Whole-Egg Diet Delays the Age-Related Impaired Glucose Tolerance of BHE/Cdb Rats (44312)

CAROLYN D. BERDANIER,* KRYSTYNA M. KRAS,* KATHIE WICKWIRE* AND D. GREG HALL†
Department of Foods and Nutrition and Veterinary Pathology,† University of Georgia, Athens, Georgia 30602*

Abstract. **The effects of dietary egg on the age-related progression of impaired glucose tolerance and glomerulonephropathy in diabetes-prone BHE/Cdb rats were studied. This rat strain mimics the human with NIDDM. The development of impaired glucose tolerance was delayed in rats fed the whole-egg diet, however, feeding this diet resulted in elevated hepatic weight but had no effect on the age-related changes in renal lesions or renal function. We conclude that in this animal model for NIDDM, the development of glomerulonephropathy is independent of the development of impaired glucose tolerance and that diet can affect the time course for impaired glucose tolerance without affecting renal disease development.** [P.S.E.B.M. 1998, Vol 219]

The BHE/Cdb rat mimics the human with noninsulin-dependent diabetes mellitus (NIDDM). Impaired glucose tolerance develops as the animal matures, and this is attributable to an age-related decline in β cell function (1, 2). BHE/Cdb rats have a point mutation in the F_oATPase 6 gene of the mitochondrial genome that we believe accounts for their somewhat decreased ATP synthesis efficiency (3). This inefficiency probably impacts protein synthesis as well as the many ATP-requiring processes in the body. It may also explain the increased lipogenic and gluconeogenic characteristics of this rat (4–6). Sprague-Dawley rats made inefficient through treatment with a low dose of a mitochondrial uncoupling agent mimic the BHE/Cdb rat with a significant increase in lipogenesis (7).

In recent papers we reported the age changes in glucose tolerance and renal function in male BHE/Cdb rats fed purified diets from weaning (8–10). We found that the amount and source of the dietary fat could affect the time course of development of impaired glucose tolerance and diabetic nephropathy. For example, we reported that rats fed a 1% corn oil-9% menhaden oil diet developed diabetic nephropathy sooner than rats fed a 1% corn oil-9% beef tallow diet (8, 9). At the same time, the menhaden oil diet postponed the development of impaired glucose tolerance. In a subsequent lifelong study (10), we used twice as much fat and made periodic measurements to assess glomerular function and glucose tolerance. In addition, we documented a diet fat effect on hepatic and renal membrane composition as well as on the activity of the renal Na^+K^+ATPase, and we suggested that the diet fat effect on renal lesions had to do with membrane composition and function (11). A second explanation had to do with the possibility that diet could influence free radical formation that in turn would stimulate renal lesion formation. A short-term study provided a measure of support for this hypothesis (12), however, a lifelong study of age changes in free radical production and renal lesion development denied this hypothesis (10). This lifelong study used twice as much fat (as corn oil) as the earlier longevity study, and even though this fat was corn oil rather than menhaden oil, assessments of both lesion development and free radical formation showed that the lesion development occurred far earlier than measurable rises in free radical production in the kidney.

Since we have already observed a diet fat effect on the age progression of renal lesions and glucose intolerance, the question arose as to whether specific foods could influence this progression. To answer this question, we used dehydrated whole egg. This material was freeze-dried, and its crude composition was matched by that of a control, semipurified diet. Whole egg was selected because of its ease of use and because it could be obtained in a reasonably pure

[1] To whom requests for reprints should be addressed at Department of Foods and Nutrition, University of Georgia, Athens, GA 30602. E-mail: cberdani@fcs.uga.edu

Received October 10, 1997. [P.S.E.B.M. 1998, Vol 219]
Accepted April 20, 1998.

0037-9727/98/2191–0028$10.50/0

form. We found that feeding whole egg, unlike feeding beef tallow, did not ameliorate renal lesion development. Whole egg–fed animals did not evidence the expected lifespan extension. However, impaired glucose tolerance did not develop as early in these rats as in the rats fed the control diet. Thus, we showed a delay in glucose intolerance development but no delay in renal disease development.

