Overexpression of Monocarboxylate Transporter-1 (*Slc16a1*) in Mouse Pancreatic β-Cells Leads to Relative Hyperinsulinism During Exercise

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Exercise-induced hyperinsulinism (EIHI) is an autosomal dominant disorder characterized by inappropriate insulin secretion in response to vigorous physical exercise or pyruvate injection. Activating mutations in the monocarboxylate transporter-1 (MCT1, *SLC16A1*) promoter have been linked to EIHI. Expression of this pyruvate transporter is specifically repressed (disallowed) in pancreatic β-cells, despite nearly universal expression across other tissues. It has been impossible to determine, however, whether EIHI mutations cause MCT1 expression in patient β-cells. The hypothesis that MCT1 expression in β-cells is sufficient to cause EIHI by allowing entry of pyruvate and triggering insulin secretion thus remains unproven. Therefore, we generated a transgenic mouse capable of doxycycline-induced, β-cell–specific overexpression of MCT1 to test this model directly. MCT1 expression caused isolated islets to secrete insulin in response to pyruvate, without affecting glucose-stimulated insulin secretion. In vivo, transgene induction lowered fasting blood glucose, mimicking EIHI. Pyruvate challenge stimulated increased plasma insulin and smaller excursions in blood glucose in transgenic mice. Finally, in response to exercise, transgene induction prevented the normal inhibition of insulin secretion. Forced overexpression of MCT1 in β-cells thus replicates the key features of EIHI and highlights the importance of this transporter's absence from these cells for the normal control of insulin secretion. ***Diabetes*** **61:1719–1725, 2012**

Exercise-induced hyperinsulinism (EIHI) is an autosomal dominant disorder in which strenuous physical exercise causes inappropriate insulin secretion in affected individuals, leading to hypoglycemia (1). Insulin secretion can also be induced in patients with EIHI by injection of pyruvate, a procedure that does not cause insulin secretion in unaffected individuals. Therefore, it has been proposed that the disorder results from an action of pyruvate, generated along with lactate by muscle during exercise, to stimulate insulin secretion from the pancreatic β-cell inappropriately and despite low blood glucose levels (1,2).

Through linkage analysis and sequencing of two pedigrees, we have recently identified mutations within the monocarboxylate transporter-1 promoter (*MCT1/SLC16A1*) (2) as the likely underlying cause of EIHI in these families. Each of the mutations (in one case a 25-bp duplication with a point mutation, in the other a single G>A change) increased the expression of *MCT1* mRNA in patient fibroblasts and promoter activity in luciferase assays. MCT1 is a plasma membrane proton-linked monocarboxylate transporter that conveys lactate, pyruvate, and other short-chain monocarboxylates across this membrane in a freely reversible manner (3). Although MCT1 expression is essentially ubiquitous across all mammalian tissues, *MCT1* mRNA and MCT1 protein are detected only at vanishingly low levels in pancreatic islets, and neither is found in highly purified β-cells (2,4). In a corresponding manner, an earlier study from this laboratory (5) demonstrated that the transport of exogenous lactate into β-cells is unusually slow. That study also revealed that lactate dehydrogenase A (LDHA) is expressed at very low levels in β-cells, despite nearly universal expression elsewhere (5). These findings, subsequently confirmed by several groups (6,7), suggest that pyruvate derived from glycolysis is preferentially directed toward mitochondrial oxidation, consistent with the remarkable capacity of these cells for the oxidative metabolism of glucose (5,7).

We have more recently identified an additional 37 genes whose expressions are selectively disallowed in pancreatic islets (8). These results, and similar findings by other groups (9), suggest that the metabolic configuration of β-cells is highly adapted to couple glucose metabolism to insulin secretion, not only through the expression of key glucose-sensing genes (e.g., *GLUT2/SLC2A2* and *GCK*) but also by the suppression of genes providing alternative metabolic pathways for glucose that could interfere with this stimulus-secretion coupling.

Increased ATP and ADP generated by the mitochondrial catabolism of pyruvate, derived from glycolysis, is critical to trigger insulin exocytosis (10). Both LDHA and MCT1 catalyze processes that represent alternative fates for pyruvate as follows: LDHA through conversion to lactate and MCT1 through allowing pyruvate to leave the cell, potentially decreasing the efficiency of glucose-stimulated insulin secretion. In contrast, the presence of MCT1 would also allow circulating pyruvate to enter β-cells, allowing pyruvate to fuel the Krebs cycle and producing ATP to stimulate insulin secretion inappropriately. Although in vitro evidence from experiments performed on isolated islets has been presented to support a role for MCT1 and LDHA suppression in restricting pyruvate-induced insulin secretion from β-cells (4,11,12), the physiological relevance of disallowing expression of these genes in the islet has not previously been examined in vivo.

We have recently proposed that the mutations in the *MCT1* promoter linked to EIHI are sufficient to overcome

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the β-cell–specific block on the expression of the transporter (2). Inappropriate upregulation of *MCT1* in β-cells would render the plasma membrane permeable to pyruvate, allowing the latter to stimulate insulin secretion and cause the symptoms of EIHI. Although this represents a compelling hypothesis, there is currently no direct evidence that *MCT1* overexpression in β-cells is the chief cause of EIHI. Indeed, although the mutations linked to EIHI cause increased *MCT1* expression (2), this effect is not tissue-specific and *MCT1* expression is increased in patient fibroblasts and presumably in other tissues as well. Moreover, the difficulty of obtaining human pancreatic tissue has to date prevented confirmation that *MCT1* is indeed overexpressed in the islets of affected individuals.

