Renal Function and Glucose Transport in Male and Female Mice with Diet-Induced Type II Diabetes Mellitus (44568)

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Abstract. **The aim of this study was to measure cardiovascular and renal function, including the renal transport capacity for glucose, in male and female C57BL/6J mice with diet-induced Type II diabetes mellitus. Typical of Type II diabetes, mice fed a high-fat, high-simple carbohydrate diet for 3 months were obese (45–65 g), hyperglycemic (138–259 mg%), and hyperinsulinemic (1.8–15.06 ng/ml); significant gender differences were observed in all cases. Based on systolic pressure measurements in conscious mice and arterial blood pressure measurements in anesthetized mice, no diet-induced hypertension was observed in either male or female mice. Urine flow rate, sodium, potassium, osmolar, and protein excretion rates were significantly increased ($P < 0.05$) in male mice fed the high-fat, high-simple carbohydrate diet compared with female mice fed the same diet. However, no differences in the excretion variables existed between male and female mice fed the control diet. The glomerular filtration rate (ml min^{-1} g kw^{-1}), determined by FITC-inulin, in male and female mice fed the control diet (0.87 ± 0.01 and 0.90 ± 0.1, respectively) and high-fat, high-simple carbohydrate diet (0.96 ± 0.1 and 0.93 ± 0.2, respectively) was not different between the groups. These hyperglycemic mice were also not glucosuric. Infusions of progressive amounts of glucose in male mice fed either diet for 3 or 6 months demonstrated that the renal threshold for glucose was 400 mg% for all these mice, well above the fasting plasma glucose concentrations observed in this study. Thus, C57BL/6J mice were valuable tools for studying diet-induced obesity, hyperglycemia, and hyperinsulinemia; however, no hypertension or kidney dysfunction was apparent within the time frame of the current study.** [P.S.E.B.M. 2000, Vol 225:221–230]

Over 16 million cases of diabetes mellitus have been diagnosed in the United States, and Type II (non-insulin-dependent) diabetes mellitus is the form responsible for 90% of those cases. Even more alarming is the fact, according to the San Antonio Heart Study (1), that new cases of Type II diabetes mellitus rose an average of 9% per year between 1987 and 1996. Diabetes is the leading cause of blindness, renal failure, and lower limb amputations in adults and is an important factor in other forms of cardiovascular disease and stroke (2). Type II diabetes mellitus is a polygenic disease characterized by defects in both insulin action and insulin secretion. Diet, physical activity, and age interact with genetic predisposition to affect disease prevalence.

There has been considerably less experimental investigation in Type II diabetes mellitus than in Type I (insulin-dependent) diabetes mellitus due in part to a lack of satisfactory animal models. The genetically obese Zucker rat (3) is a spontaneous model of Type II diabetes mellitus that has a missense mutation in the leptin receptor gene (4, 5). However, wide interanimal variation in obesity and associated changes in renal function exist in this strain (6), and the animals are quite costly. Other examples of spontaneous genetic mutations include the diabetic *db/db* mouse that contains a mutation in the leptin receptor gene (7) and the *ob/ob* mouse, a model for obesity that lacks the leptin (8). Genetically engineered models are now becoming the fore-

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front of animal research. The IRS-1 knockout causes growth retardation and mild insulin resistance but not overt diabetes because insulin secretion increases to compensate for the resistance (9, 10). Studies on other genetically engineered models such as the IRS-2 knockout indicate that disruption of this single gene causes defects in both insulin action and insulin secretion (11). Disruption of IRS-2 causes a progressive deterioration in glucose homeostasis because of insulin resistance in liver and skeletal muscle as well as a lack of β-cell compensation for this insulin resistance. A diet-induced mouse model that strongly resembles the metabolic abnormalities of the human disease has also been studied. Specifically, the C57BL/6J mouse is reported to become obese, hyperglycemic, and hyperinsulinemic when fed a high-fat, high-simple carbohydrate (HFHSC) diet for 3 months (12). Another feature that makes this mouse especially useful is that the development of hyperglycemia and hyperinsulinemia have divergent time courses. Elevated plasma glucose occurs typically after 1 month on the diet, whereas hyperinsulinemia occurs around 3 months. C57BL/6J mice also were reported to develop hypertension when placed on an HFHSC diet containing either a low (0.06%) or normal (0.4%) sodium content (13).

Considerable species variation exists in glucose transport by the kidney (14). Evidence suggests an approximate inverse correlation between body size and the renal capacity to reabsorb glucose. Moreover, a study in humans with insulin-dependent diabetes showed a significant increase in the renal transport maximum for glucose, the so-called T_mG (15). No reports of the T_mG have occurred in either healthy mice or in mice with Type II diabetes mellitus.

The goal of the current study was to determine to what extent cardiovascular and/or renal function, including the renal reabsorptive capacity for glucose, are altered in C57BL/6J mice fed an HFHSC diet for 3 or 6 months, a time course reported to cause Type II diabetes mellitus as early as 3 months in male C57BL/6J mice (12).

Materials and Methods

This study followed the principles of animal care as outlined by the NIH (publication no. 85–23, revised 1985) and was approved by the institutional animal care and use committee (IACUC) at the University of Cincinnati.

Protocol I: Baseline Renal Function in Male and Female Mice. *Mice and diets.* Twenty-four male and 24 female C57BL/6J mice were obtained from Jackson Laboratories (Bar Harbor, ME) at 4 weeks of age and housed four to a cage. After 1 week, 12 male mice and 12 female mice were fed a control diet (Teklad LM-485 Mouse/Rat Sterilizable Diet; Madison, WI) for 3 months that consisted of 5% fat, 54% carbohydrate (ground corn), 19% protein, 5% fiber, 0.31% sodium, and 0.85% potassium (4.1 kcal/g). The remaining mice (n = 12 males and n = 12 females) were fed a high-fat, high-simple carbohydrate diet (HFHSC; Diet F2685; Bioserv Frenchtown, NJ) for 3 months that contained 35% fat (lard), 35% simple carbohydrate (sucrose), 20% protein, 0.1% fiber, 0.39% sodium, and 0.56% potassium (5.4 kcal/g). All animals were allowed access to food and water *ad libitum*. Each week mice were weighed, and the amount of food and water consumed was determined. Animals were monitored for the presence of glucose and protein in the urine with a Uristix (Bayer, Elkhart, IN). At this stage of the study, the Uristix was used because of the small amount of urine obtained when the animals were stimulated to urinate by lifting the tail.

