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ORIGINAL ARTICLE

Reduced aldehyde dehydrogenase activity and arginine vasopressin receptor 2 expression in the kidneys of male TALLYHO/JngJ mice of prediabetic age

Noriko Nakamura

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Abstract The TALLYHO/JngJ (TH) mouse is a novel polygenic model of type 2 diabetes and exhibits obesity, hyperglycemia (males), hyperinsulinemia, hyperlipidemia, and enlarged pancreatic islets. Since the kidney is damaged by hyperglycemia in other animal models, the present study aimed to determine the kidney phenotype of TH mice using immunoblot and histological analyses of the kidneys of 6-week-old (prediabetic) and 16-week-old TH mice. Interestingly, even 6-week-old male TH mice showed significant increases in kidney weight, compared to C57BL/B6 (B6) mice. Cuboidal parietal epithelium was observed in the Bowman's capsule in male TH mice at the prediabetic age. Water accumulated inside the kidneys of male TH mice in an age-dependent manner, but not in B6 mice. Since Swr/J mice are reported to develop diabetes insipidus and share 86.8% genotype homology with TH mice, the expression level of arginine vasopressin receptor 2 (AVPR2), a candidate protein for diabetes insipidus, was examined and determined to be significantly reduced in the kidneys of prediabetic male TH mice, compared to B6 mice. Aldehyde dehydrogenase (ALDH) activity in the kidneys of prediabetic male TH mice was significantly lower than that in age-matched male B6 mice, while there were no differences between female TH and B6 mice. These results suggest that the kidney phenotype of prediabetic TH mice occurs only in males, accompanied by a reduction in ALDH activity and AVPR2 expression. The kidney phenotype of male TH mice at a prediabetic age becomes evident before the onset of diabetes.

Keywords Aldehyde dehydrogenase activity · TALLYHO/JngJ mice · Arginine vasopressin receptor 2 · Kidney · Swr/J mice

Introduction

Type 2 diabetes is the most common human disease worldwide, and its prevalence is predicted to increase 2-fold by 2030, over the prevalence in 2000 [1]. Diabetes results in kidney disease, and diabetic nephropathy is currently a major cause of end-stage renal failure. Data from the United States Renal Data System [2] show that diabetes was the primary cause of end-stage renal failure in 54% of new patients in 2007 and a more common cause of end-stage renal failure (619 per million in the population) than hypertension (407 per million in the population).

Several monogenic animal models exhibit both obesity and diabetes, including C57BLKS/J^{Lepr} (*db/db*) mice, B6.V-Lep^{ob}/J (*ob/ob*) mice, and Zucker fatty (*fa/fa*) rats [3]. The *db/db* mice are well studied in terms of diabetic kidney diseases [4, 5]. Polygenic animal models of type 2 diabetes, such as the Goto Kakizaki (GK) rat, KK mouse, Nagoya-Shibata-Yasuda (NSY) mouse, Otsuka Long-Evans Tokushima fatty (OLETF) rat, and Israeli sand rat, have also been studied. Since human diseases, such as

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N. Nakamura (✉)
Department of Pharmacology, Physiology and Toxicology, Joan C. Edwards School of Medicine, Marshall University, Huntington, WV 25755, USA
e-mail: Noriko.Nakamura@fda.hhs.gov

Present Address:
N. Nakamura
Division of Personalized Nutrition and Medicine, National Center for Toxicological Research, Food and Drug Administration, Jefferson, AR 72079, USA

obesity, atherosclerosis, diabetes, and hypertension, show phenotypic variation and a predominantly polygenic basis, it is important to study polygenic animal models. Mice are especially advantageous to use, because of their ease of breeding, tremendous genetic variation, high homology with human genes, and well-characterized gene markers [6].

The TALLYHO/JngJ (TH) mouse is a novel polygenic model of diabetes [7] and exhibits hyperglycemia (males), obesity, hyperlipidemia, hyperinsulinemia, and enlarged pancreatic islets [7, 8]. Although the kidney is a very important organ, whose function is impaired by hyperglycemia as demonstrated in other animal models [9–11], it has not yet been examined in TH mice.

The present study used histological and physiological approaches to examine the kidneys of TH mice fed a standard chow diet, especially those at a prediabetic age. We found that the kidney phenotype of TH mice is only seen in male mice and is accompanied by reduced aldehyde dehydrogenase (ALDH) activity and reduced arginine vasopressin receptor 2 (AVPR2) expression in the kidney at a prediabetic age. Thus, in TH mice, this kidney phenotype may be influenced by the genetic background of the mouse strain, and male TH mice exhibit these phenotypes before the onset of type 2 diabetes.

