Rapid Publications

Hyperglycemia Activates Glucose Transport in Rat Skeletal Muscle via a Ca^{2+}-Dependent Mechanism

Lorraine A. Nolte, Jorge Rincón, Erica Odegaard Wahlström, Bruce W. Craig, Juleen R. Zierath, and Harriet Wallberg-Henriksson

We investigated the acute effect of hyperglycemia on 3-*O*-methylglucose transport in isolated rat epitrochlearis muscles. High levels of glucose (20 mmol/l) induced an approximately twofold increase in the rate of glucose transport when compared with muscles exposed to a low level of glucose (8 mmol/l) ($P < 0.001$). The hyperglycemic effect was additive to the effects of both insulin and exercise on the glucose transport rates. Dantrolene (25 μmol/l), a potent inhibitor of Ca^{2+} release from the sarcoplasmic reticulum, blocked the ability of hyperglycemia to increase glucose transport by 73% ($P < 0.01$). Although dantrolene had no effect on the non-insulin-stimulated or the insulin-stimulated glucose transport rates during normoglycemic conditions, the effect of exercise was completely blocked in the presence of dantrolene ($P < 0.01$). Inhibition of phosphatidylinositol (PI) 3-kinase by wortmannin (500 nmol/l) had no effect on the activation of glucose transport by hyperglycemia, whereas the insulin-stimulated glucose transport was completely abolished ($P < 0.001$). These findings suggest that hyperglycemia activates glucose transport by a Ca^{2+}-dependent pathway. The signaling system for this Ca^{2+}-dependent activation of glucose transport does not involve the activation of PI 3-kinase and is separate from the mass-action effect of glucose on glucose transport.
***Diabetes* 44:1345–1348, 1995**

Two independent pathways for regulating skeletal muscle glucose transport have been described; one stimulated by insulin, insulin-like growth factors and insulin-mimicking agents, and one activated by exercise, muscle contractions and hypoxia (1–4). The demonstration of an additive effect of a maximal dose of insulin and a maximal bout of exercise on the glucose transport rate (1,4) and on the translocation of the insulin-regulatable glucose transporter GLUT4 to the plasma membrane (5,6), suggests that there are two separate pathways for the activation of skeletal muscle glucose transport. Recently, phosphatidylinositol (PI) 3-kinase has been identified as an important signaling intermediate involved in insulin-stimulated activation of glucose transport (7) and GLUT4 translocation (8). Exposure of skeletal muscle to wortmannin, an inhibitor of PI 3-kinase activity, has been shown to inhibit the insulin-stimulated activation of glucose transport (9). Conversely, the exercise-stimulated pathway for activation of glucose transport has been shown to be insensitive to wortmannin, i.e., independent of PI 3-kinase activation (10). Thus, a distinct separation of the signaling pathways by insulin and contraction on glucose transport appear to exist in rat skeletal muscle. In skeletal muscle, an increase in cytosolic Ca^{2+}, in the absence or presence of depolarization, results in an enhanced permeability of the cell membrane to glucose (11–14). Thus, the muscle contraction–induced increase in glucose transport has been suggested to be initiated by an increase in cytosolic Ca^{2+}, independent of muscle contraction (14).

The increase in the rate of glucose uptake in response to high levels of extracellular glucose has been attributed to the mass-action effect of glucose rather than to an activation of glucose transport by specific glucose transporter proteins (15,16). Recently, acute hyperglycemia was demonstrated to induced an approximately twofold increase in plasma membrane GLUT4 content (17), suggesting that skeletal muscle displays an insulin-independent autoregulation of the glucose transport system. Thus, hyperglycemia per se appears to activate glucose transport in skeletal muscle by a transporter-mediated process, in addition to the increase in glucose transport that can be attributed to the mass-action effect of glucose. The present investigation was designed to test the hypothesis that hyperglycemia stimulates skeletal muscle glucose transport via a Ca^{2+}-dependent mechanism.

RESEARCH DESIGN AND METHODS

Male Wistar rats were purchased from B&K Universal (Sollentuna, Sweden) and housed for 1 week in the animal facility at the Karolinska Hospital. All rats were maintained on a 12:12 h light-to-dark schedule and received a diet of standard rat food and water ad libitum. Overnight-fasted rats were anesthetized with an intraperitoneal injection of pentobarbital sodium (5 mg/100 g body wt) before muscle dissection. The intact epitrochlearis muscle was used for the in vitro incubations. The procedure for dissection and the suitability of the epitrochlearis muscle for in vitro incubation has been previously described by Wallberg-Henriksson (18).