Cardiovascular measurements in conscious mice. Systolic blood pressure and heart rate were measured in conscious mice 2 weeks prior to surgery using a tail cuff device designed for mice and BP-2000 software (Visitech Systems, Apex, NC). Briefly, four mice were restrained individually with holders on the specimen platform with their tails exposed and placed through the tail cuff device. The specimen platform sits atop the control unit that contains a heating unit. The temperature was maintained at 37°C during the measurement period. The pressure transducer inside the unit was calibrated daily. Each day 10 preliminary measurements were made to allow the mice to become acclimated to the cuff inflating around its tail; this procedure also served to thermally challenge the mice with a resultant increase in tail blood flow. The preliminary period was followed by 10 recorded measurements for each mouse. Systolic blood pressure and heart rate were measured for 10 days total, the first 5 days were considered a conditioning period, and the actual measurements were obtained during the last 5 days.

Metabolism cage studies. One week prior to the surgical portion of the study (see below), mice from each dietary group were placed in metabolism cages (four mice per metabolism cage) for 24 hr. The following variables were measured: urine flow rate (V), sodium excretion rate ($U_{Na}V$), potassium excretion rate (U_KV), osmolar excretion rate ($U_{OS}V$), and protein excretion rate (U_PV). The urine sample was also tested for the presence of glucose. Data are from three cages of mice (n = four per cage) per dietary group and represent measurements either in duplicate (n = six) or triplicate (n = nine). Animals were placed in the metabolism cages every other day.

Plasma glucose, insulin, and percentage glycohemoglobin. Two days prior to the surgical portion of the study (see below), a fasting plasma glucose concentration was determined. Mice were fasted for 6–8 hr during the day, and a blood sample (about 75 μl) was obtained from a nick in a tail vein (12). The plasma glucose concentration was determined immediately, and the remaining plasma was frozen at −80°C until the insulin radioimmunoassay was performed. At the end of the surgical portion of the study, a blood sample (200 μl) was obtained for determination of the percentage glycohemoglobin in the sample.

Surgical and other experimental procedures. On the day of the experiment, mice were anesthetized with separate intraperitoneal injections of ketamine (50 mg/kg) and thiobutabarbital (Inactin, 100 mg/kg, Research Bio-

chemicals International, Natick, MA). During the experiment, supplemental Inactin was administered as needed. A tracheotomy was performed with PE-90 to facilitate breathing. Heat stretched PE tubing (approximating PE-10) was inserted into a femoral vein, and to measure the glomerular filtration rate (GFR) a solution of 1% fluoroscein isothiocyanate (FITC) inulin (Sigma St. Louis, MO) in 150 m*M* NaCl was infused (16). Mean arterial pressure (MAP) and arterial blood samples were obtained from a similar catheter inserted into a femoral artery. This catheter was connected to a fixed-dome pressure transducer (model CDXIII; COBE Cardiovascular, Arvada, CO), an amplifier, a MacLab 2E (ADI Instruments, Boston, MA), and the signal displayed and recorded on a computer using MacLab software. Finally, after exposing the urinary bladder through an abdominal incision, a cuffed catheter (PE-10) was inserted into the bladder. Following surgery, an infusion of 1% FITC-inulin was initiated at 0.1 μl/g body wt/min. An hour recovery period ensued, followed by two urine collections for 30 min each. At the end of the collections, a blood sample (60 μl) was obtained for determination of the plasma inulin concentration, and a 100-μl blood sample was obtained for determination of the percentage of glycohemoglobin in the blood. The animals were then sacrificed with an overdose of the anesthetic.

Protocol II: Glucose Titration Experiments in Male Mice. *Mice and diets.* Forty-eight male C57BL/6J mice were evaluated in this protocol. Mice were housed four to a cage. Twenty-four male mice were fed the control diet, and 24 were fed the high-fat, high-simple carbohydrate diet. Mice in this protocol were fed the diets for either 3 or 6 months; therefore, $n = 12$ in each dietary group. Animals were allowed access to food and water *ad libitum*. Each week mice were weighed, and the amount of food and water consumed was determined. Animals were monitored for the presence of glucose and protein in the urine with a Uristix (Bayer, Elkhart, IN). Again, at this stage of the study, the Uristix was used because of the small amount of urine obtained when the animals were stimulated to urinate by lifting the tail.

Cardiovascular measurements in conscious mice, metabolism cage studies, and plasma glucose and insulin determinations. In this portion of the study, systolic blood pressure and heart rate (tail cuff method) measurements in conscious mice and 24-hr excretion profiles were only measured on the mice ingesting the HFHSC diet for 6 months; plasma glucose and insulin concentrations were measured on all mice as detailed above. Percentage glycohemoglobin was not determined in this portion of the study.

Surgical and other experimental procedures. The surgical procedure was followed as outlined above. However, following surgery, animals were infused with 150 m*M* NaCl plus 1% FITC-inulin at 0.5 ml kg^{-1} min^{-1} for 1 hr. This infusion rate is similar to the volume expansion used by Cervenka *et al.* (17) and was chosen because it provided for rapid increases in plasma glucose concentrations. After the recovery period, urine was collected for 30 min (baseline clearance). Next, 10% glucose was added to the 150 m*M* NaCl plus 1% FITC-inulin solution and infused at 0.5 ml kg^{-1} min^{-1} for 15 min, followed by a 30-min urine collection. This procedure was continued with 20%, 30%, and in some cases 40% glucose. At the end of the study, animals were sacrificed with an overdose of the anesthetic.