Materials and Methods

Two groups of male 21-day-old weanling SPF[1] BHE/Cdb rats[2] (UGA colony) were used. All of the animals were homeoplasmic with respect to the ATPase 6 mutation (3). This colony was rederived by cesarean section six years ago and has been maintained at the University of Georgia as a closed, isolated colony since that time. The rats were randomly allocated to the diet groups and, likewise, randomly selected at the stated ages for sampling. We made every effort to avoid the exclusive use of full sibs at each sampling time. That is, we avoided using six full sibs at, for example, the 100-day sampling time. At the 480- and 560-day sampling time, the number of rats remaining was quite small. We examined sibships at that time and found no full sibs in the two diet groups. One group (n = 58) was fed a whole-egg diet whereas the other group (n = 59) was fed an isoenergetic semisynthetic diet that matched the fat and protein content of the whole egg. Feeding commenced at weaning. The fat used in the control diet was corn oil (gift of Best Foods, Union, NJ), and the protein was a 1:1 mix of casein and lactalbumin. Both diets were designed to contain 20% protein and 18.5% fat (the approximate composition of dehydrated whole egg). The energy value for the two diets was 18 KJ/g. Dehydrated whole egg was obtained from National Egg Products (Social Circle, GA). The remaining ingredients in percentages were: AIN 93 vitamin mix, 1.5; AIN 93 mineral mix, 4.9; fiber (celufil), 3.2; sucrose, 48.3; and dl-all rac-α tocopherol (Sigma, St. Louis, MO), 0.4. Except as noted, diet ingredients were obtained from US Biochemical (Cleveland, OH).

Animals were housed individually in hanging wire mesh cages in a room controlled for temperature (21° ± 1°C), humidity (40%–50%), and light (lights on, 0600–1800). The animals were fed diets that contained all the needed nutrients in the appropriate amounts. Humane animal care followed the guidelines set forth in the NRC *Guide for the Care and Use of Laboratory Animals* (Publication #85-23). Unless otherwise noted, food and water were always available. Although the AIN Vitamin Mix used in the diets is supposed to provide sufficient vitamin E, earlier work (13) suggested that BHE/Cdb rats require significantly more vitamin E than do normal rats. For this reason, a vitamin E (all rac dl-α tocopherol) supplement (4 g/kg diet) was added to the diet. In addition, every effort was made to prevent autooxidation of the diet. The diet was mixed and stored (at −5°C) under a layer of nitrogen, and fresh food was provided daily just before the onset of the dark period. Body weights and food intakes were determined each week to be sure that no differences in energy intake occurred. The daily food intake was adjusted for body weight change and expressed as g/100 g body weight.

Five to seven animals were sacrificed at 100, 200, 300, 400, 480, and 560 days of age. The rats were selected randomly from each diet group with the exception of the last group sacrificed. At that time point, all remaining rats were sacrificed. One week prior to that time, glucose tolerance was assessed. The rats were starved for 14–16 hr and gavaged with a 25% glucose solution. Tail blood glucose (Sigma kit #510, Sigma Chemical Co., St. Louis, MO) was determined before and at 30, 60, and 120 min after the glucose challenge (2.5 g glucose/kg body weight, *per os*).

Prior to sacrifice, renal function was determined. At 100 and 200 days of age, renal function was assessed by determining urea and creatinine clearance. Urine was collected using metabolic cages over a 24-hr period using glycerol as a preservative. Urine volume was measured. Both blood and urine were used for the determination of urea (14) and creatinine (Sigma kit #555); protein content was determined using the biuret reaction. Serum and urine were used for the determination of sodium and potassium by flame photometry. In addition, at 300, 400, 480, and 560 days of age, glomerular filtration rates were determined in anesthetized (0.15:1 mg xylazine:ketamine/100 g body weight) rats using the methods described by Michels *et al.* (15). Inulin levels in the blood and urine were determined by the anthrone method (16).

At the end of the procedures used for determining renal function, the animals were sacrificed by pneumothorax, and the kidneys, liver, and heart were excised and weighed. Portions of kidney and heart were placed in buffered formalin for histological evaluation. The severity of each lesion type was scored from 1 (no lesion) to 5 (severe lesion). Renal tissue was examined for the following lesions: glomerulosclerosis, tubular atrophy, tubular casts, interstitial mineralization, inflammatory cells, and fibrosis. An overall rating of the lesions in each kidney was given. Heart tissue was scored for degeneration of myofibers, inflammatory cells, mineralization of the myofibers, and mineralization of the intima of the aorta. An overall rating of the lesions in each heart was given.

