wistar rats were randomized into the following four groups with 5 animals each: 7 weeks of exercise, 7 weeks of sedentary control, 11 weeks of exercise, and 11 weeks of sedentary control. The exercise regimen consisted of running on a treadmill at 25 m/min for 1 h each day on 5 days a week. After each period of exercise, the bone mineral content (BMC) of the tibia and fifth lumbar spine was measured by dual-energy X-ray absorptiometry, using a Lunar DPX-L instrument. The femoral length and levels of bone markers and calciotropic hormones were also assessed. Seven and 11 weeks of exercise increased the serum osteocalcin and 1,25-dihydroxyvitamin D_3 levels, and decreased the serum parathyroid level. Seven weeks of exercise decreased the urinary deoxypyridinoline level, and 11 weeks of exercise increased the serum alkaline phosphatase level and decreased the serum tartrate-resistant acid phosphatase level. As a result, 7 and 11 weeks of exercise increased the femoral length and tibial BMC, but did not alter the lumbar BMC. The present study demonstrates that treadmill exercise stimulates bone formation and suppresses bone resorption, increases the serum 1,25-dihydroxyvitamin D_3 level, and decreases the serum parathyroid hormone level, resulting in an increase in bone mass with stimulation of longitudinal bone growth, especially at weight-bearing sites, in young growing rats. Further studies with long-term exercise may be needed to obtain a positive effect on the lumbar BMC.

Key words exercise · bone mineral content (BMC) · bone formation · parathyroid hormone · vitamin D_3

Offprint requests to: J. Iwamoto
(e-mail: jiwamoto@sc.itc.keio.ac.jp)
Received: November 29, 2002 / Accepted: April 21, 2003

Introduction

Current strategies for the prevention of osteoporosis focus on maximizing bone mass early in life during growth and maturation and minimizing bone loss later in life [1,2]; maximal bone mass at skeletal maturity, in particular, is considered to be the best protection against osteoporotic fractures [3]. Because physical activity during childhood and adolescence may be one of the most important determinants of peak bone mass, exercise during this period should be emphasized to maximize peak bone mass.

Several experimental studies have shown the effect of exercise on bone mass during the growth period [4–7], and it has been confirmed that exercise increases bone mass in young rats. However, a few studies have reported the effect of exercise on both bone markers and calciotropic hormones in young growing rats [6–8]. It is accepted that exercise promotes a positive calcium balance and increases skeletal mass largely as a result of an increase in 1,25-dihydroxyvitamin D_3 and enhancement of intestinal calcium absorption in rats [8]. However, the response of parathyroid hormone (PTH) to exercise is not consistent [9–15]. Thus, the mechanism by which exercise increases bone mass during the growth period is not fully understood. In the present study, in young growing rats, we examined the effects of treadmill exercise on bone mass, bone markers, and calciotropic hormones to clarify the mechanism by which exercise increases bone mass.

Materials and methods

Animal care and exercise program

Twenty female Wistar rats, aged 3 weeks, were purchased from Clea Japan (Tokyo, Japan), and housed in individual cages (25 × 18 × 34 cm^3) in a specific

pathogen-free room with a temperature of 23 ± 2°C, humidity of 55 ± 5%, and a 12-h on/off light cycle. They were allowed free access to water and a pelleted chow diet (CE-2; Funabashi Farm, Chiba, Japan). After 3 weeks of adaptation to this diet and the new environment, the 6-week-old rats were randomized, using the stratified weight method, into the following four groups with 5 animals each; 7 weeks of exercise (7EX), 7 weeks of sedentary control (7CON), 11 weeks of exercise (11EX), and 11 weeks of sedentary control (11CON). The exercise regimen consisted of daily running on a flat-bed treadmill (Shinano Instrument, Tokyo, Japan). During the first 2 weeks, the speed of the treadmill and duration of each running session were gradually increased from 8 m/min for 5 min to 14 m/min for 45 min. The running speed and duration were gradually increased to 25 m/min for 60 min each day in the third week, and this speed and duration were maintained for 5 days a week for the remaining period. The experiment was performed at Kitasato Institute Hospital, and the protocols were approved by the Research Animal Resource Committee of Kitasato Institute Hospital.

Measurement of bone markers and calciotropic hormones

After the exercise regimen had been completed, 24-h urine was collected, using a metabolic cage. A blood sample was taken from the vena cava with the animals under pentobarbital sodium anesthesia (100 mg/100 g body weight, intraperitoneal injection). The urinary deoxypyridinoline (DPD) level was measured by enzyme immunoassay. The serum osteocalcin (OC) level was measured by radioimmunoassay. The serum tartrate-resistant acid phosphatase (TRAP) level was measured by colorimetric assay, using the substrate naphthyl-phosphatase. The serum PTH and 1,25-dihydroxyvitamin D_3 levels were also measured by radioimmunoassay.

Measurement of gastrocnemius muscle weight, length of femur, and bone mineral content (BMC) and bone mineral density (BMD) of tibia and lumbar spine

After the urine and blood samples had been collected, the animals were killed by exsanguination from the vena cava. The right gastrocnemius muscle was dissected, and weighed immediately. The right femur and tibia and fifth lumbar (L5) spine were dissected free of soft tissue. The total length of the femur was measured three times, using a dial caliper, and the mean value was taken as the length of the femur. With regard to the right tibia and L5 spine, each bone was put on an acryl plate (20-mm-thick) in air and scanned three times by dual-energy X-ray absorptiometry (DXA), using a regular Lunar DPX-L instrument (Madison, WI, USA) adapted for measurement in small animals. A high-resolution mode (voltage of 76.0 kVp; current of 150 μA, collimation of fine, sample size of 0.15 × 0.3, sample interval of 1/64) was used with scan width of 15 mm and scan length of 50 mm for the tibia and 20 mm for the L5 spine. The BMC and BMD of the whole tibia, and the proximal, middle, and distal thirds of the tibia, as well as the L5 spine were analyzed in each scan, and the mean values of three analyses were taken. The reproducibility of the data was evaluated by measuring the coefficient of variation (CV = 100 × SD/mean) of measurements, performed within 24 h using specimens from five animals. The CV of these measurements was less than 2.0%.

Statistical analysis

All data values were expressed as means ± SD. Analysis of variance (ANOVA) with Fisher's protected least significant difference (PLSD) test was used to compare data among the groups. All statistical analyses were performed using the Stat View J-5.0 program (SAS Institute, Cary, NC, USA) on a Macintosh computer. A significance level of $P < 0.05$ was used for all comparisons.