Therefore, in this study, we used the power of mouse transgenesis to dissect the causes of EIHI. We sought to determine whether a single alteration allowing the controllable upregulation of *Mct1* expression in the β-cell of the adult mouse is sufficient to recapitulate the key features of this disorder. In particular, we tested whether rendering β-cells sensitive to pyruvate would be sufficient to inhibit the normal exercise-induced suppression of insulin secretion (13).

RESEARCH DESIGN AND METHODS

Generation of *Mct1-Luc* transgenic mice. The murine *Mct1* coding sequence was amplified from *pUC18-Mct1* (14), with the addition of a single COOH-terminal c-Myc epitope tag by PCR (primer sequences in Supplementary Table 1), and inserted between the *Nhe*I and *Eco*RV sites of plasmid pBI-L Tet (Clontech). This generated a plasmid with a bidirectional tetracycline-regulated promoter driving expression of both *Mct1::Myc* and firefly luciferase.

The expression cassette was excised from the plasmid backbone by *Aat*II and *Ase*I digestion and transferred by pronuclear microinjection into C57Bl/6 CBA F1 hybrid mouse oocytes (Geneta, Leicester, U.K.). Three successful integrants were identified by PCR screening of DNA extracted from ear biopsies by the HotSHOT method (15) (primer sequences in Supplementary Table 2). Founders were backcrossed five times with C57Bl/6 mice to generate the *Mct1-Luc* strain. *RIP7-rtTA* mice on a C57Bl/6 background (16), expressing the reverse tetracycline transactivator under the control of the rat insulin promoter, were crossed with *Mct1-Luc* mice to permit β-cell–specific, tetracycline-inducible expression of *Mct1::Myc* and luciferase. Of three founder lines, two produced transgenic offspring, one of which was propagated for subsequent experiments. Heterozygous *Mct1-Luc* mice were crossed with heterozygous *RIP7-rtTA* mice to produce littermates of four genotypes as follows: wild-type mice bearing no transgenes (−/−) and both single-transgenic (ST, *RIP7-rtTA*/− and −/*Mct1-Luc*), and double-transgenic (DT, *RIP7-rtTA/Mct1-Luc*) mice. All offspring were genotyped for both transgenes. Of these, only DT mice were expected to express the transgenes on induction with doxycycline. All procedures with animals received ethical approval and were compliant with both U.K. (Animals Scientific Procedures Act, 1986) and Danish legislation.

Analysis of isolated islets. Pancreatic islets were isolated by collagenase digestion (17) from noninduced DT animals. Islets from each mouse were divided into two dishes containing approximately equal distributions of variously sized islets. Islets were cultured for 48 h in the presence or absence of 5 μg/mL doxycycline and then used for luciferase assay to measure transgene expression or used for insulin secretion assays. Luciferase activity was determined with the Luciferase Assay System (Promega) on a Lumat LB9507 luminometer (Berthold Technologies). Three batches of islets from each induction state were assayed from each of three mice. Insulin secretion from isolated islets was assessed essentially as described elsewhere (18).

Expression profiling and immunohistochemistry. Transgene expression was induced by administering 1 g/L doxycycline in autoclaved distilled water for at least 5 days. RNA was extracted from islets and other tissues from three DT mice after doxycycline treatment and from three control DT mice not treated with doxycycline. *Mct1* mRNA was quantified relative to cyclophilin A by quantitative RT-PCR.

MCT1 expression in islets was assessed by immunofluorescence of pancreatic sections prepared from ST and DT mice after doxycycline treatment as described previously (19).

Intraperitoneal pyruvate and glucose tolerance tests. Littermates of ST (*RIP7-rtTA*/-) and DT (*RIP7-rtTA/Mct1-Luc*) animals were fasted overnight (16 h), and fasting blood glucose levels were measured in tail blood samples. Sodium pyruvate (0.5 g/kg) was administered by intraperitoneal injection, and blood glucose was measured at 15, 30, 60, 90, and 120 min after injection with the Accu-Chek Compact Plus glucometer (Roche). Transgene expression was induced by administering doxycycline in drinking water as before, and the pyruvate tolerance test was repeated on induced animals 1 week after the previous test. Doxycycline treatment was maintained for an additional week before glucose tolerance tests were performed (1 g/kg glucose, same procedure as pyruvate tolerance test).

Exercise protocol. All mice were acclimatized to treadmill exercise before experiments on days 1, 3, and 5 by being placed on the inactive treadmill for 10 min, followed by running for 5 min at 10 m/min and 4 min at 16 m/min (5% incline). Maximum running capacity was determined on day 7. Predoxycycline blood glucose levels were measured after a 4-h fast on day 10. Mice were administered with doxycycline as previously described for 2 weeks. Maximum running capacity after doxycycline treatment was determined 3 days before the exercise experiment. Because of limitations on the volume of blood taken in a single day, blood samples were taken from tail vein 2 days before exercise experiment after a 4-h fast to determine basal levels of plasma insulin, pyruvate, and lactate. On the experimental day, mice were fasted for 4 h and then exercised for 20 min at 80% maximum running speed at 5% incline. Blood glucose was determined by tail vein sampling 2 min before exercise and after 10- and 20-min periods of exercise. Blood was also collected directly after exercise to determine plasma levels of insulin, pyruvate, and lactate.

