Exercise in Transgenic Mice Overexpressing GLUT4 Glucose Transporters: Effects on Substrate Metabolism and Glycogen Regulation

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We assessed the effects of GLUT4 glucose transporter expression on substrate metabolism and glycogen regulation during exercise. Transgenic mice overexpressing human (h)GLUT4 in muscle and fat (TG) and their wild-type littermates (WT) were studied by indirect calorimetry at rest and during acute treadmill exercise (30 minutes) and recovery (30 minutes). The rate of carbon dioxide production ($\dot{V}CO_2$) increased to a greater degree in TG during exercise, whereas resting $\dot{V}CO_2$, resting oxygen production ($\dot{V}O_2$), and exercise-induced increments in $\dot{V}O_2$ were similar in TG and WT. As a result, the respiratory quotient (RQ) was increased by .03 to .05 in TG during exercise, due to greater consumption of carbohydrate (up to ~64% more) and less consumption of lipid (up to ~40% less) compared with WT, without differences in overall energy expenditure. These differences in substrate metabolism were observed despite relative hypoglycemia and elevated free fatty acids (FFAs) in TG that persisted throughout resting, exercise, and recovery periods. To further assess substrate availability, glycogen content and glycogen synthase activity were measured in skeletal muscle and liver. At rest, muscle glycogen content was 50% higher and glycogen synthase I was 40% lower in TG compared with WT. During exercise and recovery, muscle glycogen was more profoundly depleted in TG than in WT, and glycogen synthase I increased to levels observed in WT, with no change in total glycogen synthase. In the liver, glycogen content and total glycogen synthase were similar in TG and WT under resting conditions, while glycogen synthase I was reduced by 48%. Exercise and recovery induced a more profound depletion of liver glycogen (76% *v* 30%) and greater increments in both I-form and total glycogen synthase in TG. In conclusion, (1) TG overexpressing GLUT4 exhibit greater muscle glycogen content at rest than WT; (2) during exercise, TG metabolize more carbohydrate, made possible by increased glycogenolysis in muscle and liver, and this predominates as a fuel source despite hypoglycemia and increased availability of FFA; (3) increased carbohydrate metabolism is linked to a decrease in lipid metabolism such that there is no change in overall energy expenditure; and (4) glycogen synthase I activity is inversely proportional to tissue glycogen content despite differences in circulating glucose, insulin, and FFA concentrations, indicating that glycogen content has an overriding regulatory influence on glycogen synthase.

THE GLUT4 GLUCOSE TRANSPORTER is a member of the facilitative monosaccharide transporter gene family,[1] and is expressed exclusively in insulin target tissues (fat and skeletal and cardiac muscle). Upon acute insulin stimulation, GLUT4 transporters rapidly translocate from an intracellular vesicular compartment to the plasma membrane, and mediate the bulk of glucose transport activity.[2,3] The level of GLUT4 expression is a major determinant of insulin sensitivity.[4-6] Transgenic mice carrying 11.5 kilobases of human GLUT4 genomic DNA exhibited increased glucose tolerance compared with nontransgenic littermates,[7] due to enhanced activity of the insulin-responsive glucose transport system.[8] On the other hand, decreased expression of GLUT4 is associated with insulin resistance in both skeletal muscle and adipocytes.[9] The effects of GLUT4 expression on glucose transport activity have been well studied, but less is known regarding the effects on substrate metabolism in vivo.

Exercise training is known to augment muscle GLUT4 content and insulin sensitivity.[10-12] However, how this biochemical event affects substrate metabolism, energy expenditure, and glycogen regulation is unclear. In this study, we addressed these issues in human (h) GLUT4-11.5 transgenic mice (TG) and their nontransgenic wild-type littermates (WT). To study the effects of GLUT4 overexpression on substrate metabolism, TG and WT were studied under resting conditions and then exercised on a treadmill for 30 minutes followed by 30 minutes of recovery. The rates of oxygen consumption ($\dot{V}O_2$) and carbon dioxide production ($\dot{V}CO_2$) were continuously monitored, and the respiratory quotient (RQ), energy production rate, and substrate utilization rate were then calculated and analyzed. The results of indirect calorimetry indicated that TG used more carbohydrate and less lipid as an energy source for acute exercise despite hypoglycemia and high blood free fatty acids (FFAs) in comparison to WT. This was made possible by dramatic glycogenolysis and regulatory effects on glycogen synthase in liver and muscle.

MATERIALS AND METHODS

TG

TG were kindly supplied by J.E. Pessin (University of Iowa, Iowa City, IA). Heterozygous transgenic progeny were bred for study by mating the male founder animals with C57/BL6 female mice, and TG animals were identified by polymerase chain reaction amplification of tail DNA using primers specific for the hGLUT4-11.5 construct as previously described.[13] Animals were studied between 6.5 and 9 weeks of age (body weight, 18 to 28 g).

Acute Exercise Protocol and Indirect Calorimetry

TG and WT were acutely exercised by forced running on a treadmill (Columbus Instruments, Columbus, OH) for 30 minutes at 30 m/min and 0° incline. All mice performed well at this level of exercise. Before exercise, they were first accommodated to the treadmill chamber over 30 minutes, at which point $\dot{V}O_2$ and $\dot{V}CO_2$ were stable (resting state).