Analytical and Statistical Procedures. Blood and urine glucose concentrations were measured with Sigma Diagnostics Glucose (Trinder) reagent (St. Louis, MO), a method previously shown to provide values that are in good agreement with the hexokinase and Beckman Glucose Analyzer methods (18). Insulin levels were measured with a sensitive rat insulin radioimmunoassay from Linco (100% cross-reactivity with mouse insulin; St. Louis, MO). Glycohemoglobin concentrations were measured using a kit from StanBio (San Antonio, TX). FITC-inulin concentrations in the blood and urine were measured with an MTX Labsystems Fluorskan II (Vienna, VA) using the method of Lorenz and Gruenstein (16). Urine volumes and kidney weights were determined gravimetrically. Sodium and potassium concentrations in the urine (U_{Na} and U_K, respectively) were measured using a flame photometer (Corning 480 Flame Photometer, Medfield, MA). Osmolality of the urine was measured by freezing point depression (Fiske 110 Osmometer, Norwood, MA). Protein in the urine was measured with the StanBio liquicolor total protein assay (San Antonio, TX).

A mixed, three-factor analysis of variance (ANOVA) with repeated measures of body weight, food consumption, and water consumption, was used to determine statistical differences between the groups [gender × diet × (weeks × subjects)]. All other comparisons were made with two-factor ANOVA. Where necessary, individual comparisons of group means were accomplished using individual contrasts. Data are expressed as means ± SEM, and differences were accepted as significant when $P < 0.05$.

Results

Protocol I: Baseline Renal Function in Male and Female Mice. *Body weight and food and water consumed.* Body weights of the mice fed a control or the high-fat, high-simple carbohydrate diet for 3 months were measured each week and are presented in Figure 1. There was a significant gender-diet interaction ($P = 0.0001$) on body weight, demonstrating that all groups gained weight at different rates. At Week 12, a significant gender-diet interaction occurred ($P = 0.0005$) on the final body weight of male and female mice fed either the control or HFHSC diets. Compared with female mice fed the control diet (21.3 ± 0.2 g), a significant increase occurred in the weight of male mice fed the control diet (26.1 ± 0.5 g). The final body weight of male and female mice fed the HFHSC diet (36.5 ± 0.8 and 47.2 ± 1.2 g, respectively) was significantly increased compared with the corresponding gender fed the control diet. Lastly, the final body weight of male mice fed

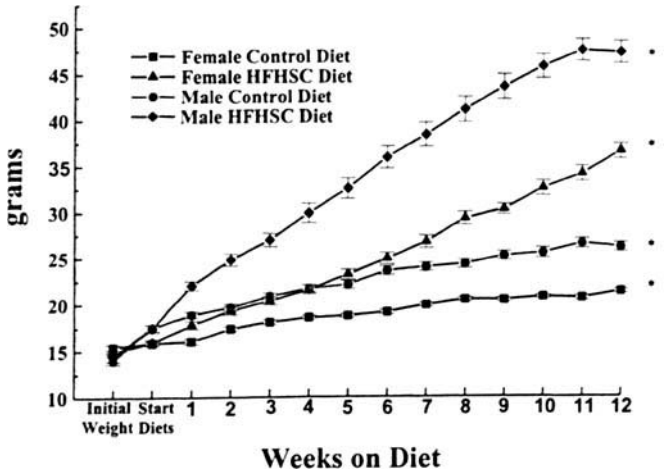

Figure 1. Body weight of male and female C57BL/6J mice fed either a control diet or a high-fat, high-simple carbohydrate diet for 3 months. $n = 12$ for all groups. Data are expressed as means ± SEM. *Significant gender-diet interaction.

the HFHSC diet was significantly increased compared with female mice fed the same diet.

Table I summarizes the amount of food and water consumed by the mice over the 12 weeks. A significant gender-diet interaction ($P = 0.0001$) was detected on the amount of food consumed, thus demonstrating that all groups consumed food at different rates. Although there was no difference in food consumption between the groups fed the control diet, mice fed the HFHSC diet consumed significantly more than the corresponding gender fed the control diet. In addition, female mice fed the HFHSC diet consumed significantly more than male mice fed the same diet. However, it should be noted that the first lot of the HFHSC diet used for the female portion of the study was not tightly compacted and fell apart easily, with some falling to the bottom of the cage. Therefore the significance of the large amount of food consumed by the female mice must be interpreted with caution.

Table I. Average Weekly Food and Water Consumption of Male and Female C57BL/6J Mice Fed Either the Control Diet or the HFHSC Diet

	Average Weekly Food Consumption (g)	Average Weekly Water Consumption (ml)
Female control diet	84 ± 2	107 ± 4
Male control diet	81 ± 1	106 ± 3
Female HFHSC diet	149 ± 5[a]	98 ± 3
Male HFHSC diet	102 ± 3[a,b]	85 ± 2

Note. Since there was no significant gender-diet interaction on the average weekly water consumption data, individual comparisons were not made; however, there were significant effects of gender and diet on water consumption and are reported in the text.
For all groups, $n = 36$ total measurements (12 weeks × 3 cages). Data are expressed as means ± SEM. [a] Denotes $P < 0.05$ compared with same gender, control diet and [b] denotes $P < 0.05$ compared with female, same diet.

Significant effects of gender ($P = 0.03$) and diet ($P = 0.0001$) were detected on water consumption; however, there was no significant gender-diet interaction. Female mice consumed more water than male mice, and animals fed the control diet consumed more water than animals fed the HFHSC diet.

Cardiovascular measurements in conscious and anesthetized mice. Table II summarizes cardiovascular measurements in conscious and anesthetized mice. There was a significant gender-diet interaction ($P = 0.0001$) on systolic pressure in conscious mice. The systolic blood pressure of female mice fed the control diet was significantly elevated compared with male mice fed the same diet. Relative to female mice on the control diet, female mice fed the HFHSC diet had a significant decrease in systolic pressure, whereas systolic blood pressure in male mice fed the HFHSC diet was unchanged compared with male mice on the control diet. Finally, there was no difference in systolic pressure between conscious male and female mice fed the HFHSC diet.