Statistical analysis was performed using Statistical Analysis Software (SAS Institute, Cary, NC). Differences

[1] SPF status monitoring involves the use of sentinel animals in each animal room. These animals are sacrificed at monthly intervals and examined for evidence of chronic and acute disease. Serological (ELISA) testing for the presence of RCV/SDAV, Sendai, PVM, REO3 TMEV, GDVII, M. pulmonis, LCM, and PARVO is done on each sentinel. To date, there have been no significant serological titers found.

[2] Animals in the colony are fed a basal commercial diet (Purina Chow, #5012). They seldom live beyond 700 days. The median lifespan is 450 days. Less than 1% attain the age of 800 days. The majority of deaths occur between 325 and 425 days. When fed purified diets, this time frame is compressed (see Ref. 8 and the present data). Colony rats are routinely sacrificed 300 days of age at the end of their reproductive life. At this time they are glucose tolerance tested, and their tissues are evaluated for evidence of disease. Random serological testing (as in the sentinel animals) is also performed.

with a probability of 0.05 or less were accepted as being significant. Two-way analysis of variance was used to determine the effect of age and diet on each of the measurements made. When two groups were compared a Students *t* test was used.

Results

Figure 1 shows the body weight changes and food intakes for the rats. The food intakes of the two groups of animals were similar throughout the experiment; however, after maturity (~100 days of age) the rats fed the whole-egg diet began to plateau whereas rats fed the control diet continued to gain weight. Peak mature weight was attained at about 400 days of age. Thereafter there were small losses in weight followed by large losses as the rats sickened and became moribund. Animals that were obviously ill were humanely euthanized, and their organs were excised and examined.

Table I provides information about the sampling of the groups and the spontaneous deaths. Because we expected a number of spontaneous deaths, we designed the experiment to allow us to have sufficient numbers of animals in the older groups for meaningful comparisons. Despite this precaution, it became apparent that the animals were not going to survive in sufficient numbers to 600 days of age. Thus, we decided to collect data at 480 days of age and 560 days of age. A number of animals died in the 1-week interval between glucose tolerance testing and glomerular filtration rate (GFR) determination, and several animals did not survive the anesthesia needed for the latter test. Those that did not survive the glucose tolerance and/or GFR were included in the spontaneous death number. Among the typical features of the animals that died spontaneously was gangrene in the tail, rear feet, and legs and hair loss. Of the 117 rats used in this experiment, five of the control group and nine of the group fed the whole-egg diet had evidence of respiratory disease.[3] Two of the control group and one of the whole-egg group had a large growth in the abdominal cavity. Almost all of the animals that died spontaneously had renal disease, however, because of postmortem changes in the vital organs, a true evaluation of the histology of some of these tissues was not always possible. Furthermore, because so many rats died in the last two age intervals, the data shown in Tables II–VI must be regarded with caution since these data came from the "survivors." Those for whom diabetes and its complications were mortal offenses, had already died or were sacrificed for humane reasons.

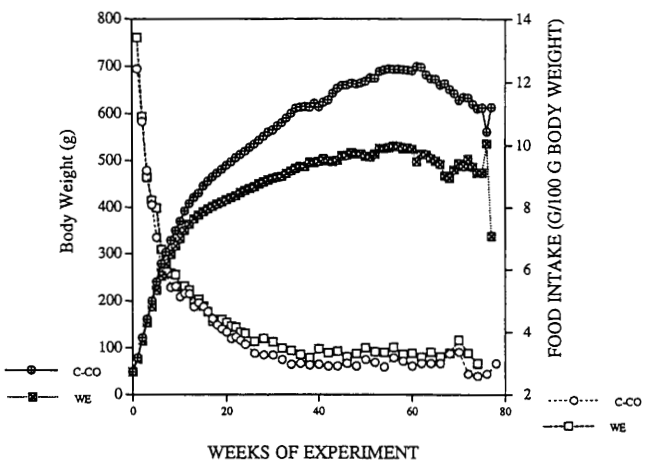

Figure 1. Growth and food intake of male BHE/Cdb rats from weaning. Mean values are presented. Number of rats decreased as the experiment progressed as rats were removed for assessment or died prematurely.

