Increased muscle fatigability in GLUT-4-deficient mice

M. Gorselink,1 M. R. Drost,2 K. F. J. De Brouwer,1 G. Scharta,2 G. P. J. Van Kranenburg,2 T. H. M. Roemen,1 M. Van Bilser,1 M. J. Charron,3 and G. J. Van Der Vusse1

Departments of 1Physiology and 2Movement Sciences, University of Maastricht, 6200 MD Maastricht, The Netherlands; and 3Department of Biochemistry, Albert Einstein College of Medicine, Bronx, New York 10461

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Increased muscle fatigability in GLUT-4-deficient mice. Am J Physiol Endocrinol Metab 282: E348–E354, 2002; 10.1152/ajpendo.00085.2001.—GLUT-4 plays a predominant role in glucose uptake during muscle contraction. In the present study, we have investigated in mice whether disruption of the GLUT-4 gene affects isometric and shortening contractile performance of the dorsal flexor muscle complex in situ. Moreover, we have explored the hypothesis that lack of GLUT-4 enhances muscle fatigability. Isometric performance normalized to muscle mass during a single tetanic contraction did not differ between wild-type (WT) and GLUT-4-deficient [GLUT-4(−/−)] mice. Shortening contractions, however, revealed a significant 1.4-fold decrease in peak power per unit mass, most likely caused by the fiber-type transition from fast-glycolytic fibers (IIB) to fast-oxidative fibers (IIA) in GLUT-4(−/−) dorsal flexors. In addition, the resting glycogen content was significantly lower (34%) in the dorsal flexor complex of GLUT-4(−/−) mice than in WT mice. Moreover, the muscle complex of GLUT-4(−/−) mice showed enhanced susceptibility to fatigue, which may be related to the decline in the muscle carbohydrate store. The significant decrease in relative work output during the steady-state phase of the fatigue protocol suggests that energy supply via alternative routes is not capable to compensate fully for the lack of GLUT-4.

GLUCOSE IS A major fuel for contracting muscle fibers (6, 20). This substrate is supplied to the muscle fiber from extra- and intracellular sources, i.e., blood glucose pool and intracellular glycogen (12, 25). The uptake of glucose by skeletal muscle cells is facilitated by a family of membrane-associated glucose transporters (GLUTs; see Refs. 1, 6, and 16). Basal glucose uptake is mediated via the GLUT-1 isoform, whereas the bulk of glucose is primarily transported across the sarcolemma by the insulin- and contraction-regulatable glucose transporter GLUT-4 (5, 9, 18, 24, 28, 29). After uptake, glucose is metabolized to generate ATP or is stored as glycogen (2). During the initial phase of moderate-intensity exercise, skeletal muscle uses the intracellular glycogen store to meet its energy demand (23). It was recently shown that, during electrical stimulation, blood-borne glucose also serves as a suitable substrate during moderate-intensity exercise in mice (27). To enable detailed studies on the specific role of GLUT-4 in glucose homeostasis in general and in muscle mechanical performance in particular, mice with the GLUT-4 gene disrupted [GLUT-4(−/−)] were generated (17, 36).

Despite the importance of regulatable glucose transporters for muscle energy metabolism, information on the impact of GLUT-4 deficiency on muscle contractile behavior is scarce. It was recently reported that developed isometric tension during in vitro electrical stimulation of isolated extensor digitorum longus (EDL) muscle did not differ between wild-type (WT) and GLUT-4(−/−) mice (27). Extrapolation of these findings to the in vivo situation, however, should be done with caution, since the muscle fibers were studied isolated from their natural surroundings, and only isometric contractile function was studied.

