

# Ablating the protein TBC1D1 impairs contraction-induced sarcolemmal glucose transporter 4 redistribution but not insulin-mediated responses in rats

Received for publication, July 14, 2017, and in revised form, August 10, 2017 Published, Papers in Press, August 14, 2017, DOI 10.1074/jbc.M117.806786

Jamie Whitfield<sup>‡</sup>, Sabina Paglialunga<sup>‡</sup>, Brennan K. Smith<sup>§</sup>, Paula M. Miotto<sup>‡</sup>, Genevieve Simnett<sup>‡</sup>, Holly L. Robson<sup>‡</sup>, Swati S. Jain<sup>‡</sup>, Eric A. F. Herbst<sup>‡</sup>, Eric M. Desjardins<sup>§</sup>, David J. Dyck<sup>‡</sup>, Lawrence L. Spriet<sup>‡</sup>, Gregory R. Steinberg<sup>§¶1</sup>, and Graham P. Holloway<sup>‡2</sup>

From the <sup>‡</sup>Department of Human Health and Nutritional Sciences, University of Guelph, Guelph, Ontario N1G 2W1, Canada and <sup>§</sup>Division of Endocrinology and Metabolism, Department of Medicine, and <sup>¶</sup>Department of Biochemistry and Biomedical Sciences, McMaster University, Hamilton, Ontario L8N 3Z5, Canada

Edited by Jeffrey E. Pessin

TBC1 domain family member 1 (TBC1D1), a Rab GTPaseactivating protein and paralogue of Akt substrate of 160 kDa (AS160), has been implicated in both insulin- and 5-aminoimidazole-4-carboxamide ribonucleotide formyltransferase/IMP cyclohydrolase-mediated glucose transporter type 4 (GLUT4) translocation. However, the role of TBC1D1 in contracting muscle remains ambiguous. We therefore explored the metabolic consequence of ablating TBC1D1 in both resting and contracting skeletal muscles, utilizing a rat TBC1D1 KO model. Although insulin administration rapidly increased (p < 0.05) plasma membrane GLUT4 content in both red and white gastrocnemius muscles, the TBC1D1 ablation did not alter this response nor did it affect whole-body insulin tolerance, suggesting that TBC1D1 is not required for insulin-induced GLUT4 trafficking events. Consistent with findings in other models of altered TBC1D1 protein levels, whole-animal and ex vivo skeletal muscle fat oxidation was increased in the TBC1D1 KO rats. Although there was no change in mitochondrial content in the KO rats, maximal ADP-stimulated respiration was higher in permeabilized muscle fibers, which may contribute to the increased reliance on fatty acids in resting KO animals. Despite this increase in mitochondrial oxidative capacity, run time to exhaustion at various intensities was impaired in the KO rats. Moreover, contraction-induced increases in sarcolemmal GLUT4 content and glucose uptake were lower in the white gastrocnemius of the KO animals. Altogether, our results highlight a critical role for TBC1D1 in exercise tolerance and contractionmediated translocation of GLUT4 to the plasma membrane in skeletal muscle.

Skeletal muscle glucose uptake in response to either insulin or contraction is a highly regulated process that results in facilitated diffusion through glucose transporter 4 (GLUT4).<sup>3</sup> The effects of these metabolic stimuli are additive (1, 2), ultimately resulting in the translocation of GLUT4 from intracellular depots to the sarcolemmal membrane. Akt2 (also known as protein kinase B (PKB)) is a proximal member of the insulin signaling cascade, and its phosphorylation is an essential step in insulin-stimulated glucose transport (3), whereas contractionmediated translocation of GLUT4 is believed to occur via a distinct molecular mechanism (2, 4–7). Although the terminal downstream factors of both of these cascades are currently not fully understood, the binding of Rab-GTPases to GLUT4-containing vesicles is thought to be critical for translocation in both processes (8).

Rab GTPase-activating proteins (GAPs) are members of the Ras superfamily of monomeric G-proteins that regulate numerous membrane trafficking events, including vesicle formation and movement along actin and tubulin networks, as well as membrane fusion. Two protein members of the TBC1 (Tre-2, BUB2, CDC16) domain family member 1 (TBC1D1) and TBC1D4 (also known as AS160) are functional GAPs that are believed to prevent GLUT4 translocation by maintaining Rabs in their inactive GDP-bound state (8). Although AS160 and TBC1D1 are only 61% similar in sequence over their entire length, their Rab GAP domains are 91% similar and have the same Rab substrate specificity (9). Upstream intracellular signaling events (e.g. insulin stimulation or contraction) result in the phosphorylation of AS160 and TBC1D1 and binding of these proteins to the molecular adaptor protein 14-3-3. Ultimately, these events enable GAPs to return to an active GTPbound state and initiate vesicle exocytosis and trafficking of GLUT4 transporters (8).

This work was supported in part by the Natural Sciences and Engineering Research Council of Canada (NSERC) (to L. L. S. and G. P. H.), NSERC graduate scholarships (to P. M. M. and E. A. F. H.), and Canadian Institute for Health Research Grant 125980-1 (to G. R. S.). Infrastructure was purchased with the assistance of the Canadian Foundation for Innovation (to G. P. H.) as well as the Ontario Research Fund (to G. P. H.). The authors declare that they have no conflicts of interest with the contents of this article.

<sup>&</sup>lt;sup>1</sup> Supported by a Canada Research Chair in Metabolism and Obesity and the J. Bruce Duncan Chair in Metabolic Diseases.

<sup>&</sup>lt;sup>2</sup> To whom correspondence should be addressed: Human Health and Nutritional Sciences, University of Guelph, 50 Stone Rd. East, Guelph, Ontario N1G 2W1, Canada. Tel.: 519-824-4120 (ext. 53688); Fax: 519-763-5902; E-mail: ghollowa@uoguelph.ca.

<sup>&</sup>lt;sup>3</sup> The abbreviations used are: GLUT4, glucose transporter type 4; GAP, GTPase-activating protein; TBC1D1, TBC1 (Tre-2, BUB2, CDC16) domain family member 1; AS160, Akt substrate of 160 kDa; AMPK, 5'-AMP-activated protein kinase; β-HAD, β-hydroxyacyl-CoA dehydrogenase; FAT, fatty acid transporter; GTT, glucose tolerance test; ITT, insulin tolerance test; RER, respiratory exchange ratio; EDL, extensor digitorum longus; P-CoA, palmitoyl-CoA; ACC, acetyl-CoA carboxylase; 2-DG, 2-deoxyglucose; PCr, phosphocreatine.

Table 1			
Animal characterization	on and tissue screen o	of WT and TBC1D	1 KO animals

The data are expressed as means  $\pm$ S.E. (error bars) (n = 3-5). WAT, white adipose tissue.