A significant gender-diet ($P = 0.0139$) interaction occurred on heart rate in conscious mice. The heart rate of female mice fed the control diet was significantly elevated compared with male mice fed the same diet. Heart rate did not change in female mice when they were fed the HFHSC diet. By contrast, male mice maintained on the HFHSC had a significant increase in conscious heart rate compared with male mice on the control diet. Compared with male mice fed the HFHSC diet, the conscious heart rate of female mice fed the HFHSC diet was significantly elevated.

There were no significant effects of gender ($P = 0.5796$), diet ($P = 0.0976$), or a gender-diet interaction ($P = 0.3716$) on mean arterial blood pressure (Table II). Heart rate in anesthetized mice was also similar in all groups.

Metabolism cage studies. Several 24-hr electrolyte (including protein) excretion variables are summarized in Table III. A significant gender-diet interaction ($P = 0.0001$) occurred on 24-hr urine flow rate in male and female mice fed either control or HFHSC diets for 3 months. There was no difference in urine flow rate between the animals fed the control diet. However, female mice fed the HFHSC diet had significantly decreased urine flow rates compared with female mice fed the control diet. By contrast, male mice maintained on the HFHSC diet had significantly increased urine flow rates compared with male mice on the control diet. Male mice on the HFHSC diet also had urine flow rates that were significantly greater that female mice fed the same diet. A significant gender-diet interaction ($P = 0.002$) occurred on sodium excretion. Male mice fed the HFHSC diet had significant increases in sodium excretion compared with male mice fed the control diet and female mice fed the HFHSC diet. There was a significant gender-diet interaction ($P = 0.01$) on potassium excretion. Potassium excretion was significantly decreased in the female mice fed the HFHSC diet compared with female mice fed the control diet as well as male mice maintained on the

Table II. Systolic Arterial Blood Pressure and Heart Rate (beats per minute, bpm), in Conscious Male and Female Mice and Mean Arterial Blood Pressure and Heart Rate in Anesthetized Male and Female Mice Fed Either a Control or High-Fat, High-Simple Carbohydrate Diet for 3 Months

	Conscious Systolic Pressure (mm Hg)	Conscious Heart Rate (bpm)	Anesthetized Mean Arterial Pressure (mmHg)	Anesthetized Heart Rate (bpm)
Female control diet	123 ± 2 (n = 12)	663 ± 11 (n = 12)	95 ± 2 (n = 11)	522 ± 20 (n = 11)
Male control diet	109 ± 2[a] (n = 12)	581 ± 9[a] (n = 12)	95 ± 1 (n = 10)	495 ± 17 (n = 10)
Female HFHSC diet	102 ± 3[b] (n = 12)	653 ± 3 (n = 12)	101 ± 4 (n = 8)	496 ± 14 (n = 8)
Male HFHSC diet	107 ± 3 (n = 12)	624 ± 13[a,b] (n = 12)	97 ± 3 (n = 7)	469 ± 9 (n = 7)

Note. Data are expressed as means ± SEM.
[a] Denotes $P < 0.05$ compared with female, same diet, and [b] denotes $P < 0.05$ compard with same gender, control diet.

HFHSC diet. There was a significant gender-diet interaction ($P = 0.008$) on osmolar excretions rates. The osmolar excretion rates showed a pattern similar to the potassium excretion rates. Osmolar excretion rates were significantly decreased in the female mice fed the HFHSC diet compared with female mice fed the control diet as well as male mice fed the HFHSC diet. Lastly, a significant gender-diet interaction ($P = 0.001$) occurred on protein excretion rates. Again, protein excretion rates were significantly reduced in female mice maintained on the HFHSC diet compared with female mice fed the control diet and male mice fed the HFHSC diet. Additionally, compared with male mice fed the control diet, male mice maintained on the HFHSC diet had significantly increased protein excretion rates.

Plasma glucose and insulin concentrations and percentage glycohemoglobin. Plasma glucose and insulin concentrations and the glycohemoglobin concentration (expressed as a percentage) are summarized in Table IV. There were significant gender-diet interactions ($P < 0.05$) on glucose concentrations and insulin levels in animals fed the control or HFHSC diet for 3 months. The plasma glucose level of male mice fed the control diet was significantly elevated compared with female mice fed the same diet. Plasma glucose concentrations were significantly increased in female mice fed the HFHSC diet. In addition, male mice maintained on the HFHSC for 3 months had a significant increase in levels compared with male mice on the control diet. Finally, plasma glucose levels of male mice fed the HFHSC diet were significantly elevated compared with female mice fed the same diet. Nonetheless, at no time during this study did any of the mice present with glucosuria as measured with Uristix.

There were no differences in insulin levels between the male and female mice fed the control diet. Significant increases were seen in plasma insulin levels in male and female mice fed the HFHSC diet compared with the corresponding gender fed the control diet. Finally, the plasma insulin levels of male mice maintained on the HFHSC diet were significantly compared with female mice fed the same diet.

Percentage glycohemoglobin data are summarized in Table IV. There was a significant gender-diet interaction ($P = 0.0001$) on percentage glycohemoglobin. Compared with female mice fed the control diet, percentage glycohemoglobin was significantly decreased in male mice fed the control

Table III. Urine Flow Rate and Excretion Variables per 24 hr in Female and Male C57BL/6J Mice Fed Either a Control or High-Fat, High-Simple Carbohydrate Diet for 3 Months

	Urinary Excretions				
	Volume ml/day/4 Mice	Sodium mEq/day/4 Mice	Potassium mEq/day/4 Mice	Osmolar Osm/day/4 Mice	Protein mg/day/4 Mice
Female control diet (n = 6 cages)	4.7 ± 0.3	0.6 ± 0.1	1.2 ± 0.1	9.3 ± 0.9	39.8 ± 2.8
Male control diet (n = 9 cages)	5.1 ± 0.5	0.6 ± 0.1	1.1 ± 0.1	8.7 ± 0.9	42.5 ± 2.7
Female HFHSC diet (n = 9 cages)	2.0 ± 0.2[a]	0.6 ± 0.1	0.7 ± 0.1[a]	6.6 ± 0.5[a]	22.7 ± 1.7[a]
Male HFHSC diet (n = 9 cages)	6.4 ± 0.4[a,b]	1.1 ± 0.1[a,b]	1.0 ± 0.1[b]	10.6 ± 0.7[b]	61.0 ± 8.2[a,b]

Note. Data are from three cages of mice per group (n = four mice per cage) per dietary group and represents either duplicate (n = 6) or triplicate (n = nine) determinations. Data are expressed as means ± SEM.
[a] Denotes $P < 0.05$ compared with same gender, control diet and [b] denotes $P < 0.05$ compared with female, same diet.