Results

Body weight, gastrocnemius weight, femoral length, and tibial and lumbar BMC and BMD

Table 1 and Figs. 1 and 2 show the body weight, gastrocnemius weight, femoral length, and tibial and lumbar BMC and BMD. There were no significant differences in the initial body weight among the four groups. Maturation-related increases in the final body weight, and in tibial and lumbar BMC and BMD were observed. Seven and 11 weeks of exercise increased the femoral length and tibial BMC, but did not alter the body weight, tibial BMD, and lumbar BMC and BMD. With more detailed data for tibial BMC, although 7 and 11 weeks of exercise did not increase the proximal, middle, or distal tibial BMD, 7 weeks of exercise increased the middle and distal tibial BMC, and 11 weeks of exercise increased the proximal, middle, and distal tibial BMC. The mean percent increase in the proximal, middle, and distal tibial BMC obtained through 11 weeks of exercise was 12.2%, 25.6%, and 30.8%, respectively. The response of BMC to exercise was greatest in the distal tibia and least in the proximal tibia, when the mean percent increase in BMC obtained through 11 weeks of exercise was compared among the proximal, middle, and distal tibiae.

Table 1. Body weight, gastrocnemius weight, femoral length, and tibial and lumbar BMC and BMD

	7 Weeks			11 Weeks		
	7EX	7CON	*P* value	11EX	11CON	*P* value
Initial body weight (g)	149.0 ± 6.8	154.0 ± 3.7	NS	150.8 ± 5.4	152.0 ± 5.8	NS
Final body weight (g)	231.6 ± 18.9	236.0 ± 8.5	NS	248.0 ± 13.6	258.0 ± 14.4*	NS
Gastrocnemius weight (g)	5.87 ± 0.79	5.85 ± 0.44	NS	6.39 ± 0.44	6.36 ± 0.47	NS
Femoral length (cm)	3.22 ± 0.08	3.14 ± 0.03	<0.05	3.26 ± 0.10	3.16 ± 0.02	<0.05
Tibial BMC (g)						
Whole	0.176 ± 0.012	0.142 ± 0.007	<0.05	0.218 ± 0.009	0.182 ± 0.010**	<0.05
Proximal	0.084 ± 0.004	0.076 ± 0.002	NS	0.101 ± 0.002	0.090 ± 0.001**	<0.05
Middle	0.035 ± 0.002	0.024 ± 0.003	<0.05	0.049 ± 0.006	0.039 ± 0.001*	<0.05
Distal	0.056 ± 0.007	0.042 ± 0.007	<0.05	0.068 ± 0.003	0.052 ± 0.003*	<0.05
Tibial BMD (g/cm²)						
Whole	0.175 ± 0.006	0.177 ± 0.008	NS	0.194 ± 0.003	0.193 ± 0.004**	NS
Proximal	0.190 ± 0.006	0.187 ± 0.004	NS	0.216 ± 0.009	0.219 ± 0.004**	NS
Middle	0.145 ± 0.006	0.142 ± 0.004	NS	0.155 ± 0.003	0.160 ± 0.008**	NS
Distal	0.171 ± 0.008	0.167 ± 0.009	NS	0.194 ± 0.015	0.182 ± 0.020	NS
Lumbar BMC (g)	0.059 ± 0.005	0.053 ± 0.012	NS	0.073 ± 0.009	0.076 ± 0.007**	NS
Lumbar BMD (g/cm²)	0.204 ± 0.007	0.201 ± 0.008	NS	0.226 ± 0.018	0.224 ± 0.012**	NS

* $P < 0.05$; ** $P < 0.01$ vs 7CON group
Data values are expressed as means ± SD. Data comparison was performed by unpaired *t*-test
NS, not significant; BMC, bone mineral content; BMD, bone mineral density; EX, exercise; CON, control

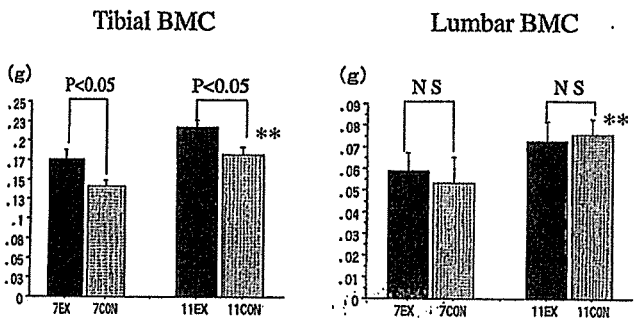

Fig. 1. Tibial and lumbar bone mineral content (*BMC*). All data values are expressed as means ± SD. Analysis of variance (ANOVA) with Fisher's protected least significant difference (PLSD) test was used to compare data among the groups. Maturation-related increases in the tibial and lumbar BMC were observed. Seven and 11 weeks of exercise increased the tibial BMC, but did not alter the lumbar BMC. **P* < 0.05; ***P* < 0.01 vs 7CON group. *NS*, not significant; *7EX*, 7 weeks of exercise; *7CON*, 7 weeks of sedentary control; *11EX*, 11 weeks of exercise; *11CON*, 11 weeks of sedentary control

Bone markers and calciotropic hormones

Table 2 and Fig. 3 show the levels of bone markers and calciotropic hormones. Maturation-related decreases in serum OC, ALP, and TRAP levels, and in urinary DPD levels, were observed, without any alterations shown in the serum PTH and 1,25-dihydroxyvitain D_3 levels. Seven and 11 weeks of exercise increased the serum OC and 1,25-dihydroxyvitamin D_3 levels, and decreased the serum PTH level. Seven weeks of exercise decreased the urinary DPD level, and 11 weeks of exercise increased the serum ALP level and decreased the serum TRAP level.

Discussion

It is generally accepted that, although bone formation and bone resorption take place actively in young bone, they decline with maturation. In the present study, maturation-related increases in the final body weight, and in tibial and lumbar BMC and BMD, were observed, with decreases in the serum OC, ALP, and TRAP levels and in urinary DPD levels. These findings suggest that the animals experienced maturation-related bone gain with a decline in bone turnover.

Treadmill exercise increased the tibial BMC and length of the femur, but did not increase the lumbar vertebral BMC. The tibia and femur are likely to receive much more mechanical loading than the lumbar spine during treadmill running in rats, as they are tetrapedal animals [16,17]. Our findings suggest that treadmill exercise increases bone mass and stimulates longitudinal bone growth, especially at weight-bearing sites, in young growing rats. It is well documented that weight-bearing bones like the tibia and femur may have a higher sensitivity to treadmill exercise than less weight-bearing bone like the lumbar spine in rats [7,16–18]. Our findings are consistent with the results of these previous studies, and also support the generally accepted concept that weight-bearing activity has a positive influence on bone health [19].