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Immediately after exercise, the mice were allowed to recover for 30 minutes in the same treadmill chamber. Open-circuit indirect calorimetry was performed at rest and throughout the exercise and recovery periods using the Oxymax-ECO system (Columbus Instruments). The system monitors oxygen and carbon dioxide gas fractions at both the inlet and output ports of a sealed treadmill chamber through which a known flow of air is passing. The gas fraction and flow measurements are used to compute $\dot{V}O_2$ and $\dot{V}CO_2$. The measurement, display, and storage of data are performed with the help of a personal computer using software provided with the system. In our experiments, $\dot{V}O_2$ and $\dot{V}CO_2$ were recorded at 2.5 minute intervals by the computer and used to calculate the following items by standard formulas: (1) RQ ($\dot{V}CO_2/\dot{V}O_2$); (2) energy production rate, which is calculated using the formula of Lusk[14] as modified by Weir[15]; and (3) substrate utilization rate. According the formula used by Ferrannini,[16] the rate of carbohydrate consumption is G (g/min) = 4.55 $\dot{V}CO_2$ (L/min) − 3.21 $\dot{V}O_2$ (L/min) − 2.87N (mg/min) and the rate of lipid oxidation is L (g/min) = 1.67 ($\dot{V}O_2$ − $\dot{V}CO_2$) − 1.92N, where N is the rate of urinary nitrogen excretion used to estimate protein oxidation. Considering that only a small portion of resting and exercise energy expenditure arises from protein oxidation,[15,17] protein metabolism is frequently neglected in published indirect calorimetry studies. For our purpose, we have assumed that there is no difference in urinary nitrogen between WT and TG, or that any existing difference is not sufficient to affect conclusions regarding substrate utilization. Differences in carbohydrate and lipid consumption rates between WT and TG can then be calculated based on $\dot{V}O_2$ and $\dot{V}CO_2$ values.

Serum Analysis

Mice blood was collected via orbital sinus bleeding. Serum glucose and lactate levels were measured using the YSI 2300 STAT PLUS glucose analyzer (YSI, Yellow Springs, OH). Serum FFA levels were determined spectrophotometrically by the method of Novak.[18]

Immunoblot Analysis

Total postnuclear membrane proteins were prepared from gastrocnemius muscle and epididymal fat. Tissues were homogenized at 4°C in a pH 7.4 buffer containing 20 mmol/L HEPES, 255 mmol/L sucrose, 1 mmol/L EDTA, and protease inhibitors including 5 μg/mL leupeptin, 1 μg/mL pepstatin, and 5 μg/mL aprotinin. The homogenates were cleared by centrifugation at 1,000 × *g* for 15 minutes followed by centrifugation of the supernatant at 388,000 × *g* at 4°C for 100 minutes. The final pellets containing the total postnuclear membranes were then resuspended in homogenization buffer. For immunoblot analysis, membrane proteins (35 μg) were fractionated on 7% reducing sodium dodecyl sulfate–polyacrylamide gels and then transferred to nitrocellulose filters. The nitrocellulose filters were then incubated with a 1:1,000 dilution of a GLUT4-specific polyclonal antibody (East Acres Biologicals, Southbridge, MA) followed by ^{125}I-protein A. Relative levels of immunoreactive GLUT4 were determined by densitometric analysis of autoradiographs.

Determination of Tissue Glycogen Content and Glycogen Synthase Activity

Gastrocnemius muscle and liver tissue from WT and TG were surgically removed and frozen in liquid nitrogen. Liver glycogen content was determined by enzymatic digestion of liver glycogen[19] with amylo-α-1,4-α-1,6-glucosidase (Sigma, St Louis, MO) followed by measurement of liberated glucose with the YSI glucose analyzer. Muscle glycogen content was determined spectrophotometrically by the Anthrone method.[20] The active (I) form of glycogen synthase activity was assayed at low glucose-6-phosphate concentration (0.01 mmol/L) and total glycogen synthase activity was measured at high glucose-6-phosphate concentration (10 mmol/L) by a modification of the method of Thomas et al[21] as previously described.[22]

Statistical Analysis

Statistical comparisons of $\dot{V}O_2$, $\dot{V}CO_2$, RQ, and energy production rates between WT and TG were made with repeated-measures ANOVA. Other statistical comparisons were made by paired *t* test. Data are presented as the mean ± SE.

RESULTS

Characterization of TG and WT

TG expressing the human glucose transporter gene demonstrated normal rates of growth and no difference in total body weight compared with gender-matched WT (Table 1). To quantify overexpression of GLUT4 in TG, we measured GLUT4 protein levels in muscle and fat from male TG and their WT littermates by immunoblot analysis. GLUT4 protein levels in gastrocnemius and epididymal fat in male TG were 145% and 86% higher, respectively, than in male WT ($P < .05$; Fig 1). Analysis of blood metabolites in TG demonstrated reduced blood glucose (29% to 33%) and increased blood lactate (20% to 25%) and FFAs (20% to 48%) in comparison to WT (Table 1). These results were similar to previous observations made by other investigators with the same line of TG.[7,23]

Substrate and Energy Metabolism

To examine the effects of GLUT4 overexpression on substrate utilization and energy metabolism, we performed indirect calorimetric studies on WT and their TG littermates at rest and during 30 minutes of acute exercise followed by 30 minutes of recovery. Figure 2 shows the comparison of $\dot{V}O_2$ and $\dot{V}CO_2$ between WT and TG. Resting values for $\dot{V}O_2$ and $\dot{V}CO_2$ were similar between WT and TG (P = nonsignificant [NS]). In both groups, $\dot{V}O_2$ and $\dot{V}CO_2$ increased dramatically in the first 10 minutes of exercise and then plateaued near the peak value during the remaining 20 minutes. After exercise, $\dot{V}O_2$ and $\dot{V}CO_2$ decreased gradually to baseline values in the recovery period. There was no significant difference in $\dot{V}O_2$ curves between WT and TG (P = NS). However, TG produced more CO_2 than WT during exercise ($P < .05$). Differences in $\dot{V}CO_2$ between TG and WT were consistently observed in both males and females. Male mice did tend to consume less O_2 and produce less CO_2 than female mice for the same amount of exercise (data not shown); however, the gender difference did not interfere with the group difference between WT and TG ($P < .05$), and the data from male and female mice have therefore been pooled.