In the present study, we have investigated whether disruption of the GLUT-4 gene affects muscular function in situ. To this end, we recently developed an experimental model to monitor isometric and shortening parameters of intact mouse skeletal muscle (10, 11). These parameters include torque development of the dorsal flexors during and the rate of relaxation after a single isometric tetanic contraction on the one hand and peak power and optimal and maximal shortening velocity on the other. Mass of the dorsal flexor complex, its fiber composition, and the tissue content of high-energy phosphates have been assessed to establish possible differences in muscle contractile behavior between WT and GLUT-4(−/−) mice. Moreover, we have explored the hypothesis that lack of GLUT-4 results in enhanced fatigability of the dorsal flexor complex subjected to a contraction protocol of moderate intensity. This hypothesis was based on the notion that, under normal conditions, glucose derived from both blood plasma and intracellular glycogen is an
important fuel for muscles contracting at moderate intensity (13, 23).

To assess muscle fatigability, the dorsal flexors of the anesthetized mouse were subjected to a series of 150 shortening contractions. The muscle complex was electrically stimulated at 125 Hz for 200 ms every 2,000 ms (tour duty cycle, 1). Moreover, the muscle glycogen content has been measured before and after the contraction protocol.

The present study showed that the dorsal flexors of GLUT-4(−/−) mice are more susceptible to failure. This phenotypic change was associated with a shift from fast-twitch glycolytic IIB fibers to fast-twitch oxidative IIA fibers and a substantial decline in resting glycogen content of the muscle complex under investigation.

METHODS

Animals. Male GLUT-4(−/−) mice, as described by Katz et al. (17), were used in the present study. Twelve-week-old male littermates (C57Bl/6) served as age-matched controls. Genotyping of the WT and GLUT-4(−/−) mice was performed via PCR analysis. Briefly, isolation of mouse tail DNA was performed with the DNeasy tissue kit (Qiagen, Hilden, Germany) according to the manufacturer’s instructions.

DNA (75 ng) was analyzed in a total volume of 25 μl containing 1× PCR buffer, 0.2 mM of each dNTP, 1.5 mM MgCl2, 24 nmol of each oligonucleotide primer, and 2.5 units Taq DNA polymerase, recombinant (Life Technologies, Rockville, MD). Two separate reactions were performed to establish the genotype. Primers against the neomycin cassette (forward primer, 5′-TGACTGCGAACAAGACAATCGGC-3′ and reverse primer, 5′-GTAGCCAAAGCTATGTCCTGATAGC-3′) were used to detect disrupted GLUT-4 allele(s). Amplification of this sequence (35 cycles of 30 s at 95°C, 30 s at 63°C, and 1 min at 72°C) resulted in a 650-bp fragment. To detect the (uninterrupted) WT GLUT-4 allele(s), primers were used spanning the target region, i.e., forward primer 5′-TCTTGTAGCCCGTGACT-3′ in exon 9 and reverse primer 5′-GCCGCAATAGTACACG-3′ in exon 10. For the WT allele, this resulted in an amplified fragment of 750 bp (35 cycles of 30 s at 95°C, 30 s at 60°C, 1 min at 72°C).

Surgical procedure. During the measurements of contractile properties, the animals were anesthetized with Halothane (Fluothane; Zeneca, Ridderkerk, The Netherlands), supplied in O2 and N2O (3:1, 1.5–2.0%) via a facemask through a flowmeter system (4.0 l/min; Medec, Montvalle, NJ). All experimental procedures were approved by the Institutional Animal Care and Use Committee of the University Maastricht and complied with the principles of laboratory animal care.

The anesthetized mice were positioned on a thermostatic platform (38.5 ± 0.1°C). After the skin was depilated locally, a small incision in the lateral part of the knee was made to expose the peroneal nerve.

A bipolar platinum electrode was carefully attached to the nerve to electrically stimulate the dorsal muscle complex (tibialis anterior muscle and EDL muscle) via a pulse generator (HSE 215/IZ). The position of the electrode was changed if a current of >1.0 mA was needed to obtain supramaximal muscle contraction. The electrode was attached to the skin with cyanoacrylate glue to prevent electrode displacement during the experimental period.