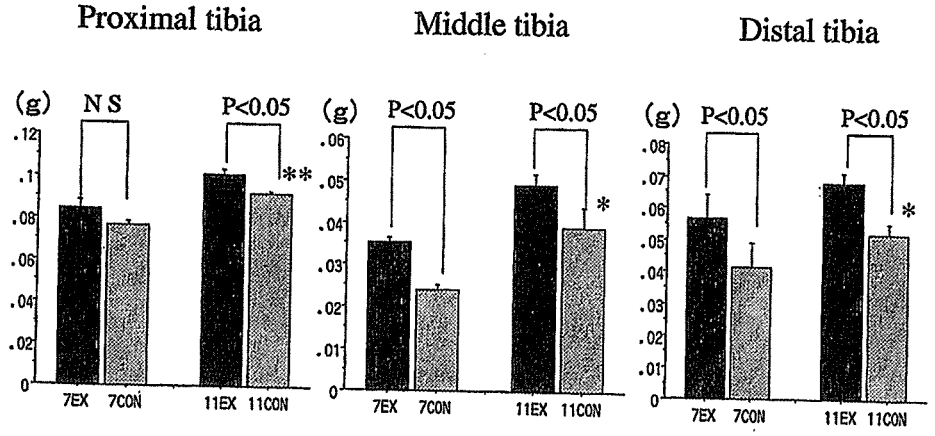

Fig. 2. Proximal, middle, and distal tibial BMC. All data values are expressed as means ± SD. ANOVA with Fisher's PLSD test was used to compare data among the groups. Seven weeks of exercise increased the middle and distal tibial BMC, and 11 weeks of exercise increased the proximal, middle, and distal tibial BMC. The mean percent increases in the proximal, middle, and distal tibial BMC obtained through 11 weeks of exercise were 12.2%, 25.6%, and 30.8%, respectively. $*P < 0.05$; $**P < 0.01$ vs 7CON group

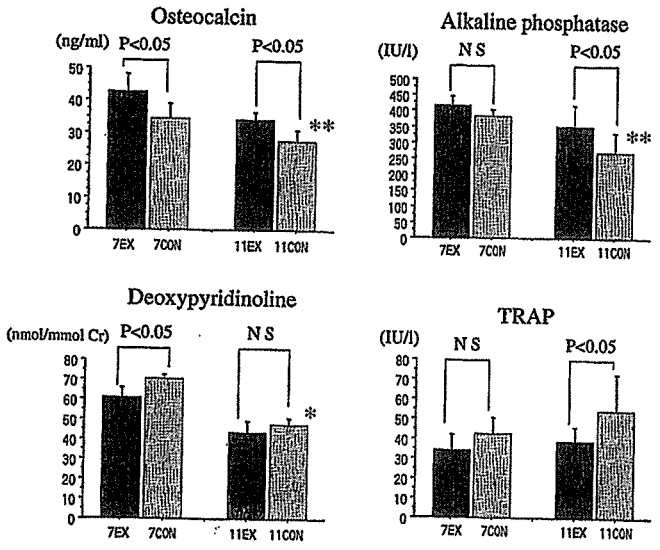

Fig. 3. Bone markers. All data values are expressed as means ± SD. ANOVA with Fisher's PLSD test was used to compare data among the groups. Maturation-related decreases in the serum osteocalcin, alkaline phosphatase, and tartrate-resistant acid phosphatase (*TRAP*), and urinary deoxypyridinoline levels were observed. Seven and 11 weeks of exercise increased the serum osteocalcin level. Seven weeks of exercise decreased the urinary deoxypyridinoline level and 11 weeks of exercise increased the serum alkaline phosphatase level and decreased the serum TRAP level. $*P < 0.05$; $**P < 0.01$ vs 7CON group. *Cr*, creatinine

The response of BMC to exercise was greatest in the distal tibia and least in the proximal tibia. Iwamoto et al. [5] also clearly demonstrated that treadmill exercise increased the proximal and distal cancellous bone mass in young growing rats, but did not alter the lumbar cancellous bone mass, and that the response of cancellous bone to treadmill exercise was greater in the distal tibia than in the proximal tibia. The mechanism for this greater response of cancellous bone mass or BMC in the distal tibia than in the proximal tibia remains uncertain; however, the location and diameter of the bone tissue area may contribute to this result to some extent. The distal tibia is likely to receive more mechanical loading than the proximal tibia during treadmill exercise because the distal tibia is situated farther away from the body mass of the rat. This distal location increases the total load on the distal tibia as compared with the proximal tibia. Additionally, the total diameter of the distal tibia is smaller than that of the proximal tibia, and therefore the distal tibia supports greater weight per surface area during treadmill exercise. These findings are supported by the concept that high strain magnitude may be more effective than low strain magnitude in exerting a positive effect on bone mass [19,20].

In our study, treadmill exercise stimulated longitudinal bone growth and increased BMC at weight-bearing sites, but did not alter BMD. These findings suggest that treadmill exercise during the growth period increased bone size in the longitudinal direction, maintaining bone density. In contrast, in mature or aged rats, treadmill exercise increases BMD at weight-bearing sites without stimulation of longitudinal bone growth [16,21]. Thus, in young growing rats, longitudinal bone growth is sensitive to gravitational mechanical loading; treadmill exercise stimulates longitudinal bone growth. However, because of the lack of bone histomorphometric and peripheral quantitative computed tomography (pQCT) analyses of cortical bone, it remains uncertain whether treadmill exercise stimulated radial bone growth. Recently, Yeh et al. [22] developed a noninvasive animal model of circular motion exercise to evaluate the effect of isometric exercise on bone in rats. They demonstrated that circular motion exercise (isometric exercise) under force without additional weight-loading caused bone-modeling drift in the radial direction, with an increase in bone turnover to reconstruct the bone shape as an adaptation to the demand for strength, and they showed that circular motion exercise did not enhance longitudinal bone growth. Gravitational force caused by treadmill exercise can at least enhance longitudinal bone growth, while horizontal

Table 2. Bone markers and calciotropic hormones

	7 Weeks			11 Weeks		
	7EX	7CON	*P* value	11EX	11CON	*P* value
Serum						
Calcium (mg/dl)	10.1 ± 0.1	10.0 ± 0.4	NS	10.2 ± 0.3	9.8 ± 0.4	NS
Phosphorus (mg/dl)	7.5 ± 0.5	7.6 ± 0.2	NS	7.1 ± 0.4	7.5 ± 0.9	NS
ALP (IU/l)	417.0 ± 28.3	382.6 ± 22.3	NS	351.8 ± 68.6	270.4 ± 62.0**	<0.05
Osteocalcin (ng/ml)	42.8 ± 5.5	34.4 ± 4.6	<0.05	34.0 ± 2.3	27.6 ± 3.4**	<0.05
TRAP (IU/l)	34.0 ± 8.0	42.6 ± 7.9	NS	38.9 ± 7.0	54.2 ± 18.2	<0.05
PTH (pg/ml)	29.8 ± 2.7	51.0 ± 24.1	<0.05	40.0 ± 3.7	58.0 ± 6.0	<0.05
1,25 $(OH)_2$ D_3 (pg/ml)	37.6 ± 1.4	22.0 ± 7.0	<0.001	25.8 ± 4.1	18.9 ± 4.2	<0.05
Urine						
DPD (nmol/mmol Cr)	61.0 ± 4.7	70.4 ± 1.9	<0.05	43.2 ± 5.9	47.5 ± 3.4*	NS