HOMA2 of β-cell function. Insulin levels relative to glucose were calculated as homeostasis model assessment 2 (HOMA2) of β-cell function (HOMA2-B%) according to the HOMA2 model by means of HOMA Calculator software (20). The original HOMA1 model corrects for the nonproportional response of β-cells to glucose, according to the following equation:

$$\text{HOMA1-B\%} = \frac{20 \times \text{Insulin (mU/L)}}{\text{Glucose (mmol/L)} - 3.5}$$

The updated HOMA2 model (21) provides a further refinement of this calculation (which cannot be reduced to a simple formula).

Statistical analyses. Differences were determined by Student *t* test and one- or two-way ANOVA, with Dunnett or Bonferroni post hoc test as appropriate. A P value of <0.05 was considered statistically significant.

RESULTS

Generation of transgenic mice with β-cell–specific, doxycycline-inducible MCT1 expression. *RIP7-rtTA* mice have previously been used successfully to create a number of transgenic mouse strains in which β-cell–specific expression of transgenes is tightly regulated by doxycycline (22). In this experiment, we generated a *Mct1-Luc* mouse strain harboring the coding sequences of COOH-terminally epitope-tagged MCT1 and firefly luciferase under the control of a bidirectional, tetracycline-regulated promoter. *Mct1-Luc* mice were crossed with *RIP7-rtTA* mice producing offspring of four genotypes as follows: −/−, *RIP7-rtTA*/−, −/*Mct1-Luc*, and *RIP7-rtTA/Mct1-Luc* (Fig. 1*A*). Only doxycycline-treated DT mice (*RIP7-rtTA/Mct1-Luc*) were expected to express *Mct1* and luciferase in the β-cell, so other genotypes were used as controls. In initial experiments, the two ST and wild-type genotypes all produced similar results, so only results from the *RIP7-rtTA*/− ST genotype are presented here as controls.

To assess the effectiveness of transgene regulation by the tetracycline analog doxycycline, luciferase expression was compared between noninduced and induced islets from the same mouse. Islets were isolated from DT mice and cultured in the presence or absence of 5 μg/mL doxycycline for 48 h. Batches of six size-matched islets were lysed, and luciferase activity was measured in the lysate (Fig. 1*B*). Luciferase activity increased more than tenfold on induction with doxycycline. This result also gives a good indication of the regulation of the *Mct1* transgene, because both genes are expressed from the same promoter (22).

Overexpression of MCT1 in vivo was confirmed by immunofluorescence of pancreatic sections from doxycycline-treated mice. Strong staining with an anti-MCT1 antibody