We next calculated the RQ (Fig 3). The RQ in WT increased from 0.78 to 0.81 during the first 5 minutes of exercise. After reaching the peak value at 5 minutes, RQ decreased gradually toward the baseline and stayed slightly below the resting value for most of the exercise and all of the recovery period. The resting RQ in TG was similar to that in WT (P = NS); however, the RQ in TG increased more dramatically from 0.78 to 0.86 during the first 5 minutes of exercise, an increment that was twofold greater than that in WT. As exercise continued, the RQ remained above baseline values and was consistently greater than that observed in WT ($P < .05$), with a mean absolute difference of 0.04 between the TG and WT RQs. During

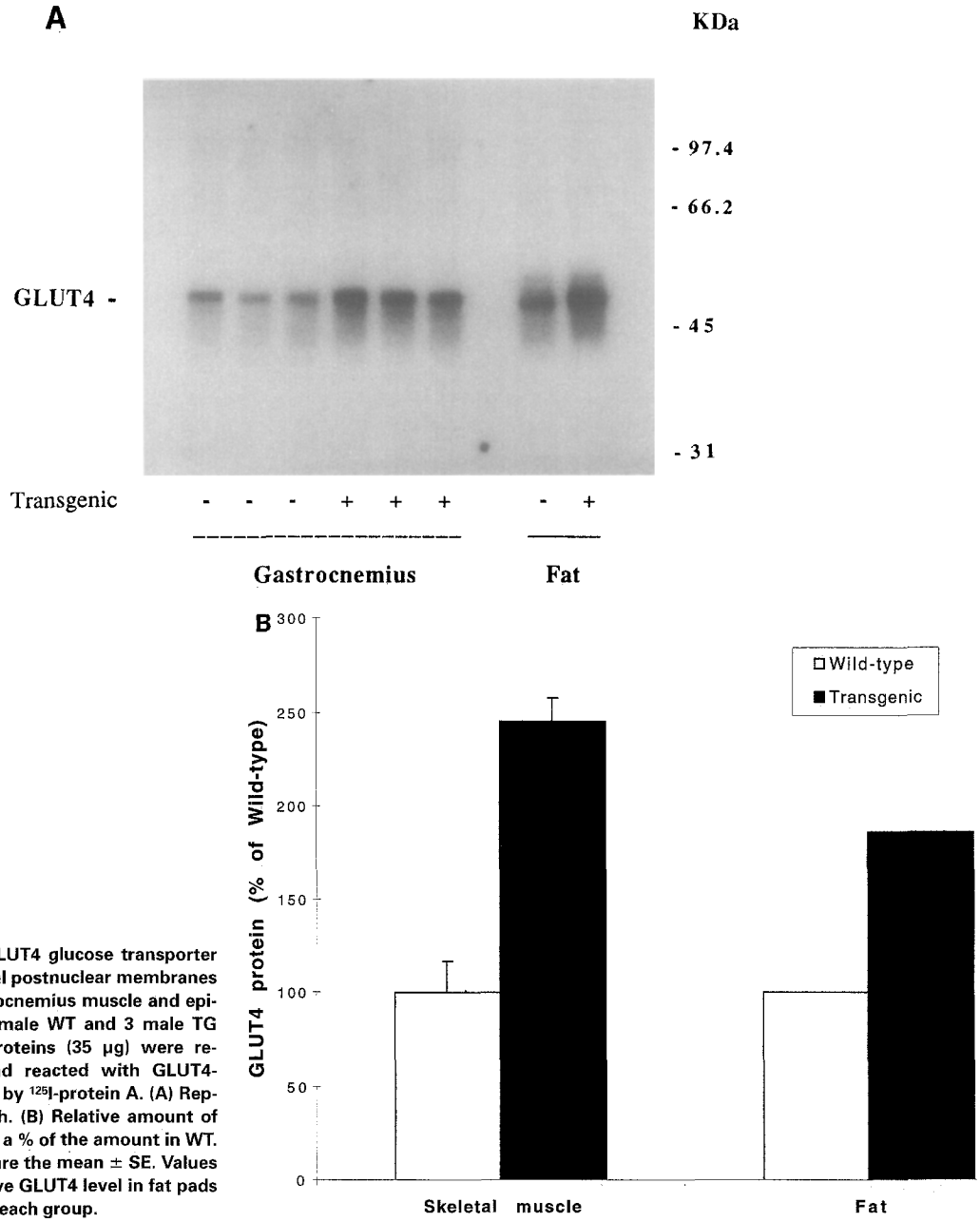

Fig 1. Expression of GLUT4 glucose transporter protein in WT and TG. Total postnuclear membranes were prepared from gastrocnemius muscle and epididymal fat pads from 3 male WT and 3 male TG littermates. Membrane proteins (35 μg) were resolved by SDS/PAGE and reacted with GLUT4-specific antibody followed by ^{125}I-protein A. (A) Representative autoradiograph. (B) Relative amount of GLUT4 in TG expressed as a % of the amount in WT. Values for muscle GLUT4 are the mean ± SE. Values for fat represent the relative GLUT4 level in fat pads pooled from 3 mice within each group.

Table 1. Comparison of Body Weight and Circulating Metabolites Between WT and TG

Parameter	Male		Female	
	WT	TG	WT	TG
Body weight (g)	24.6 ± 0.5	25.8 ± 0.5	19.3 ± 0.3	19.7 ± 0.3
Glucose (mg/dL)	135.4 ± 4.8	90.2 ± 8.9*	114.8 ± 11.4	84.7 ± 4.9*
Lactate (mmol/L)	16.1 ± 1.0	20.2 ± 0.6*	16.3 ± 1.0	19.0 ± 0.7*
FFA (μmol/L)	1,748 ± 177	2,204 ± 98*	1,559 ± 71	2,315 ± 84*

NOTE. Data (mean ± SE) were obtained from resting animals: 14 WT and 14 paired TG littermates (6 male and 8 female) were used for each analysis.

*$P < .05$ for WT *v* TG.

recovery, the RQ in TG decreased gradually, and the mean value was similar to the RQ in WT at the end of the recovery period.