* $P < 0.01$; ** $P < 0.001$ vs 7CON group
Data values are expressed as means ± SD. Data comparison was performed by unpaired *t*-test
NS, not significant; ALP, alkaline phosphatase; TRAP, tartrate-resistant acid phosphatase; PTH, parathyroid hormone; 1,25$(OH)_2D_3$, 1,25-dihydroxyvitamin D_3; DPD, deoxypyridinoline; Cr, creatinine

force caused by circular motion exercise can enhance radial bone growth.

The mechanism by which treadmill exercise increases bone mass, especially at weight-bearing sites, remains uncertain. There are a few studies that have focused on the effects of exercise on bone formation and bone resorption in young rats by assessing cellular activities [4–7]. Exercise increases cancellous bone mass through increased bone formation and/or decreased bone resorption, and increases cortical bone mass as a result of increased periosteal bone formation [4–7]. In particular, exercise in young growing rats is associated with an initial increase and then a decrease in bone resorption, while active bone formation is sustained [6]. Exercise increases bone formation and prolonged exercise decreases bone resorption. In the present study, bone gain through exercise in young rats was suggested to be attributable to increased bone formation and decreased bone resorption in response to increased mechanical loading. We detected a decrease in bone resorption, probably following an initial increase, and sustained active bone formation.

With regard to the effect of exercise on calciotropic hormones, it is accepted that exercise promotes a positive calcium balance and increases skeletal mass, largely as a result of an increase in 1,25-dihydroxyvitamin D_3 and enhancement of intestinal calcium absorption in rats [8]. However, reports concerning the response of PTH to exercise are conflicting; it has been shown to be unchanged, decreased, or increased [9–15]. In the present study, treadmill exercise increased the serum 1,25-dihydroxyvitamin D_3 level and decreased the serum PTH level. It is speculated that treadmill exercise stimulated bone formation and suppressed bone resorption, resulting in an increased demand for minerals that was satisfied by an increase in serum 1,25-dihydroxyvitamin D_3 level and increased intestinal absorption of calcium; it is also speculated that the increase in calcium absorption suppressed the serum PTH level.

Despite the changes in the levels of calciotropic hormones, treadmill exercise altered the tibial BMC but not the lumbar BMC. These findings suggest that an increase in bone mass through short-term exercise appears to be achieved by the combined actions of local mechanical loading and general calciotropic hormones, and that weight-bearing activity appears to be important in the promotion of longitudinal bone growth and bone gain. However, it is possible that long-term exercise could increase the lumbar BMC through the actions of general calciotropic hormones. Thus, further studies with long-term exercise are needed to clarify whether treadmill exercise can alter the mass of less weight-bearing bones through the actions of calciotropic hormones.

The limitation of the present study is the lack of bone histomorphometric analysis. Therefore, the alterations in local bone formation and bone resorption, cellular activities, and bone architecture in the tibia and lumbar spine remain uncertain. Further studies will be needed to clarify the effects of treadmill exercise on these parameters in the tibia and lumbar spine.

In conclusion, the present study demonstrates that treadmill exercise stimulates bone formation and suppresses bone resorption, increases the serum 1,25-dihydroxyvitamin D_3 level, and decreases the serum PTH level, resulting in an increase in bone mass with stimulation of longitudinal bone growth, especially at weight-bearing sites, in young growing rats. Further studies with long-term exercise may be needed to obtain a positive effect on the lumbar BMC.

References

1. Kulak CA, Bilezikian JP (1998) Osteoporosis: preventive strategies. Int J Fertil Womens Med 43:56–64
2. Rozenberg S, Vandromme J, Ayata NB, Filippidis M, Kroll M (1999) Osteoporosis management. Int J Fertil Womens Med 44:241–249
3. Matkovic V, Fontana D, Tominac C, Goel P, Chesnut CH III (1990) Factors that influence peak bone mass formation: a study of calcium balance and the inheritance of bone mass in adolescent females. Am J Clin Nutr 52:878–888
4. Bourrin S, Palle S, Pupier R, Vico L, Alexsandre C (1995) Effects of physical training on bone adaptation in three zones of the rat tibia. J Bone Miner Res 10:1745–1752
5. Iwamoto J, Yeh JK, Aloia JF (1999) Differential effect of treadmill exercise on three cancellous bone sites in the young growing rat. Bone 24:163–169
6. Yeh JK, Liu CC, Aloia JF (1993) Effects of exercise and immobilization on bone formation and resorption in young rats. Am J Physiol 264:E182–E189
7. Yeh JK, Aloia JF (1990) Effect of physical activity on calciotropic hormones and calcium balance in rats. Am J Physiol 258:E263–E268
8. Yeh JK, Aloia JF, Yasumura S (1989) Effect of physical activity on calcium and phosphorus metabolism in the rat. Am J Physiol 256:E1–E6
9. Aloia JF, Rasulo P, Deftos L, Vaswani A, Yeh JK (1985) Exercise-induced hypercalcemia and the calciotropic hormones. J Lab Clin Med 106:229–232
10. Blum JW, Bianca W, Naf F, Kunz P, Fisher JA, Da Prada M (1979) Plasma catecholamine and parathyroid hormone responses in cattle during treadmill exercise at simulated high altitude. Hormone Metab Res 11:246–251
11. Bell N, Godsen RN, Henry DP, Shary J, Epstein S (1998) The effects of muscle-building exercise on vitamin D and mineral metabolism. J Bone Miner Res 3:369–373
12. Cunningham J, Segre GV, Slatopolsky E, Avioli LV (1985) Effect of heavy exercise on mineral metabolism and calcium regulating hormones in humans. Calcif Tissue Int 37:598–601
13. Ljunghall S, Joborn H, Roxin LE, Rostad J, Wide L, Akerstrom G (1986) Prolonged low-intensity exercise raises the serum parathyroid hormone levels. Clin Endocrinol 25:535–542
14. Nelson ME, Meredith CN, Dawson-Hughes B, Evans WJ (1988) Hormone and bone mineral status in endurance-trained and sedentary postmenopausal women. J Clin Endocrinol Metab 66:927–933
15. Vora NM, Kukreja SC, York PAJ, Bowser EN, Hargis GK, Williams GA (1983) Effect of exercise on serum calcium and parathyroid hormone. J Clin Endocrinol Metab 57:1067–1069
16. Yeh JK, Aloia JF, Tierney JM, Sprintz S (1993) Effect of treadmill exercise on vertebral and tibial bone mineral content and bone mineral density in the aged adult rat: determined by dual energy X-ray absorptiometry. Calcif Tissue Int 52:234–238
17. Yeh JK, Aloia JF, Chen MM (1994) Growth hormone administration potentiates the effect of treadmill exercise on long bone formation but not on the vertebrae in middle-aged rats. Calcif Tissue Int 54:38–43
18. Yeh JK, Aloia JF, Chen MM, Tierney JM, Sprintz S (1993) Influence of exercise on cancellous bone of the aged female rat. J Bone Miner Res 8:1117–1125
19. Bennell KL, Malcolm SA, Khan KM, Thomas SA, Reid SJ, Brukner PD, Ebeling PR, Wark JD (1997) Bone mass and bone turnover in power athletes, endurance athletes, and controls: a 12-month longitudinal study. Bone 20:477–484
20. Frost HM (1997) Perspective: on our age-related bone loss: insights from a new paradigm. J Bone Miner Res 12:1539–1546
21. Iwamoto J, Takeda T, Ichimura S (1998) Effects of exercise on bone mineral density in mature osteopenic rats. J Bone Miner Res 13:1308–1317
22. Yeh JK, Niu Q, Evans JF, Iwamoto J, Aloia JF (2001) Effect of circular motion exercise on bone modeling and bone mass in young rats: an animal model of isometric exercise. J Musculoskel Neuron Interact 1:235–240