Materials and methods

Materials

All reagents were purchased from Fisher Scientific (Pittsburgh, PA, USA), MP Biomedicals (Solon, OH, USA), and Acros Organics USA (Morris Plains, NJ, USA), unless otherwise indicated.

Animals

Establishment and breeding of TALLYHO/JngJ (TH) mice have been described previously [7, 8]. C57BL/B6 (B6) and Swr/J (Swr) mice were used as control mice. The mice studies were approved by the Marshall University Committee on Animal Care and Use. Animals were maintained under controlled ambient temperature, humidity, and light cycles. Animals were fed a standard rodent chow (Purina 5001; Purina Mills LLC, St. Louis, MO, USA).

Sample collection

TH and B6 mice (6 or 16 weeks old) were euthanized with carbon dioxide, followed by exsanguination and tissue collection. Body, liver, and kidney weights were recorded.

Measurement of plasma glucose, triglycerides, and cholesterol

Blood was collected into glass tubes with sodium heparin (BD, Franklin Lakes, NJ, USA), and plasma was obtained by centrifugation at 1200×*g* for 10 min at 4°C. Plasma glucose levels were measured using a glucose oxidase reagent (Thermo Fisher Scientific, Waltham, MA, USA).

Morphological analysis

Kidneys from B6 and TH mice were fixed in 4% paraformaldehyde in phosphate-buffered saline, dehydrated, embedded in paraffin, and cut into 4–6 μm sections. After deparaffinization, slides were stained with hematoxylin QS (Vector Laboratories, Burlingame, CA, USA) for 30 s, washed in running water, and stained with a 0.5% eosin/alcohol solution (Polyscience Inc., Warrington, PA, USA) for 2 min. The stained slides were dehydrated and mounted with Poly-Mount (Polyscience Inc.).

Sample preparation procedure

Kidneys were homogenized in lysis buffer (20 mM TBS, 150 mM NaCl, 1 mM EDTA [pH 8.0], 1% Triton X-100, 0.1% SDS, 0.1% sodium deoxycholate, 0.2 mM NaF, 0.2 mM sodium orthovanadate, and 0.2 mM phenylmethanesulfonyl fluoride [PMSF]), incubated for 30 min on ice, and centrifuged at 15,000×*g* for 30 min at 4°C. The supernatants were collected as samples. To prepare the samples for sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE), supernatants were adjusted to 50 μg and added to an equal volume of 2× sample buffer (4% SDS, 100 mM Tris–HCl [pH 6.8], 20% glycerol, 2% 2-mercaptoethanol, and 0.001% bromophenol blue).

Immunoblotting

Proteins extracted from tissues were separated by SDS–PAGE on 10% ready gels (Bio-Rad Laboratories, Hercules, CA, USA) and transferred to Immobilon-P PVDF membranes (Millipore, Billerica, MA, USA). The membranes were immunoblotted with AVPR2 (T5168; Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA) and GAPDH antibodies (T5168; Santa Cruz Biotechnology Inc.) and then detected using an ECL Western Blotting Detection system (GE Healthcare, Piscataway, NJ, USA) according to the manufacturer's instructions. Band intensity was quantified using Image J software (Version 1.38, NIH, Bethesda, MD).

ALDH activity

Livers and kidneys were homogenized in lysis buffer (100 mM Tris [pH 8.0], 100 mM KCl, 1 mM EDTA, 0.02% BSA, 1% Triton X-100, 0.2 mM NaF, 0.2 mM sodium orthovanadate, and 0.2 mM PMSF), incubated on ice for 10 min, and centrifuged at 15,000×*g* for 30 min at 4°C. The supernatants were used as samples. ALDH activity was measured as described by Bostian and Betts [12]. Briefly, 0.1 ml of sample was assayed at room temperature in a reaction buffer containing 100 mM Tris (pH 8.0), 100 mM KCl, 0.67 mM βNAD+, 2 mM acetaldehyde, and 10 mM 2-mercaptoethanol. Enzyme activity was determined using a UV spectrometer (Thermo Scientific, Madison WI, USA) set at 340 nm to measure the increasing amount of NADH. The protein concentration in the samples was measured using a Total Protein Kit (TP0300; Sigma-Aldrich) or Bradford reagents.

