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

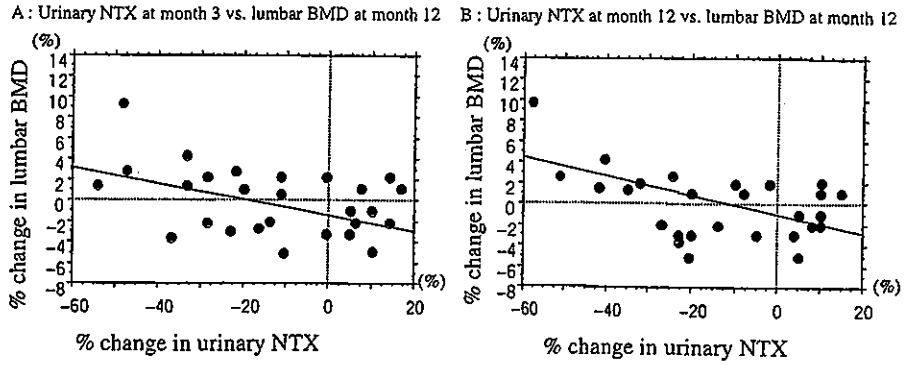

Fig. 2. Correlation between percent changes in urinary NTX level at months 3 and 12 (**A**) and lumbar BMD at month 12 in the exercise group (**B**). Single regression analysis was used to examine the correlation between percent changes in the urinary NTX level and lumbar BMD in the exercise group. A 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-

pausal women with or without osteopenia/osteoporosis, several well-controlled studies have reported the effect of physical activity on bone markers. Dalsky et al. [17] demonstrated that weight-bearing exercise increased lumbar BMD as a result of decreased serum OC levels. Hatori et al. [16] also showed that walking exercise at an anaerobic threshold level increased lumbar BMD as a result of decreased urinary calcium excretion. Danz et al. [18] showed that combined aerobic and anaerobic exercise decreased radial BMD as a result of increased serum OC levels. Brooke-Wavell et al. [19] reported that 20 min walking a day for 2 years increased calcaneal broadband ultrasonic attenuation without any significant changes in bone markers in contrast to increased urinary DPD in the sedentary group. In these studies, the assessment of bone markers has not always been adequate. However, despite inconsistency in the results of exercise on bone metabolism, it may be possible that the positive effect of exercise on bone mass in postmenopausal women with or without osteopenia/osteoporosis might result from a suppression of bone turnover, bone resorption, or urinary calcium excretion. We also found using the measurements of bone markers including urinary NTX (more specific for bone than urinary DPD) and serum BAP that walking exercise, like bisphosphonates, decreased bone turnover in postmenopausal women with osteoporosis, although its efficacy was apparently less than that of bisphosphonates. This antiresorptive effect of exercise can be confirmed by a couple of experimental studies using ovariectomized rats [48,49].

The normal range of the urinary NTX level in Japanese women (30–44 years of age) is 9.3–54.3 nmol BCE/mmol Cr [50]. Thus, the subjects of this study did not always show extremely high bone turnover. In this population, the more the urinary NTX level decreased, the more lumbar BMD increased. It is also known that the more the bisphosphonate alendronate decreases the urinary NTX level, the more lumbar BMD increases in postmenopausal women with osteoporosis [20,51]. The change in the urinary NTX level at month 6 correlated with the long-term lumbar BMD change ($r = -0.41$) in treatment with alendronate in elderly women [20]. The correlation coefficient of the percent change in the urinary NTX level at month 3 and that in lumbar BMD at month 12 in our study ($r = -0.409$) was similar to the effect of alendronate in elderly women. Thus, the decrease in the urinary NTX level might play an important role for the maintenance or increase of lumbar BMD in intervention with exercise as well as bisphosphonates. To date, very few studies have demonstrated a significant association between physical activity and urinary NTX level in postmenopausal women with osteopenia/osteoporosis. Urinary NTX appeared to be a more responsive and useful bone marker than serum BAP and OC in an intervention consisting of moderate walking exercise in postmenopausal women with osteopenia/osteoporosis, and an early change in the urinary NTX level may be useful to predict the long-term response of lumbar BMD to exercise, although its efficacy for increasing lumbar BMD may be quite modest.