Experimental model. For proper determination of isotropic contractile properties of the intact dorsal flexor complex, the anesthetized mouse was attached to the measurement device via the hip and foot, as previously described in detail (11). Supramaximal stimulation current, required for full recruitment of the fibers of the muscle complex under investigation, was first assessed using between three and five isotropic double-twitch contractions (5-ms interval) with increasing current. Resting periods between the double twitches were 60 s. Optimal muscle length at optimal ankle angle was determined using nine double-twitch contractions at ankle angles between 30° dorsal flexion and 30° plantar flexion. All measurements were determined at optimal (isotropic contractions) or around (shortening contractions) optimal ankle angle. Maximal tetanic torque was determined during a 150-ms pulse train (125-Hz stimulation frequency). Torque signals were digitized and analyzed for maximal tetanic torque and half-relaxation time. Maximal torque was determined from the filtered torque signals (11 point moving average filter). The half-relaxation time, i.e., the time taken for torque to decline from 50 to 25% of its maximal value (7), was determined from the unfiltered torque signals. Thereafter, the isometric measurement device was replaced by the mouse ergometer, i.e., the device appropriate for measuring the characteristics of the skeletal muscle during shortening contractions (10). During this procedure, the optimal ankle angle was maintained. Shortening velocity was adjusted stepwise via variation in angular stroke and rotation frequency as indicated in Table 1. Stimulation frequency was adjusted to angular velocity (26).

Maximal torque during subsequent shortening contractions was defined as torque at the optimal ankle angle. The torque-velocity relation was fitted to a hyperbola described by the Hill (14) equation. Power was determined from the Hill curve and was calculated as the product of angular velocity and torque generated at this angular velocity. Optimal shortening velocity was defined as the value of shorten-

Table 1. Input parameters of the stimulation protocol for assessment of the torque-velocity relationship of intact dorsal flexors and velocity measured at optimal angle

<table>
<thead>
<tr>
<th>Input parameters</th>
<th>Chronological Order of Stimulation Conditions</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Frequency, Hz</td>
<td>1</td>
</tr>
<tr>
<td>Angular stroke, °</td>
<td>20</td>
</tr>
<tr>
<td>Stimulation frequency, Hz</td>
<td>125</td>
</tr>
<tr>
<td>Stimulation phase, °</td>
<td>145</td>
</tr>
<tr>
<td>Stimulation duration, ms</td>
<td>150</td>
</tr>
<tr>
<td>Velocity measured at optimal angle, °/s</td>
<td>129 ± 3</td>
</tr>
</tbody>
</table>

Values are means ± SD; n = 10 experiments.
increasing velocity at peak power. Maximal shortening velocity was determined as the value of the intercept of the Hill curve with the velocity axis. Data acquisition was performed at 1,000 Hz with an Apple Macintosh 7100 PowerPC with an 8-channel, 12-bit Lab-NB analog-to-digital conversion board (National Instruments, Mopac Expwy, Austin, TX) under Labview 3.1. Postprocessing of the torque data was performed with Matlab 5.2.1 (The Math Works, Natick, MA).

**Fatigue protocol.** After a 10-min recovery period after the determination of basal isometric and shortening properties, dorsal flexors were subjected to a series of 150 shortening contractions. The muscle complex was electrically stimulated at 125 Hz for 200 ms every 2,000 ms (tour duty cycle, 1), at 1 Hz angular frequency with a 20° stroke. In this way, electrically stimulated and nonstimulated strokes alternated.

The torque output measured during electrical stimulation equals the sum of frictional torque, inertial torque, passive muscle torque, and the active muscle torque. The active torque of the muscle contraction was calculated by subtraction of the torque measured during a nonstimulated stroke (frictional torque, inertial torque, and passive torque) from the torque signal measured in a stimulated stroke (total torque). Absolute work output of the individual shortening contractions was calculated by integrating active torque over angle. Specific work is defined as absolute work per unit dorsal flexor muscle mass. Relative work during the course of the fatigue protocol is work normalized to work output during the first contraction of the series.