Table IV. Fasting Plasma Glucose Concentration, Insulin Levels, and the Percentage Glycohemoglobin in Female and Male C57BL/6J Mice Fed Either a Control or High-Fat, High-Simple Carbohydrate Diet for 3 Months

	Plasma Glucose (mg%)	Insulin (ng/ml)	Glycohemoglobin (%)
Female control diet	128.8 ± 4.2	0.8 ± 0.1	7.0 ± 0.2[a]
Male control diet	175.2 ± 4.2*	1.2 ± 0.1	4.2 ± 0.2*,[b]
Female HFHSC diet	166.8 ± 7.9†	4.5 ± 0.3†	8.0 ± 0.3†,[c]
Male HFHSC diet	250.6 ± 15.9*,†	12.0 ± 2.3*,†	10.2 ± 0.5*,†,[d]

Note. Data are expressed as mean ± SEM. Except as noted, $n = 12$. * Denotes $P < 0.05$ compared with female, same diet, and † denotes $P < 0.05$ compared with same gender, control diet.
[a] Denotes $n = 11$, [b] denotes $n = 10$, [c] denotes $n = 8$, [d] denotes $n = 7$).

diet. Significant increases were seen in percentage glycohemoglobin in male and female mice fed the HFHSC diet compared with the corresponding gender fed the control diet. Lastly, percentage glycohemoglobin was significantly higher in male mice fed the HFHSC diet compared with female mice fed the same diet.

Baseline renal function. Table V summarizes kidney weight and glomerular filtration rate data in female and male mice fed the control and HFHSC diet for 3 months. There were significant effects of gender ($P = 0.0001$) and diet ($P = 0.0001$), but no gender-diet interaction on kidney weight. Animals (males and females) fed the control diet for 3 months had an average kidney weight of 0.31 ± 0.01 g, significantly less than male and female mice fed the HFHSC diet for the same amount of time (0.37 ± 0.01 g). Female mice on either diet had an average kidney weight of 0.30 ± 0.02 g, significantly less than male mice on either diet (0.37 ± 0.01 g). There were no significant effects of diet or gender on the absolute glomerular filtration rate (GFR). Accordingly, no significant diet-gender interactions existed between the groups. Since significant differences in kidney weight existed between the groups, the GFR was then normalized per gram kidney weight (also shown in Table V). However, there was no diet, gender, or diet-gender interaction on the normalized GFR data.

Table V. Effect of Feeding Male and Female C57BL/6J Mice Either a Control Diet or a High-Fat, High-Simple Carbohydrate Diet for 3 Months on Kidney Weight and the Glomerular Filtration Rate in Male and Female C57BL/6J Mice

	Kidney Weight (g)	GER (ml/min)	GFR (ml/min/gkw)
Female control diet ($n = 11$)	0.28 ± 0.03	0.26 ± 0.01	0.90 ± 0.05
Male control diet ($n = 10$)	0.35 ± 0.01[a]	0.32 ± 0.05	0.93 ± 0.15
Female HFHSC diet ($n = 8$)	0.33 ± 0.10[b]	0.29 ± 0.03	0.87 ± 0.08
Male HFHSC diet ($n = 7$)	0.42 ± 0.01[a,b]	0.32 ± 0.04	0.96 ± 0.07

Note. Data are expressed as means ± SEM.
[a] Denotes $P < 0.05$ compared with female, same diet, and [b] denotes $P < 0.05$ compared with same gender, control diet.

Protocol II: Glucose Titration Experiments in Male Mice. *Body weight and food and water consumed.* A significant diet–length-of-time-on-diet interaction ($P = 0.0001$) occurred on the final body weight of male mice at the end of either 3 or 6 months. Males fed the control diet for 6 months weighed significantly more than males fed the control diet for 3 months (29.7 ± 0.4 vs 27.5 ± 0.6 g). Males fed the HFHSC diet for 3 and 6 months (48.0 ± 0.6 and 58.8 ± 1.2 g, respectively) weighed significantly more than corresponding mice fed the control diet. Finally, males fed the HFHSC diet for 6 months weighed significantly more than males fed the same diet for 3 months.

Differences were also present in the amount of food and water consumed at the end of 3 and 6 months. Statistical analysis detected a significant effect ($P = 0.02$) of length of time on diet on food consumption during the last week of the study. During the last week of the study, animals fed either the control or HFHSC diets for 6 months consumed 87 ± 2 g of food compared with 103 ± 4 g for animals maintained on the control or HFHSC diets for 3 months.

A significant ($P = 0.05$) diet–length-of-time-on-diet interaction occurred on water consumption during the last week of the study. During the final week of the study, male mice fed the HFHSC diet for 3 months consumed 92 ± 3 ml of water, significantly less than male mice fed the control diet for 3 months (118 ± 11 ml). On the other hand, male mice fed the HFHSC diet for 6 months consumed more water than males fed the control diet for 6 months (108 ± 10 vs 97 ± 6 ml, respectively); however, the difference was not significant.

Cardiovascular measurements in conscious mice. In protocol II, systolic pressure and heart rate were only measured on conscious male mice maintained on either the control or HFHSC diets for 6 months. However, to evaluate the effect of the length of time on the diet on these variables, the results were compared back with male mice in protocol I fed the control or HFHSC diets for 3 months. There was only a significant effect ($P = 0.0001$) of length of time on diet on conscious systolic pressure. Compared with animals maintained on either the control or HFHSC diets for 3 months, there was a significant increase in conscious systolic pressure in animals fed both diets for 6 months (108 ±

2 and 122 ± 2 mmHg, respectively). Male mice fed either the control or HFHSC diet for 3 months had average conscious systolic pressures of 109 ± 2 and 107 ± 3 mmHg, respectively, whereas male mice maintained on the control or HFHSC diets for 6 months had average conscious systolic pressures of 121 ± 2 and 123 ± 3 mmHg, respectively.