		Animal characteristic						
Genotype	Weight	Soleus	EDL	Heart	Heart/tibial length	Liver	WAT	
	g	g	g	g	$gcm^{-1}$	g	g	
WT	$409.80 \pm 13.42$	$0.195 \pm 0.04$	$0.170 \pm 0.01$	$1.09 \pm 0.03$	$0.249 \pm 0.01$	$9.48 \pm 0.78$	$5.89 \pm 0.39$	
KO	$353.80 \pm 40.52$	$0.167\pm0.01$	$0.155\pm0.01$	$1.10\pm0.09$	$0.254\pm0.02$	$9.69 \pm 1.06$	$6.34 \pm 1.28$	

Work in cell lines has shown that deletion of TBC1D1 increases GLUT4 translocation to the plasma membrane (10), whereas overexpression inhibits it (9, 11), further supporting the notion that TBC1D1 plays a role in the control of GLUT4 translocation. Paradoxically, results in animals lacking TBC1D1 show no difference in whole-body glucose or insulin tolerance, whereas in vivo maximal insulin-stimulated skeletal muscle glucose uptake is impaired (12, 13), which is contradictory to the proposed mechanism of action for this protein in glucose handling and GLUT4 trafficking. However, both congenic mice lacking TBC1D1 (12) and conventional TBC1D1 knock-out (KO) mice (13, 14) display an  $\sim$ 50% reduction in total GLUT4 protein, which by itself has been shown to impair in vitro glucose uptake without altering whole-body glucose tolerance (15). Therefore, one possible explanation for the decrease in glucose transport in TBC1D1-deficient mice is the decrease in GLUT4 protein content, making interpretations on the functional role of TBC1D1 with respect to intracellular trafficking difficult. Nevertheless, the decreased GLUT4 content suggests that TBC1D1 influences glucose homeostasis and may represent a compensatory attempt by the cell to maintain glucose homeostasis and limit glucose flux into the muscle.

More recently, work in both rodents (16–18) and humans (17, 19) has shown that TBC1D1 may play a critical role in the signaling cascade in response to skeletal muscle contraction with TBC1D1 a downstream target of 5'-AMP-activated protein kinase (AMPK). Contraction, but not insulin stimulation, has been shown to increase binding of 14-3-3 to TBC1D1 in an AMPK-dependent manner in both rodents (16) and humans (17), a process that has been shown to be critical in the regulation of GLUT4 trafficking by AS160 (20, 21). Furthermore, phosphorylation of TBC1D1 via AMPK leads to a greater decrease in electrophoretic mobility compared with Akt, suggesting that AMPK is a more robust regulator of this protein than insulin (18).

Although the precise role of TBC1D1 in glucose transport appears uncertain, one consistent finding among cell and animal studies is its effect on lipid oxidation. Several models have shown alterations in lipid oxidation as a result of TBC1D1 deficiency. Specifically, TBC1D1 ablation *in vitro* and *in vivo* increases fatty acid oxidation (13, 22), and conversely, overexpression of TBC1D1 reduces skeletal muscle palmitate oxidation (22, 23) and  $\beta$ -hydroxyacyl-CoA dehydrogenase ( $\beta$ -HAD) activity (23). However, the mechanism of action resulting in up-regulated fatty acid oxidation remains unknown. The fatty acid transporter FAT/CD36 has been shown to translocate in response to both insulin and contraction (24) and is critical in the regulation of skeletal muscle fuel selection (25), but whether this occurs via a vesicle-dependent mechanism involving TBC1D1 remains to be determined.

Altogether, state-of-the art studies have provided compelling evidence that TBC1D1 ablation impairs glucose uptake and increases fatty acid oxidation. However, the current KO mouse models display a marked reduction in GLUT4 content, which by itself may also convey a similar phenotype (*i.e.* impaired glucose uptake, greater reliance on fatty acids, and impairment in exercise capacity), making interpretations difficult. Therefore, we aimed to characterize the role of TBC1D1 in a previously unexamined KO rat model that lacks a compensatory reduction in GLUT4 content. Using this model, we provide evidence that TBC1D1 regulates contraction but not insulin-mediated GLUT4 translocation in glycolytic muscles and subsequently decreases exercise capacity. These results appear specific to GLUT4 as FAT/CD36 trafficking was not altered. Taken together, these results provide support for a role of TBC1D1 in intracellular GLUT4 trafficking in response to contraction but not insulin stimulation.

#### Results

#### Tissue screen and animal characterization

We first characterized TBC1D1 KO animals to determine whether they displayed phenotypic differences compared with wild-type (WT) animals (Table 1). There were no differences in animal or tissue weights or in heart size normalized to tibial length. TBC1D1 deficiency was confirmed by screening for TBC1D1 protein in skeletal muscle homogenate. KO animals did not express TBC1D1 protein in red or white muscle; however, they had similar levels of AS160, Akt2, and FAT/CD36 (Fig. 1*A*). We also assessed GLUT4 protein content, which was not different compared with WT rats in red or white muscle (Fig. 1, *B* and *C*).

#### Skeletal muscle response to insulin

Given the unaltered GLUT4 content in this KO model and the proposed role of TBC1D1 in glucose homeostasis, we first examined the effect of ablating this protein on glucose handling at the whole-body level. In support of previous work demonstrating that TBC1D1 influences beta cell mass/stability (26, 27), TBC1D1 KO animals displayed impaired glucose clearance during an intraperitoneal glucose tolerance test (GTT) (Fig. 2*A*) as demonstrated by an increase in the area under the curve (p <0.05; Fig. 2*B*). However, there was no difference between TBC1D1 WT and KO animals during an intraperitoneal insulin tolerance test (ITT), suggesting that peripheral insulin sensitivity is intact in this model (Fig. 2, *C* and *D*). Consistent with similarwhole-bodyinsulinsensitivity,insulinincreasedthephosphorylation of Akt2 by ~3-fold in both red and white muscles in both WT and KO animals (Fig. 3, *A* and *B*). Total muscle





Figure 1. Tissue screen for various protein targets (A) and quantified GLUT4 protein content in skeletal muscle homogenate (B and C) (n = 6). The data are expressed as means  $\pm$  S.E. (*error bars*). RG, red gastrocnemius; WG, white gastrocnemius.



**Figure 2. TBC1D1 animals display impaired glucose tolerance.** Glucose levels during an intraperitoneal glucose (*A*) or insulin (*C*) tolerance test are shown. Area under the curve (*AUC*) during glucose (*B*) and insulin (*D*) tolerance tests was calculated for WT and KO animals (n = 5). The data are expressed as means  $\pm$  S.E. M. (*error bars*). \*, p < 0.05; \*\*, p < 0.01;  $\pm, p < 0.001$  versus WT.

GLUT4 expression and insulin-mediated GLUT4 trafficking in giant sarcolemmal vesicles was also unaltered between genotypes (Fig. 3, *C* and *D*). Collectively, these data suggest that TBC1D1 is not required for insulin-stimulated glucose uptake in skeletal muscle.