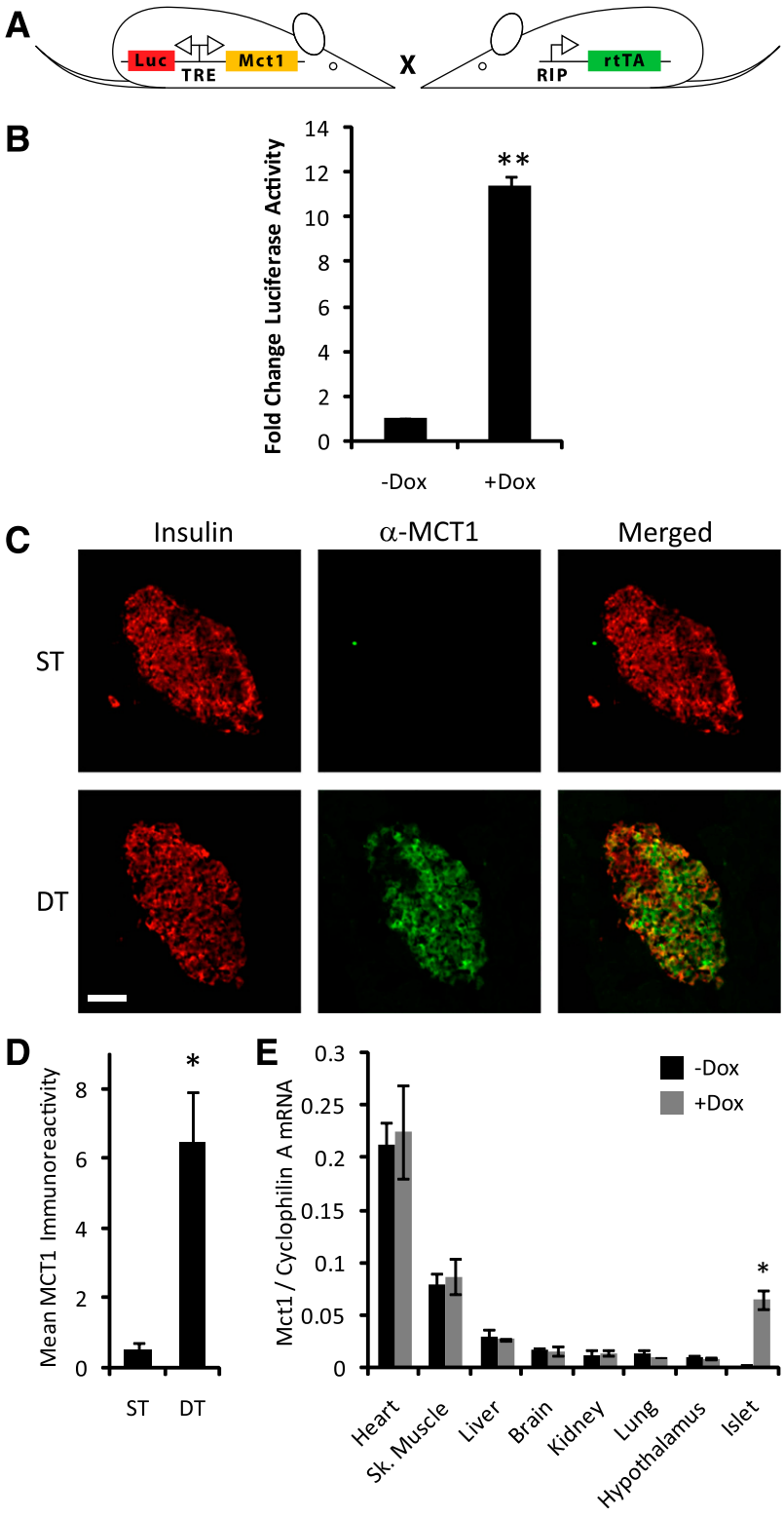

FIG. 1. ***A*****: Transgenic mouse strain for β-cell–specific, inducible expression of MCT1. MCT1 transgenic mice bearing *Mct1* and firefly luciferase (Luc) cDNA under the control of a bidirectional tetracycline-regulated promoter (TRE) (22) were crossed with *RIP7-rtTA* mice in which the reverse tetracycline transactivator (rtTA) is expressed under control of the rat insulin promoter (RIP). In DT offspring, treatment with doxycycline induces expression of luciferase and MCT1 selectively in β-cells. *B*: Regulation of transgene expression is shown in islets isolated from DT mice. Islets from a single mouse were divided and cultured in the presence (+Dox) or absence (−Dox) of 5 μg/mL doxycycline for 48 h. Six size-matched islets were lysed, and luciferase activity was measured. Data are presented as mean ± SEM (n = 3). *C*: Immunofluorescence of pancreatic slices showing islets from ST control and DT mice, both treated with doxycycline, visualized with anti-insulin antibodies. Increased MCT1 staining is visible in DT mice. Scale bar = 50 μm. *D*: Quantification of immunofluorescence showing intensity of α-MCT1 signal within insulin-positive regions as mean ± SEM. *E*: *Mct1* mRNA quantified by quantitative RT-PCR in tissues prepared from DT mice treated with (+Dox) or without (−Dox) doxycycline (n = 3). *P < 0.05 by Student t test; **P < 0.01 by Student t test. Sk., skeletal. (A high-quality digital representation of this figure is available in the online issue.)**

was observed in the islets of DT mice but was absent from islets of ST control mice (Fig. 1*C*). Quantification of the anti-MCT1 signal intensity within insulin-positive areas of the islets showed significant upregulation (Fig. 1*D*) in DT mice. An anti-MCT1 signal was observed in 68 ± 9% (mean ± SEM) of insulin-positive cells in DT islets ($n = 3$), and no MCT1 was detected in glucagon-positive cells (Supplementary Fig. 1).

The tissue-specificity of *Mct1* overexpression was determined by quantitative RT-PCR in tissues extracted from DT mice treated or not with doxycycline in vivo (Fig. 1*E*). A significant increase in *Mct1* expression was detected in islets, whereas doxycycline had no effect on *Mct1* expression in any other tissue examined.

***Mct1* expression renders insulin secretion from isolated islets sensitive to pyruvate.** After confirmation of transgene expression, we next studied its effects on insulin secretion from isolated islets. It is noteworthy that the inducible transgenic model developed here allowed islets from a single animal to be compared with and without transgene induction, thus excluding interanimal variation. Islets isolated from a single animal were divided and cultured in the presence or absence of doxycycline for 48 h before being subjected to insulin secretion assays (Fig. 2). MCT1 overexpression exerted no clear effect on insulin secretion in response to glucose at either 8 or 17 mmol/L. This suggests that the expected increased permeability of the β-cell plasma membrane to pyruvate in response to MCT1 induction does not lead to any loss of glycolysis-generated pyruvate from the cell. Whereas the addition of 1 mmol/L or 10 mmol/L pyruvate exerted no effect on noninduced islets, the monocarboxylate significantly and substantially stimulated insulin secretion from islets overexpressing *Mct1*. Although the effect of 0.1 mmol/L pyruvate was not statistically significant, a tendency toward increase insulin secretion was observed (Fig. 2). These effects were specific to pyruvate, with lactate having no significant stimulatory effect at the same concentrations, consistent with previous findings (11). Having confirmed that *Mct1* expression renders insulin secretion specifically sensitive to pyruvate in vitro, we went on to study its effects on glucose homeostasis in the context of the whole animal.