Table I. Spontaneous Deaths and Animal Removal for Assessment in Groups of Male BHE/Cdb Rats Fed Either a Control Diet or a Whole-Egg Diet from 21 Days of Age

Spontaneous death			Sample removal		
Age	Control[1]	Whole egg[2]	Age	Control	Whole egg
21–100	0	0	100	5	5
101–200	0	0	200	5	5
201–300	3	3	300	7	6
301–400	6	6	400	5	6
401–480	8	8	480	7	7
480–560	5	5	560	6	5
TOTAL	24	24		35	34

[1] $n = 59$ at initiation of study.
[2] $n = 58$ at initiation of study.

Final body weights and organ weights expressed as g/100 g body weight are shown in Table II. Rats fed the whole-egg diet were not as heavy as rats fed the control diet. Relative organ weights adjust for the changes in body size (shown in Fig. 1) so that the effect of age alone can be assessed. Note that the hearts were larger in the 100-day-old animals compared to animals at 200, 300, 400, and 480 days of age but were not larger than the 560-day-old rats. The mean relative liver weights followed a similar pattern in the control group but not in the whole-egg group. Diet significantly affected the relative liver weight with the rats consuming the whole-egg diet having larger livers than rats consuming the control diet. The kidney weights were greater in the oldest animals than in the younger ones and reflected a gradual increase in kidney weight due to hyperplasia as the rats aged. The rats consuming the whole-egg diet tended to have larger kidneys at the younger ages and smaller kidneys at 560 days of age than rats fed the control diet. Two-way analysis of variance revealed that diet had a significant effect on final body weight, relative kidney weight, and relative liver weight whereas age had a signifi-

[3] Respiratory disease included chronic pulmonary disease (loss of elasticity) as well as inflammatory changes as indicated by loss of color in the tissue. No evidence of acute infectious pulmonary disease was noted. There were no pockets of pus nor were cultures of the lungs found positive to infectious agents.

Table II. Final Body Weights and Relative Organ Weights of Aging BHE/Cdb Rats Fed a Control or Whole-Egg Diet

Age Days	*n* C[1]	*n* WE	Final wt (g) Body weight C	Body weight WE	Relative weight (g/100 g body weight) Heart C	Heart WE	Kidneys C	Kidneys WE	Liver C	Liver WE
100	5	5	330 ± 11[2]	310 ± 23	0.35 ± 0.01	0.39 ± 0.02	0.76 ± 0.02	0.86 ± 0.04	3.96 ± 0.14	5.12 ± 0.29[a]
200	5	5	509 ± 8	444 ± 22[a]	0.29 ± 0.02	0.34 ± 0.03	0.61 ± 0.05	0.79 ± 0.11	3.24 ± 0.22	5.65 ± 0.88[a]
300	7	6	712 ± 32	516 ± 19[a]	0.25 ± 0.01	0.26 ± 0.02	0.68 ± 0.03	0.80 ± 0.04[a]	3.20 ± 0.12	4.95 ± 0.31[a]
400	5	6	619 ± 32	508 ± 20[a]	0.27 ± 0.01	0.28 ± 0.02	0.98 ± 0.19	1.12 ± 0.20	3.25 ± 0.20	5.74 ± 0.34[a]
480	7	7	687 ± 38	568 ± 19[a]	0.27 ± 0.01	0.30 ± 0.01[a]	0.84 ± 0.05	1.16 ± 0.16[a]	3.68 ± 0.23	5.61 ± 0.25[a]
560	6	5	629 ± 47	512 ± 24[a]	0.32 ± 0.01	0.30 ± 0.01	1.54 ± 0.33	1.00 ± 0.05[a]	3.78 ± 0.34	6.00 ± 0.22[a]
ANOVA										
Age			.05		ns		.05		ns	
Diet			.05		ns		.05		.05	
Age-diet interaction			ns		ns		ns		.05	

[1] Abbreviations used: C, control; WE, whole-egg diet.
[2] Mean ± SEM; [a]Effect of diet at this age is significant ($P < 0.05$).

cant effect on final body weight, relative kidney weight, and relative liver weight.