Statistical analysis

Values are presented as means ± SD. Statistical analyses were performed using the Student's *t* test (2 samples, assuming unequal variances) to calculate the means and SD.

Results

Increased kidney weight and reduced arginine vasopressin receptor expression in 6-week-old male TH mice

Body weight was significantly higher in 6-week-old (prediabetic age) TH mice of both sexes, as compared to age-matched B6 mice (Table 1). This result is consistent with previous results [8]. Interestingly, the kidney weight/10 g body weight was significantly higher in male TH mice at 6 and 16 weeks of age than in the same age groups of B6 mice, while liver weights were comparable between these strains (Table 1). There were no differences in kidney or liver weights between female TH and B6 mice at 6 weeks of age.

Next, the possible causes of the differences in kidney weights between 6-week-old TH and B6 mice were examined. Water accumulated inside the kidneys of male TH mice in an age-dependent manner was observed, but not in female TH or male or female B6 mice (data not shown). The genetic quality control annual report from the Jackson Laboratory indicates that the genotype homology of Swr and TH mice is 86.8% [13]. The kidney weight/10 g body weight in 6-week-old male TH mice was almost identical to that in Swr mice (data not shown). Swr mice are reported to have renal deficiency associated with nephrogenic diabetes insipidus (NDI) [14]. NDI occurs as a result of an *Avpr2* gene mutation in humans [15] and is most common in male mice because the *Avpr2* gene is located on the X chromosome [16]. Another animal model of diabetes insipidus, the Brattleboro rat, is also reported to have reduced vasopressin gene expression levels [17, 18].

It was hypothesized that both TH and Swr mice develop the NDI phenotype. To test this hypothesis, Western blot analysis was conducted to determine whether the AVPR2 expression level was reduced in the kidneys of TH mice. AVPR2 protein expression was significantly lower in male TH mice than in male B6 mice (Fig. 1). Although we have not yet conducted a functional analysis of the kidneys in TH mice to determine whether the phenotype shows polyuria or polydipsia [19], their blood urea nitrogen (BUN) levels were significantly reduced compared with those of B6 mice [8]. In humans, patients with diabetes insipidus are reported to have low serum BUN levels [20, 21]. Collectively, these findings suggest that the kidneys of TH mice exhibit a phenotype characteristic of NDI.

Table 1 Body and organ weights in B6 and TH mice at 6 and 16 weeks of age

		6 weeks		16 weeks	
		TH	B6	TH	B6
Body weight (g)	Male	26.22 ± 1.40‡ ($n = 8$)	19.85 ± 1.43 ($n = 10$)	35.88 ± 4.01‡ ($n = 8$)	27.59 ± 1.40 ($n = 11$)
	Female	21.95 ± 0.70‡ ($n = 9$)	17.88 ± 1.50 ($n = 9$)	26.67 ± 1.93** ($n = 6$)	22.09 ± 1.36 ($n = 5$)
Kidney weight (g/10 g body weight)	Male	0.109 ± 0.009‡ ($n = 12$)	0.073 ± 0.003 ($n = 11$)	0.098 ± 0.008‡ ($n = 5$)	0.072 ± 0.004 ($n = 8$)
	Female	0.080 ± 0.008 ($n = 7$)	0.074 ± 0.008 ($n = 8$)	0.071 ± 0.006 ($n = 5$)	0.068 ± 0.002 ($n = 5$)
Liver weight (g/10 g body weight)	Male	0.647 ± 0.051 ($n = 6$)	0.671 ± 0.021 ($n = 8$)	0.546 ± 0.055 ($n = 5$)	0.559 ± 0.013 ($n = 6$)
	Female	0.562 ± 0.044 ($n = 7$)	0.593 ± 0.029 ($n = 8$)	0.462 ± 0.022 ($n = 5$)	0.552 ± 0.020‡ ($n = 5$)