There were significant effects of diet ($P = 0.0001$) and length of time on diet ($P = 0.0085$) on heart rate but no significant diet–length-of-time-on-diet interaction. The heart rate of male mice fed the HFHSC diet for 3 and 6 months was significantly elevated compared with animals fed the control diet for 3 and 6 months (639 ± 8 and 592 ± 7 beats per minute (bpm), respectively). Additionally, animals fed either diet for 6 months had significantly elevated heart rates compared with animals fed either diet for 3 months (629 ± 8 and 602 ± 9 bpm, respectively).

Metabolism cage studies. In protocol II, metabolism studies (Table VI) were only measured on male mice maintained on either the control or HFHSC diets for 6 months. However, to evaluate the effect of the length of time on the diet on excretion variables, the results were compared back with male mice in protocol I fed the control or HFHSC diets for 3 months (results are also reproduced in Table VI). There were significant effects of diet ($P = 0.0024$) and length of time on diet ($P = 0.0306$) on urine flow rate. Animals fed the HFHSC diet for 3 and 6 months had significantly elevated urine flow rates compared with animals fed the control diet for 3 and 6 months (6.3 ± 0.4 vs 4.4 ± 0.9 ml/day/four mice, respectively). The urine flow rates of mice maintained on either diet for 6 months were significantly decreased compared with urine flow rates of mice maintained on either diet for 3 months (4.2 ± 0.9 vs 5.7 ± 0.4 ml/day/four mice, respectively). There was only a significant effect of diet ($P = 0.0001$) on sodium excretion. Animals fed the HFHSC diet for 3 and 6 months excreted 1.1 ± 0.1 mEq/day/four mice of sodium compared with 0.5 ± 0.1 mEq/day/four mice of sodium in animals fed the control diet for 3 and 6 months. A significant effect of diet ($P = 0.0053$) and the length of time on the diet ($P = 0.05$) was detected on osmolar excretion rates. Animals fed the HFHSC diet for 3 and 6 months had significantly increased osmolar excretion rates compared with animals fed the control diet for 3 and 6 months (10.4 ± 0.6 vs 7.7 ± 0.9 Osm/day/four mice, respectively). The osmolar excretion rate of mice maintained on either diet for 6 months were significantly decreased compared with the mice maintained on the diets for 3 months (7.3 ± 1.5 vs 9.6 ± 0.6 Osm/day/four mice, respectively). Finally, a significant effect of length of time on diet ($P = 0.003$) was observed for protein excretion. Animals fed either diet for 6 months had protein excretion rates that were significantly below animals fed either diet for 3 months (25.1 ± 2.0 vs 51.8 ± 4.8 mg/day/four mice).

Glucose and insulin concentrations. There were significant effects of diet ($P = 0.002$) and length of time on diet ($P = 0.05$) on fasting plasma glucose concentrations in male mice fed either a control or HFHSC diet for 3 or 6 months; however, a significant diet–length-of-time-on-diet interaction did not exist. Animals fed the HFHSC diet for 3 and 6 months had significantly elevated plasma glucose levels compared with animals fed the control diet for 3 and 6 months. Animals fed the diets for 6 months had fasting plasma glucose concentrations that were significantly decreased compared with animals fed the diets for 3 months. Male mice fed the control diet for 3 months had a fasting plasma glucose concentration of 162 ± 5.3 mg%, whereas male mice fed the HFHSC diet for 3 months had fasting plasma glucose concentrations of 217 ± 19.7 mg percentage. Male mice fed the HFHSC diet for 6 months had fasting plasma glucose concentrations of 177 ± 9.0 mg%, whereas male mice fed the control diet for 6 months had fasting plasma glucose concentrations of 155 ± 5.0 mg%. Again, at no time during the present study did the mice present with glucosuria as measured by Uristix.

There was a significant effect of diet ($P = 0.0001$) on insulin levels. Animals fed the HFHSC diet for 3 and 6

Table VI. Urine Flow Rate and Excretion Variables per 24 hr in Female and Male C57BL/6J Mice Fed Either a Control or High-Fat, High-Simple Carbohydrate Diet for 3 Months

	Urinary Excretions				
	Volume ml/day/4 Mice	Sodium mEq/day/4 Mice	Potassium mEq/day/4 Mice	Osmolar Osm/day/4 Mice	Protein mg/day/4 Mice
Male control diet 3 months ($n = 9$ cages)	5.1 ± 0.5	0.6 ± 0.1	1.1 ± 0.1	8.7 ± 0.9	42.5 ± 2.7
Male control diet 6 months ($n = 3$ cages)	2.5 ± 0.8	0.3 ± 0.01	0.7 ± 0.1	4.6 ± 1.0	24.9 ± 1.0
Male HFHSC diet 3 months ($n = 9$ cages)	6.4 ± 0.4	1.1 ± 0.1	1.0 ± 0.1	10.6 ± 0.7	61.0 ± 8.2
Male HFHSC diet 6 months ($n = 3$ cages)	5.8 ± 0.8	1.0 ± 0.2	0.9 ± 0.2	10.0 ± 1.6	25.3 ± 4.4

Note. Since there were no significant diet–length-of-time-on-diet interactions on the urinary excretion data, individual comparisons were not made; however, there were significant effects of diet and length of time, and these are reported in the text.
Data are from three cages of mice per group ($n = 4$ mice per cage) per dietary group and represents either single ($n = 3$) or triplicate ($n = 9$) determinations. Data are expressed as means ± SEM.

months had significantly increased insulin levels compared with animals fed the control diet for 3 and 6 months (12.6 ± 0.7 vs 1.2 ± 0.1 ng/ml, respectively). Insulin levels for male mice fed either the control diet or HFHSC diet for 3 months were 1.0 ± 0.1 and 11.7 ± 0.7 ng/ml, respectively. Similarly, male mice fed either the control diet or HFHSC diet for 6 months had insulin levels of 1.5 ± 0.2 and 13.5 ± 1.1 ng/ml, respectively.