The RQ or respiratory exchange ratio is an indicator of substrate utilization and can be used to quantify the amount of energy derived from fat and carbohydrate.[16,24] RQs for pure fat and carbohydrate metabolism are 0.707 and 1.000, respectively.[24] In our study, the RQ in TG was significantly higher than in WT during exercise, indicating that TG used more carbohydrate and less lipid as an energy source in comparison to WT. The absolute difference in carbohydrate and lipid consumption rates between TG and WT was calculated using the formula of Ferrannini[16] and is shown in Fig 4. During exercise, TG metabolize up to 23 mg/kg/min more carbohydrate and up to 8 mg/kg/min less lipid than WT. If the small amount of protein

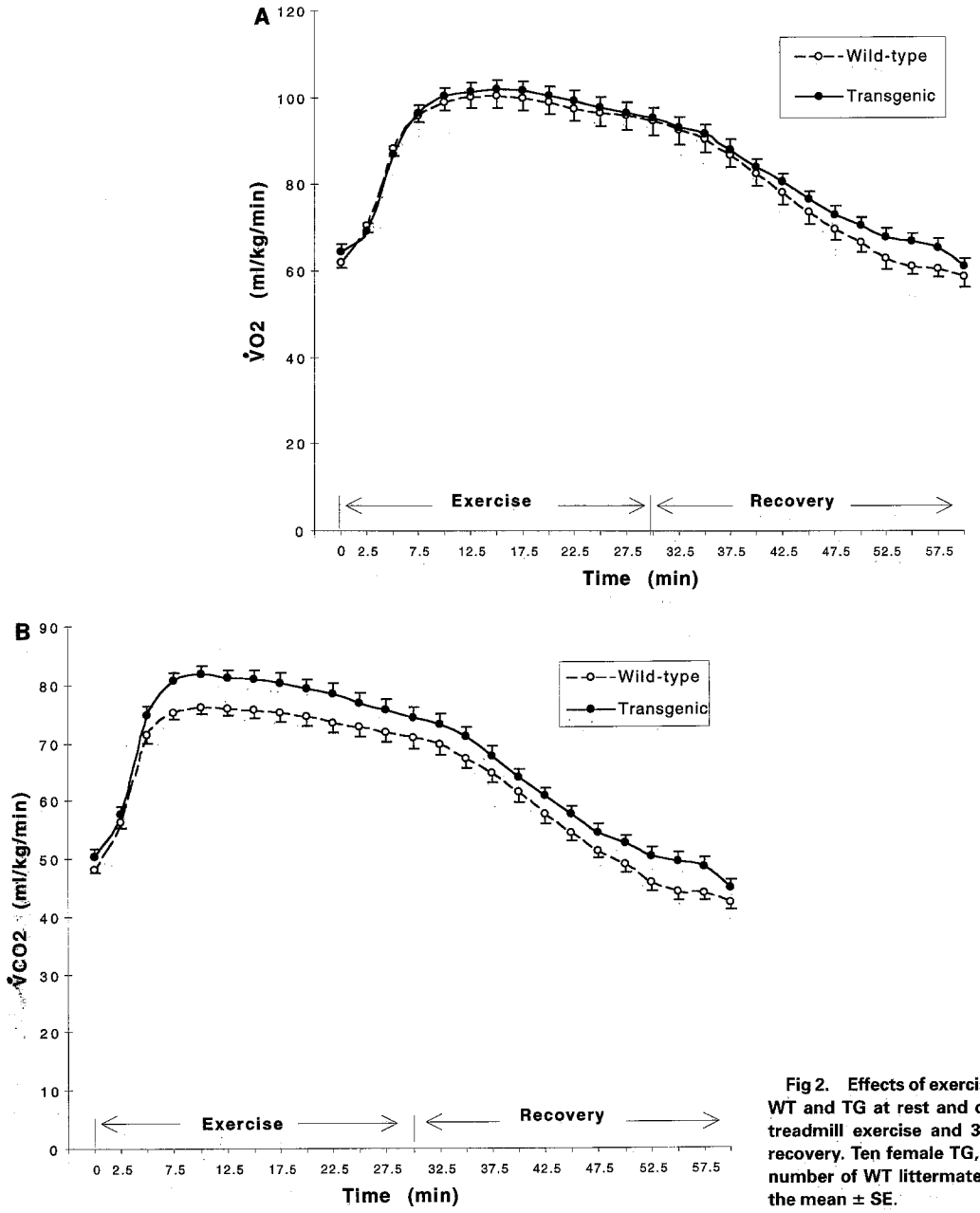

Fig 2. Effects of exercise on (A) $\dot{V}O_2$ and (B) $\dot{V}CO_2$ in WT and TG at rest and during 30 minutes of acute treadmill exercise and 30 minutes of postexercise recovery. Ten female TG, 11 male TG, and the same number of WT littermates were studied. Values are the mean ± SE.

metabolism is neglected, the rate of carbohydrate metabolism is up to 64% higher (57.7 *v* 35.2 mg/kg/min at the 7.5-minute time point) and as much as 40% lower (20.2 *v* 28.2 mg/kg/min at the 5.0-minute time point) in TG.

We then calculated energy production rates using the formula of Weir[15] based on $\dot{V}O_2$ and $\dot{V}CO_2$ values. The energy production rate in WT increased from 339 to 472 cal/kg/min in the first 10 minutes of acute exercise, remained at this plateau throughout exercise, and then decreased gradually to baseline during recovery. TG followed the same pattern, and there was no statistical difference in energy production rates between WT and TG (P = NS). Although TG consumed more carbohydrate during exercise, this was linked to a decrease in lipid utilization such that overall energy expenditure between WT and TG was not significantly different (Fig 5).