Glucose tolerance is shown in Figure 2, and fasting blood glucose and urine glucose are shown in Table III. Both diet and age affected the fasting glucose whereas diet alone affected glucose tolerance. There was a significant diet effect on the fasting glucose level at 480 days of age. Glucose in the urine increased as the animals aged with large amounts in the urine of 560-day-old rats fed the control diet. This is reflective of their concurrent hyperglycemia during the time the urine was collected. Random, nonfasting, blood glucose levels ranged from 7–10 mmol/l. Comparing panel A to panel B, the whole-egg diet appeared to delay the age-related impaired glucose tolerance in this group when the age-related intolerance was compared to that of the control group. By 200 days of age, animals in the control group failed to return to the pre-challenge glucose value. Even at 100 days of age an impairment could be observed in this return. By 300 days of age glucose tolerance was definitely abnormal. In the whole-egg group, marked impairment was not observed until 480 days of age.

As in earlier studies, renal disease was a prevalent feature of these rats as shown in Table IV. Although we also examined the other vital organs, no significant lesions were found. The aorta, heart, lungs, and liver were not unusual in these rats (data not shown). We observed gangrene and necrotic change in the tail and feet in the rats that either died spontaneously or were sacrificed after the determination of glomerular filtration rate. Rats that were found to have these features in their severe form at whatever age were sacrificed for humane reasons. Rats were also sacrificed if they lost weight very rapidly and were polyuric. These rats were not assessed with respect to renal function or blood and urine components and were included in the number of rats listed as having died spontaneously.

Glomerulosclerosis was indicated by an increased mesangial matrix and thickening of the mesangial basement membrane. Tubular atrophy was scored if foci of tubules had diminished lumens and decreased epithelium. Scores for tubular casts indicated foci of hyaline casts within tubules. Inflammatory cell score indicated the presence of foci of leukocytes in the interstitial space. Fibrosis refers to connective tissue deposition in the interstitial areas. Although the lesion score is a subjective one and not quantitative, it is based on a set of standardized definitions. A score of 1 meant that no lesions were present whereas a score of 5 indicated the presence of severe lesions. None of the lesions reported in Table IV were more severe in one diet group than in the other. All the lesions were progressively more severe as the animals aged.

Despite the presence of significant lesions in the kidney, the measures of renal function were unchanged until 560 days of age (Table V). At this age, there was a marked reduction in creatinine and urea clearance, which (at 480 days of age) was preceded by an increase in clearance, hyperfiltration. The glomerular filtration rate increased with age as did urine volume and urine protein. There was a marked increase in urine volume and protein between 300 and 400 days of age which suggests a marked change in filtration capacity with respect to membrane permeability. This change is also reflected in the urinary electrolytes (Table VI). With age, urinary sodium and potassium rose whereas the plasma levels of sodium remained relatively unchanged. Urinary sodium loss was generally greater in the older groups of whole-egg-fed rats than those fed the control diet. This was also true for the 300- and 400-day-old rats with respect to urinary potassium.

Discussion

The purpose of the present work was to determine whether the metabolic attributes of the diabetes-prone BHE/Cdb rat could be manipulated by the choice of dietary ingredients. We elected to use a diet composition that mimics that consumed by humans with respect to the distribution of energy from carbohydrate, protein, and fat. In this respect, we wanted to mimic the human situation *vis-à-vis* the role of diet in the phenotypic expression of the diabetes genotype. In part, we were successful. Feeding a whole-egg diet did