J Bone Miner Metab (2004) 22:500–508
DOI 10.1007/s00774-004-0514-2

Journal of
Bone and
Mineral
Metabolism

Effect of walking exercise on bone metabolism in postmenopausal women with osteopenia/osteoporosis

Satoshi Yamazaki[1], Shoichi Ichimura[2], Jun Iwamoto[3], Tsuyoshi Takeda[3], and Yoshiaki Toyama[1]

[1]Department of Orthopaedic Surgery, Keio University School of Medicine, 35 Shinanomachi, Shinjuku-ku, Tokyo 160-8582, Japan
[2]Department of Orthopaedic Surgery, Kyorin University School of Medicine, Tokyo, Japan
[3]Department of Sports Medicine, Keio University School of Medicine, Tokyo, Japan

Abstract The purpose of this prospective study was to determine whether moderate walking exercise in postmenopausal women with osteopenia/osteoporosis would affect bone metabolism. Fifty postmenopausal women, aged 49–75 years, with osteopenia/osteoporosis were recruited: 32 women entered the exercise program (the exercise group) and 18 served as controls (the control group). The exercise consisted of daily outdoor walking, the intensity of which was 50% of maximum oxygen consumption, with a duration of at least 1 h with more than 8000 steps, at a frequency of 4 days a week, over a 12-month period. Lumbar (L2–L4) bone mineral density (BMD) was measured at the baseline and every 6 months with dual-energy X-ray absorptiometry (DXA) in both groups. Serum bone-specific alkaline phosphatase (BAP) and urinary cross-linked N-terminal telopeptides of type I collagen (NTX) levels were measured at baseline and at months 1, 3, 6, 9, and 12 by EIA and ELISA, respectively, in the exercise group, and urinary NTX level was measured at the baseline and every 6 months in the control group. There were no significant differences in baseline characteristics including age, height, body weight, bone mass index, years since menopause, lumbar BMD, and urinary NTX level between the two groups. Although no significant changes were observed in lumbar BMD and the urinary NTX level in the control group, lumbar BMD in the exercise group was increased as compared with the control group, but was sustained from the baseline. In the exercise group, the urinary NTX level rapidly responded to walking exercise from month 3, and this reduction was sustained until month 12, followed by reduction in the serum BAP level. A moderately negative correlation was found between the percent change in the urinary NTX level at month 3 and that in lumbar BMD at month 12 in the exercise group. This study clearly demonstrates that the mechanism for the positive response of lumbar BMD to moderate walking exercise in postmenopausal women with osteopenia/osteoporosis appears to be the suppression of bone turnover, and that an early change in the urinary NTX level may be useful to predict the long-term response of increasing lumbar BMD to exercise, although its efficacy for lumbar BMD may be quite modest.

Key words walking exercise · bone metabolism · bone mineral density · postmenopausal women · osteoporosis

Offprint requests to: S. Yamazaki
(e-mail: serm.y@cello.ocn.ne.jp)
Received: October 31, 2003 / Accepted: February 12, 2004

Introduction

Osteoporosis primarily affects postmenopausal women, because estrogen deficiency caused by menopause induces marked bone loss. Physical activity plays an important role in the prevention of osteoporosis [1]; increased physical activity increases bone mass [2–4], while decreased physical activity such as bed rest causes bone loss [5]. Although a number of studies have reported the effect of exercise on bone mass in postmenopausal women with or without osteopenia/osteoporosis [3,6–15], the results were not always consistent, probably because the age of the subjects, skeletal sites assessed, and the mode, intensity, duration, and frequency of exercise varied from study to study. However, it may be accepted that high-intensity aerobics and weight-bearing exercise as well as resistance exercise seem to be more effective to increase bone mass than low- to moderate-intensity walking exercise.

In regard to the mechanism by which exercise brings about the positive effect on bone mass in postmenopausal women with or without osteopenia/osteoporosis, several well-controlled studies have reported the effect of physical activity on bone markers [16–19]; some studies showed that walking at an anaerobic threshold level or weight-bearing exercise decreased serum osteocalcin (OC) level or urinary calcium excretion [16,17], while other studies showed that combined aerobic and anaerobic exercise increased serum OC level, and brisk walking did not significantly affect bone

markers [18,19]. Thus, the effect of exercise on bone metabolism in postmenopausal women is not yet established.

An exercise program for the elderly should be safe and easy to perform and continue. Although high-intensity exercise is suitable for young athletes, moderate aerobic exercise, i.e., walking, may be safe and effective to maintain general health in elderly women with osteoporosis. Recently, bone marker measurements have been developed, and a bone resorption marker, the urinary cross-linked N-terminal telopeptides of type I collagen (NTX), which is more specific for bone than urinary deoxypyridinoline (DPD), has become available. In particular, the usefulness of urinary NTX measurement in the treatment with bisphosphonates for osteoporosis has been established; an early dynamic decrease in the urinary NTX level can be used to monitor and predict the long-term response to bisphosphonate therapy in elderly women [20]. The purposes of this prospective study were to determine whether moderate walking exercise in postmenopausal women with osteopenia/osteoporosis would affect bone metabolism as measured by bone markers including urinary NTX and to confirm the usefulness of urinary NTX measurement in intervention with exercise for osteopenia/osteoporosis.