Throughout the entire incubation protocol, all muscles were contained in sealed glass flasks, which were continuously gassed with 95% O_2:5% CO_2 and held in a shaking water bath maintained at 30°C. After dissection, muscles were incubated (15 min) in 2 ml of Krebs-Henseleit buffer (KHB) (19) supplemented with 5 mmol/l HEPES, 0.1% essentially fatty acid–free bovine serum albumin, and 40 mmol/l mannitol in the

From the Department of Clinical Physiology, Karolinska Hospital, Karolinska Institute, Stockholm, Sweden.

Address correspondence and reprint requests to Dr. Harriet Wallberg-Henriksson, Department of Clinical Physiology, Karolinska Hospital, S-171 76 Stockholm, Sweden.

Received for publication 26 June 1995 and accepted in revised form 24 August 1995.

ANOVA, analysis of variance; KHB, Krebs-Henseleit buffer; PI, phosphatidylinositol.

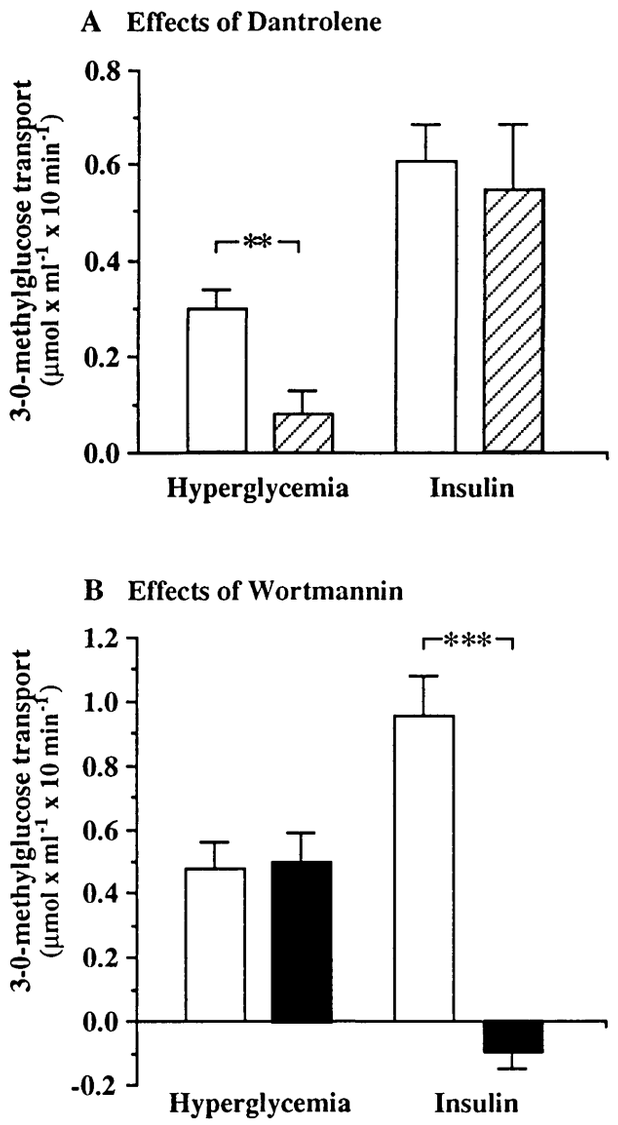

FIG. 1. Effects of dantrolene (*A*) or wortmannin (*B*) on the hyperglycemia-induced and insulin-stimulated increases in 3-*O*-methylglucose transport above basal rates in rat skeletal muscle. Epitrochlearisis muscles were incubated in KHB containing 40 mmol/l mannitol in the absence (□) or presence of 25 μmol/l dantrolene (▨) or 500 nmol/l wortmannin (■). Thereafter, the muscles were transferred to new vials with KHB supplemented with 8 mmol/l or 20 mmol/l glucose, mannitol, the absence or presence of 6,000 pmol/l insulin, and the same concentration of dantrolene or wortmannin as present in the previous step. The 3-*O*-methylglucose transport capacity was assessed as described in the METHODS. Results are presented as increase above basal glucose transport (mean ± SE) for 5–14 muscles per group. **$P < 0.01$ or ***$P < 0.001$ vs. nontreated muscles.

absence or presence of 25 μmol/l dantrolene. Dantrolene is an agent capable of preventing Ca^{2+} release from the sarcoplasmic reticulum of skeletal muscle (14,20). Thereafter, muscles were transferred to a new flask and preincubated (1 h) in 2 ml KHB containing 8 or 20 mmol/l glucose and insulin or dantrolene in the concentrations described in Fig. 1 and Table 1.

