Elevated Kidney Glucosyltransferase Activity in Genetic Prediabetic Mice

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Summary. Glucosyltransferase activity in the renal cortex of genetic diabetic KK mice was significantly increased at 40 days of age when compared to that of Swiss albino and F_1 hybrid mice. This increase in enzyme activity in the absence of glucose intolerance can be regarded as an earlier genetic marker for the diagnosis of diabetic microangiopathy.

In recent years, the importance of genetics in diabetes mellitus has been well appreciated. Persons with a family history of diabetes mellitus were found to have not only hormonal derangement[2,3] but also hypertrophy of the muscle capillary basement membranes[4,5]. These abnormalities become manifest before these individuals develop carbohydrate intolerance (genetic prediabetics). Although the hormonal derangement is less consistent and varies from person to person, a much more consistent defect appears to be the thickening of the muscle capillary basement membrane. For these reasons, the capillary basement membrane hypertrophy has been considered a likely genetic marker for diabetes mellitus[6].

Biochemical studies have shown that basement membranes are glycoprotein in nature[7,8]. In recent years, the nature of the glomerular basement membranes has been the subject of several investigations, both in normal and diabetic states. Spiro and Spiro[9] reported a significant increase in kidney glucosyltransferase activity of alloxan-diabetic rats. This enzyme facilitates the attachment of glucose to hydroxylysine-linked galactose to complete the disaccharide units of the basement membrane. The present report describes the glucosyltransferase activity in the renal cortices and its relationship to blood sugar levels of normal control Swiss albino, genetically diabetic KK and their F_1 hybrid mice.

Material and methods. The Swiss albino (SA) and KK mice have been inbred in our laboratory for 30 and 40 generations, respectively. Male KK and female SA mice were mated and the offspring were considered F_1 hybrids. All mice were maintained under similar laboratory conditions and fed the Old Guilford mouse chow ad libitum.

The mice were sacrificed at 40 days of age between 10.00 h and 12.00 h. They were fasted for 18 h with free access to water. Oral glucose tolerance tests were performed at 38 days of age. Blood was drawn from the orbital plexus. After collecting the fasting blood sample for glucose determination, the mice were given 3 g/kg of glucose by a gastric catheter, and the subsequent samples were collected at 30, 60 and 120 min. For the determination of glucosyltransferase activity, the mice were sacrificed on the 40th day under light ether anesthesia. Kidneys were isolated, cortices separated and homogenized in 0.15 *M Tris*- acetate buffer, pH. 6.8, containing 0.002 *M* 2-mercaptoethanol. The homogenates were centrifuged at 10,000 $\times$ *g* for 20 min, and the supernatant was used as the enzyme source. Blood glucose was determined by the ferricyanide method of Hoffman on the Technicon Autoanalyzer. Glucose areas were calculated by the method of Chiles and Tzagournis[10]. Glucosyltransferase activity was assayed

Table I. Response of blood glucose to oral glucose load and glucose area in Swiss albino (SA) F_1 hybrid and KK mice at 38 days of age after an 18-h fast

		Blood glucose (mg/100 ml)				Glucose area (mg/h/100 ml)
		Time after glucose load (min)				
Mice	*N*	0	30	60	120	
SA	5	68±4	303±8	292±6	182±28	479±14
F_1	5	68±1	287±10	295±4	275±9	519±11
KK	5	69±3	304±9	303±6	233±21	503±21

Values shown are means ± SE.

Table II. Kidney glucosyltransferase activity in Swiss albino (SA), F_1 hybrid and KK mice at 40 days of age

Mice	*N*	Enzyme activity (cpm/mg protein)
SA	5	2319±255
F_1	5	2933±157
KK	5	3808±253
Significance	*p*	
SA vs. KK:	<0.005	
SA vs. F_1	<0.1	
KK vs. F_1	<0.02	

Values shown are means ± SE.

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according to the method of BARBER and JAMIESON.[11] as modified by CAMERINI-DAVALOS et al.[12] Protein was determined by the biuret method[13] using serum albumin as standard.