Subjects and methods

Subjects

Fifty postmenopausal women, aged 49–75 years, with osteopenia/osteoporosis [21,22] were recruited in our outpatient clinic during a 1-year period between July 1999 and June 2000. Thirty-two women entered the exercise program (the exercise group) and 18 served as controls (the control group), according to the wish of the participants. Because exercise training requires effort on the part of the patients, the subjects who hoped to perform walking exercise were placed into the exercise group and those who did not so wish were placed into the control group.

Preliminary screening included medical history, physical examination, blood and urine examination, plain X-ray examination of the thoracic and lumbar spine, and measurement of lumbar bone mineral density (BMD). Serum calcium, phosphorus, and alkaline phosphatase (ALP) levels were measured by standard automated laboratory techniques. Bone markers and lumbar BMD were measured as described below. Vertebral fractures were determined by the Japanese criteria [21,22]. None of the subjects had a history of hormone (estrogen) replacement therapy or had ever taken medication that affects bone metabolism, such as glucocorticoid, warfarin, methotrexate, bisphosphonate, vitamin D, vitamin K, and calcitonin, before this trial. None of them were past or current smokers. None of the women suffered from cardiopulmonary disease or severe osteoarthritis and osteopathy that might have affected physical activity. In addition, none of the subjects had participated in a sporting activity with a frequency of one or more times a week for at least the previous 5 years and none of them participated in such activity during this trial. All subjects were strictly encouraged to consume 800 mg calcium daily in their food, according to the instruction of dietitians.

The exercise program consisted of daily outdoor walking. After the target heart rate (THR) of each subject was calculated with Karvonen's formula [23], corresponding to 50% of maximal oxygen consumption, which was determined with Blackburn's formula [24], each subject learned a walking speed that corresponded to their corresponding THR by using an exercise treadmill at the beginning of the exercise program, and was instructed to walk for at least 1 h with more than 8000 steps a day for at least 4 days a week for 12 months at the determined walking speed. During walking exercise, they checked their heart rate with a handheld heart rate meter every 10 min to sustain the THR. The daily step count and heart rate were monitored every month in the exercise group. All data of daily step count and heart rate were recorded in a notebook by each subject in the exercise group. Informed consent was obtained from all subjects, and this protocol was approved by the Ethical Committee of Keio University School of Medicine. Five subjects in the exercise group and three in the control group discontinued this trial without any specific reason.

Bone marker measurements

Blood samples were collected after an overnight fast and serum samples were obtained by centrifugation at baseline and months 1, 3, 6, 9, and 12 in the exercise group. Urine samples were collected from the second voiding at baseline and months 1, 3, 6, 9, and 12 in the exercise group and at baseline and every 6 months in the control group. Both serum and urine samples were stored at −80°C until the measurement of serum bone-specific alkaline phosphatase (BAP) and OC and urinary NTX levels.

Serum BAP and OC were measured using an enzyme immunoassay (EIA) [25] and a two-site immunoradiometric assay (IRMA) [26], respectively. The former was measured with monoclonal antibody anti-BAP (Metra Biosystems, Mountain View, CA, USA), and the latter was specific for intact human OC and was measured with a BGP-IRMA kit (Mitsubishi Chemical, Tokyo, Japan) [27]. These assays were carried out by Mitsubishi

Kagaku Bio-Clinical Laboratories (Tokyo, Japan). The urinary NTX level was quantified by an enzyme-linked immonosorbent assay (ELISA) (MOS-19; Mochida Pharmaceutical, Tokyo, Japan) using a specific monoclonal antibody [28].

Measurement of lumbar BMD

Lumbar (L2–L4) BMD was measured at the baseline and every 6 months by dual-energy X-ray absorptiometry (DXA) using a Norland XR-36 (Atkinson, WI, USA) in the exercise and control groups. The coefficient of variation (CV) value of five measurements each time with repositioning within 72 h was less than 1.1% in three persons.

Statistical analysis

Data are expressed as the mean ± standard error (SE). Data comparisons were performed by the Mann–Whitney U test. The correlation between percent changes in bone markers and lumbar BMD was examined by single regression analysis. A significance level of $P < 0.05$ was used for all comparisons. All statistical analyses were performed using BMDP statistical software on an NEC computer.

Results

Characteristics of study subjects

Table 1 shows the baseline characteristics of the study subjects who completed this trial, 27 subjects in the exercise group and 15 in the control group. There were no significant differences in mean age, height, body weight, body mass index, years since menopause, serum calcium, phosphorus, ALP, BAP, OC, and estradiol levels, urinary NTX level, and lumbar BMD between the two groups. All subjects showed normal ranges of serum calcium, phosphorus, and ALP levels, but a low (≦20 pg/ml) serum estradiol level. None of the subjects revealed any evidence of thoracic or lumbar vertebral fractures.

The baseline characteristics of the 8 subjects who discontinued this trial did not significantly differ from those of the 42 subjects who completed the trial.

Daily step count and heart rate

Table 2 shows the daily step count and heart rate in the exercise or control group. In the exercise group, the mean baseline THR was 108 beats/min, and the mean baseline daily step count was 4256. In the control group,

Table 1. Characteristics of study subjects

Characteristic	Exercise ($n = 27$)	Control ($n = 15$)
Number of subjects		
Osteopenia	11	6
Osteoporosis	16	9
Age (years)	64.2 ± 2.9	65.7 ± 2.7
Height (cm)	155.4 ± 1.3	155.7 ± 1.2
Weight (kg)	51.2 ± 1.4	50.1 ± 1.6
Body mass index (kg/m²)	21.2 ± 0.7	21.1 ± 1.1
Years since menopause (years)	16.6 ± 1.7	14.6 ± 1.6
Serum values		
Calcium (mg/dl)	9.2 ± 0.3	9.5 ± 0.3
Phosphorus (mg/dl)	3.4 ± 0.4	3.6 ± 0.4
ALP (U/l)	236 ± 18	212 ± 12
BAP (U/l)	28.9 ± 1.8	ND
OC (ng/μl)	6.6 ± 0.5	ND
Estradiol (pg/ml)	≦20	≦20
Urine		
NTX (nmol BCE/mmol Cr)	49.5 ± 4.5	49.0 ± 5.6
Lumbar BMD (g/cm²)	0.699 ± 0.082	0.728 ± 0.078
T score (%)	67.2 ± 7.25	69.9 ± 6.71

Data are expressed as mean ± SE
Mann–Whitney U test was used to compare the data between the two groups; there were no significant differences in all parameters between the two groups
BMI, body mass index; ALP, alkaline phosphatase; BAP, bone-specific alkaline phosphatase; OC, osteocalcin; NTX, cross-linked N-terminal telopeptides of type I collagen; BCE, bone collagen equivalent; BMD, bone mineral density; ND, no data