In a separate set of experiments, the effect of exercise to exhaustion and hyperglycemia were investigated. Rats swam for 15 min, and subsequently a weight consisting of ~3% of their body weight was tied to the base of the tail and six additional 30-min swimming bouts were completed. Each swimming bout was separated by a 5-min rest period. Immediately after the final swimming bout, rats were anesthetized and the epitrochlearis muscles were dissected out. The isolated muscles were preincubated for 20 min in the presence of 8 or 20 mmol/l 3-*O*-methylglucose rather than 60 min in the presence of glucose to minimize the reversal of the exercise effect (21). The changes in preincubation time (20 vs. 60 min) and substrate (3-*O*-methylglucose versus glucose) did not affect the absolute rates of glucose transport.

TABLE 1
The additive effect of hyperglycemia and insulin or exercise on the 3-*O*-methylglucose transport rate in isolated rat epitrochlear muscle

Group	3-*O*-methylglucose transport rate ($\mu mol \cdot ml^{-1} \cdot 10\ min^{-1}$)	
	8 mmol/l	20 mmol/l
Basal	0.38 ± 0.04	0.65 ± 0.04*
Insulin-stimulated	1.27 ± 0.10‡	2.68 ± 0.19*
Exercise-stimulated	0.87 ± 0.10§	1.46 ± 0.15†

Results are means ± SE for 6–12 muscles per group. Muscles were incubated in the presence of a maximal insulin concentration (6,000 pmol/l). Muscles from sedentary or maximally exercised rats were preincubated, and the rate of 3-*O*-methylglucose transport was assessed as indicated in METHODS. *$P < 0.001$, †$P < 0.01$ vs. 8 mmol/l in the same treatment group. ‡$P < 0.001$, §$P < 0.01$ vs. basal (8 mmol/l).

Wortmannin (500 nmol/l), an inhibitor of PI 3-kinase activation (22), was used to determine the role of PI 3-kinase in insulin-stimulated or glucose-mediated glucose transport. Epitrochlearis muscles were incubated (10 min) in 2 ml of KHB including 40 mmol/l mannitol and a further addition or omission of wortmannin (500 nmol/l). Thereafter, the muscles were preincubated for (2 × 20 min) in new flasks containing 8 or 20 mmol/l glucose, 0 or 6,000 pmol/l insulin, and the same concentration of wortmannin as in the previous step.

After preincubation, all muscles were rinsed (3 × 3 min) of glucose before measurement of the 3-*O*-methylglucose transport rate. Muscles were incubated in 2 ml of glucose-free KHB medium with 40 mmol/l mannitol and the same concentrations of insulin, dantrolene, or wortmannin as in the previous steps.

Glucose transport was assessed using the nonmetabolizable glucose analog 3-*O*-methyl-D-glucose as previously described by Wallberg-Henriksson et al. (23). Briefly, muscles were transferred to a new flask containing 1.5 ml KHB with 8 or 20 mmol/l 3-*O*-methyl-D-[^{3}H]-glucose (437 μCi/mmol), 32 mmol/l or 20 mmol/l [^{14}C]mannitol (8 μCi/mmol), and the same concentrations of insulin, dantrolene, or wortmannin as in the previous steps and incubated for 10 min. Immediately after the 10-min incubation period, muscles were quickly frozen in liquid nitrogen and analyzed as described previously (23).

All chemicals of the highest quality were purchased from Sigma (St. Louis, MO). Radionucleotides were purchased from DuPont-NEN (Boston, MA). The insulin was a gift from Novo Nordisk (Copenhagen, Denmark).

Multiple comparisons were analyzed using a one-way analysis of variance (ANOVA) test. When the ANOVA indicated significance, a Fisher least significant difference was performed for post hoc analysis. An unpaired Student's *t* test was used to determine significant differences when only two groups were compared.