We could demonstrate a significant antiresorptive effect of walking exercise on bone in postmenopausal women with osteopenia/osteoporosis in a prospective study. However, there are limitations that we have to discuss before we conclude that walking exercise is efficacious. First, even though we performed a prospective observation, the subjects were not randomly divided into the exercise and nonexercise (control) groups. Because exercise training requires effort, the subjects who hoped to perform walking exercise were assigned to the exercise group. Further randomized controlled studies are needed to confirm our results. Second, one can say that the duration of the study might be too short to evaluate the effect of exercise on lumbar BMD as well as bone metabolism. In fact, lumbar BMD increased in the exercise group as compared with the control group, but the increase from the baseline was modest. However, the main purpose of this study was to examine the effect of exercise on bone formation and resorption markers, especially on the urinary NTX level. We could obtain a rapid exercise-related reduction in urinary NTX level, followed by its steady state. Therefore, we believe that the duration of this study might be sufficient, if we focus on the effect of exercise on bone resorption markers such as urinary NTX.

In conclusion, 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 lumbar BMD to exercise, although its efficacy for increasing lumbar BMD may be quite modest. Further studies are needed to elucidate the efficacy of walking exercise on the bone quality and the risk of falls as an exercise-related effect.

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References

1. Nguyen TV, Sambrook PN, Eisman JA (1998) Bone loss, physical activity, and weight change in elderly women: the Dubbo osteoporosis epidemiology study. J Bone Miner Res 13:1458–1467
2. Chilibeck PD, Sale DG, Webber CE (1995) Exercise and bone mineral density. Sports Med 19:103–122