‡ $P < 0.001$ compared to B6 in each ages

** $P < 0.01$ compared to B6 in each ages

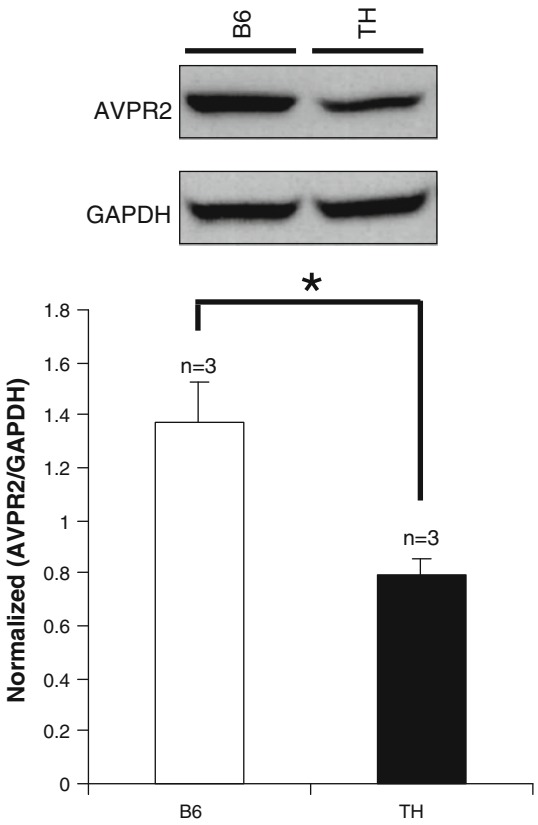

Fig. 1 Arginine vasopressin receptor 2 (AVPR2) expression in the kidneys of TH and B6 mice at 6 weeks of age. Immunoblotting was done using an AVPR2 antibody and a GAPDH antibody as loading controls (*upper panel*). Expression levels are shown as normalized values (*lower panel*). Data are expressed as mean ± SD of 3 different experiments (*$P < 0.05$ compared with B6 mice)

Cuboidal epithelia-like parietal epithelium of Bowman's capsule in kidneys of TH mice at 6 and 16 weeks of age

Histology of the kidneys of TH mice was almost normal, with the exception that the parietal epithelium of Bowman's capsule exhibited cuboidal epithelia-like epithelial cells forming the proximal tubules in 6-week-old male TH mice (Fig. 2b, arrow), compared to normal parietal epithelia in age-matched B6 mice (Fig. 2a). At 16 weeks of age, most parietal epithelia of Bowman's capsule in male TH mice (Fig. 2d, arrows) had greater amounts of cuboidal cells than those seen in 6-week-old male TH mice. Interestingly, this change in the parietal epithelium of Bowman's capsule was not observed in female TH mice at either 6 or 16 weeks of age (data not shown). In addition, this histological change was also observed in kidneys of male Swr mice (data not shown). There was no evidence of macrophage invasion in the renal glomeruli in male TH mice at 6 or 16 weeks of age (Fig. 2b, d). In inbred mice strains, cuboidal epithelium has been observed with greater frequency in Bowman's capsule in adult male mice and in mice treated with testosterone [22]. As with the spontaneously hypertensive rat [23], susceptibility to renal tube injury and histological changes in the parietal epithelium of Bowman's capsule may be correlated [24] in GK rats [25] and *db/db* mice [26].

The factors that contribute to formation of cuboidal parietal epithelium in Bowman's capsule in the kidneys of 6-week-old male TH mice were next considered. It was hypothesized that high blood glucose levels caused this phenotype in male TH mice, because high glucose levels induce reactive oxygen species (ROS) generation, and ROS damages the glomerulus of the kidneys in *db/db* mice [9–11]. However, plasma glucose concentrations did not differ between male TH and male B6 mice at 6 weeks of age (Supplementary Table 1). These results suggest that morphological changes in the parietal epithelium of Bowman's capsule in 6-week-old male TH mice are not be caused by glucose. Thus, this observation (Fig. 2) of the kidney morphology in male TH mice may be species or strain dependent [24]; however, it may also occur due to factors that are present in mice and are unlike those in rats.

Reduced ALDH activity in kidneys of 6-week-old male TH mice

We next measured kidney ALDH activity to determine which factors cause morphological changes in the kidneys of 6-week-old male TH mice. Aldehydes induce lipid peroxidation, which causes oxidative degradation of lipids in the cellular membrane, leading to cell damage [27]. Most isozymes of the ALDH family are distributed in the kidney [28] and catalyze aldehyde oxidation with $NAD(P)^+$, which protects cells from ROS [29]. In addition, some ALDH isozymes protect against osmotic stress [30]. At both 6 and 16 weeks of age, ALDH activity in the kidneys of male TH mice was significantly lower than that in B6 male mice (0.018 ± 0.006 units/mg protein in B6 mice vs. 0.004 ± 0.002 units/mg protein in TH mice at 6 weeks of age; 0.0022 ± 0.0009 units/mg protein in B6 mice vs. 0.0007 ± 0.0008 units/mg protein in TH mice at 16 weeks of age, Fig. 3a). However, there was no difference in kidney ALDH activity between 6-week-old female TH and B6 mice (Fig. 3a). There was also no difference in liver ALDH activity among male and female TH and B6 mice (Fig. 3b). ALDH activity in the kidneys of 6-week-old Swr male mice was also significantly lower than that in B6 mice (0.006 ± 0.0001 units/mg protein in Swr mice at 6 weeks of age; $n = 3$), but was similar to that in TH mice.