#### Substrate utilization

As alterations in TBC1D1 protein have been associated with changes in fat metabolism, we next examined fuel utilization *in vivo*. In the present study and consistent with previous reports in murine models (13, 22), TBC1D1 KO rats displayed a





**Figure 3. GLUT4 translocation in response to insulin.** Basal and insulin stimulated phosphorylated Akt (Ser-473) protein content in red (*A*) and white (*B*) gastrocnemius muscle. Basal and insulin stimulated plasma membrane GLUT4 in (*C*) red and (*D*) white gastrocnemius muscle. Ponceau staining of the membrane was used as a loading control. The data are expressed as means  $\pm$  S.E. M. (*error bars*). \*, *p* < 0.05, main effect *versus* basal. *pAkt*, phosphorylated Akt; *tAkt*, total Akt.

decreased respiratory exchange ratio (RER), and therefore calculated fat oxidation was increased whereas carbohydrate oxidation was concomitantly decreased in both the light and dark cycle (Fig. 4, A-C; p < 0.05). These responses represent a shift in fuel preference as there was no change in total energy expenditure (Fig. 4*D*). The increase in fatty acid oxidation was also observed in *ex vivo* experiments in isolated EDL muscle strips. Specifically, palmitate oxidation was increased by 36% (Fig. 4*E*; p < 0.05). In contrast, there was no change in glucose oxidation (Fig. 4*F*) in KO animals.

#### Mitochondrial respiration

Given this alteration in skeletal muscle fatty acid oxidation, we next examined whether the KO animals displayed a change in mitochondrial content or respiratory function. TBC1D1 KO animals displayed increased ADP-stimulated oxygen consumption in permeabilized muscle fibers (Fig. 5*A*; p < 0.05); however, there was no change in lipid-supported respiration (palmitoyl-CoA (P-CoA); Fig. 5*A*, *right y axis*). To determine whether this functional increase was due to a change in mitochondrial protein content, we digested the fibers used for oxygen consumption measurements for assessment via Western blotting. Similar to previous work in TBC1D1 KO mice (14), mitochondrial content in both digested fibers (Fig. 5*B*) and muscle homogenate (Fig. 5, *C* and *D*) was similar between WT and KO animals. Furthermore, there was no difference in basal  $\beta$ -HAD enzyme activity in white (WT, 5.10  $\pm$  1.04

 $\mu$ mol·min<sup>-1</sup>·g<sup>-1</sup> wet weight; KO, 4.33 ± 0.63  $\mu$ mol·min<sup>-1</sup>·g<sup>-1</sup> wet weight; n = 6, p = 0.30) or red muscle (WT, 14.56 ± 1.77  $\mu$ mol·min<sup>-1</sup>·g<sup>-1</sup> wet weight; KO, 13.95 ± 1.37  $\mu$ mol· min<sup>-1</sup>·g<sup>-1</sup> wet weight; n = 6, p = 0.77) between genotypes. To further examine the effects of ablating TBC1D1 on oxidative phosphorylation, we determined the kinetic properties of ADP to stimulate respiration in the presence of saturating substrates (Fig. 6, *A* and *C*). Maximal respiration was higher in KO animals when ADP was titrated both with and without creatine (~37%, p < 0.05 versus WT; Fig. 6, *A* and *C*, respectively); however, this did not alter estimated ADP sensitivity as assessed by the apparent  $K_m$  (Fig. 6, *B* and *D*, respectively). Similarly, maximal respiration in KO animals was also increased (~30%, p < 0.05) when pyruvate was titrated in the presence of saturating ADP concentrations with no effect on the apparent  $K_m$  (Fig. 6, *E* and *F*).

#### Skeletal muscle contraction

We next sought to determine whether the up-regulation of fatty acid oxidation seen at both the whole-body and skeletal muscle levels in KO animals could be explained by altered substrate delivery and/or metabolic inflexibility in response to contraction. Following electrical stimulation, AMPK and ACC were phosphorylated regardless of genotype or muscle (Fig. 7, *A* and *B*), confirming successful contraction of the muscles and suggesting that these signaling cascades remained intact in the absence of TBC1D1. We therefore isolated giant sarcolemmal vesicles for the determination of plasma membrane transport-





**Figure 4. TBC1D1 KO animals display an increased reliance on fatty acid oxidation.** Indirect calorimetry measurements of (A) RER, (B) fat oxidation, (C) carbohydrate oxidation and total energy expenditure (D) were determined in light and dark cycles for WT or KO animals (n + 8). (E) Palmitate oxidation and (F) glucose oxidation in EDL strips (n=5). The data are expressed as means  $\pm$  S.E. M. (*error bars*). \*, p < 0.05 versus WT.



**Figure 5. TBC1D1 KO animals display improved mitochondrial function.** *A*, mitochondrial oxygen consumption in permeabilized fibers prepared from EDL muscle. Mitochondrial respiration was determined in the absence (state 4) and presence of ADP (5 mM; state 3) supported by complex I (pyruvate + malate + glutamate; 5 mM each), complex II (succinate; 10 mM), and lipid-supported (P-CA; 60  $\mu$ M) substrates. Uncoupled respiration was measured in the presence of 2,4-dinitrophenol (50  $\mu$ M). Respiratory control ratios (state 3/state 4) were calculated at ~5.9 for WT and ~7.3 for TBC1D1 KO rat fibers bundles. *B*, representative digested fiber blot for mitochondrial subunits of the electron transport chain. Mitochondrial content in red and white gastrocnemius muscles was determined by an OXPHOS antibody, which recognizes subunits of the electron transport chain complexes (*CI-CV*) (*C*), and a representative blot is shown (*D*). The data are expressed as means ± S.E. M. (*error bars*) (*n* = 8). \*, *p* < 0.05 *versus* WT. *P*, pyruvate; *M*, malate; *D*, ADP; *G*, glutamate; *S*, succinate.

ers. Although FAT/CD36 increased in red muscle following contraction (Fig. 7*C*), there was no difference between geno-types and no effect of contraction in either group in white mus-

cle (Fig. 7*D*). This suggests that the increase in whole-body fatty acid oxidation in KO animals is not due to increased transport into skeletal muscle. We next sought to determine whether





**Figure 6. Kinetic properties of mitochondria.** Shown are ADP titrations in permeabilized fibers prepared from EDL muscle in the presence (*A* and *B*) and absence (*C* and *D*) of creatine with pyruvate and malate present in the respiration buffer. Pyruvate titrations (*E* and *F*) were performed with ADP and malate present in the buffer. The data are expressed as means  $\pm$  S.E. M. (*error bars*) (n = 8). \*, p < 0.05 versus WT.

there was an impairment in contraction-mediated glucose uptake and GLUT4 trafficking. Electrically induced contraction resulted in a significant increase in 2-deoxyglucose (2-DG) uptake in both genotypes in red and white muscles (p < 0.05; Fig. 8, A and B); however, compared with WT, this effect was significantly reduced in KO animals in white muscle (p < 0.05; Fig. 8*B*). The increase in glucose uptake from basal levels ( $\Delta$ change) following stimulation was also significantly reduced in white muscle (p < 0.05; Fig. 8A, *inset*) and tended to be lower in red muscle (p = 0.07; Fig. 8B, inset) of KO animals. These effects were mirrored in sarcolemmal GLUT4 protein content as there was a main effect for contraction to increase GLUT4 in red muscle with a strong trend for a decreased response to contraction in KO animals (p = 0.10; Fig. 8*C*, *inset*). In addition, both absolute plasma membrane GLUT4 content and the ability of contraction to stimulate plasma membrane GLUT4 content were lower in white muscle of KO animals following contraction (Fig. 8, *D* and *inset*), further suggesting that ablation of TBC1D1 impairs intracellular trafficking and glucose uptake. Given this finding, we analyzed glycogen utilization during contraction to determine whether there was a compensatory increase in endogenous substrate utilization. However, there was no change in glycogen content or in skeletal muscle metabolite production (Table 2) between genotypes following contraction.