3. Walker M, Klentrou P, Chow R, Plyley M (2000) Longitudinal evaluation of supervised versus unsupervised exercise programs for the treatment of osteoporosis. Eur J Appl Physiol 83:349–355
4. Chesnut CH (1933) Bone mass and exercise. Am J Med 95:34–36
5. Zerwekh JE, Ruml LA, Gottschalk F, Pak CYC (1998) The effects of twelve weeks of bed rest on bone histology, biochemical markers of bone turnover, and calcium homeostasis in eleven normal subjects. J Bone Miner Res 13:1594–1601
6. White MK, Martin RB, Yeater RA, Butcher RL, Radin EL (1984) The effects of exercise on bones of postmenopausal women. Int Orthop 7:209–214
7. Krall EA, Dawson-Hughes B (1994) Walking is related to bone density and rates of bone loss. Am J Med 96:20–26
8. Cavanaugh DJ, Cann CE (1988) Brisk walking dose not stop bone loss in postmenopausal women. Bone (NY) 9:201–204
9. Iwamoto J, Takeda T, Ichimura S (2001) Effect of exercise training and detraining on bone mineral density in postmenopausal women with osteoporosis. J Orthop Sci 6:128–132
10. Martin D, Notelovitz M (1993) Effects of aerobic training on bone mineral density of postmenopausal women. J Bone Miner Res 8:931–936
11. Sandler RB, Cauley JA, Hom DL, Saahin D, Kriska AM (1987) The effect of walking on the cross-sectional dimensions of the radius in postmenopausal women. Calcif Tissue Int 41:65–69
12. Nelson ME, Fisher EC, Dilmanian FA, Dallal GE, Evans WJ (1991) A 1-y walking program and increased dietary calcium in postmenopausal women: effects on bone. Am J Clin Nutr 53: 1304–1311
13. Cussler EC, Lohman TG, Going SB, Houtkooper LB, Metcalfe LL, Flint-Wagner HG, Harris RB, Teixeira PJ (2003) Weight lifted in strength training predicts bone change in postmenopausal women. Med Sci Sports Exerc 35:10–17
14. Kemmler W, Engelke K, Lauber D, Weineck J, Hensen J, Kalender WA (2002) Exercise effects on fitness and bone mineral density in early postmenopausal women: 1-year EFOPS results. Med Sci Sports Exerc 34:2115–2123
15. Qin L, Au S, Choy W, Leung P, Neff M, Lee K, Lau M, Woo J, Chan K (2002) Regular Tai Chi Chuan exercise may retard bone loss in postmenopausal women: a case-control study. Arch Phys Med Rehabil 83:1355–1359
16. Hatori M, Hasegawa A, Adachi H, Shinozaki A, Hayashi R, Okano H, Mizunuma H, Murata K (1993) The effects of walking at the anaerobic threshold level on vertebral bone loss in postmenopausal women. Calcif Tissue Int 52:411–414
17. Dalsky GP, Stocke KS, Ehsani AA, Slatopolsky E, Lee WC, Birge SJ (1998) Weight-bearing exercise training and lumbar bone mineral content in postmenopausal women Ann Intern Med 108:824–828
18. Danz AM, Zittermann A, Schiedermaier U, Klein K, Hotzel D, Schonau E (1998) The effect of a specific strength-development exercise on bone mineral density in perimenopausal and postmenopausal women. J Women Health 7:701–709
19. Brooke-Wavell K, Hardman AE, Tsuritani I, Yamada Y (2001) Commencing, continuing and stopping brisk walking: effect on bone mineral density, quantitative ultrasound of bone and markers of bone metabolism in postmenopausal women. Osteoporos Int 12:581–587
20. Greenspan SL, Parker RA, Ferguson L, Rosen NH, Maitland-Ramsey L, Karpf DB (1998) Early changes in biochemical markers of bone turnover predict the long-term response to alendronate therapy in representative elderly women: a randomized clinical trial. J Bone Miner Res 13:1431–1438
21. Orimo H, Sugioka Y, Fukunaga M, Mutoh Y, Hotokebuchi T, Gorai I, Nakamura T, Kushida K, Tanaka H, Igai T, Oh-hashi Y (1997) Diagnostic criteria for primary osteoporosis: year 1996 version. J Bone Miner Metab 14:219–233
22. Orimo H, Hayashi Y, Fukunaga M, Sone T, Fujiwara S, Shiraki M, Kushida K, Miyamoto S, Soen S, Nishimura J, Oh-hashi Y, Hosoi T, Gorai I, Tanaka H, Igai T, Kishimoto H (2001) Diagnostic criteria for primary osteoporosis: year 2000 version. J Bone Miner Metab 19:331–337
23. Karvonen J, Vuorimaa T (1988) Heart rate and exercise intensity during sports activities practical application. Sports Med 5:303–312
24. Blackburn HW (1969) Developments in exercise electrocardiography. Proc Annu Meet Med Sect 57:145–170
25. Miki T, Nakatsuka K, Ezawa I, Satoh K (1997) Measurement of serum bone-ALP concentration with enzyme immunoassay. Clin Endocrinol 45:999–1004
26. Kawaguchi H, Matsumoto T, Kurokawa T, Orimo H, Mizunashi K, Takuwa Y, Niimi H, Shiraki M, Ohara T, Shishiba Y, Tsuchiya Y, Takahashi H, Takatsuki K, Seino Y, Morii H, Fujita T, Okamoto S, Ogata E (1990) Serum levels of BGP determined by two-site immuno-radiometric assay (IRMA) using monoclonal antibodies. Clin Endocrinol 38:1291–1296
27. Masatomi C, Imai K, Wang DH, Ikeda S, Taketa K, Takata S, Kira S (1999) Urinary excretion of type 1 collagen cross-linked N-telopeptides, bone mass and related lifestyle in middle-aged women. Acta Med Okayama 53:133–140
28. Gorai I, Chaki O, Nakayama M, Minaguchi H (1995) Urinary biochemical markers for bone resorption during the menstrual cycle. Calcif Tissue Int 57:100–104
29. Shiraki M, Kushida K, Fukunaga M, Kishimoto H, Taga R, Nakamura T, Kaneda K, Minaguchi H, Inoue T, Morii H, Tomita A, Yamamoto K, Nagata Y, Nakajima M, Orimo H (1999) A double-masked multicenter comparative study between alendronate and alfacalcidol in Japanese patients with osteoporosis. Osteoporos Int 10:183–192
30. Fukunaga M, Kushida K, Kishimoto H, Shiraki, Taketani Y, Minaguchi H, Inoue T, Morita R, Morii H, Yamamoto K, Ohashi Y, Orimo H (2002) A comparison of the effect of risedronate and etidronate on lumbar bone mineral density in Japanese patients with osteoporosis: a randomized controlled trial. Osteoporos Int 13:971–979
31. Maddalozzo GF, Snow CM (2000) High intensity resistance training: effects on bone in older men and women. Calcif Tissue Int 66:399–404
32. Heinonen A, Kannus P, Sievanen H, Oja P, Pasanen M, Rinne M, Uusi-Rasi K, Vuori I (1996) Randomized controlled trial of effect of high-impact exercise on selected risk factors for osteoporotic fractures. Lancet 348:1343–1347
33. Chien MY, Wu AT, Hsu AT, Yang RS, Lai JS (2000) Efficacy of a 24-week aerobic exercise program for osteopenic postmenopausal women. Calcif Tissue Int 67:443–448
34. Sinaki M, Wahner HW, Offord KP, Hodgson SH (1989) Efficacy of nonloading exercises in prevention of vertebral bone loss in postmenopausal women: a controlled trial. Mayo Clin Proc 64: 762–769
35. Specker BL (1996) Evidence for an interaction between calcium intake and physical activity on changes in bone mineral density. J Bone Miner Res 11:1539–1544
36. Kelley GA, Kelley KS, Tran ZV (2002) Exercise and lumber spine bone mineral density in postmenopausal women: a meta-analysis of individual patient data. J Gerontol A Biol Sci Med Sci 57:559–604
37. Cononie CC, Graves JE, Pollock ML, Phillips MI, Sumners C, Hagberg JM (1991) Effect of exercise training on blood pressure in 70- to 79-yr-old men and women. Med Sci Sports Exerc 23:505–511
38. Sunami Y, Motoyama M, Kinoshita F, Mizooka Y, Sueta K, Matsunaga A, Sasaki J, Tanaka H, Shindo M (1999) Effect of low-intensity aerobic training on the high-density lipoprotein cholesterol concentration in healthy elderly subjects. Metabolism 48:984–988