RESULTS

Additive effect of hyperglycemia on insulin- or exercise-stimulated glucose transport. In the absence of insulin and exercise, hyperglycemia (20 mmol/l) induced a 1.7-fold increase in the glucose transport rate compared with the rate in muscles incubated in the presence of normoglycemia (8 mmol/l) ($P < 0.001$) (Table 1). When muscles were stimulated with a maximal dose of insulin (6,000 pmol/l) and 20 mmol/l glucose, the rate of glucose transport was 2.1-fold higher than in insulin-stimulated muscles exposed to 8 mmol/l glucose ($P < 0.001$) (Table 1). Exercise increased the glucose transport rate 2.3-fold compared with the rate in nonexercised muscles incubated in the presence of 8 mmol/l glucose ($P < 0.01$) (Table 1). The glucose transport rate of muscles stimulated by a maximal exercise bout and 20 mmol/l glucose was higher than the separate effects of hyperglycemia or exercise (Table 1).

Effect of dantrolene. Dantrolene, an inhibitor of Ca^{2+} release from the sarcoplasmic reticulum (14,20), was used to assess the role of cytosolic Ca^{2+} on glucose transport in the presence of hyperglycemia or insulin (Fig. 1*A*). The increase in the rate of 3-*O*-methylglucose transport above basal (8 mmol/l glucose) induced by hyperglycemia (0.30 ± 0.04 $\mu mol \cdot ml^{-1} \cdot 10\ min^{-1}$) was inhibited in the presence of 25 μmol/l dantrolene (0.08 ± 0.05 $\mu mol \cdot ml^{-1} \cdot 10\ min^{-1}$, $P < 0.01$) (Fig. 1*A*). The glucose transport rate assessed in muscles exposed to dantrolene and 20 mmol/l glucose was not significantly different from the glucose transport rate in the presence of 8 mmol/l glucose. The exercise effect on glucose transport (0.36 ± 0.03 vs. 0.57 ± 0.09 $\mu mol \cdot ml^{-1} \cdot 10\ min^{-1}$, basal and exercise, respectively) was also inhibited in muscles incubated in the presence of dantrolene (0.28 ± 0.06 $\mu mol \cdot ml^{-1} \cdot 10\ min^{-1}$, $P < 0.01$). Dantrolene had no effect on the insulin-stimulated (6,000 pmol/l) glucose transport rate assessed in muscles incubated in the presence of 8 mmol/l glucose (Fig. 1*A*). The additive effect of hyperglycemia on the maximal insulin–stimulated glucose transport rate (Table 1) was completely abolished in muscles incubated in the presence of dantrolene (1.15 ± 0.13 $\mu mol \cdot ml^{-1} \cdot 10\ min^{-1}$ for hyperglycemia + insulin + dantrolene, $P < 0.001$).

Effect of wortmannin. To determine whether or not the increase in the glucose transport rate in the presence of high glucose was dependent on PI 3-kinase, the agent wortmannin was used (Fig. 1*B*). Wortmannin (500 nmol/l) completely blocked the insulin stimulation over basal glucose transport ($P < 0.001$) (Fig. 1*B*). The same concentration of wortmannin had no effect on the stimulatory effect of hyperglycemia on glucose transport (Fig. 1*B*) or on basal glucose transport (0.20 ± 0.01 vs. 0.18 ± 0.03 $\mu mol \cdot ml^{-1} \cdot 10\ min^{-1}$, 8 mmol/l glucose in the absence and presence of wortmannin, respectively; NS).

DISCUSSION

The increase in glucose transport or uptake due to hyperglycemia has been attributed to the mass action of glucose (15,16). However, elevated levels of glucose have recently been demonstrated to give rise to a translocation of GLUT4 to the cell surface in rat skeletal muscle (17), suggesting that glucose per se may activate its own transport system. Here we demonstrate that the increase in glucose transport induced by hyperglycemia can be inhibited by blocking the release of Ca^{2+} from the sarcoplasmic reticulum. These data imply that, in addition to the mass action of glucose, the hyperglycemic-induced increase in glucose transport may be mediated via a Ca^{2+}-dependent signaling system.