#### Time to exhaustion

Given our finding of reduced GLUT4 trafficking and glucose uptake in KO animals in response to contraction and the prominent role of exogenous substrates during contraction in rats, we next examined whether this would result in impaired running performance. Consistent with reductions in glucose uptake *in situ*, running time decreased in TBC1D1 KO animals by  $\sim$ 18% at low intensity and  $\sim$ 25% at the higher intensity (Fig. 9).





**Figure 7. AMPK signaling and FAT/CD36 translocation in response to contraction.** Total (*tAMPK*) and phosphorylated AMPK (*pAMPK*) and total (*tACC*) and phosphorylated ACC (*pACC*) in skeletal muscle homogenate from red (*A*) and white (*B*) gastrocnemius muscles in response to electrical stimulation (*Stim*) of the sciatic nerve is unaltered in TBC1D1 KO animals (n = 4). Plasma membrane FAT/CD36 was measured in giant sarcolemmal vesicles isolated from red (*C*) and white (*D*) muscles (n = 6). Ponceau staining of the membrane was used as a loading control. The data are expressed as means  $\pm$  S.E. M. (*error bars*). \*, p < 0.05 versus basal.

#### Discussion

In this study, we utilized a novel TBC1D1 KO rat model to elucidate the role of this protein in intracellular GLUT4 trafficking. We have shown that, in contrast to the TBC1D1-deficient murine model, there is no decrease in total GLUT4 protein content or impairment in GLUT4 accumulation on the plasma membrane of muscle in response to insulin. These data therefore suggest that TBC1D1 is not required for insulin-mediated GLUT4 trafficking. In contrast, we provide evidence that contraction-mediated glucose uptake and GLUT4 translocation in KO animals are impaired in TBC1D1 KO animals, and this is associated with compromised exercise capacity. These findings demonstrate that TBC1D1 plays an important role in contraction-mediated but not insulin-stimulated GLUT4 translocation in skeletal muscle.

#### TBC1D1 KO animals display intact insulin signaling

In the current study, we have shown that TBC1D1 ablation results in impaired glucose tolerance, which is consistent with previous reports implicating this protein in maintaining beta

cell mass (26, 27). However, the current work also demonstrates that TBC1D1 is not required for intracellular GLUT4 trafficking in response to insulin as there were no differences in ITT or in total or phosphorylated (Ser-473) Akt2 or plasma membrane GLUT4 content. Our findings are supported by previous work showing that mutation of the canonical Akt phosphorylation motif on TBC1D1 (Thr-596) to Ala had no effect on insulinstimulated glucose transport (28). We have also shown that total GLUT4 protein is unaltered between WT and KO rats, which is critical to the interpretations of the functional role of TBC1D1. This is in contrast to previous work utilizing TBC1D1-deficient mice that showed impaired insulin-stimulated glucose transport (12, 13). However, these studies were confounded by an  $\sim$ 50% reduction in GLUT4 content, thus making it difficult to ascertain the exact role of TBC1D1 in glucose transport. It is therefore possible that the reduced glucose uptake in TBC1D1-deficient mouse models is a conseguence of lowered GLUT4 content as opposed to a direct role of TBC1D1 in mediating GLUT4 trafficking.





**Figure 8. 2-DG uptake and GLUT4 translocation in response to contraction.** 2-DG uptake in red (*A*) and white (*B*) gastrocnemius in WT and KO animals (n = 8 for WT and n = 5 for KO) is shown. Plasma membrane GLUT4 was measured in giant sarcolemmal vesicles isolated from red (*C*) and white (*D*) gastrocnemius muscles (n = 6) in response to electrical stimulation (*Stim*) of the sciatic nerve. The *insets* within each figure depict the absolute changes ( $\Delta$ ) in response to contraction (*i.e.* contraction minus sedentary). Ponceau staining of the membrane was used as a loading control. The data are expressed as means  $\pm$  S.E. M. (*error bars*). \*, p < 0.05 versus basal; †, p < 0.05 versus WT stimulated (Stim).

#### Table 2

Skeletal metabolite content in red and white gastrocnemius muscle following electrical stimulation of the sciatic nerve

The data are expressed as means  $\pm$ S.E. (error bars) (n = 6). Cr, creatine; Stim, stimulated.

	Red muscle			White muscle				
	Control		Stim		Control		Stim	
Metabolites	WT	КО	WT	КО	WT	КО	WT	КО
ATP (mmol·kg <sup><math>-1</math></sup> dry wt)	$24.87\pm0.87$	$25.66 \pm 1.41$	$22.41 \pm 1.32$	$25.31\pm0.61$	$28.27 \pm 2.24$	$27.99 \pm 0.76$	$28.95 \pm 1.98$	$28.66 \pm 1.01$
PCr (mmol·kg <sup>-1</sup> dry wt)	$68.4 \pm 3.4$	$69.5 \pm 5.9$	$58.4 \pm 3.9$	$64.5 \pm 4.1$	$82.4 \pm 7.1$	$81.9 \pm 2.1$	$61.7 \pm 6.5^{a}$	$65.7 \pm 7.0^{a}$
Cr (mmol·kg <sup><math>-1</math></sup> dry wt)	$57.5 \pm 2.7$	$61.9 \pm 3.6$	$55.5 \pm 2.5$	$63.6 \pm 4.1$	$64.5 \pm 4.7$	$69.8 \pm 3.7$	$85.2 \pm 10.2^{a}$	$97.2 \pm 13.4^{a}$
Total Cr (mmol·kg <sup>-1</sup> dry wt)	$125.9 \pm 4.9$	$131.3 \pm 6.5$	$113.9 \pm 5.9$	$128 \pm 4.24$	$146.9\pm8.5$	$151.7 \pm 2.4$	$147.0\pm13.2$	$162.9 \pm 7.9$
Lactate (mmol·kg <sup>-1</sup> dry wt)	$2.3 \pm 0.3$	$2.7 \pm 0.7$	$4.1 \pm 1.2$	$3.27 \pm 0.5$	$5.3 \pm 1.1$	$5.6 \pm 0.4$	$10.6 \pm 1.8^{a}$	$17.7 \pm 5.0^{a}$
Glycogen (mmol·kg <sup><math>-1</math></sup> dry wt)	$111.24\pm19.36$	$114.85\pm24.38$	$50.58\pm6.78^a$	$60.90 \pm 10.43^{a}$	$96.03\pm13.88$	$106.32 \pm 10.57$	$40.32\pm11.46^a$	$50.13 \pm 11.35^a$

<sup>*a*</sup> p < 0.05, main effect *versus* control.