**Tissue sampling.** A subset of mice was used to determine glycogen content in the dorsal flexor complex after the 150 shortening contractions. The muscle complex of the nonstimulated contralateral leg was used as a control. These contralateral muscles were also used for analysis of resting high-energy phosphate levels. A second set of contralateral muscles was frozen in melting isopentane for light microscopic analysis, i.e., myosin ATPase staining and determination of the cross-sectional areas of the muscle fibers. The tissue samples were stored at −80°C until analysis.

**Biochemical and histochemical assays.** Before glyco
gen and high-energy phosphate analysis, muscle complexes were freeze-dried overnight at −30°C. High-energy phosphates and related compounds were assessed by HPLC (31, 33). Glycogen content was measured fluorimetrically in HCl extracts of the dorsal flexors and was expressed as micromole glycogen unit per gram dry weight (19). For fiber-type distribution and fiber diameter analysis, the midbelly regions of the dorsal flexor complex were frozen in melting isopentane. Frozen dorsal flexor sections were cryosectioned (10 μm) at −20°C, thaw-mounted on glass slides, and air-dried until analysis for myosin ATPase staining (4) and fiber diameter measurements.

Briefly, sections were immersed in acetate buffer at pH 4.5, washed in Tris buffer (pH 7.8), and subsequently rinsed in distilled water. Sections were incubated in fresh ATP-glycine buffer (pH 9.4), quenched in CaCl₂ (1%), washed in distilled water, and incubated in CoCl₂ (1%). Thereafter, the sections were incubated in ammonium sulfite and rinsed in distilled water. Slides were dehydrated in an ethanol xylol series and embedded in entellan. Sections were photographed microscopically (Leica, Rijswijk, The Netherlands), and a distinction was made between the tibialis anterior and EDL muscle. One hundred fibers were counted for each muscle, and fibers were classified as type I, IIA, or IIB (4). Fibers were classified miscellaneous when no differentiation could be made between type IIA and IIB fibers. Cross-sectional area of the distinct fibers was determined from the smallest diameter, assuming ellipsoid cross-sections.

**Statistics.** Data are expressed as means and SD. Differences in mechanical parameters, high-energy phosphates, and glycogen content and fiber characteristics between GLUT-4(−/−) mice and WT were analyzed using the nonparametric Mann-Whitney U-test. Differences in work output at individual times during the fatigue protocol were analyzed using repeated-measures ANOVA with Scheffe’s post hoc analyses to identify differences between GLUT-4(−/−) and WT mice. Increased fatigability in GLUT-4(−/−) mice was tested as the difference in mean relative work output over the last 40 contractions of the fatigue protocol by a one-sided Mann-Whitney U-test. Differences were considered significant at P < 0.05. SPSS 9.0 (SPSS Benelux, Gorinchem, The Netherlands) was used for statistical analyses.

**RESULTS**

The values of body, heart, and dorsal flexor mass are shown in Table 2. Body mass of GLUT-4(−/−) mice was significantly lower than WT littermates. Dorsal flexor mass of GLUT-4(−/−) mice amounted to 59.2 ± 5.8 mg, which was significantly lower than the dorsal flexors of the WT (69.3 ± 9.7 mg). In contrast, heart mass of the GLUT-4(−/−) mice showed a significant 1.4-fold increase compared with WT.

**Single isometric and shortening contraction.** Maximal torque developed during a single isometric tetanic contraction of the dorsal flexors at a 125-Hz stimulation frequency was 1.3-fold higher (P < 0.05) in WT than in GLUT-4(−/−) mice (Table 3). However, when the maximal torque was normalized to dorsal flexor mass, the values of GLUT-4(−/−) mice did not differ from their WT littermates. Half-relaxation time after a maximal tetanic contraction was not significantly different between GLUT-4(−/−) and WT.

Shortening properties of the dorsal flexors were characterized in terms of absolute and specific peak power, optimal shortening velocity, and maximal shortening velocity (Table 3). Absolute peak power was significantly lower in GLUT-4(−/−) muscles than in WT. Even after normalization to muscle mass, specific peak power was significantly reduced in GLUT-4(−/−) mice. Optimal and maximal shortening velocity of the muscle complex did not differ between GLUT-4(−/−) and WT.