Availability of Blood Glucose and FFAs for Substrate Metabolism

Characterization of TG and WT has demonstrated that TG had lower blood glucose and higher FFA levels compared with WT at rest (Table 1). Table 2 delineates serum glucose and FFA levels at the end of the exercise and recovery periods. Serum glucose for both WT and TG increased as a function of exercise, and then tended to decrease during recovery. During exercise, the mean glucose increased to a lower absolute level in TG (132 mg/dL) compared with WT (170 mg/dL). At the end of the recovery period, serum glucose in TG (71.9 ± 8.6 mg/dL) had decreased to initial resting values (80.5 ± 10.1 mg/dL, P = NS); however, in WT, the decrease in blood glucose was slower and the level (149.6 mg/dL) was still 31% higher ($P < .05$) than the

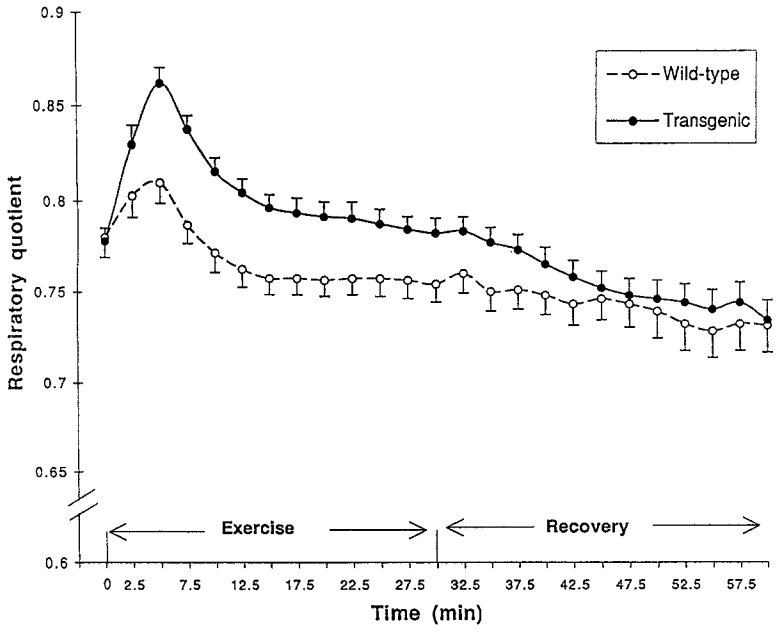

Fig 3. Effects of exercise on the RQ in WT and TG. Data were calculated from $\dot{V}O_2$ and $\dot{V}CO_2$ values shown in Fig 2 and are the mean ± SE.

baseline value (114 mg/dL). At the end of exercise and recovery, serum FFA levels in TG remained 20% to 30% higher than in WT ($P < .05$).

Effects on Glycogen Metabolism

The substantial energy requirements of exercise are primarily met by oxidation of skeletal muscle glycogen and triglyceride, as well as bloodborne glucose and FFAs derived from hepatic glycogen and adipocyte triglyceride, respectively.[17] Since we found that TG consumed more carbohydrate during exercise than the WT littermates (Fig 4), we further studied differences in glycogen metabolism between WT and TG. Table 3 shows the comparison of muscle and liver glycogen content and glycogen synthase activity between WT and TG at rest and at the end of exercise and recovery periods. The major findings are as follows. (1) Under resting conditions, muscle glycogen content was significantly higher ($P < 0.05$) and both I-form and total glycogen synthase activity were significantly lower ($P < .05$) in TG than in WT. The same tendencies were observed in the liver, although differences in liver glycogen content and total glycogen synthase activity between WT and TG were not statistically significant (P = NS). (2) Glycogen content was relatively more profoundly depleted in TG as a consequence of exercise and recovery than in WT. In muscle, glycogen content decreased 30% in TG, whereas the decrease in WT was only 5% by the end of the recovery period. In the liver, glycogen content decreased 76% in TG, compared with 30% in WT. (3) In TG, the depletion in muscle glycogen content

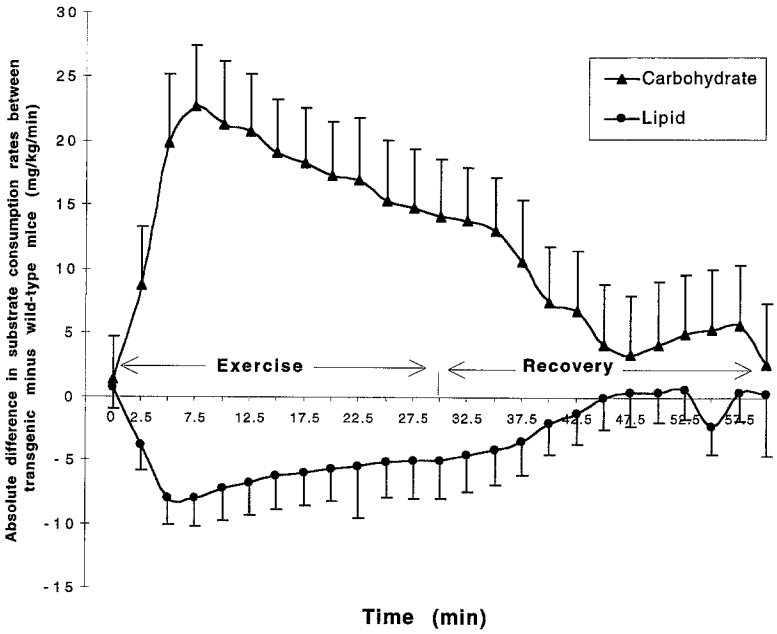

Fig 4. Effects of exercise on substrate utilization rates in WT and TG. Carbohydrate and lipid utilization rates were assessed by indirect calorimetry (see Fig 2), and absolute differences between WT and TG are shown at each time point. Data are the mean ± SE.

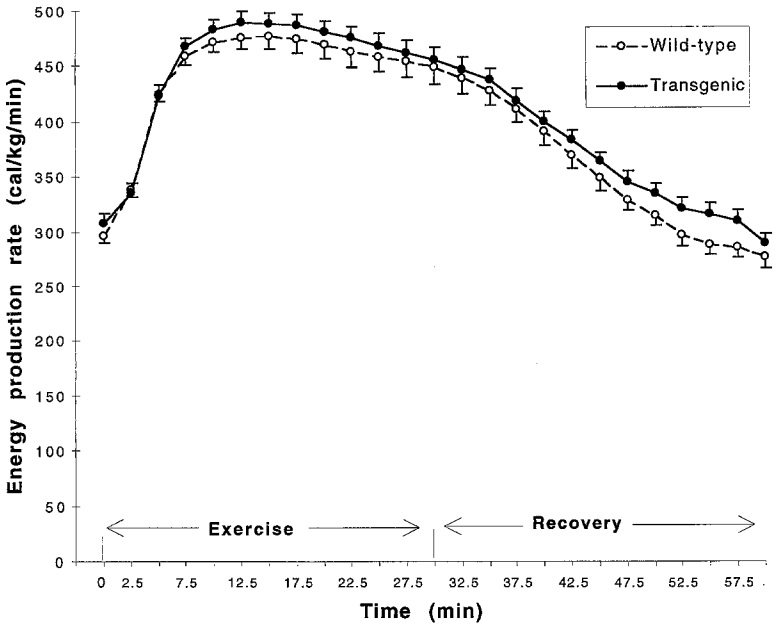

Fig 5. Effects of exercise on energy production rates in WT and TG. Data were calculated from $\dot{V}O_2$ and $\dot{V}CO_2$ values shown in Fig 2 and are the mean ± SE.