**Figure 9. Impaired exercise capacity in TBC1D1 KO animals.** Time to exhaustion (min) in WT and TBC1D1 KO animals during treadmill running at low (*LO*; 20 m·min<sup>-1</sup>, 5% grade) and high (*HI*; 20 m·min<sup>-1</sup>, 15% grade) intensities. The data are expressed as means  $\pm$  S.E. M. (*error bars*) (n = 6).\*, p < 0.05, main effect for intensity and genotype.

#### Increased fatty acid oxidation in TBC1D1 KO animals

We have shown that ablation of TBC1D1 resulted in an increase in whole-body fat oxidation, which occurred in the absence of any change in energy expenditure. This is consistent with previous studies utilizing TBC1D1-deficient mice (13, 22) as well as cell line models with reduced TBC1D1 (22). We have also shown an increase in palmitate oxidation in KO animals in isolated EDL strips; however, there was no change in glucose oxidation between genotypes. This suggests that, in the absence of other substrates, the ability to utilize fatty acids is increased in TBC1D1 KO animals, whereas the basal capacity to oxidize carbohydrate is not impaired.

To elucidate the mechanisms underlying the change in substrate utilization, we evaluated a proximal step in lipid oxidation, FAT/CD36 trafficking to the plasma membrane. FAT/ CD36 has previously been shown to respond to similar metabolic stimuli as GLUT4, including insulin and skeletal muscle contraction (24), and is a key component for the regulation of skeletal muscle fuel selection (25). It was therefore possible that TBC1D1 plays an important role in intracellular trafficking of this transporter and that ablation of the TBC1D1 gene would result in a compensatory increase in localization of FAT/CD36 to the plasma membrane. However, there was no difference between KO and WT animals in FAT/CD36 protein content measured in sarcolemmal vesicles at rest (p = 0.45) and following contraction in red or white muscle. This therefore indicates that increases in fatty acid transport cannot explain the shift in fuel selection in these animals.

Previous work utilizing TBC1D1 overexpression in mice showed reduced activity of  $\beta$ -HAD concomitant with a decrease in fatty acid oxidation (23). However, neither  $\beta$ -HAD activity nor rates of P-CoA supported mitochondrial respiration were altered in KO rats and therefore cannot explain the observed increase in fatty acid oxidation seen at the wholebody or muscle level. In contrast, TBC1D1 KO animals display increased mitochondrial respiration in the absence of any change in mitochondrial protein content, suggesting improved respiratory function. This cannot be explained by increased sensitivity to substrates as there was no change in the apparent  $K_m$  for pyruvate or for ADP either in the presence or absence of creatine. It is therefore possible that the increase in mitochondrial function is a compensatory adaptation in response to TBC1D1 ablation; however, further work is required to elucidate the exact mechanism behind this. An increase in maximal mitochondrial respiration supports our finding of increased reliance on fatty acid oxidation and may indicate tighter metabolic control as increased respiration, and therefore ATP production, should result in decreased accumulation of mitochondrial-free ADP, although this remains speculative and requires further examination.

#### Impaired contraction-mediated GLUT4 translocation

Consistent with previous reports, we have shown that run time to exhaustion is impaired in TBC1D1 KO animals (14). However, in the previous study, total GLUT4 protein was decreased, again making it difficult to determine whether the impairment in exercise capacity was due to a decrease in transporter protein content or the ablation of TBC1D1. In the current study, we have shown that total GLUT4 protein content was not reduced in the KO animals, and therefore the exercise impairment is likely a result of decreased contraction-mediated GLUT4 trafficking and therefore decreased glucose uptake. This clearly highlights TBC1D1 as having a critical role in glucose homeostasis during exercise, especially given the increased reliance on exogenous glucose as a fuel source in rodents. Interestingly, the decrease in contraction-mediated glucose uptake and plasma membrane GLUT4 content did not result in increased intramuscular glycogen breakdown or an increase in cellular energy perturbation as measured by metabolite accumulation. This suggests that despite the apparent improvement in mitochondrial respiratory function TBC1D1 KO animals are not able to adequately compensate for the decrease in glucose uptake to rescue exercise capacity.

#### Conclusion

Altogether, we have shown that TBC1D1 KO rats displayed increased mitochondrial respiratory function, which may explain the increase in fatty acid oxidation seen both *ex vivo* and at the whole-body level. Despite these alterations, KO animals displayed decreased exercise capacity likely due to the decrease in GLUT4 translocation and glucose uptake seen following contraction. Combined with the findings that TBC1D1 expression is highly specific to skeletal muscle (11, 18) and appears to be more robustly regulated by AMPK than Akt-mediated phosphorylation (18), these results support the theory that TBC1D1 is downstream of AMPK and represents a likely terminus for the contraction.

#### **Experimental procedures**

#### Chemicals

All chemicals were purchased from Sigma-Aldrich unless stated otherwise.



#### Rodents

TBC1D1 KO rats on a Sprague-Dawley background were provided by Dr. Hamra (29). Animals were housed in a temperature- and humidity-regulated room on a 12/12-h light– dark cycle. Rats were maintained on a chow diet (Harlan Laboratories Inc.) with *ad libitum* access to food and water. All protocols were approved by and performed in accordance with the Committee on Animal Care guidelines at the University of Guelph.

#### Glucose and insulin tolerance tests

Four-hour-fasted rats underwent intraperitoneal GTT (2 g/kg of body weight) and ITT (1 unit/kg of body weight; Humulin, Lilly) tolerance tests. Blood glucose was measured using a glucose meter (Freestyle Lite, Abbott Diabetes Care) for both GTT and ITT before and up to 120 and 45 min post-injection, respectively.

#### Indirect calorimetry

Oxygen consumption  $(VO_2)$ , carbon dioxide  $(VCO_2)$ , and RER were measured over a 24-h period in a CLAMS (Comprehensive Laboratory Animal Monitoring System) open circuit system (Columbus Instruments). Fat and carbohydrate oxidation and total energy expenditure were calculated as described previously (30).

#### Substrate oxidation

To determine the rates of substrate oxidation, glucose and palmitate oxidation assays were performed in EDL muscle strips as described previously (31, 32). Briefly, palmitate oxidation was measured in pregassed (95% O2, 5% CO2) modified Krebs-Henseleit buffer supplemented with 4% BSA and palmitate (0.5 mm; 0.5 µCi/µl [1-14C]palmitate) (PerkinElmer Life Sciences) at 30 °C for 40 min by measuring the <sup>14</sup>CO<sub>2</sub> released in addition to determining the water-soluble <sup>14</sup>C-labeled intermediates. Glucose oxidation was determined in a separate set of experiments as described previously (33). Briefly, muscle strips were incubated in modified Krebs-Henseleit buffer supplemented with 5.5 mM glucose and 0.2  $\mu$ Ci D-[U-<sup>14</sup>C]glucose (American Radiolabeled Chemicals) at 30 °C for 40 min. Gaseous <sup>14</sup>CO<sub>2</sub> was captured as above and counted using standard liquid scintillation techniques. Substrate oxidation rates were calculated using the specific activity of labeled palmitate or glucose within the Krebs-Henseleit buffer and normalized to muscle weight.