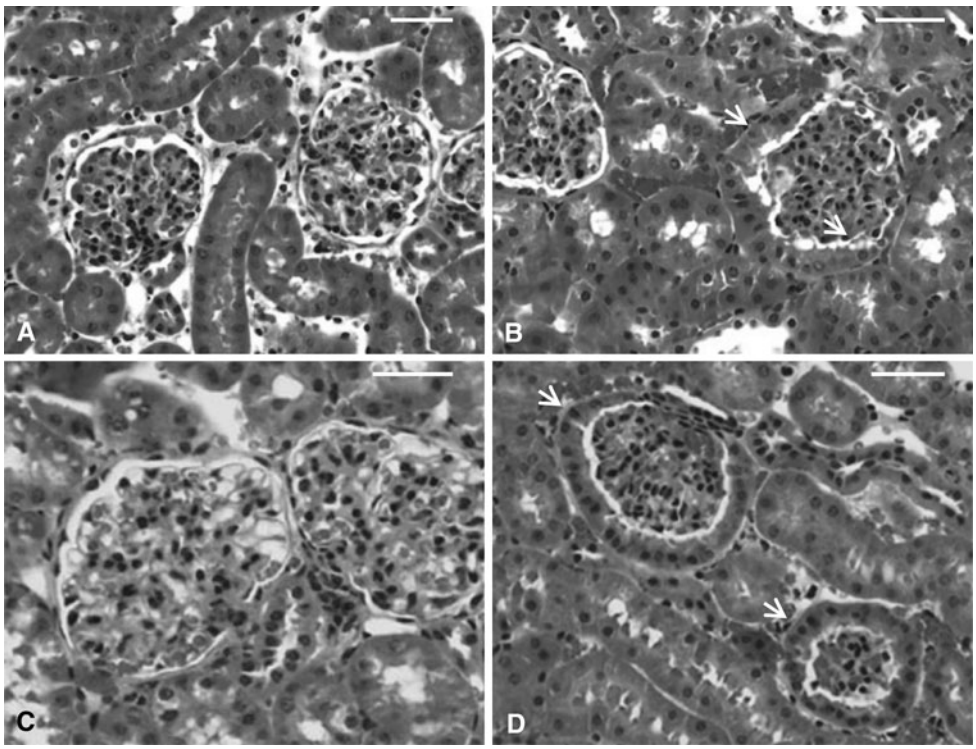

Fig. 2 Kidney histology in B6 and male TH mice at 6 and 16 weeks of age. Glomerulus of a kidney is shown from 6-week-old (**a**, **b**) and 16-week-old (**c**, **d**) B6 (**a**, **c**) and TH (**b**, **d**) mice. The cuboidal parietal epithelia of Bowman's capsule are shown in the kidneys of TH mice at 6 and 16 weeks of age (**b**, **d**; *arrows*). The kidney histology was performed using three different B6 and TH mice at each age with identical results. *Bars*: 25 μm

Discussion

The present study of kidney phenotype in TH mice found that even at a prediabetic age, kidney weight/10 g body weight was increased, AVPR2 protein expression level was reduced, and kidney ALDH activity was decreased. These findings suggest that the kidney phenotype occurs before the onset of type 2 diabetes. The present findings also suggest that the kidney phenotype of TH mice is similar to that of Swr mice at a young age. Reduced AVPR2 expression levels and ALDH activity in the kidneys were observed in male TH mice, indicating that male TH mice may be able to develop NDI like Swr mice. The kidneys may be affected by genetic background rather than by the disease at the prediabetic age. As TH mice age, the kidneys may be affected and damaged by high glucose levels and obesity, due to increasing TNF-α expression levels in the kidneys of male TH mice (data not shown). The findings of this study indicate that the described kidney phenotype of male TH mice should also be considered, independent of diabetes disease state, whenever interpreting results from this particular mouse model.