Dantrolene prevents the efflux of Ca^{2+} from the sarcoplasmic reticulum (14,20). The effect of hyperglycemia on glucose transport, measured in the absence or presence of a maximal dose of insulin, was blocked when dantrolene was present in the incubation medium. Similar to the hyperglycemic effect, the exercise effect on glucose transport was inhibited in the presence of dantrolene. The muscle contraction–induced increase in glucose transport has been demonstrated to be associated with the release of Ca^{2+} from the sarcoplasmic reticulum (12). The increased cytosolic Ca^{2+} is presumed to be an early signal in the exercise-mediated glucose transport pathway (14). Although both exercise and hyperglycemia appear to activate glucose transport by increasing the level of cytosolic Ca^{2+}, the effect of exercise and hyperglycemia on glucose transport is additive, suggesting that the two pathways diverge at some point in the signaling pathway.

Dantrolene had no effect on insulin-stimulated glucose transport in the presence of 8 mmol/l glucose. This result is consistent with the fact that calcium is not directly involved in the ability of insulin to mediate its action on glucose transport (12). However, at a dantrolene concentration twofold higher than that used in the present study, an inhibition of the insulin-stimulated increase in glucose transport in the rat epitrochlearis muscle has been demonstrated (24). Nevertheless, here we demonstrate a distinct difference in the response to 25 μmol/l dantrolene on the insulin-stimulated increase in glucose transport, which did not respond, and on the hyperglycemia- and exercise-induced increase in glucose transport, which in both cases was significantly inhibited. This implies that skeletal muscle contains several Ca^{2+}-dependent regulatory proteins, which display different sensitivities to changes in intracellular Ca^{2+} concentrations, and that these Ca^{2+}-dependent regulatory proteins are involved in separate activation pathways of the glucose transport system.

Recently, the PI 3-kinase inhibitor wortmannin was used to display the divergence between the insulin- and contraction-activated glucose transport pathways in rat skeletal muscle (10). Wortmannin induced a dose-dependent decrease in 2-deoxyglucose uptake in the insulin-stimulated muscles, whereas no effect was noted on the contraction-mediated 2-deoxyglucose uptake (10). In the present investigation, wortmannin did not effect the hyperglycemia-mediated glucose transport rate and, consistent with the work of Yeh et al. (10), the insulin-stimulated glucose transport rate was completely inhibited.

In conclusion, the present report demonstrates that it is possible to selectively inhibit the stimulation of glucose transport by hyperglycemia in skeletal muscle without affecting the insulin-induced increase in glucose transport. The exact mechanism for this Ca^{2+}-dependent PI 3-kinase–independent activation of the glucose transport system by hyperglycemia requires further elucidation.

ACKNOWLEDGMENTS

This work was supported by grants from the Swedish Medical Research Council (9517, 10627, and 11135), The Bank of Sweden Tercentenary Foundation, The Swedish Diabetes Association, The Nordisk Insulin Foundation, Gustav V's Research Foundation, and Novo Nordisk A/S.

REFERENCES

1. Constable SH, Favier RJ, Cartee GD, Young DA, Holloszy JO: Muscle glucose transport: interactions of in vitro contractions, insulin, and exercise. *J Appl Physiol* 64:2329–2332, 1988
2. Nesher R, Karl IE, Kipnis DM: Dissociation of effects of insulin and contraction on glucose transport in rat epitrochlearis muscle. *Am J Physiol* 249:C226–C232, 1985
3. Wallberg-Henriksson H, Holloszy JO: Activation of glucose transport in diabetic muscle: responses to contraction and insulin. *Am J Physiol* 249:C233–C237, 1985
4. Wallberg-Henriksson H, Constable SH, Young DA, Holloszy JO: Glucose transport into rat skeletal muscle: interaction between exercise and insulin. *J Appl Physiol* 65:909–913, 1988
5. Ploug T, Galbo H, Ohduwa T, Tranum-Jensen J, Vinten J: Kinetics of glucose transport in rat skeletal muscle membrane vesicles: effects of insulin and contractions. *Am J Physiol* 262:E700–E711, 1992
6. Gao J, Ren J, Gulve E, Holloszy JO: Additive effect of contractions and