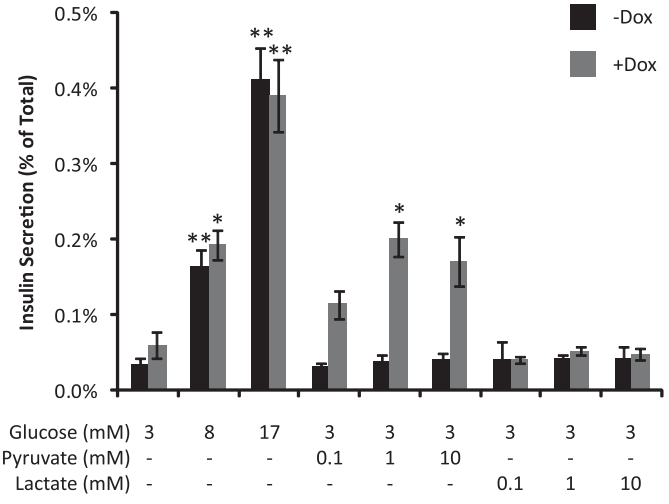

FIG. 2. Abnormal stimulation of insulin secretion by pyruvate from islets in vitro. Islets isolated from a DT mouse were isolated, divided, and cultured for 48 h in the presence (+Dox) or absence (−Dox) of 5 μg/mL doxycycline. Batches of six size-matched islets were incubated in Krebs buffer with 3 mmol/L glucose for 60 min and then transferred to various concentrations of glucose, pyruvate, or lactate in Krebs buffer for 30 min. Secreted insulin is presented as a percentage of total insulin content of islets. Data are presented as mean ± SEM ($n = 3$–6). Data for each induction state were analyzed by ANOVA with Dunnett multiple comparison test to compare each condition with 3 mmol/L glucose *$P < 0.05$; **$P < 0.01$.

MCT1 expression in vivo causes pyruvate to lower blood glucose without affecting glucose tolerance. Without doxycycline treatment, blood glucose levels after an overnight fast were similar between ST and DT genotypes (Fig. 3*A*). After doxycycline treatment, however, DT animals displayed significantly lower fasting blood glucose by an average of 1.5 ± 0.3 mmol/L relative to ST controls ($P < 0.001$) (Fig. 3*A*). It is noteworthy that lowered fasting blood glucose has also been observed in human subjects affected by EIHI (1). Although the fasting plasma insulin levels were not significantly different between mouse genotypes, there was a nonsignificant trend toward lower insulin levels in DT animals. Because there were no significant differences between males and females, data from both sexes were combined for this assessment and for pyruvate tolerance tests. After 3 weeks of doxycycline treatment, there were no differences in behavior or body weight (Supplementary Fig. 2) between ST and DT animals.

EIHI is characterized by the ability of pyruvate to stimulate insulin secretion, lowering blood glucose (1). To test whether induced *Mct1* expression in β-cells may be sufficient to cause this effect in vivo, blood glucose was measured after intraperitoneal injection of pyruvate. Under these conditions, pyruvate is likely to be metabolized largely by the liver, serving as a gluconeogenic substrate in control animals and leading to glucose output and an increase in blood glucose levels (23). However, should pyruvate also stimulate insulin secretion, hepatic glucose output is likely to be lowered and the excursion in blood glucose diminished.

Initially, a relatively low dose (0.5 g/kg) of pyruvate was administered to avoid increasing blood glucose levels excessively. Pyruvate tolerance was first tested in mice before treatment with doxycycline (Fig. 3*C* and *D*). The drug was then administered in drinking water for 1 week, and a second pyruvate tolerance test was then performed on the induced animals (Fig. 3*C* and *D*). Before doxycycline treatment, the glucose excursion was similar in both genotypes, with no significant differences detected. After transgene induction, however, the DT animals displayed a slower initial increase in blood glucose in response to pyruvate administration, resulting in significantly lower levels at the 15- and 30-min time points. At 120 min, blood glucose levels had dropped significantly lower in DT animals than in ST animals. These effects are consistent with pyruvate stimulation of insulin secretion lowering blood glucose in DT animals relative to ST controls.

We next attempted directly to detect an increase in plasma insulin in response to injection of this level of pyruvate. Low basal plasma insulin levels after overnight fast approached the assay's detection limit, however, meaning that any increase was too small to be detected over the sample-to-sample variation. To address this, we increased the level of pyruvate administered to 2 g/kg, a level routinely used in pyruvate tolerance tests (24,25). Intraperitoneal injection at this dose led to substantial increases in both plasma pyruvate and lactate, and these changes were not affected by MCT1 overexpression (Fig. 3*F* and *G*). The increased plasma pyruvate stimulated a significant and rapid increase in plasma insulin only in DT animals (Fig. 3*E*), confirming that MCT1 expression renders insulin secretion sensitive to pyruvate in vivo.