39. Hersey WC (1994) Endurance exercise training improves body composition and plasma insulin responses in 70- to 79-year-old men and women. Metabolism 43:847–854
40. Cummings SR, Karpf DB, Harris F, Genant HK, Ensrud K, LaCroix AZ, Black DM (2002) Improvement in spine bone density and reduction in risk of vertebral fracture during treatment with antiresorptive drugs. Am J Med 112:281–289
41. Feskanich D, Willett W, Colditz G (2002) Walking exercise and leisure-time activity and risk of hip fracture in postmenopausal women. JAMA 288:2300–2306
42. Gardner MM, Robertson MC, Campbell AJ (2000) Exercise in preventing falls and fall related injuries in older people: a review of randomized controlled trials. Br J Sports Med 34:7–17
43. Robertson MC, Campbell AJ, Gardner MM, Devlin N (2002) Preventing injuries in older people by preventing falls: a meta-analysis of individual-level data. J Am Geriatr Soc 50:905–911
44. Buchner DM, Cress ME, de Lateur BJ, Esselman PC, Margherita AJ, Prince R, Wagner EH (1997) The effect of strength and endurance training on gait, balance, fall risk, and health services use in community-living older adults. J Gerontol A Biol Sci Med Sci 52:M218–M224
45. Blanchet C, Giguere Y, Prud'homme D, Turcot-Lemay L, Dumont M, Leduc G, Cote S, Lafiamme N, Rousseau F, Dodin S (2003) Leisure physical activity is associated with quantitative ultrasound measurements independently of bone mineral density in postmenopausal women (abstract). Calcif Tissue Int 73(4):339–349
46. Yamaguchi J, Truman G, Cameron ID (2000) Lifestyle factors affecting bone ultrasonometry of the calcaneus in Japanese women. Calcif Tissue Int 66:43–46
47. Greff EW, Kriska AM, Salamone LM, Wolf RL, Roberts MM, Ferrell RE, Anderson SJ, Kuller LH, Cauley JA (1999) Correlations of quantitative ultrasound in the women's healthy lifestyle project. Osteoporos Int 10:416–424
48. 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
49. Yeh JK, Liu CC, Aloia JF (1993) Additive effect of treadmill exercise and 17 beta-estradiol replacement on prevention of tibial bone loss in adult ovariectomized rats. J Bone Miner Res 8:677–683
50. Fukunaga M (2003) Jissen Kotsutaishya Marker (Bone Metabolic Marker). Medical Review, Tokyo, pp 93–107
51. Tonino RP, Meuier PJ, Emkey R, Rodriguez-Portales JA, Menkes CJ, Wasnich RD, Bone HG, Santora AC, Wu M, Desai R, Ross D (2000) Skeletal benefits of alendronate: 7-year treatment of postmenopausal osteoporotic women. J Clin Endocrinol Metab 85:3109–3115