Table 2. Daily step count and heart rate

	Exercise (n = 27)	Control (n = 15)
Heart rate (beats/min)		
Baseline (THR)	108 ± 7.5	ND
Month 6	107 ± 6.3	ND
Month 12	102 ± 5.6*	ND
Walking speed at baseline (km/h)	4.2 ± 0.4	ND
Daily step count (steps/day)		
Baseline	4256 ± 348	4025 ± 315
Month 6	8053 ± 352**	ND
Month 12	8185 ± 315**	ND
Frequency of exercise (days/week)	4.2 ± 0.3	ND

Data are expressed as mean ± SE
Data comparisons were performed by Mann–Whitney U test
In the exercise group, the mean THR decreased by 0.82% at month 6 and 5.52% at month 12, with a significant decrease at month 12, and the mean daily step count increased by 89.2% at month 6 and 92.3% at month 12, with a significant increase at months 6 and 12
*$P < 0.05$ vs baseline THR, **$P < 0.05$ vs baseline

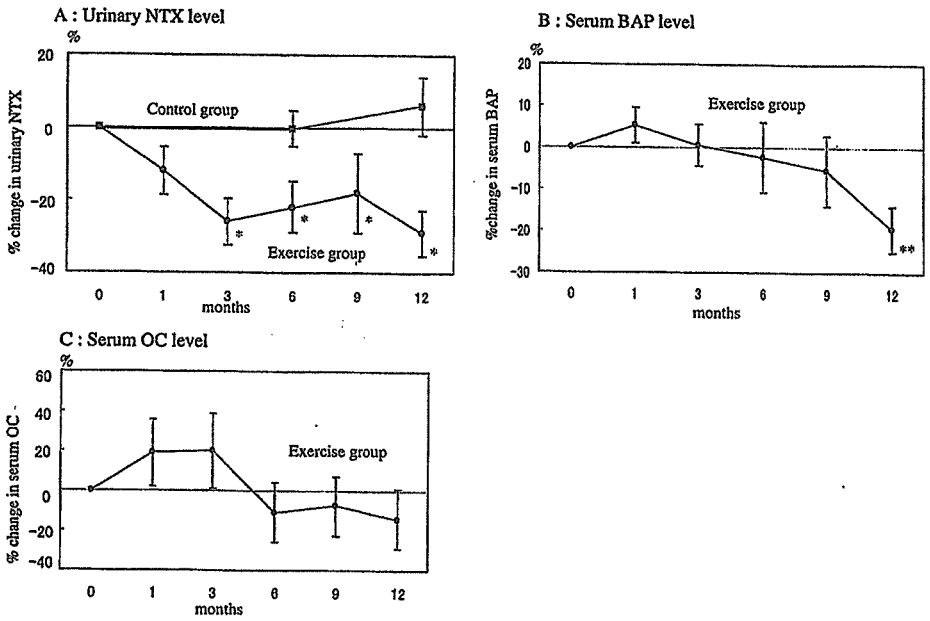

Fig. 1. Percent changes in bone markers. Data are expressed as means and SE. Data comparisons were performed by Mann–Whitney U test. In the control group, the urinary NTX level (**A**) did not significantly alter from the baseline. In the exercise group, the urinary NTX level rapidly responded to walking exercise from month 3 (25% reduction), and this reduction was sustained until month 12, followed by reduction in the serum BAP level (**B**). The serum OC level (**C**) did not significantly alter in the exercise group. *$P < 0.05$ vs baseline, **$P < 0.05$ vs baseline. *NTX*, cross-linked N-terminal telopeptides of type I collagen; *BAP*, bone-specific alkaline phosphatase; *OC*, osteocalcin

the mean daily step count was 4025 with no significant difference. In the exercise group, the mean heart rate decreased by 0.82% at month 6 and 5.52% at month 12, with a significant decrease at month 12, and the mean daily step count increased by 89.2% at month 6 and 92.3% at month 12, with a significant increase at months 6 and 12. Finally, the mean frequency of exercise was 4.2 days a week.

Changes in bone markers

Figure 1 shows the changes in bone markers in the exercise and control groups. In the control group, the urinary NTX level did not significantly alter from the baseline. In the exercise group, the urinary NTX level rapidly responded to walking exercise from month 3 (25% reduction), and this reduction was sustained until month 12, followed by reduction in the serum BAP level. The serum OC level did not significantly alter in the exercise group.

Changes in lumbar BMD

Table 3 shows the change in lumbar BMD in the exercise and control groups. The mean percent change in lumbar BMD from the baseline at months 6 and 12 was

Table 3. Change in lumbar BMD

	Baseline	Month 6	Month 12
Exercise (n = 27)			
Lumbar BMD (g/cm²)	0.699 ± 0.08	0.703 ± 0.09	0.714 ± 0.09
Percent change from baseline (%)		0.47 ± 0.21	1.71 ± 0.85*
Control (n = 15)			
Lumbar BMD (g/cm²)	0.728 ± 0.07	0.724 ± 0.07	0.712 ± 0.08
Percent change from baseline (%)		−0.45 ± 0.31	−1.92 ± 0.76

Data are expressed as mean ± SE
Data comparisons were performed by Mann–Whitney U test
Although lumbar BMD increased in the exercise group and decreased in the control group, these changes from baseline were not significant in either group. However, the percent change in lumbar BMD at 12 month in the exercise group was significantly greater than in the control group
*$P < 0.05$ vs percent change from baseline in control group at month 12

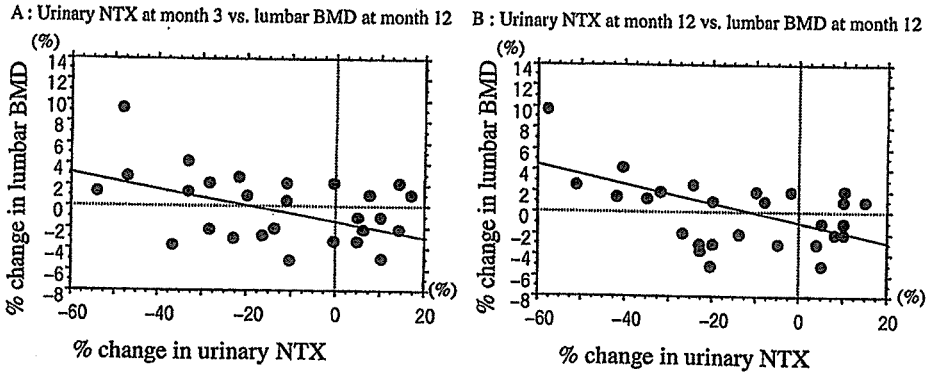

Fig. 2. Correlation between percent changes in urinary NTX level at months 3 and 12 (**A**) and lumbar BMD at month 12 in the exercise group (**B**). Single regression analysis was used to examine the correlation between percent changes in the urinary NTX level and lumbar BMD in the exercise group. A moderate negative correlation was found between percent changes in the urinary NTX level at months 3 and 12 and lumbar BMD at month 12 (r = −0.409 and −0.472, respectively, both $P < 0.05$). *NTX*, cross-linked N-terminal telopeptides of type type I collagen; *BMD*, bone mineral density

−0.45% and −1.92%, respectively, in the control group, and +0.47% and +1.71%, respectively, in the exercise group. Although lumbar BMD increased in the exercise group and decreased in the control group, these changes from the baseline were not significant in either group. However, the percent change in lumbar BMD at month 12 in the exercise group was significantly greater than that in the control group.