**Fatigue protocol.** When the dorsal flexors were subjected to a series of 150 shortening contractions, three different phases in the specific work output could be observed (Fig. 1A). In WT, specific work output re-

| Table 2. Mass characteristics of WT and GLUT-4(−/−) mice |
|-----------------|-----------------|
|                 | WT              | GLUT-4(−/−) |
| Body mass, g    | 26.2 ± 2.0      | 22.5 ± 1.2<sup>a</sup> |
| Dorsal flexor mass, mg | 69.3 ± 9.7 | 59.2 ± 5.8<sup>a</sup> |
| Heart mass, mg  | 120 ± 11        | 165 ± 16<sup>a</sup> |

Data are means ± SD; n = 10 mice in each group. WT, wild type; GLUT-4(−/−), GLUT-4-deficient. *P < 0.05, statistical difference between WT and GLUT-4(−/−).
TABLE 3. Mechanical characteristics of a single isometric contraction and of shortening contractions of intact dorsal flexors of WT and GLUT-4(−/−) mice

<table>
<thead>
<tr>
<th></th>
<th>WT</th>
<th>GLUT-4(−/−)</th>
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<tbody>
<tr>
<td><strong>Isometric contraction</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Absolute torque, mN·m</td>
<td>2.2 ± 0.4</td>
<td>1.7 ± 0.2*</td>
</tr>
<tr>
<td>Specific torque, mN·m⁻¹·g wet wt⁻¹</td>
<td>33.5 ± 3.6</td>
<td>30.2 ± 6.1</td>
</tr>
<tr>
<td>Half-relaxation time, ms</td>
<td>8.6 ± 1.6</td>
<td>9.7 ± 1.2</td>
</tr>
<tr>
<td><strong>Shortening contractions</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Absolute peak power, mW</td>
<td>25.5 ± 7.7</td>
<td>15.5 ± 6.3*</td>
</tr>
<tr>
<td>Specific peak power, mW/g wet wt</td>
<td>360 ± 90</td>
<td>260 ± 100*</td>
</tr>
<tr>
<td>V_opt, °/s</td>
<td>1,330 ± 300</td>
<td>1,120 ± 310</td>
</tr>
<tr>
<td>V_max, °/s</td>
<td>2,860 ± 830</td>
<td>2,580 ± 700</td>
</tr>
</tbody>
</table>

Data are means ± SD; n = 10 mice in each group. Isometric muscle properties were assessed at 125 Hz, 0.5 ms, and supramaximal stimulation current. Duration of the tetanic contraction was 150 ms. Shortening properties were assessed using the stimulation parameters as indicated in Table 1. V_opt, optimal angular velocity; V_max, maximal angular velocity. *P < 0.05, statistical difference between WT and GLUT-4(−/−).

Fig. 1. Dorsal flexor work output normalized to muscle mass (specific work; A) and as a percentage of initial work output (relative work; B) during 150 shortening contractions in wild-type (WT; ⋄) and GLUT-4-deficient (GLUT-4(−/−); □) mice. The total duration of the contraction protocol amounted to 300 s. The muscles were electrically stimulated at 125 Hz for 200 ms every 2,000 ms. Symbols and vertical lines refer to means and SD, respectively (n = 10 in each group). All data points of specific work (A) were statistically lower in GLUT-4(−/−) than in WT (P < 0.05). *P < 0.05, statistical differences between WT and GLUT-4(−/−). w.w., Wet weight.

Fig. 2. Glycogen content in dorsal flexor complex of WT and GLUT-4(−/−) mice. Postcontraction values (n = 6) were obtained in muscles freeze-clamped at the end of the fatigue protocol. Resting values were from the contralateral muscle group of the same animal. *Significantly different from corresponding resting values. #Significantly different from corresponding glycogen content in WT. dw, Dry weight.