The most interesting finding of the present study is that the kidney phenotype occurred only in male TH mice. Hyperglycemia was also seen only in male TH mice [7, 8]. As such, there are sex-related differences in the kidney phenotype of TH mice. Since reduced AVPR2 expression levels in male TH mice may be related to NDI and the mutation of the *Avpr2* gene on the X chromosome, the observed disease-related sex differences in TH mice may be related to the X chromosome. In a study using OLETF rats, Hirashima et al. [31] identified a candidate gene for diabetes, located on the X chromosome. In humans, the X chromosome has a potential locus for fat distribution [32]. Thus, TH mice may have a candidate locus for diabetes or obesity on the X chromosome.

This study found that ALDH activity was lower in the kidneys of male TH mice, but not in the liver of male TH mice or female TH or B6 mice. Currently, we do not know why ALDH activity was lower only in the kidneys of male TH mice and have yet to determine the correlation between the cuboidal parietal epithelium of Bowman's capsule and low ALDH activity in the kidneys. However, we found that ALDH activity in the kidneys of Swr male mice was lower than that in B6 mice. There may be an association between reduced AVPR2 expression and ALDH activity in the kidney. Further studies are needed to elucidate the reason for this finding.

In conclusion, kidney phenotypes showed the following characteristics in TH mice at a prediabetic age: (1) the kidney weight/10 g body weight of male TH mice was increased compared with that of B6 mice; (2) cuboidal parietal epithelium was observed in Bowman's capsule in male TH mice; (3) AVPR2 expression levels in the kidneys of male TH mice were significantly reduced compared with those of B6 mice; and (4) ALDH activity in the kidneys of male TH mice was significantly reduced compared with

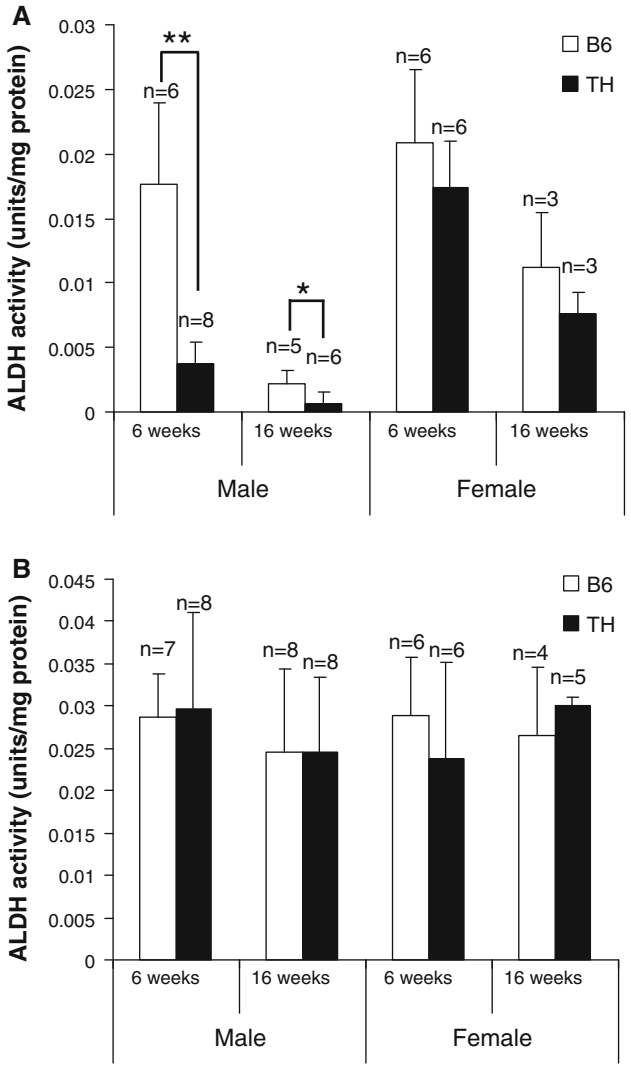

Fig. 3 Aldehyde dehydrogenase (ALDH) activity in the kidneys (**a**) and livers (**b**) of male and female TH and B6 mice at 6 and 16 weeks of age. ALDH activity was measured in the lysates from kidneys and livers of TH and B6 mice at 6 and 16 weeks of age (*white bars*, B6 mice; *black bars*, TH mice). Data are expressed as mean ± SD of separate assays, each done in duplicate (** $P < 0.01$, * $P < 0.05$ compared with B6 mice of the same age)

that in B6 mice. Thus, the kidney phenotype of TH mice is seen only in male mice and may occur before the onset of type 2 diabetes.

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Conflict of interest None.

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