#### Skeletal muscle response to insulin

To determine skeletal muscle-specific insulin signaling, rats were anesthetized (sodium pentobarbital; 60 mg/kg of body weight), and then muscle samples were excised before (basal) and 30 min after an intravenous insulin injection (1 unit/kg of body weight). Harvested tissues were collected for plasma membrane preparations or rapidly frozen in liquid nitrogen for Western blotting as described below.

#### Electrical stimulation-induced muscle contraction

For muscle contraction experiments, the sciatic nerve of the experimental limb was exposed, and stimulating electrodes

were placed around the nerve. Electrical stimulation was applied for 15 min (train delivery, 100 Hz/3 s at 6–8 V; train duration, 200 ms; pulse duration, 10 ms). Following stimulation, both experimental and control hind limb muscles (red and white gastrocnemius and red and white tibialis anterior) were excised and either immediately frozen in liquid nitrogen for further analysis or used for the preparation of giant sarcolemmal vesicles.

#### In vivo glucose clearance

Muscle-specific glucose uptake was performed by tail vain injection. Specifically, 50  $\mu$ Ci of tritiated 2-DG was injected 5 min before commencing 15 min of sciatic nerve stimulation (as described above). Thereafter, muscle was immediately taken and rates of muscle-specific glucose uptake were determined by analyzing radioactivity from red and white gastrocnemius as described previously (31, 32).

#### Muscle enzymatic activity

Red and white tibialis anterior muscles were excised from both experimental and control limbs following stimulation experiments and immediately frozen in liquid nitrogen. A small piece of frozen muscle (6–10 mg) was taken for the spectrophotometric determination of  $\beta$ -HAD at 37 °C as described previously (33).

#### Glycogen and metabolite analysis

Red and white gastrocnemius muscles from both the control and experimental limb in stimulation experiments was freezedried, dissected free of visible blood and connective tissue, and powdered for metabolite and glycogen analyses. An aliquot of freeze-dried muscle (10-12 mg) was extracted with 0.5 M perchloric acid (HClO<sub>4</sub>) containing 1 mM EDTA and neutralized with 2.2 M KHCO<sub>3</sub>. The supernatant was used to determine creatine, PCr, ATP, and lactate with enzymatic spectrophotometric assays (33, 34). Muscle glycogen content was determined from an aliquot of freeze-dried muscle (2–3 mg) as described elsewhere (33).

#### Isolation of plasma membranes

Giant sarcolemmal vesicles were prepared as described previously (24). Briefly, red and white gastrocnemius muscles excised following contraction and insulin stimulation experiments were cut into thin strips and incubated (1 h, 34 °C, 100 rpm) in 140 mM KCl, 10 mM MOPS (pH 7.4) containing collagenase VII (150 units/ml) and aprotinin (10 mg/ml). The muscle was washed with KCl/MOPS containing 10 mM Na<sub>2</sub>EDTA (pH 7.4), and the supernatants were combined. Percoll (3.5%), KCl (28 mM), and aprotinin (10  $\mu$ g/ml) were added to the supernatant. This solution was layered under 3 ml of 4% Nycodenz and 1 ml of KCl/MOPS and centrifuged (60 × g, 45 min, 25 °C). The vesicles were harvested from the interface of Nycodenz and KCl/MOPS and pelleted by centrifugation (9000 × g, 10 min, 25 °C), and the resulting pellet was resuspended in KCl/MOPS and stored at -80 °C for Western blotting.

#### Preparation of permeabilized fibers

A small portion of muscle was placed in ice-cold BIOPS (50 mm MES, 7.23 mm K\_2EGTA, 2.77 mm CaK\_2EGTA, 20 mm im-



idazole, 0.5 mM DTT, 20 mM taurine, 5.77 mM ATP, 15 mM PCr, and 6.56 mM MgCl<sub>2</sub>·H<sub>2</sub>O (pH 7.1)) and separated under a microscope into bundles using fine-tipped forceps as described previously (35). Fiber bundles were then treated with 30  $\mu$ g/ml saponin for 30 min at 4 °C and then washed for 15 min in MiR05 respiration buffer (0.5 mM EGTA, 10 mM HH<sub>2</sub>PO<sub>4</sub>, 110 mM sucrose, 1 mg/ml fatty acid-free BSA (pH 7.1)) for respiration analysis as described below.

#### Mitochondrial respiration

Measurements of O<sub>2</sub> consumption were performed in MiR05 respiration buffer on prepared permeabilized fibers using an Oxygraph-2K (Oroboros Instruments, Innsbruck, Austria) at 37 °C in the presence of 25  $\mu$ M blebbistatin as described previously (36). ADP-stimulated respiration was determined in the presence of 5 mM pyruvate and 2 mM malate as well as 60  $\mu$ M P-CoA. Maximal state III respiration was determined by the addition of 5 mM ADP with 10 mM glutamate and 10 mM succinate added to determine maximal complex I and maximal complex I + II respiration, respectively. Uncoupled respiration was measured in the presence of 50  $\mu$ M 2,4-dinitrophenol. Mitochondrial kinetics in permeabilized fibers were analyzed using three separate titration protocols. ADP kinetics were analyzed both in the presence and absence of 20 mM creatine. Both ADP titrations were initiated with 2 mM malate and 10 mM pyruvate, and ADP was titrated at various concentrations. Pyruvate kinetics were determined in the presence of 5 mM ADP, 5 mM malate, and 20 mM creatine, and pyruvate was titrated in at various concentrations. Then 10 mM glutamate and 10 mM succinate were added following the pyruvate and ADP titrations to determine maximum mitochondrial respiration. Finally, 10  $\mu$ M cytochrome c was added with <10% increase in respiration in all measurements indicating outer mitochondrial membrane integrity. The apparent  $K_m$  values for pyruvate and ADP were determined as described previously (35). All fibers were recovered from the respirometer, freezedried, and weighed for normalization to muscle bundle weight. Freeze-dried fibers were then digested in 5  $\mu$ g· $\mu$ l lysis buffer as described previously (37) for determination of mitochondrial proteins via Western blotting.

#### Western blotting

Whole muscle was homogenized in lysis buffer, diluted to 1  $\mu g \cdot \mu l^{-1}$ , and loaded equally for TBC1D1 (Abcam), total and phosphorylated (Ser-473) Akt2 (Cell Signaling Technology Inc.), total and phosphorylated (Thr-178) AMPK (Cell Signaling Technology Inc.), total and phosphorylated (Ser-79) ACC (Cell Signaling Technology Inc.), GLUT4 (Chemicon), FAT/CD36 (Santa Cruz Biotechnologies), MitoProfile Total OXPHOS antibody mixture (Mitosciences), and cytochrome *c* oxidase complex IV (Life Technologies) with both  $\alpha$ -tubulin (Abcam) and Ponceau staining used as a loading control. Proteins were separated by SDS-polyacrylamide gel electrophoresis, transferred to polyvinylidene difluoride membrane, and incubated in blocking solution, primary antibody, and the corresponding secondary antibody as specified by the supplier. Membrane proteins were detected by enhanced chemiluminescence

(ChemiGenius2 Bioimaging system, SynGene, Cambridge, UK).