The content of phosphocreatine and creatine in resting WT and GLUT-4(−/−) dorsal flexors amounted to 90.1 ± 9.3 and 91.9 ± 10.7 and to 76.8 ± 17.5 and 82.3 ± 5.6 μmol/g dry wt, respectively. The ATP, ADP, and AMP content in WT and GLUT-4(−/−) muscle amounted to 32.9 ± 2.4 and 31.4 ± 2.8, 4.9 ± 0.9 and 4.8 ± 0.6, and 0.4 ± 0.0 and 0.5 ± 0.1 μmol/g dry wt, respectively. The resting tissue content of inosine monophosphate was 0.4 ± 0.1 and 0.5 ± 0.1 μmol/g dry wt in WT and GLUT-4(−/−) mice, respectively. The differences between WT and GLUT-4(−/−) were not significant.
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Fiber-type distribution in the tibialis anterior and EDL muscle in WT and GLUT-4(-/-) mice

<table>
<thead>
<tr>
<th>Fiber Type</th>
<th>Relative Number, % WT</th>
<th>Fiber CSA, µm² WT</th>
<th>Relative Muscle CSA, % WT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tibialis anterior</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Type IIA</td>
<td>15 ± 6</td>
<td>650 ± 250</td>
<td>7 ± 3</td>
</tr>
<tr>
<td>Type IIB</td>
<td>83 ± 5</td>
<td>1,230 ± 290</td>
<td>90 ± 2</td>
</tr>
<tr>
<td>Miscellaneous</td>
<td>2 ± 1</td>
<td>740 ± 300</td>
<td>3 ± 2</td>
</tr>
<tr>
<td>EDL muscle</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Type IIA</td>
<td>8 ± 1</td>
<td>480 ± 140</td>
<td>3 ± 2</td>
</tr>
<tr>
<td>Type IIB</td>
<td>18 ± 4</td>
<td>650 ± 290</td>
<td>11 ± 7</td>
</tr>
<tr>
<td>Type Miscellaneous</td>
<td>70 ± 5</td>
<td>1,310 ± 200</td>
<td>82 ± 10</td>
</tr>
</tbody>
</table>

Data are means ± SD; n = 4 mice in each group. EDL, extensor digitorum longus. The relative number refers to the number of fibers of each type/100 fibers studied. Cross-sectional area (CSA) of each fiber type is expressed both as CSA of single fibers and as a percentage of the area occupied by each type divided by the area occupied by the hundred fibers studied. Fibers are typed miscellaneous when no distinction could be made between IIA and IIB fibers. *P < 0.05, statistical difference between WT and GLUT-4(-/-).

Fiber-type composition and fiber cross-sectional area. Both in tibialis anterior and EDL muscle, GLUT-4(-/-) mice showed a significant decline in relative number of IIB fibers compared with the fiber distribution in the corresponding WT muscles (Table 4). In contrast, the relative number of type IIA fibers increased in GLUT-4(-/-) muscle. Type IIA fibers showed a relatively higher contribution to the muscle cross-sectional area in GLUT-4(-/-) at the expense of type IIB fibers. The absolute cross-sectional area of single fibers belonging to the respective subtypes did not differ between WT and GLUT-4(-/-), neither in tibialis anterior nor in EDL muscle.

Discussion

The bulk of glucose taken up by skeletal muscle tissue is facilitated by GLUT-1 and GLUT-4, where GLUT-4 is the predominant glucose transporter in muscle cells after a contraction stimulus. Here we provide more insight into the consequence of GLUT-4 deficiency for contractile performance of the intact dorsal flexors during a single isometric and shortening contraction. Moreover, we have investigated whether GLUT-4 deficiency results in enhanced fatigability of the dorsal flexors in their natural surrounding during repetitive shortening contractions.