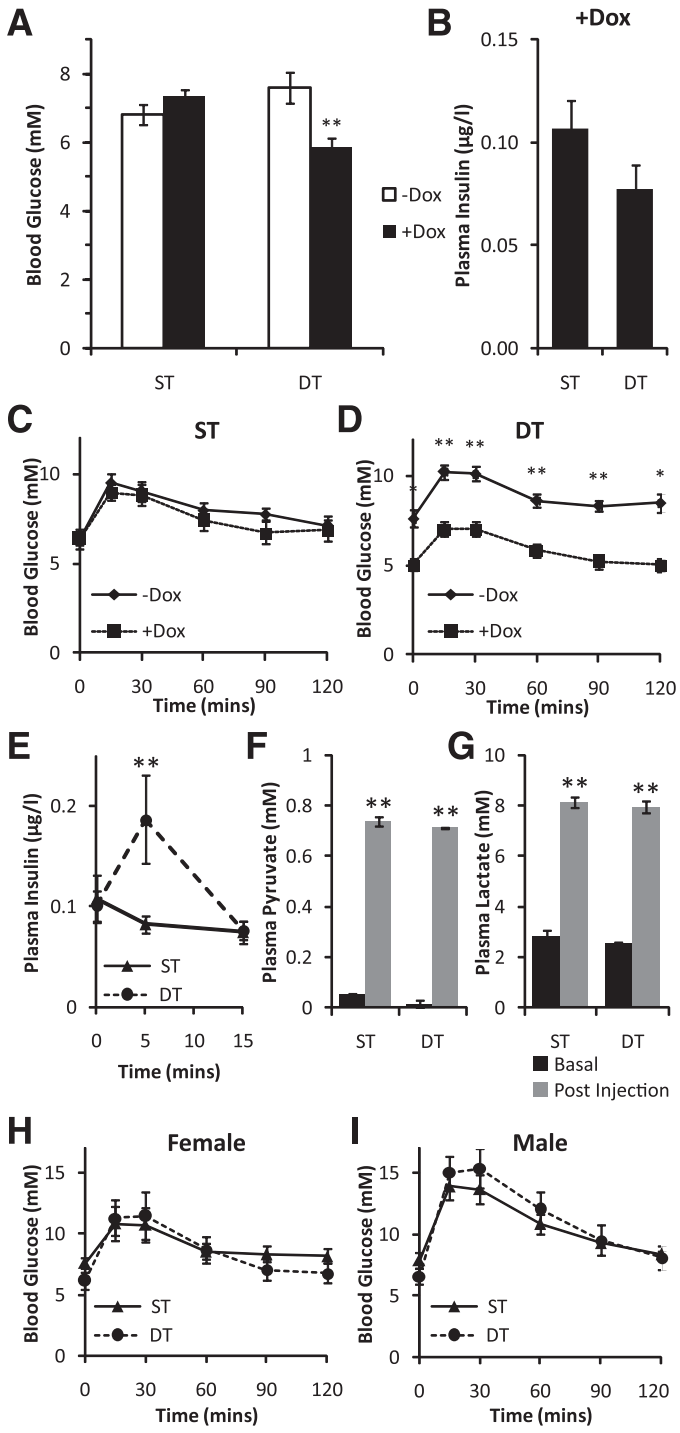

FIG. 3. Effects of *Mct1* overexpression in β-cells on blood glucose homeostasis in vivo. *A*: Blood glucose after overnight fast was not significantly different between ST and DT mice without doxycycline treatment. After treatment with 1 g/L doxycycline in the drinking water for 5 days, fasting blood glucose in DT mice was significantly lower than in ST controls (n = 13 for both groups). *B*: Fasting plasma insulin levels were not significantly different between ST and DT mice (n = 14 for both groups). *C* and *D*: Pyruvate tolerance test. ST and DT mice were tested both before (−Dox) and after (+Dox) induction with doxycycline. Pyruvate (0.5 g/kg) was injected intraperitoneally into mice after an overnight fast, and blood glucose was measured at the time points indicated. Two-way ANOVA with Bonferroni post test was performed to detect differences between genotypes for each time point (ST n = 6, DT n = 12). *E*: Pyruvate-stimulated insulin secretion. ST and DT mice were tested after induction with doxycycline. Pyruvate (2 g/kg) was injected intraperitoneally into mice after an overnight fast, and blood was collected at time points indicated. Plasma insulin was measured by enzyme-linked immunosorbent assay (n = 5 for both groups). *F* and *G*: Plasma pyruvate (*F*) and lactate (*G*) levels both before and 15 min after injection with 2 g/kg pyruvate (n = 3 for both groups). *H* and *I*: Glucose tolerance test. ST and DT mice were induced with doxycycline as previously described, fasted overnight, then injected intraperitoneally with 1 g/kg glucose. Blood glucose was measured at the time points indicated. Because of sex-specific differences in glucose tolerance, data for females (*H*) and males (*I*) are displayed separately (ST females n = 6, DT females n = 8; ST males n = 8, DT males n = 12). No significant differences between genotypes were detected by two-way ANOVA with Bonferroni post hoc test. All data are presented as mean ± SEM. *P < 0.05; **P < 0.001.

The effect of MCT1 overexpression on glucose tolerance was next studied in doxycycline-treated ST and DT mice. Because differences in glucose tolerance were observed between males and females, data from each sex were analyzed separately. Whereas ST males were less glucose tolerant than corresponding females, there was no significant effect of MCT1 expression in either case (Fig. 3*H* and *I*).

MCT1 expression causes relative hyperinsulinemia during physical exercise. The key characteristic of EIHI is inappropriate stimulation of insulin secretion during physical exercise. To test whether forced expression of MCT1 in β-cells is sufficient to replicate this phenotype, we investigated the effect of treadmill exercise on the transgenic mice. The running capacities of male DT mice and their ST littermate controls were measured both before and after doxycycline treatment. The drug exerted no significant effect on the running capacity of mice of either genotype (Fig. 4*A*). In contrast, doxycycline induced a significant decrease in blood glucose level after a 4-h fast in DT mice (Fig. 4*B*). One exercise bout at 80% of maximal running speed for 20 min induced a decrease in blood glucose level in both genotypes (Fig. 4*C*). The magnitude of the decrease (ST 1.82 ± 0.61 vs. DT 1.89 ± 0.30 mmol/L) was similar between genotypes; however, because the initial level was lower in the DT mice, the level fell to 3.3 ± 0.6 mmol/L relative to 5.4 ± 0.6 mmol/L in ST controls (Fig. 4*C*). In ST controls, the drop in blood glucose was accompanied by a significant fall in plasma insulin (Fig. 4*D*). Moreover, there was no significant change in HOMA2-B%, a measure of insulin relative to glucose (21,26), before and after exercise (93 ± 11 vs. 103 ± 22%) (Fig. 4*E*) in these mice.