insulin on GLUT4 translocation into the sarcolemma. *J Appl Physiol* 77:1597–1601, 1994
7. Jullien D, Tanti J-F, Heydrick SJ, Gautier N, Grémeaux T, Van Obberghen E, Le Marchand-Brustel Y: Differential effects of okadaic acid on insulin-stimulated glucose and amino acid uptake and phosphatidylinositol 3-kinase activity. *J Biol Chem* 268:15246–15251, 1993
8. Cheatham B, Vlahos CJ, Cheatham L, Wang L, Blenis J, Kahn CR: Phosphatidylinositol 3-kinase activation is required for insulin stimulation of pp70 S6 kinase, DNA synthesis, and glucose transporter translocation. *Mol Cell Biol* 14:4902–4911, 1994
9. Clarke JF, Young PW, Yonezawa K, Kasuga M, Holman GD: Inhibition of the translocation of GLUT1 and GLUT4 in 3T3-L1 cells by the phosphatidylinositol 3- kinase inhibitor, wortmannin. *Biochem J* 300:631–635, 1994
10. Yeh J-I, Gulve EA, Rameh L, Birnbaum MJ: The effects of wortmannin on rat skeletal muscle: dissociation of signaling pathways for insulin- and contraction-activated hexose transport. *J Biol Chem* 270:2107–2111, 1995
11. Holloszy JO, Narahara HT: Enhanced permeability to sugar associated with muscle contraction: studies of the role of Ca^{++}. *J Gen Physiol* 50:551–562, 1967
12. Clausen T, Elbrink J, Dahl-Hansen AB: The relationship between the transport of glucose and cations across cell membranes in isolated tissues. IX. The role of cellular calcium in the activation of the glucose transport system in rat soleus muscle. *Biochim Biophys Acta* 375:292–308, 1975
13. Valant P, Erlij D: K^+-stimulated sugar uptake in skeletal muscle: role of cytoplasmic Ca^{2+}. *Am J Physiol* 245:C125–C132, 1983
14. Youn JH, Gulve EA, Holloszy JO: Calcium stimulates glucose transport in skeletal muscle by a pathway independent of contraction. *Am J Physiol* 260:C555–C561, 1991
15. Edelman SV, Laakso M, Wallace P, Brechtel G, Olefsky JM, Baron AD: Kinetics of insulin-mediated and non-insulin-mediated glucose uptake in humans. *Diabetes* 39:955–964, 1990
16. Yki-Järvinen H, Young AA, Lamkin C, Foley JE: Kinetics of glucose disposal in whole body and across the forearm in man. *J Clin Invest* 79:1713–1719, 1987
17. Galante P, Mosthaf L, Kellerer M, Berti L, Tippmer S, Bossenmaier B, Fujiwara T, Okuno A, Horikoshi H, Häring HU: Acute hyperglycemia provides an insulin-independent inducer for GLUT4 translocation in C_2C_{12} myotubes and rat skeletal muscle. *Diabetes* 44:646–651, 1995
18. Wallberg-Henriksson H: Glucose transport into skeletal muscle: influence of contractile activity, insulin, catecholamines and diabetes mellitus. *Acta Physiol Scand* (Suppl. 564) 131:1–80, 1987
19. Krebs HA, Henseleit K: Untersuchungen über die Harnstoffbildung im Tierkörper. *Hoppe-Seyler's Z Physiol Chem* 210:33–66, 1932
20. Van Winkle WB: Calcium release from skeletal muscle sarcoplasmic reticulum: site of action of dantrolene sodium? *Science* 193:1130–1131, 1976
21. Young DA, Wallberg-Henriksson H, Sleeper MD, Holloszy JO: Reversal of the exercise-induced increase in muscle permeability to glucose. *Am J Physiol* 253:E331–E335, 1987
22. Arcaro A, Wymann MP: Wortmannin is a potent phosphatidylinositol 3-kinase inhibitor: the role of phosphatidylinositol 3,4,5-trisphosphate in neutrophil responses. *Biochem J* 296:7665–7671, 1987
23. Wallberg-Henriksson H, Zetan N, Henriksson J: Reversibility of decreased insulin-stimulated glucose transport capacity in diabetic muscle with in vitro incubation: insulin is not required. *J Biol Chem* 262:7665–7671, 1987
24. Youn JH, Gulve EA, Henriksen EJ, Holloszy JO: Interactions between effects of W-7, insulin, and hypoxia on glucose transport in skeletal muscle. *Am J Physiol* 267:R888–R894, 1994