Glucose reabsorptive capacity. The glucose titration experiments in male mice fed the control or HFHSC diets for 3 and 6 months are summarized in Figures 2A–D. Since significant differences in kidney weight were observed between the groups, the amount of glucose filtered, excreted, and reabsorbed were all normalized per gram kidney weight. It is apparent from the data summarized in this figure that none of the groups of mice were glucosuric until a plasma glucose concentration of greater than 400 mg% was attained.

Discussion

Results of the current study demonstrate that male and female C57BL/6J mice fed a high-fat, high-simple carbohydrate diet for 3 months, a paradigm for Type II diabetes mellitus in this strain of mice, develop obesity, hyperglycemia, and hyperinsulinemia, but do not become hypertensive. In addition, these mice do not exhibit any changes in the glomerular filtration rate. Male mice fed an HFHSC diet for 6 months also have the same profile. However, there are gender-related differences in the plasma concentrations of glucose and insulin, as well as in the electrolyte excretion variables, including total protein. Additionally, the results of the current study illustrate that both male and female mice are characterized by a relatively high renal threshold for glucose, therefore providing an explanation for the fact that these animals are not glucosuric despite the fact that they are hyperglycemic.

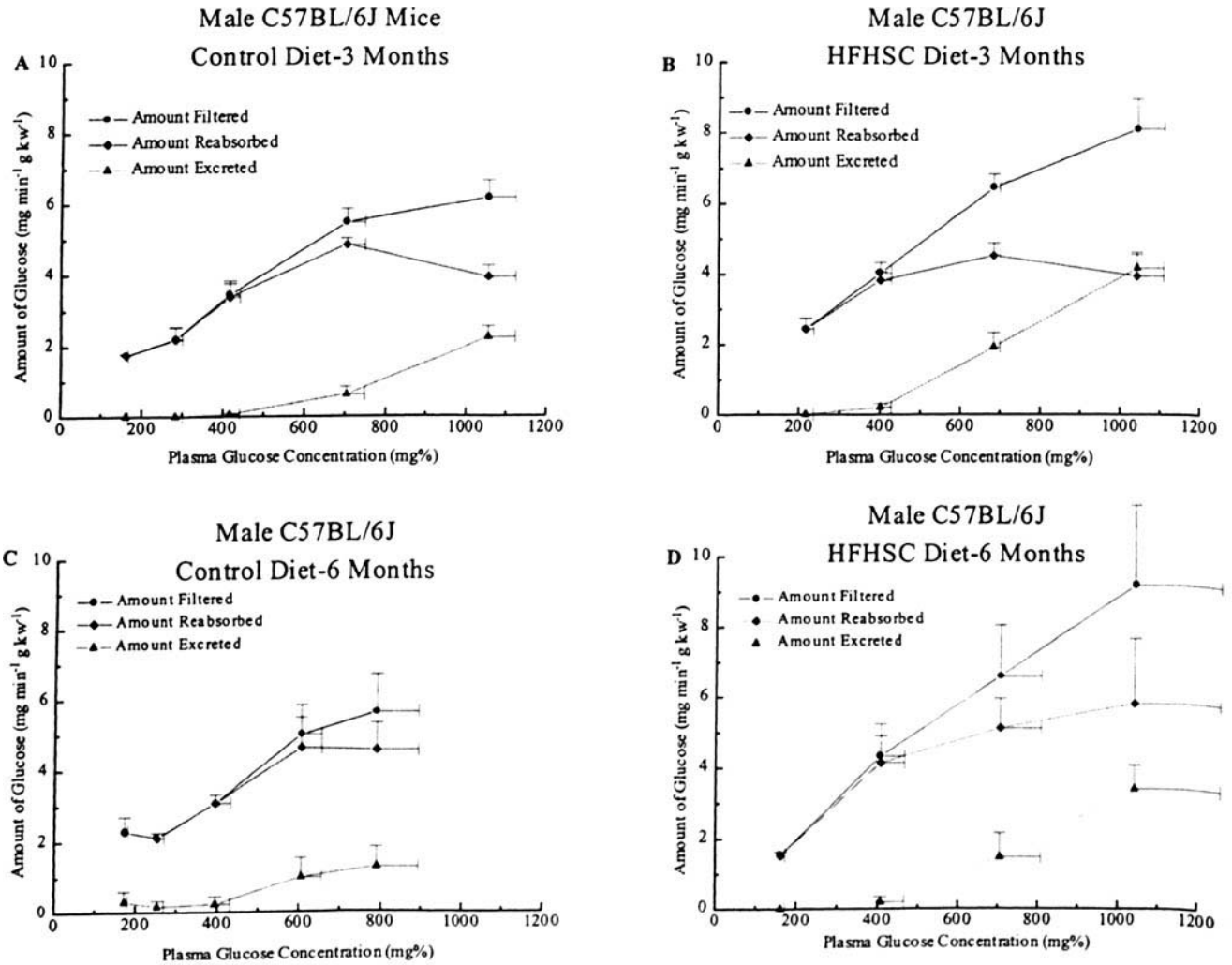

Figure 2. Glucose titration curves in (A) male C57BL/6J mice fed a control diet for 3 months ($n = 6$), (B) male C57BL/6J mice fed an HFHSC diet for 3 months ($n = 8$), (C) male C57BL/6J mice fed a control diet for 6 months ($n = 5$), and (D) male C57BL/6J mice fed an HFHSC diet for 6 months ($n = 7$). The amount of glucose filtered ($GFR \times P_{glucose}$), the amount of glucose excreted ($[U_{glucose}] \times V$), and the amount of glucose reabsorbed ($GFR \times P_{glucose} - [U_{glucose}] \times V$) are plotted as a function of the concentration before and during infusions of solutions 10%, 20%, 30%, and in some cases 40% glucose. Data are expressed as means ± SEM.