induced a 66% increase in glycogen synthase I activity ($P < .05$) without affecting the total glycogen synthase activity (P = NS). In the liver, glycogen depletion induced large increments in both I-form (threefold) and total glycogen synthase (twofold) activity ($P < .05$). In WT, similar tendencies were observed, but, changes in glycogen synthase activity were less marked and not statistically significant (P = NS).

DISCUSSION

GLUT4 is the major insulin-responsive glucose transporter isoform, and is expressed exclusively in insulin target tissues (muscle and fat). It is the most important glucose transporter in determining whole-body glucose homeostasis.[1] In adipocytes, GLUT4 comprises 90% to 95% of total glucose transporters, and GLUT4 depletion is a major cause of adipocyte insulin resistance in obesity and non–insulin-dependent diabetes mellitus.[4] In skeletal muscle, the GLUT4 level in normal individuals is directly correlated with the rate of whole-body glucose disposal,[5,25] and increased GLUT4 levels mediate insulin hypersensitivity induced by exercise training.[10-12] The importance of GLUT4 in regulating glucose homeostasis has been further studied in transgenic mice carrying 11.5 kilobases of human GLUT4 genomic DNA (TG). These mice expressed high levels of human GLUT4 protein in muscle and fat and displayed decreased blood glucose and insulin and increased blood FFA levels compared with WT. This demonstrated that an alteration in glucose transport can itself alter the glucose set-point[1]; higher expression of GLUT4 is linked to lower blood glucose. The relative hypoglycemia in TG results from an augmentation in both glucose transport and glucose metabolism.[8,23] The latter was manifested by increased glycogen synthesis and glycolysis, as TG had elevated muscle glycogen content and blood lactate levels. The high level of blood FFA in TG was attributable to increased lipolysis secondary to hypoinsulinemia.[7,8,23]

To further examine the effects of GLUT4 expression on substrate metabolism, we performed indirect calorimetric studies on TG and their WT littermates at rest and during acute exercise and recovery. GLUT4 overexpression did not affect substrate utilization in resting mice, but had dramatic effects as a function of exercise. We were able to confirm previous studies[7,8,23] showing relative hypoglycemia, elevated circulating FFA concentrations, and increased muscle glycogen content in resting TG compared with WT. Despite these differences in substrate availability, the RQ and carbohydrate and lipid oxidation rates were similar in TG and WT at rest. In addition, increased muscle glycogen was accompanied by greatly suppressed I-form glycogen synthase activity in both muscle and liver from resting TG. During exercise, TG sustained a higher

Table 2. Effects of Exercise on Serum Glucose and FFAs in WT and TG

Period	Glucose (mg/dL)		FFAs (μmol/L)	
	WT	TG	WT	TG
Resting	114.4 ± 9.7	80.5 ± 10.1*	1,733.8 ± 74.6	2,276.0 ± 120.5*
Exercise	169.7 ± 10.4†	131.6 ± 24.1†	1,826.6 ± 112.6	2,179.8 ± 138.3*
Recovery	149.6 ± 10.3‡	71.9 ± 8.6*	1,617.8 ± 64.4	2,032.6 ± 165.8*

Six WT and 6 paired TG littermates (3 male and 3 female) were studied in each condition. Since there was no significant difference in blood glucose and FFA levels between male and female mice (data not shown), data from males and females were pooled (mean ± SE).

*$P < .05$ *v* WT.

†$P < .05$, resting *v* exercise.

‡$P < .05$, resting *v* recovery.

Table 3. Effects of Exercise on Muscle and Liver Glycogen Content and Glycogen Synthase Activity in WT and TG

	Glycogen Content (mmol/kg)		Glycogen Synthase Activity (pmol/min/μg protein)			
			I-form		Total Enzyme	
	WT	TG	WT	TG	WT	TG
Skeletal muscle						
Resting	35.5 ± 2.9	53.3 ± 4.7*	5.75 ± 0.78	3.43 ± 0.46*	27.1 ± 1.0	22.4 ± 0.6*
Exercise	35.9 ± 2.2	44.6 ± 5.3†	6.77 ± 0.92	4.78 ± 1.02	23.6 ± 1.8	24.4 ± 1.7
Recovery	33.9 ± 2.1	37.4 ± 1.1‡	5.58 ± 0.42	5.69 ± 0.52‡	25.9 ± 1.9	21.0 ± 1.1*
Liver						
Resting	168.9 ± 32.6	187.1 ± 33.6	.325 ± .124	.168 ± .031*	.974 ± .254	.814 ± .110
Exercise	137.2 ± 54.0	168.8 ± 45.9	.331 ± .109	.142 ± .021	1.119 ± .208	.821 ± .124
Recovery	118.6 ± 34.1‡	45.7 ± 14.4‡	.461 ± .134	.539 ± .164‡	1.362 ± .179	1.629 ± .422‡

NOTE. Six WT and 6 paired TG littermates (3 male and 3 female) were studied in each group. Data are the mean ± SE.

*$P < .05$ *v* WT.

†$P < .05$, resting *v* exercise.

‡$P < .05$, resting *v* recovery.

RQ indicative of higher carbohydrate oxidation and lower lipid oxidation compared with WT, while overall energy production rates remained similar. TG experienced a greater degree of glycogenolysis in muscle and liver as a result of exercise, whereas circulating glucose levels remained lower and FFAs higher, than in WT. With depletion of glycogen content, TG showed increased glycogen synthase I activity in muscle and liver to a level equal to that observed in WT.