Results and discussion. Table I shows the results of oral glucose tolerance tests in SA, KK and hybrid mice. No significant difference either in the oral glucose tolerance or in the glucose area was observed among the three groups of mice. However, the kidney glucosyltransferase activity was significantly higher in the KK when compared to that of both SA and hybrid mice (Table II). The enzyme activity, although elevated in the hybrids, was not statistically significant when compared to SA mice. It should, however, be noted that the glucosyltransferase activity in the hybrids was intermediary of both parental strains.

That the KK mouse is an ideal animal model for the studies of human genetic diabetes and microangiopathy has been suggested by us and other investigators[14–18].

These mice have normal tolerance to oral glucose until 100 days (prediabetes) and impaired tolerance later on (chemical diabetes)[12]. About 60% of the mice develop glomerular lesions by 2 months of age[19] at the same time when these mice still have normal tolerance to oral glucose. Determination of glucosyltransferase activity in the renal cortices showed that the KK mice have elevated levels between the 25th and 55th days of life when compared to age-matched SA mice[12, 20]. Later on, no difference in the enzyme activity between the two groups of mice was observed. It is, therefore, understandable that the significant elevation of glucosyltransferase activity in the prediabetic KK mice precedes detectable structural lesions in the kidney and abnormal tolerance to oral glucose.

The present results indicate that changes in the kidney glucosyltransferase activity can be observed in response to gene dosage without affecting glucose tolerance, suggesting that microangiopathy and carbohydrate tolerance are independent at the stage of early diabetes. The data are compatible with changes in muscle capillary basement membrane width in human prediabetics with normal oral glucose tolerance.[4, 5] In addition, the data suggest that enzymes involved in the basement-membrane synthesis are subject to changes depending upon the genetic make-up of the animal. Such an early enzyme change can be used as a possible genetic marker for the diagnosis and prognosis of microangiopathy in those individuals with a family history of diabetes and who had not yet developed intolerance to glucose.

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Metabolism of 1,3,7-Trimethyldihydrouric Acid in the Rat: New Metabolic Pathway of Caffeine

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Summary. [1-CH_3-^{14}C] 1,3,7-trimethyldihydrouric acid which, in quantity, is the most important caffeine metabolite, was isolated and purified from the urine of rats fed with [1-CH_3-^{14}C] caffeine. The oral administration of this metabolite to rats showed that 1,3,7-trimethyldihydrouric acid was excreted unchanged in urine and was therefore an end product of caffeine metabolism. This result implies a new metabolic pathway of caffeine.

1,3,7-Trimethyldihydrouric acid was isolated[1] and then identified[2] in extracts from rats' urine. Although the exact amount of this caffeine metabolite was unknown, it was reported that about 12% of injected caffeine was excreted in this form. A quantitative study of all of the caffeine derivatives excreted in the urine has shown that this compound corresponds to 20% of caffeine administered orally[3].

The metabolism of 1,3,7-trimethyldihydrouric acid has never been studied, because this product appears to be unstable and readily dehydrates to give caffeine[1].

In this study, thin-layer chromatography was used with success to isolate and purify [1-CH_3-^{14}C] 1,3,7-trimethyldihydrouric acid from the urine of rats which had received [1-CH_3-^{14}C] caffeine orally. The metabolism of this caffeine derivative in the rat was studied, and, according to the results, a new metabolic pathway of caffeine is proposed.

Material and methods. Male Sprague-Dawley rats weighing 200 g were directly fed by stomach tube 100 μCi of [1-CH_3-^{14}C] caffeine (specific activity: 24.0 μCi/mg, New England Nuclear). Urine was collected during the 48 h after administration and immediately frozen. The urine samples were then chromatographed on preparative silica plates (Merck, 2 mm thickness) and the 1,3,7-trimethyldihydrouric acid isolated and purified by chromatography with the following solvents: 1. Chloroform-methanol (4:1, v/v), 2. Chloroform-acetone-butan-1-ol-concentrated ammonium hydroxide (3:3:4:1, v/v), 3. Chloroform-methanol (9:1 v/v). The radiochemical purity of the [1-CH_3-

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