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

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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 $ml \cdot kg^{-1} \cdot min^{-1}$ 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 $ml \cdot kg^{-1} \cdot min^{-1}$). 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 ($\dot{V}O_2$), 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

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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 ($\dot{V}o_2$). 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 $mU \cdot kg^{-1} \cdot min^{-1}$ throughout the experimental period after priming insulin infusion (0–1 min, 3.56 $mU \cdot kg^{-1} \cdot min^{-1}$; 1–2 min, 3.17 $mU \cdot kg^{-1} \cdot min^{-1}$; 2–3 min, 2.82 $mU \cdot kg^{-1} \cdot min^{-1}$; 3–4 min, 2.52 $mU \cdot kg^{-1} \cdot min^{-1}$; 4–5 min, 2.24 $mU \cdot kg^{-1} \cdot min^{-1}$; 5–6 min, 1.99 $mU \cdot kg^{-1} \cdot min^{-1}$; 6–7 min, 1.77 $mU \cdot kg^{-1} \cdot min^{-1}$; 7–8 min, 1.58 $mU \cdot kg^{-1} \cdot min^{-1}$; 8–9 min, 1.41 $mU \cdot kg^{-1} \cdot min^{-1}$; 9–10 min, 1.25 $mU \cdot kg^{-1} \cdot min^{-1}$) followed by constant insulin infusion at 1.12 $mU \cdot kg^{-1} \cdot min^{-1}$. Priming of glucose infusion with the use of a 20% glucose solution was also performed (4–10 min, 2.0 $mg \cdot kg^{-1} \cdot min^{-1}$; 10–15 min, 2.5 $mg \cdot kg^{-1} \cdot min^{-1}$; 15–16 min, 4.0 $mg \cdot kg^{-1} \cdot min^{-1}$), 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