DT mice displayed a nonsignificant trend toward higher fasting HOMA2-B% values than seen in ST mice (118 ± 28 vs. 93 ± 11%), suggestive of a higher level of insulin normalized to glucose. In response to exercise, no decrease in plasma insulin was observed despite a drop in glucose levels. This stable insulin level in the face of falling glucose in DT mice suggests that a stimulatory mechanism compensated for the expected exercise-mediated inhibition of insulin secretion. In supporting of this view and in contrast to ST controls, we observed a sharp increase in the HOMA2-B% value after exercise in DT mice (DT 486 ± 167 vs. ST 103 ± 22%; $P < 0.05$) (Fig. 4*E*).

To assess whether the imposed level of exercise might raise circulating pyruvate levels, we compared basal lactate and pyruvate levels with those immediately after exercise (Fig. 4*F* and *G*). Exercise produced a significant increase in plasma lactate, which was similar (ST 1.05 ± 0.35 vs. DT 1.46 ± 0.35 mmol/L) in the two genotypes. The expected lower levels of pyruvate rendered measurements of this parameter more difficult (Fig. 4*G*), and here no clear changes or differences between genotypes were observed. Providing confirmation that altered stress levels did not contribute to the results observed, no significant differences were observed between plasma corticosterone concentrations in ST and DT mice after exercise (Supplementary Fig. 3).

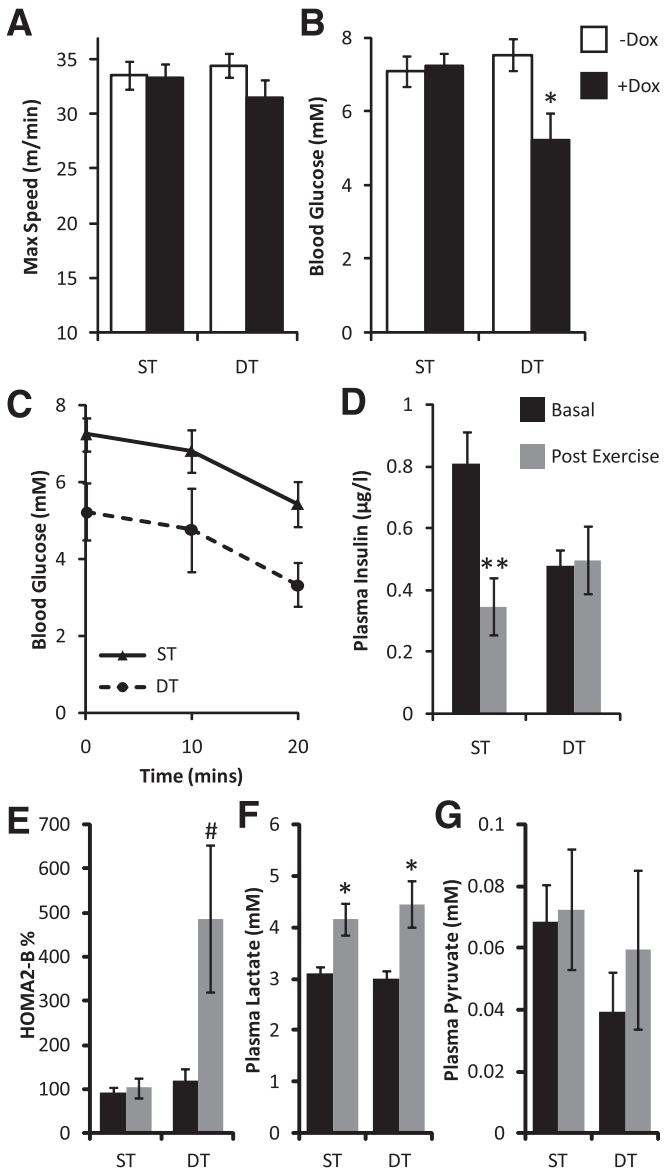

FIG. 4. DT mice display abnormal insulin secretion during treadmill exercise. Male mice were subjected to treadmill exercise at a 5% incline (see RESEARCH DESIGN AND METHODS). *A* and *B*: The maximum running speed (*A*) and blood glucose after a 4-h fast (*B*) of each genotype before (−Dox) and after (+Dox) doxycycline treatment. *C*–*G*: After fasting, mice were run at 80% of maximum running speed for 20 min. Blood glucose levels (*C*) were measured during exercise. Plasma insulin (*D*), lactate (*F*), and pyruvate (*G*) were measured before and immediately after exercise. HOMA2-B% values (*E*) were calculated from the glucose and insulin levels. Data are presented as mean ± SEM (ST n = 10, DT n = 8). *$P < 0.05$ within genotypes; **$P < 0.001$ within genotypes; #$P < 0.05$ between genotypes.