In humans, GLUT4 expression in skeletal muscle varies over fivefold in healthy individuals with normal glucose tolerance.[5,6,26-28] Furthermore, muscle GLUT4 content is positively correlated with the degree of insulin sensitivity.[5,25] One factor that can partially explain individual variability with respect to both GLUT4 expression and insulin sensitivity is exercise training. We[11] and others[10,12] have found that chronic exercise leads to an augmentation in muscle GLUT4 expression, correlated with increments in whole-body insulin sensitivity, insulin-stimulated glucose uptake in muscle, and glucose tolerance. The degree of GLUT4 overexpression in TG is well within the range of muscle GLUT4 expression in healthy humans. Therefore, the hGLUT4-11.5 TG mouse is an instructive model for studying the metabolic significance of individual differences in muscle GLUT4 expression. Specifically, muscle GLUT4 hyperexpression is a primary perturbation in these mice and can be used to identify secondary metabolic sequelae.

The current data highlight several salient points regarding metabolic effects of variable muscle GLUT4 expression. The first involves the preferred fuel used by muscle during exercise. With the increase in GLUT4 expression, muscle preferentially metabolizes carbohydrate during exercise, despite elevated FFA, hypoglycemia, and hypoinsulinemia relative to WT. Previous data have shown that the choice of substrate for energy metabolism in the working muscle varies with the duration and size of the workload, as well as different types of diets on the days preceding the muscular work.[29] With a carbohydrate-free diet, plasma FFAs increase, gluconeogenesis is stimulated, and glycolysis and glycogenolysis are inhibited. These changes lead to increased lipid metabolism.[29] With a high-carbohydrate diet, these conditions are reversed. Here, we have demonstrated that substrate metabolism can also be primarily regulated by altering GLUT4 expression. Specifically, the enhanced capacity for glucose transport is more important than the increased availability of FFA. During exercise, the increased availability of glucose in muscle, resulting from increments in muscle glycogenolysis and in muscle glucose transport activity combined with liver glycogenolysis, predominates over the increased availability of FFAs as the major determinant of whole-body substrate oxidation rates and RQ.

A second point relates to regulation of energy expenditure. During exercise, TG expired more CO_2, sustained higher RQs, and used carbohydrate at rates that were significantly increased over those observed in WT. Even so, energy expenditure throughout exercise and recovery remained similar in TG and WT because higher rates of carbohydrate utilization were linked to lower rates of lipid utilization. Furthermore, this regulation occurred despite elevated circulating FFA levels. One could hypothesize that the inverse regulation of carbohydrate and FFA oxidation occurred at the level of malonyl coenzyme A and carnitine palmitoyltransferase. This result is consistent with a recent study showing that increased glucose flux and accelerated carbohydrate oxidation may directly decrease fatty acid oxidation via inhibition of fatty acid uptake into mitochondria.[30] In any event, mechanisms that determine energy expenditure appear to be independent of forced alterations in preferred substrate utilization. Thus, changes in muscle GLUT4 expression would not be expected to alter energy expenditure even if there is resultant augmentation of carbohydrate utilization.

A third point involves regulation of tissue glycogen content and glycogen synthase activity. Glycogen synthase is the rate-limiting enzyme in the pathway of glycogen synthesis. Glycogen synthase I activity is inversely proportional to tissue glycogen content, due to a negative feedback mechanism: high glycogen content inhibits glycogen synthase activity, and low glycogen content (ie, after exercise) stimulates glycogen resynthesis by activating glycogen synthase.[31] Under resting or basal conditions, TG sustained markedly increased muscle glycogen content compared with WT, together with lower glycogen synthase I activity. These current data are in general agreement with a previous study in hGLUT4-11.5 TG mice.[8] The high glycogen tissue content is maintained despite relative hypoglycemia, hypoinsulinemia, and low I-form activity, presumably due to enhanced glucose transport activity and increased glucose flux into the cell. With acute exercise, glycogen depletion was much more profound in both muscle and liver in

TG than in WT. The enhanced liver glycogenolysis could have resulted from a more marked release of counterregulatory hormones such as catecholamines and glucagon, which become magnified in the presence of ambient hypoglycemia and hypoinsulinemia. The accelerated glycogenolysis in TG was accompanied by much greater increments in glycogen synthase I activity in the recovery period. This is a convincing demonstration of the primary importance of glycogen content as a regulator of I-form activity. In TG, lower circulating concentrations of insulin and glucose and high FFAs would act to minimize glycogen synthase activity and glycogen formation relative to these parameters in WT. Even so, the more pronounced exercise-induced decrease in tissue glycogen overrides these counteractive influences in TG to result in greater activation of glycogen synthase.

In summary, we have studied the effects of increased GLUT4 expression on substrate metabolism in response to acute exercise. These studies involved hGLUT4-11.5 TG that overexpress GLUT4 in muscle and fat, leading to enhanced insulin sensitivity and muscle glucose uptake, hypoglycemia, hypoinsulinemia, elevated blood FFAs, and increased muscle glycogen content. These changes did not significantly affect substrate utilization in the resting state, but led to marked alterations during exercise and recovery. Compared with their WT littermates, TG sustained higher RQs and carbohydrate oxidation rates during exercise at the expense of markedly increased glycogenolysis in muscle and liver. Carbohydrate predominated as a fuel source despite hypoglycemia, hypoinsulinemia, and increased circulating FFAs in TG throughout exercise and recovery. Increased carbohydrate metabolism was linked to a decrease in lipid metabolism such that there was no change in overall energy expenditure. Glycogen synthase I activity was inversely correlated with glycogen content despite counteractive differences in prevailing insulin, glucose, and FFA concentrations, indicating that glycogen content has an overriding regulatory influence on glycogen synthase activity. Thus, increased GLUT4 expression as a primary perturbation results in greater carbohydrate utilization during exercise despite increased FFA availability. In addition, energy expenditure rates are not affected by forced alterations in substrate utilization.