Single isometric tetanic contraction. The present findings show that the maximal tetanic torque output in dorsal flexors of GLUT-4(-/-) is significantly declined compared with age-matched WT. Interestingly, the mass of the dorsal flexors of GLUT-4(-/-) was also significantly declined compared with WT. After normalization of tetanic torque to muscle mass, no significant difference between GLUT-4(-/-) and WT could be observed (Table 3). These results corroborate earlier findings in an isolated muscle preparation, which showed that isometric peak tension per unit muscle mass was comparable between GLUT-4(-/-) and control mice (27). The content of high-energy phosphates was found to be comparable between resting WT and GLUT-4(-/-) dorsal flexors. This observation underlines the notion that the decrease in tetanic torque in GLUT-4(-/-) is mainly caused by decreased muscle mass rather than a decline in available high-energy phosphates.

Muscle performance during shortening contractions. In contrast to contractile performance during isometric contractions, the maximal peak power, even after normalization to muscle mass, was significantly lower in GLUT-4(-/-) muscle than in WT, i.e., 260 vs. 360 mW/g wet wt. Peak power output depends, among others, on the fiber composition of the muscle under investigation. This notion is supported by data in literature about differences in power output of distinct types of skinned fibers (3, 21, 32). Bottinelli and co-workers (3) reported that peak power of rat skinned fibers, measured at 12°C, was substantially higher for fast-twitch glycolytic (IIB) fibers than fast-twitch oxidative (IIA) fibers. Because in both the tibialis anterior and the EDL muscle of GLUT-4(-/-) mice the relative contribution of IIA fibers to the muscle cross-sectional area increased at the expense of IIB fibers, it is tempting to state that the change in fiber-type composition in GLUT-4(-/-) dorsal flexors contributes to the lower peak power per unit muscle mass in GLUT-4(-/-).

Tissue glycogen content. In the present study, an appreciably lower glycogen content was observed in the dorsal flexors, consisting of the tibialis anterior and EDL muscle, of male GLUT-4(-/-) mice compared with age-matched WT. From the literature, no consistent pattern emerges on this subject.

For instance, the glycogen content in the gastrocnemius muscle of GLUT-4(-/-) mice was unchanged (27) but declined significantly in GLUT-4(-/-) soleus muscle (35). However, in female GLUT-4(-/-) mice the glycogen content in soleus muscle was significantly increased (30). Muscle-specific GLUT-4 knockout mice showed a normal resting glycogen level in both soleus and EDL muscle (22). Similar findings in EDL muscle were made in generalized GLUT-4(-/-) mice (35). They are, however, at variance with recent observations showing that in EDL muscle of both female and male GLUT-4(-/-) mice glycogen content is decreased (30). The present findings are in line with the latter observation indicating that in GLUT-4(-/-) dorsal flex-
ors, consisting of tibialis and EDL muscle, glycogen content is significantly lower than in WT. As discussed below, the decline in resting glycogen content may influence the resistance against fatigue of contracting muscle fibers.

Fiber-type composition. A striking observation in this study is the decline in the relative number of type IIB fibers and the increase in the relative number of type IIA fibers in both EDL and tibialis anterior muscle of GLUT-4(+/−) mice. These changes resulted in an increased contribution of type IIA and a decreased contribution of type IIB fibers to the cross-sectional area of the muscle. Of interest is the finding that the cross-sectional area of individual muscle fibers did not change. At present, it is unclear what caused the change in the fiber-type composition of GLUT-4(+/−) muscles. Taking into account that the overall muscle mass is lower in GLUT-4(+/−) than in age-matched WT one may assume atrophy specifically of type IIB fibers. In the case of atrophy, one should expect a significant decline of the cross-sectional area of the affected muscle cells. Because the cross-sectional area of IIB fibers in the muscles under investigation tended to increase rather than decrease, atrophy of type IIB fiber is less likely. Alternatively, the decline observed in the number of type IIB fibers may also be caused by a decline in IIB fiber formation during prenatal development of muscle tissue or by atrophy during an early stage of postnatal life as a direct or indirect consequence of the genetic defect. Theoretically, changes in physical activity may also contribute to the altered fiber-type composition in GLUT-4(+/−) muscle. However, we consider this possible cause for the shift from type IIB to IIA fibers less likely since previous studies have shown that relatively high training levels are required to evoke a change in fiber-type composition (34), but we failed to notice a difference in spontaneous physical activity of the GLUT-4(+/−) mice in their cages (unpublished observations).