Surwit *et al.* (12) and Mills *et al.* (13) have also reported that male C57BL/6J mice fed an HFHSC diet for 3 months develop obesity, hyperglycemia, and hyperinsulinemia. The current study extends those observations illustrating that female C57BL/6J mice are also characterized by similar pathophysiological changes after 3 months of consuming an HFHSC diet. However, female mice fed the HFHSC diet did not attain the same degree of obesity, hyperglycemia, or hyperinsulinemia as male mice fed the HFHSC diet. Several studies have reported similar results for body weight in rats fed diets high in fat (19–21), and these findings are attributed to the influence of different postpubertal androgen and estrogen levels (20). In addition, when the time on the HFHSC diet was increased to 6 months in male C57BL/6J mice, a further progression of the obesity was observed compared with male mice fed the diet for 3 months.

One aspect of the current study that was different from Mills *et al.* (13) was the fact that the C57BL/6J mice did not develop hypertension when consuming the HFHSC diet. Mills *et al.* (13) had reported that C57BL/6J male mice fed an HFHSC diet for 3 months develop a significantly elevated systolic arterial pressure as measured by a tail cuff device. Although we can provide no explanation for the discrepancy between our results, there could be an intrastrain variation. On the other hand, the C57BL/6J mice used in the current study were purchased from the same supplier as those used by Mills *et al.* (13). Similarly, the diet was also purchased from the same supplier. Nonetheless, it is important to note that we observed no hypertension in animals fed the HFHSC diet for 3 or 6 months as measured by tail cuff pressures in conscious animals. In addition, there was no difference in the mean arterial blood pressure of anesthetized male or female C57BL/6J mice maintained on an HFHSC diet versus a control diet for either 3 or 6 months. Although both of these methods demonstrate a lack of diet-induced hypertension in the C57BL/6J mouse, it would be of interest to monitor mean arterial pressure in chronically instrumented conscious animals.

Although no gender-related differences in solute excretion were observed in animals fed the control diet, there were significant gender-related differences in animals fed the HFHSC diet. Both estrogens (22, 23) and insulin (24, 25) are known to promote salt and water retention, effects that would be consistent with the reductions that were observed in female mice on the HFHSC diet. However, we can provide no explanation for the increase in excretion variables observed in male mice fed the HFHSC diet.

Male mice fed the HFHSC diet for 3 months also developed proteinuria compared with male mice fed the control diet and female mice fed either the control or HFHSC diet. Typically, patients with Type II diabetes mellitus present with microalbuminuria followed by a progression to overt proteinuria (26). However, the significance of the current finding is unclear given the fact that after 6 months on the HFHSC diet, male mice did not excrete an increased amount of protein in the urine when compared with controls.

In the present study, there were no differences in the absolute glomerular filtration rate nor in the GFR normalized per gram kidney weight between the groups. Typically, glomerular hyperfiltration is observed in humans with Type II diabetes mellitus (27–29) as well as in animal models for the disease such as the obese Zucker rat (30). Two mouse models of diabetes, the C57BL/6J *db/db* and the C5BL/KSJ *db/db,* exhibit glomerular hyperfiltration as determined by the clearance of ^{51}Cr-EDTA (31). In the latter study, the hyperfiltration was present before pronounced hyperglycemia and severe adiposity (around 40 days of age). The GFR also continued to increase until 100 days of age. By contrast, in patients with short-term diabetes, glomerular hyperfiltration was observed to correlate with the fasting plasma glucose concentration (32). Micropuncture studies by Park and Kang (30) in the obese Zucker rat revealed that the increased GFR is attributable to an increase in the single nephron plasma flow and glomerular transcapillary hydraulic pressure. In summary, the diet-induced model of Type II diabetes mellitus in the C57BL/6J mouse does not exhibit kidney dysfunction after ingesting an HFHSC diet for 3 or 6 months. This is in contrast to many of the genetic models of Type II diabetes maintained on control diets.

Given the fact that the mice on the HFHSC diet had elevated fasting plasma glucose levels but were not excreting glucose, we evaluated the renal transport capacity for glucose in this strain of mice. It is apparent from the data summarized in Figure 2 that none of the groups of male mice excreted glucose until the plasma glucose concentration exceeded 400 mg%. Thus despite the fact that C57BL/6J mice fed an HFHSC diet for 3 or 6 months have elevated plasma glucose concentrations, these values were well below the renal threshold for glucose in this strain of mice.

In most mammals, there appears to be an inverse correlation between body size and the ability to reabsorb glucose (14). As illustrated in Figure 2, it appears that a tubular maximum for glucose (T_mG) was achieved in all the groups fed either control diet or HFHSC diets. It is of interest to note that the impact of infusing progressively greater amounts of glucose during the experiments designed to measure the renal transport capacity for glucose resulted in much higher plasma glucose concentrations in the mice maintained on the HFHSC diet. This fact correlates with the insulin resistance (hyperinsulinemia) observed in the mice fed the HFHSC diet.

Animal models for Type II diabetes mellitus represent invaluable tools for studying complex polygenic diseases such as Type II diabetes mellitus. The C57BL/6J mouse is an example of a diet-induced model of Type II diabetes mellitus. This animal model presents with all the characteristic traits of the disease: obesity, hyperglycemia, and hyperinsulinemia. However, based on the results of the current

study, animals fed the HFHSC diet for up to 6 months do not develop hypertension as measured by tail cuff estimates on conscious animals or with an arterial catheter in anesthetized mice. These findings are in contrast to a previous report (13) that C57BL/6J male mice fed an HFHSC diet for 3 months are hypertensive. Additionally, after 3 or 6 months on an HFHSC diet, these mice do not present with any overt kidney dysfunction, another characteristic trait of Type II diabetes mellitus. Thus, C57BL/6J mice are of value for studying obesity, hyperglycemia, and hyperinsulinemia, but would not be recommended for evaluating the kidney dysfunction characteristic of Type II diabetes mellitus, at least within the time frame of the current study.

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