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REFERENCES

1. Mueckler M: Facilitative glucose transporters. Eur J Biochem 219:713-725, 1994

2. Cushman SW, Wardzala LJ: Potential mechanisms of insulin action on glucose transport in the isolated rat adipose cell. J Biol Chem 255:4758-4762, 1980

3. Suzuki K, Kono T: Evidence that insulin causes translocation of glucose transport activity to the plasma membrane from an intracellular storage site. Proc Natl Acad Sci USA 77:2542-2545, 1980

4. Garvey WT, Maianu L, Huecksteadt TP, et al: Pretranslational suppression of a glucose transporter protein causes insulin resistance in adipocytes from patients with noninsulin-dependent diabetes mellitus and obesity. J Clin Invest 87:1072-1081, 1991

5. Garvey WT, Maianu L, Brechtel G, et al: Muscle GLUT4 expression is a familial trait and determines insulin sensitivity in humans independent of obesity. Diabetes 43:69A, 1994 (suppl 1, abstr)

6. Garvey WT: GLUT-4 glucose transporters and insulin action in humans, in Draznin B, LeRoith D (eds): Molecular Biology of Diabetes. Totowa, NJ, Humana, 1994, pp 437-471

7. Liu ML, Gibbs EM, McCoid SC, et al: Transgenic mice expressing the human GLUT4/muscle-fat facilitative glucose transporter protein exhibit efficient glycemic control. Proc Natl Acad Sci USA 90:11346-11350, 1993

8. Hansen PA, Gulve EA, Marshall BA, et al: Skeletal muscle glucose transport and metabolism are enhanced in transgenic mice overexpressing the GLUT4 glucose transporter. J Biol Chem 270:1679-1684, 1995

9. Garvey WT, Huecksteadt TP, Birnbaum MJ: Pretranslational suppression of an insulin-responsive glucose transporter in rats with diabetes mellitus. Science 245:60-63, 1989

10. Andersen PH, Lund S, Schmitz O, et al: Increased insulin-stimulated glucose uptake in athletes: The importance of GLUT4 mRNA, GLUT4 protein, and fibre type composition of skeletal muscle. Acta Physiol Scand 149:393-404, 1993

11. Hardin DS, Azzarelli B, Edwards J, et al: Mechanisms of enhanced insulin sensitivity in endurance-trained athletes: Effects on blood flow and differential expression of GLUT4 in skeletal muscles. J Clin Endocrinol Metab 80:2437-2446, 1995

12. Houmard JA, Egan PC, Neufer PD, et al: Elevated skeletal muscle glucose transporter levels in exercise-trained middle-aged men. Am J Physiol 261:E437-443, 1991

13. Olson AL, Liu ML, Moye-Rowley WS, et al: Hormonal/metabolic regulation of the human GLUT4/muscle-fat facilitative glucose transporter gene in transgenic mice. J Biol Chem 268:9839-9846, 1993

14. Lusk G: Animal calorimetry: Analysis of the oxidation of mixtures of carbohydrate and fat. J Biol Chem 59:41-42, 1924

15. de V Weir JB: New methods for calculating metabolic rate with special reference to protein metabolism. J Physiol 109:1-9, 1949

16. Ferrannini E: The theoretical bases of indirect calorimetry: A review. Metabolism 37:287-301, 1988

17. Evans WJ: Exercise and protein metabolism. World Rev Nutr Diet 71:21-33, 1993

18. Novak M: Calorimetric ultramicro method for the determination of free fatty acids. J Lipid Res 6:431-433, 1965

19. Chan TM, Exton JM: A rapid method for the determination of glycogen content and radioactivity in small quantities of tissue or isolated hepatocytes. Anal Biochem 71:96-105, 1976

20. Hassid WZ, Abraham S: Chemical procedures for analysis of polysaccharides. Methods Enzymol 3:34-35, 1959

21. Thomas JA, Schlender KK, Larner J: A rapid filter paper assay for UDP glucose-glycogen glusyltransferase, including an improved biosynthesis of UDP[^{14}C]glucose. Anal Biochem 25:486-499, 1968

22. Lima FB, Bao S, Garvey WT: Biological actions of insulin are differentially regulated by glucose and insulin in primary cultured adipocytes. Diabetes 43:53-62, 1994

23. Treadway JL, Hargrove DM, Nardone NA, et al: Enhanced peripheral glucose utilization in transgenic mice expressing the human GLUT4 gene. J Biol Chem 269:29956-29961, 1994

24. Lusk G: The Elements of the Science of Nutrition (ed 3). Philadelphia, PA, Saunders, 1917

25. Koranyi LI, Bourey RE, Vuorinen-Markkola H, et al: Level of skeletal muscle glucose transporter protein correlates with insulin-stimulated whole body glucose disposal in man. Diabetologia 34:763-765, 1991

26. Dohm GL, Elton CW, Friedman JE, et al: Decreased expression of glucose transporter in muscle from insulin-resistant patients. Am J Physiol 260:E459-E463, 1990

27. Garvey WT, Maianu L, Hancock JA, et al: Gene expression of GLUT4 glucose transporters in skeletal muscle from insulin resistant patients with obesity, IGT, GDM, and NIDDM. Diabetes 41:465-475, 1992

28. Handberg A, Vaag A, Damsbo P, et al: Expression of insulin regulatable glucose transporters in skeletal muscle from type 2 (noninsulin-dependent) diabetic patients. Diabetologia 33:625-627, 1990

29. Hultman E: Studies on muscle metabolism of glycogen and active phosphate in man with special reference to exercise and diet. Scand J Clin Invest 19:1-63, 1967

30. Sidossis LS, Stuart CA, Shulman GI, et al: Glucose plus insulin regulate fat oxidation by controlling the rate of fatty acid entry into the mitochondria. J Clin Invest 98:2244-2250, 1996

31. Munger R, Temler E, Jallut D, et al: Correlations of glycogen synthase and phosphorylase activities with glycogen concentration in human muscle biopsies: Evidence for a double feedback mechanism regulating glycogen synthesis and breakdown. Metabolism 42:36-43, 1993