It cannot be excluded that the change in fiber-type composition is related to the change in glucose metabolism. The fast-twitch, glycolytic type IIB fibers heavily rely on glucose as a substrate for energy conversion. Because of impaired glucose uptake during physical exercise, an unfavorable condition might be created for glycolytic type IIB muscle cells. Because the oxidative type IIA fibers also oxidize alternative substrates for energy conversion, they may be less sensitive to the decline in capacity to extract glucose from extracellular sources. It is tempting to speculate that the differences in metabolic properties between type IIA and IIB fibers benefits the former, resulting in a relative increase in the number of type IIA fibers in GLUT-4(+/−) muscle.

Fatigue protocol. When subjected to the fatigue protocol, WT muscles showed a typical pattern in both specific and relative work output. After an initial phase of stable work output during the first 25 shortening contractions, work output declined rapidly (phase 2) until another phase of steady-state work output was reached approximately at the 110th contraction (phase 3). GLUT-4(+/−) muscles were found to lack the first phase of relatively stable work output. In contrast to WT, in GLUT-4(−/−) signs of fatigue were immediately apparent after the onset of the shortening contraction protocol. On average, the rate of decline in work output was comparable between GLUT-4(−/−) and WT mice (phase 2). Numerous studies have been performed to elucidate the mechanisms underlying muscular fatigue (8). Among others, evidence is accumulating that the amount of endogenous glycogen available for energy conversion and the occurrence of fatigue are closely related (1, 4, 6). One may therefore speculate that the low glycogen content in GLUT-4(−/−) dorsal flexors at the start of the shortening contraction protocol and the apparent inability of the muscle cells to break into the carbohydrate store contribute to the absence of the initial phase of constant work output and the early onset of fatigue.

Of interest is the observation that the steady-state work output, both specific and relative, is significantly lower in GLUT-4(+/−) than in WT mice. This finding is not in line with the observation of Jarvis and colleagues (15) that muscles with a high IIA-to-IIB ratio are relatively more fatigue resistant, since the contribution of IIA fibers is enhanced and that of type IIB is decreased in GLUT-4(−/−) dorsal flexors (Table 4). Obviously, other factors are overruling the effect of fiber-type composition on the susceptibility toward fatigue in GLUT-4(+/−) muscles. One of these factors may be the level of energy conversion in the contracting muscle cell during the third phase of steady work output. Recent studies of Ryder and coworkers (27) indicated that the uptake of 2-deoxyglucose is substantially lower in contracting GLUT-4(+/−) muscle than in WT. Extrapolating this finding to the present study indicates that energy supply is most likely reduced in GLUT-4(+/−) muscles during the third phase of the fatigue protocol because of impaired extraction of glucose from the extracellular compartment. The uptake and utilization of alternative substrates such as fatty acids are most likely insufficient to compensate fully for the impact of GLUT-4 deficiency on muscle glucose extraction during shortening contractions.

The observation that absolute work output during the whole course of the contraction series was significantly lower in GLUT-4(−/−) than in WT strongly suggests a decrease in exercise capacity in GLUT-4(−/−) animals. This notion is in support of a preliminary report that muscle-specific GLUT-4 knockout mice perform significantly less in a treadmill test than age-matched WT mice (22).

In summary, the present findings indicate that disruption of the GLUT-4 gene, encoding for the predominant glucose transporter in muscle tissue, affects the mechanical performance of the dorsal flexors during shortening contractions. Peak power output showed a significant decline when normalized to muscle mass. The relative number of glycolytic IIB fibers decreased and that of oxidative IIA fibers increased in GLUT-4(−/−) muscle. The resistance toward fatigue was substantially decreased in GLUT-4(−/−) dorsal flexors. The reduced contractile performance may be related to
the low glycogen content in the GLUT-4(--/--) muscle 
before contraction.

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