DISCUSSION

The principal aim of this study was to determine whether controlled overexpression of MCT1 selectively in pancreatic β-cells recapitulates the key features of EIHI, notably pyruvate- and exercise-induced insulin secretion and hypoglycemia. This has seemed important in view of the fact that mutations linked to the disease are likely to increase the expression of MCT1 in all tissues (2), raising the possibility that multiple tissue interactions may be involved in altered glucose homeostasis.

Although pyruvate and lactate are reportedly metabolized by islets (27), it seems likely that this occurs largely in the non–β-cells comprising 20–30% of the islet mass, as well as in endothelial and possibly contaminating acinar cells (see Ishihara et al. [11] for discussion). Previous studies (11) have demonstrated that pyruvate-induced insulin secretion can be unmasked by adenovirus-mediated overexpression of MCT1 in isolated islets. It is noteworthy that in these earlier studies, lactate failed to stimulate insulin release even after MCT1 overexpression, consistent with the absence of significant levels of LDHA activity in the β-cell. These earlier in vitro findings, in which the overexpression is likely only to have affected peripheral islet cells (28), were recapitulated here by means of transgene induction after MCT-1 overexpression throughout the islet β-cell mass with the small-molecule regulator doxycycline.

It is noteworthy that we now extend these findings to the in vivo case and show that MCT1 induction in the β-cell markedly improves pyruvate-induced excursions in blood glucose as a result of enhanced insulin release. By contrast, neither the studies reported by Ishihara et al. (11) nor our findings revealed any differences in glucose-induced insulin secretion in vitro or in glucose tolerance in vivo after MCT1 induction. This observation indicates that strict MCT1 suppression is not essential for the normal triggering of secretion by glucose. We note that although the mouse model that we used was generated by random insertion of the transgene, an off-target effect resulting from disruption of a nontargeted gene seems unlikely given that the metabolic phenotype was only apparent after gene induction with doxycycline.

One of the common characteristics of human patients with EIHI and the doxycycline-induced DT mice described here is a significantly lowered fasting blood glucose level. In both cases, this seems likely to be a reflection of the fact that normal (low) levels of circulating pyruvate constantly augment insulin secretion, thus suppressing hepatic glucose output and stimulating glucose clearance by other tissues. In the mouse model, however, fasting insulin levels (Figs. 3*B* and 4*D*) were not significantly different between ST and DT mice, although a slight trend toward lowered insulin in the DT mice was observed. Although this result may appear surprising, two points should be made. First, the observed lowering in insulin was proportionately less than the lowering in glucose level, such that β-cell function (as gauged in our calculated value of HOMA2-B%) was enhanced in the DT relative to the ST mice (Fig. 4*E*). Second, our measurements from tail-vein samples reflect systemic insulin levels. In mice of either genotype, concentrations of the hormone in the portal vein are likely to be higher than those in the general circulation, reflecting significant hepatic extraction (29). Indeed, portal vein insulin concentrations may conceivably be higher in DT mice than in ST mice after induction and might exert a more powerful inhibitory effect on hepatic glucose output in the former group.

One of the main differences between our rodent model relative to human EIHI was the degree to which physical exercise raised lactate and pyruvate levels. Moderately strenuous bicycle exercise for 10 min in human patients with EIHI and control subjects caused lactate levels to rise from a basal value of <1 mmol/L to ~6 and 7 mmol/L, respectively (1). In contrast, exercising the mice at 80% of their maximum running speed for 20 min caused a far more modest increase, from 3 to 4.1–4.5 mmol/L. Although this change was still significant, it was clearly far less than that seen in the human subjects. Moreover, the bicycle exercise increased pyruvate levels in humans in a similar manner, from ~50 to ~200 µmol/L (1). Although the basal pyruvate level in the mice was similar at ~50 µmol/L, the combination

of a low concentration of pyruvate in blood and the considerably smaller volumes of blood we were able to obtain from mice relative to human subjects introduced considerable variability into our pyruvate measurements. Nonetheless, we anticipated that that a significant increase in plasma lactate would be accompanied by an increase in plasma pyruvate broadly similar in magnitude, as was the case with human exercise. Given the relatively modest increase in lactate, the expected similarly modest increase in pyruvate may explain why glucose levels did not drop further in DT mice in response to exercise. Although it is possible that we would have observed higher plasma lactate and pyruvate levels if we had run the mice at higher speeds, we note that the mice were already running at 80% of their maximum speed, and an increased exercise level thus would have been difficult to maintain for more than a few minutes without causing considerable stress to the animals.

Despite the relatively modest increase in lactate (and presumably pyruvate) in response to exercise, it appears that this increase was sufficient to affect β-cell function in DT mice. Thus, although blood glucose dropped in both genotypes during exercise, only in DT mice did the insulin level remain stable. We conclude that pyruvate stimulation of insulin secretion allowed by MCT1 expression compensates in DT mice for the exercise-induced inhibition of insulin secretion observed in ST mice. Thus, overexpression of MCT1 in β-cells is sufficient to replicate the key symptom of EIHI, highlighting the importance of maintaining the β-cell–specific block on the expression of this gene.

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T.J.P. researched data and wrote the manuscript. L.S., G.S., and E.A.R. researched data. A.P.H. provided reagents and reviewed the manuscript. G.A.R. wrote the manuscript, coordinated the study, and is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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