Correlation between percent changes in bone markers and lumbar BMD in exercise group

A significant negative correlation was found between the percent change in the urinary NTX level at months 3 and 12 and that in lumbar BMD at month 12 (r = −0.409 and r = −0.472, respectively, both $P < 0.05$) (Fig. 2). However, no significant correlation was found between the percent changes in serum BAP and OC levels and lumbar BMD at month 12.

Incidence of vertebral fractures

No new vertebral fractures occurred in any subjects during the 12-month period of study, and none of the subjects suffered from any other fractures in the hip, wrist, and shoulder joints.

Discussion

This study demonstrated that moderate walking exercise under adequate calcium intake sustained lumbar BMD via a suppression of bone turnover in postmenopausal women with osteopenia/osteoporosis. The more the urinary NTX level decreased, the more lumbar BMD increased. These results suggest that exercise has an antiresorptive effect on bone in postmenopausal women with osteopenia/osteoporosis, but its effect on lumbar BMD seems to be quite modest. Because treatment with alendronate or risedronate for 1 year reduces urinary bone resorption marker level by 45.0% (DPD) or 41.3% (NTX), respectively, and increases lumbar

BMD by 6.2% or 4.9%, respectively, in Japanese patients with osteoporosis [29,30], moderate walking exercise seems to be less efficacious for improving bone resorption and lumbar BMD than these bisphosphonates.

Numerous studies have reported the effect of exercise on BMD in postmenopausal women with or without osteopenia/osteoporosis [3,6–15]. However, the results are not always consistent, probably because the age of the subjects, skeletal sites assessed, and the mode, intensity, duration, and frequency of exercise varied from study to study. In particular, Cussler et al. [13] reported that evidence of a linear relationship between BMD change and total and exercise-specific weight lifted in a 1-year strength-training program reinforces the positive association between this type of exercise and BMD in postmenopausal women. Maddalozzo et al. [31] demonstrated that high-intensity training was more effective than moderate-intensity exercise. Heinonen et al. [32] and Chien et al. [33] suggested that exercise intensity should be above the aerobic threshold, corresponding to 60% to 70% of maximal oxygen uptake. On the other hand, Sinaki et al. [34] showed that nonloading muscle exercise was ineffective in vertebral bone loss. Furthermore, a meta-analysis by Specker et al. [35] showed that exercise appeared to be useful in increasing BMD in postmenopausal women when adequate intensity of exercise was applied under adequate calcium intake. Thus, it may be accepted that high-intensity aerobics and weight-bearing exercise as well as resistance exercise seem to be more effective to increase lumbar BMD than low- to moderate-intensity walking exercise, and adequate calcium supplementation may be needed to produce a positive effect of exercise on BMD. However, a meta-analysis by Kelley et al. [36] demonstrated that the effect of exercise in postmenopausal women was equivalent to an approximate 2% benefit in lumbar BMD (exercise + 1% versus control −1%). These results suggest that the effect of exercise on lumbar BMD in postmenopausal women seems to be quite modest, even if it brings about a positive effect, which supports our results.

In this study, we applied moderate outdoor walking exercise corresponding to 50% of maximal oxygen consumption, because it might be safe and easy to perform and be maintained even in elderly women with osteopenia/osteoporosis. Moderate walking exercise has been shown to be useful for treatment of diseases induced by deterioration of lifestyle, such as high blood pressure, ischemic heart disease, hyperlipidemia, and diabetes mellitus [37–39]. Conversely, it might be difficult to force the continuation of intense or vigorous exercise for elderly women. Before this study, we speculated that even if no significant increase in BMD was seen, it might have the potential to affect bone metabolism. Regulation of bone metabolism appears to be important to prevent the deterioration of bone mechanical properties, because it is confirmed that the use of antiresorptive agents in patients with osteoporosis can reduce the risk of vertebral fracture by 25%, even though it does not increase lumbar BMD [40]. In our study, the sample size was too small and study period was too short to detect the effect of exercise on the risk of vertebral fracture. Further studies are needed to examine the effect of walking exercise on the incidence of osteoporotic fractures, including vertebral fractures, in postmenopausal women.

The effect of walking exercise on lumbar BMD was modest. However, the other benefits of walking exercise should be discussed. First, recently, Feskanich et al. [41] investigated the effect of walking on the incidence of hip fractures caused by low or moderate trauma in 61200 postmenopausal women. It was reported that walking for at least 4 h per week was associated with a 41% lower risk of hip fracture compared with less than 1 h per week. This result suggests that moderate levels of activity, including walking, are associated with substantially lower risk of hip fracture caused by low or moderate trauma in postmenopausal women. Meta-analyses in the elderly also showed that exercise was effective in lowering the risk of falls and fall-related injuries in selected people, reducing health care costs [42,43]. However, Buchner et al. [44] reported that the combination of aerobic and anaerobic training in the elderly with mild gait disturbance prevented falls, despite no significant improvement of muscle strength and body balance. Thus, exercise, including walking exercise, may have the potential to prevent falls and fall-related injuries in postmenopausal women, although its effect of on muscle strength and body balance has yet to be established.

Second, even though lumbar BMD was not markedly increased by walking exercise, the bone quality, as evaluated by a quantitative ultrasound bone densitometer (QUS), might also be affected. Several studies have shown the relationship between physical activity or lifestyle and QUS parameters of the heel in postmenopausal women [45–47]. In particular, Blanchet et al. [45] reported that leisure-time physical activity level was a predictor of the heel QUS parameters, independently of femoral neck BMD in postmenopausal women. Thus, exercise, including walking exercise, may have the potential to affect bone quality in postmenopausal women. Taking the effect of exercise on the risk of falls together, walking exercise may have the potential to prevent both falls and deterioration of bone quality in postmenopausal women with osteopenia/osteoporosis. Further studies are needed to confirm these effects.

In regard to the mechanism by which exercise brings about the positive effect on bone mass in postmeno-