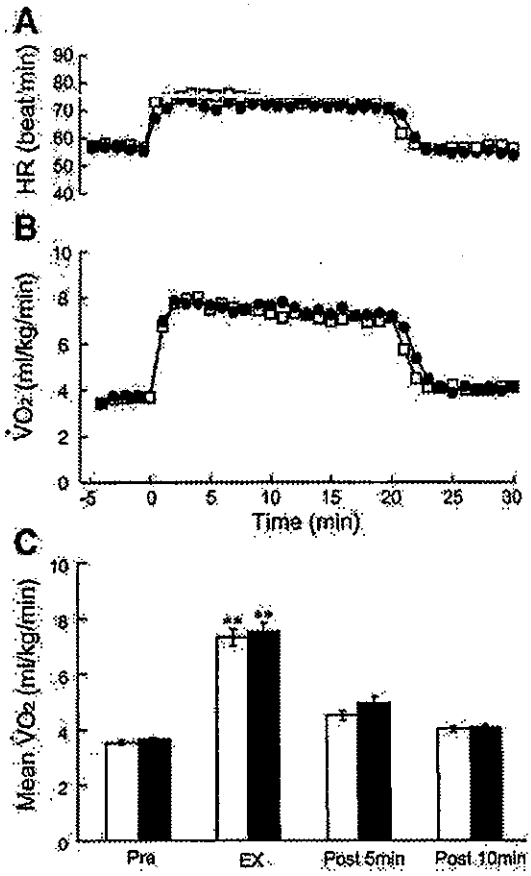

Fig. 1. Time course of changes in heart rate (HR; *A*) and whole body oxygen uptake ($\dot{V}o_2$; *B*). $\dot{V}o_2$ was continuously determined by respiratory gas exchange analysis in electrical stimulation (ES) of lower extremities (□) and voluntary cycle exercise (VE, ●). Values are means ± SE for 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

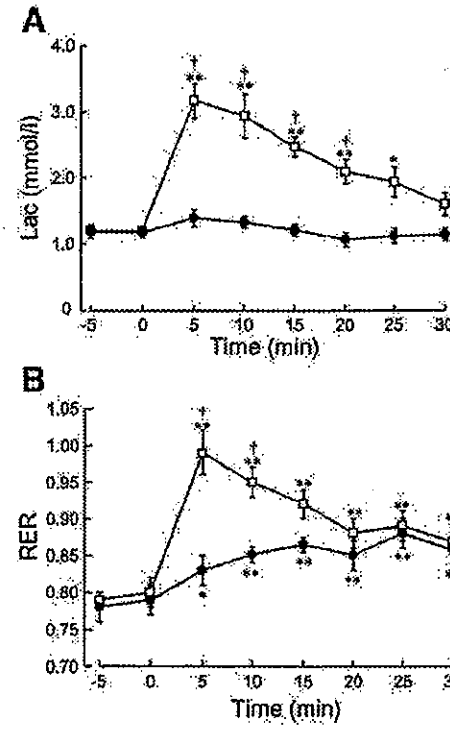

Fig. 2. Comparison of time course of changes in blood lactate concentration (Lac; *A*) and respiratory gas exchange ratio (RER; *B*). Data are indicated in a time point of every 5 min before, during, and after ES (open bars) and VE (solid bars). Values are means ± SE for 8 subjects. **Significantly different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

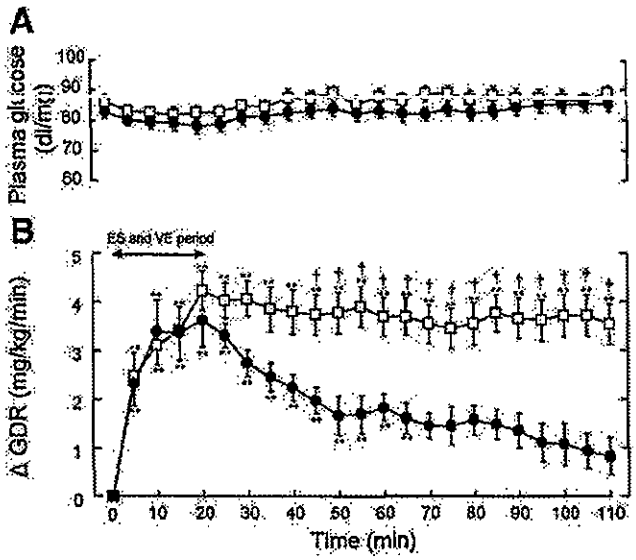

Fig. 3. Mean time course of changes in plasma glucose (*A*) and glucose disposal rate (GDR; *B*) during steady-state euglycemic clamp in both ES (open bars) and VE (solid bars) conditions. Change in GDR (ΔGDR) data determined every 5 min are indicated as increasing time point from preexercise period (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.

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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.