#### Time to exhaustion

Animals were familiarized to the treadmill at slow speeds (10 m·min<sup>-1</sup>) for 3 consecutive days followed by a 48-h rest before the start of the exercise bout. Animals completed a time to exhaustion trial at two different intensities (low; 20 m·min<sup>-1</sup>, 5% grade, and high, 20 m·min<sup>-1</sup>, 15% grade) separated by  $\geq$ 48 h.

#### Statistics

Results are expressed as means  $\pm$  S.E. and were analyzed by Student's *t* test or one- or two-way analysis of variance with a Fisher's least significant difference post hoc test where appropriate. Significance was set at p < 0.05 where NS indicates not significant. All graphs and statistical analyses were performed using GraphPad Prism 5.0 (GraphPad Software).

*Author contributions*—All authors (J. W., S. P., B. K. S., P. M. M., G. S., H. L. R., S. S. J., E. A. F. H., E. M. D., D. J. D., L. L. S., G. R. S., and G. P. H.) performed experiments and analyzed the data. J. W. primarily wrote the manuscript. G. P. H. designed the study and contributed to writing the manuscript. All authors edited and approved the final version of the manuscript.

#### References

- Gao, J., Ren, J., Gulve, E. A., and Holloszy, J. O. (1994) Additive effect of contractions and insulin on GLUT-4 translocation into the sarcolemma. *J. Appl. Physiol.* 77, 1597–1601
- Lund, S., Holman, G. D., Schmitz, O., and Pedersen, O. (1995) Contraction stimulates translocation of glucose transporter GLUT4 in skeletal muscle through a mechanism distinct from that of insulin. *Proc. Natl. Acad. Sci.* U.S.A. 92, 5817–5821
- 3. Gonzalez, E., and McGraw, T. E. (2006) Insulin signaling diverges into Akt-dependent and -independent signals to regulate the recruitment/ docking and the fusion of GLUT4 vesicles to the plasma membrane. *Mol. Biol. Cell.* **17**, 4484–4493
- Lee, A. D., Hansen, P. A., and Holloszy, J. O. (1995) Wortmannin inhibits insulin-stimulated but not contraction-stimulated glucose transport activity in skeletal muscle. *FEBS Lett.* 361, 51–54
- Goodyear, L. J., Giorgino, F., Balon, T. W., Condorelli, G., and Smith, R. J. (1995) Effects of contractile activity on tyrosine phosphoproteins and PI 3-kinase activity in rat skeletal muscle. *Am. J. Physiol. Endocrinol. Metab.* 268, E987–E995
- Goodyear, L. J., King, P. A., Hirshman, M. F., Thompson, C. M., Horton, E. D., and Horton, E. S. (1990) Contractile activity increases plasma membrane glucose transporters in absence of insulin. *Am. J. Physiol. Endocrinol. Metab.* 258, E667–E672
- Ploug, T., Galbo, H., and Richter, E. A. (1984) Increased muscle glucose uptake during contractions: no need for insulin. *Am. J. Physiol. Endocrinol. Metab.* 247, E726 – E731
- Fukuda, M. (2011) TBC proteins: GAPs for mammalian small GTPase Rab? *Biosci. Rep.* 31, 159–168
- Roach, W. G., Chavez, J. A., Minea, C. P., and Lienhard, G. E. (2007) Substrate specificity and effect on GLUT4 translocation of the Rab GT-Pase-activating protein Tbc1d1. *Biochem. J.* 403, 353–358
- Ishikura, S., and Klip, A. (2008) Muscle cells engage Rab8A and myosin Vb in insulin-dependent GLUT4 translocation. *Am. J. Physiol. Cell Physiol.* 295, C1016–C1025
- Chavez, J. A., Roach, W. G., Keller, S. R., Lane, W. S., and Lienhard, G. E. (2008) Inhibition of GLUT4 translocation by Tbc1d1, a Rab GTPase-ac-



tivating protein abundant in skeletal muscle, is partially relieved by AMPactivated protein kinase activation. *J. Biol. Chem.* **283**, 9187–9195

- Szekeres, F., Chadt, A., Tom, R. Z., Deshmukh, A. S., Chibalin, A. V., Björnholm, M., Al-Hasani, H., and Zierath, J. R. (2012) The Rab-GTPaseactivating protein TBC1D1 regulates skeletal muscle glucose metabolism. *Am. J. Physiol. Endocrinol. Metab.* 303, E524–E533
- Dokas, J., Chadt, A., Nolden, T., Himmelbauer, H., Zierath, J. R., Joost, H.-G., and Al-Hasani, H. (2013) Conventional knockout of Tbc1d1 in mice impairs insulin- and AICAR-stimulated glucose uptake in skeletal muscle. *Endocrinology*. **154**, 3502–3514
- Stöckli, J., Meoli, C. C., Hoffman, N. J., Fazakerley, D. J., Pant, H., Cleasby, M. E., Ma, X., Kleinert, M., Brandon, A. E., Lopez, J. A., Cooney, G. J., and James, D. E. (2015) The RabGAP TBC1D1 plays a central role in exerciseregulated glucose metabolism in skeletal muscle. *Diabetes.* 64, 1914–1922
- Zisman, A., Peroni, O. D., Abel, E. D., Michael, M. D., Mauvais-Jarvis, F., Lowell, B. B., Wojtaszewski, J. F., Hirshman, M. F., Virkamaki, A., Goodyear, L. J., Kahn, C. R., and Kahn, B. B. (2000) Targeted disruption of the glucose transporter 4 selectively in muscle causes insulin resistance and glucose intolerance. *Nat. Med.* 6, 924–928
- Pehmøller, C., Treebak, J. T., Birk, J. B., Chen, S., Mackintosh, C., Hardie, D. G., Richter, E. A., and Wojtaszewski, J. F. (2009) Genetic disruption of AMPK signaling abolishes both contraction- and insulin-stimulated TBC1D1 phosphorylation and 14-3-3 binding in mouse skeletal muscle. *Am. J. Physiol. Endocrinol. Metab.* 297, E665–E675
- Frøsig, C., Pehmøller, C., Birk, J. B., Richter, E. A., and Wojtaszewski, J. F. (2010) Exercise-induced TBC1D1 Ser237 phosphorylation and 14-3-3 protein binding capacity in human skeletal muscle. *J. Physiol.* 588, 4539–4548
- Taylor, E. B., An, D., Kramer, H. F., Yu, H., Fujii, N. L., Roeckl, K. S., Bowles, N., Hirshman, M. F., Xie, J., Feener, E. P., and Goodyear, L. J. (2008) Discovery of TBC1D1 as an insulin-, AICAR-, and contraction-stimulated signaling nexus in mouse skeletal muscle. *J. Biol. Chem.* 283, 9787–9796
- Treebak, J. T., Pehmøller, C., Kristensen, J. M., Kjøbsted, R., Birk, J. B., Schjerling, P., Richter, E. A., Goodyear, L. J., and Wojtaszewski, J. F. (2014) Acute exercise and physiological insulin induce distinct phosphorylation signatures on TBC1D1 and TBC1D4 proteins in human skeletal muscle. *J. Physiol.* 592, 351–375
- Ramm, G., Larance, M., Guilhaus, M., and James, D. E. (2006) A role for 14-3-3 in insulin-stimulated GLUT4 translocation through its interaction with the RabGAP AS160. *J. Biol. Chem.* 281, 29174–29180
- Stöckli, J., Davey, J. R., Hohnen-Behrens, C., Xu, A., James, D. E., and Ramm, G. (2008) Regulation of glucose transporter 4 translocation by the Rab guanosine triphosphatase-activating protein AS160/TBC1D4: role of phosphorylation and membrane association. *Mol. Endocrinol.* 22, 2703–2715
- Chadt, A., Leicht, K., Deshmukh, A., Jiang, L. Q., Scherneck, S., Bernhardt, U., Dreja, T., Vogel, H., Schmolz, K., Kluge, R., Zierath, J. R., Hultschig, C., Hoeben, R. C., Schürmann, A., Joost, H.-G., *et al.* (2008) Tbc1d1 mutation in lean mouse strain confers leanness and protects from diet-induced obesity. *Nat. Genet.* 40, 1354–1359
- Maher, A. C., McFarlan, J., Lally, J., Snook, L. A., and Bonen, A. (2014) TBC1D1 reduces palmitate oxidation by inhibiting β-HAD activity in skeletal muscle. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **307**, R1115–R1123
- Jain, S. S., Chabowski, A., Snook, L. A., Schwenk, R. W., Glatz, J. F., Luiken, J. J., and Bonen, A. (2009) Additive effects of insulin and muscle contrac-

tion on fatty acid transport and fatty acid transporters, FAT/CD36, FAB-Ppm, FATP1, 4 and 6. *FEBS Lett.* **583**, 2294–2300

- 25. McFarlan, J. T., Yoshida, Y., Jain, S. S., Han, X. X., Snook, L. A., Lally, J., Smith, B. K., Glatz, J. F., Luiken, J. J., Sayer, R. A., Tupling, A. R., Chabowski, A., Holloway, G. P., and Bonen, A. (2012) *In vivo*, fatty acid translocase (CD36) critically regulates skeletal muscle fuel selection, exercise performance, and training-induced adaptation of fatty acid oxidation. *J. Biol. Chem.* 287, 23502–23516
- Paglialunga, S., Simnett, G., Robson, H., Hoang, M., Pillai, R., Arkell, A. M., Simpson, J. A., Bonen, A., Huising, M., Joseph, J. W., and Holloway, G. P. (2017) The Rab-GTPase activating protein, TBC1D1, is critical for maintaining normal glucose homeostasis and β-cell mass. *Appl. Physiol. Nutr. Metab.* 42, 647–655
- Rütti, S., Arous, C., Nica, A. C., Kanzaki, M., Halban, P. A., and Bouzakri, K. (2014) Expression, phosphorylation and function of the Rab-GTPase activating protein TBC1D1 in pancreatic β-cells. *FEBS Lett.* 588, 15–20
- An, D., Toyoda, T., Taylor, E. B., Yu, H., Fujii, N., Hirshman, M. F., and Goodyear, L. J. (2010) TBC1D1 regulates insulin- and contractioninduced glucose transport in mouse skeletal muscle. *Diabetes* 59, 1358-1365
- Izsvák, Z., Fröhlich, J., Grabundzija, I., Shirley, J. R., Powell, H. M., Chapman, K. M., Ivics, Z., and Hamra, F. K. (2010) Generating knockout rats by transposon mutagenesis in spermatogonial stem cells. *Nat. Methods* 7, 443–445
- Péronnet, F., and Massicotte, D. (1991) Table of nonprotein respiratory quotient: an update. *Can. J. Sport. Sci.* 16, 23–29
- Henriksbo, B. D., Lau, T. C., Cavallari, J. F., Denou, E., Chi, W., Lally, J. S., Crane, J. D., Duggan, B. M., Foley, K. P., Fullerton, M. D., Tarnopolsky, M. A., Steinberg, G. R., and Schertzer, J. D. (2014) Fluvastatin causes NLRP3 inflammasome-mediated adipose insulin resistance. *Diabetes* 63, 3742–3747
- Smith, B. K., Ford, R. J., Desjardins, E. M., Green, A. E., Hughes, M. C., Houde, V. P., Day, E. A., Marcinko, K., Crane, J. D., Mottillo, E. P., Perry, C. G., Kemp, B. E., Tarnopolsky, M. A., and Steinberg, G. R. (2016) Salsalate (salicylate) uncouples mitochondria, improves glucose homeostasis, and reduces liver lipids independent of AMPK-β1. *Diabetes* 65, 3352–3361
- Bergmeyer, H. U. (1974) Methods of Enzymatic Analysis, pp. 474–476, Elsevier, New York, NY
- Harris, R. C., Hultman, E., and Nordesjö, L.-O. (1974) Glycogen, glycolytic intermediates and high-energy phosphates determined in biopsy samples of musculus quadriceps femoris of man at rest. Methods and variance of values. *Scand. J. Clin. Lab. Invest.* 33, 109–120
- 35. Perry, C. G., Kane, D. A., Herbst, E. A., Mukai, K., Lark, D. S., Wright, D. C., Heigenhauser, G. J., Neufer, P. D., Spriet, L. L., and Holloway, G. P. (2012) Mitochondrial creatine kinase activity and phosphate shuttling are acutely regulated by exercise in human skeletal muscle. *J. Physiol.* 590, 5475–5486
- Perry, C. G., Kane, D. A., Lin, C.-T., Kozy, R., Cathey, B. L., Lark, D. S., Kane, C. L., Brophy, P. M., Gavin, T. P., Anderson, E. J., and Neufer, P. D. (2011) Inhibiting myosin-ATPase reveals a dynamic range of mitochondrial respiratory control in skeletal muscle. *Biochem. J.* 437, 215–222
- Herbst, E. A., Paglialunga, S., Gerling, C., Whitfield, J., Mukai, K., Chabowski, A., Heigenhauser, G. J., Spriet, L. L., and Holloway, G. P. (2014) ω-3 supplementation alters mitochondrial membrane composition and respiration kinetics in human skeletal muscle. *J. Physiol.* **592**, 1341–1352



# Ablating the protein TBC1D1 impairs contraction-induced sarcolemmal glucose transporter 4 redistribution but not insulin-mediated responses in rats

Jamie Whitfield, Sabina Paglialunga, Brennan K. Smith, Paula M. Miotto, Genevieve Simnett, Holly L. Robson, Swati S. Jain, Eric A. F. Herbst, Eric M. Desjardins, David J. Dyck, Lawrence L. Spriet, Gregory R. Steinberg and Graham P. Holloway

J. Biol. Chem. 2017, 292:16653-16664. doi: 10.1074/jbc.M117.806786 originally published online August 14, 2017

Access the most updated version of this article at doi: 10.1074/jbc.M117.806786

Alerts:

- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 36 references, 21 of which can be accessed free at http://www.jbc.org/content/292/40/16653.full.html#ref-list-1