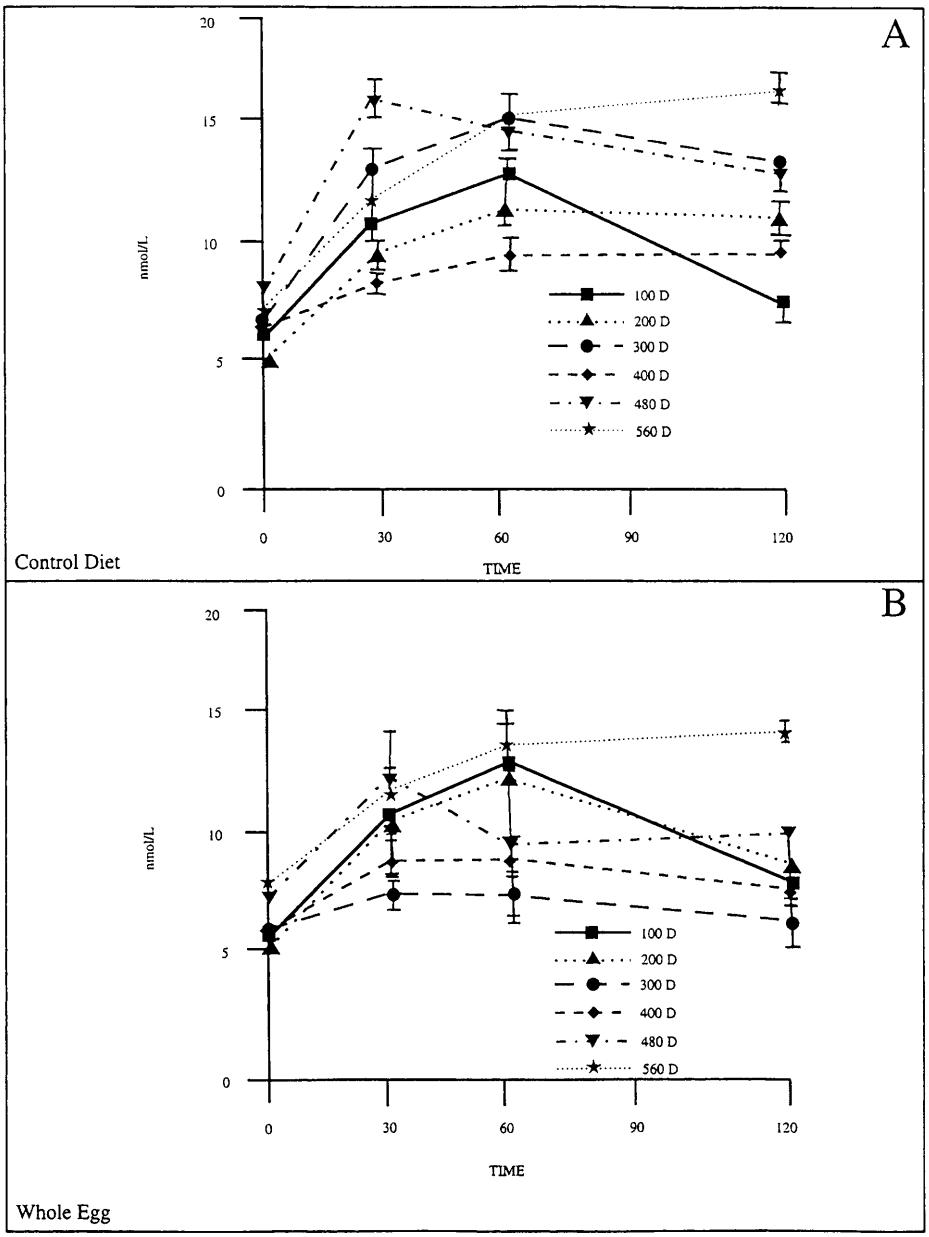

Figure 2. Glucose tolerance at 100, 200, 300, 400, 480, and 560 days of age of BHE/Cdb rats fed either a control diet (panel A) or a whole diet (panel B). Each time point represents mean ± SEM. N is provided in Table II.

delay the onset of the impaired glucose tolerance that is the hallmark of NIDDM in this rat strain (1, 2, 6). However, diet had little effect on renal lesion development. Whereas diet had modest effects on some of the functional attributes of the kidney, in the final analysis, we must report that the diabetic nephropathy was just as severe in this group as it was in the control group.

The fact that the whole-egg diet delayed the development of impaired glucose tolerance is of interest with respect to the mechanism of this effect and with respect to the hypothesis that diabetic nephropathy is secondary to impaired glucose tolerance and hyperglycemia. Several explanations of the first phenomena can be offered. First, note in Fig. 1 and Table II that rats fed the whole-egg diet were not as heavy as those fed the control diet. Their food intake was similar, but their weight gain (after the rapid gain for growth) was less. Because of the many reports in the literature that have related food intake to longevity of rats and mice, we monitored the *ad libitum* intake very carefully to be sure that the two groups did not differ. The differences in body weight at maturity were surprising because there were no differences in food intake. The differences in body weight might be related to regulation of glucose homeostasis.

In the human experience, those that are heavier than normal have a greater tendency to develop abnormal glucose tolerance. This probably occurs in rats as well and, in part, may be due to peripheral insulin resistance. Unfortunately, we did not measure insulin tolerance or adipocyte glucose uptake in these rats as they aged so we do not know whether peripheral resistance can explain the diet differences in the age-related impaired glucose tolerance observed here. In another study, using rats fed a 6% fat diet

Table III. Aspects of Glucose Tolerance in Aging BHE/Cdb Rats Fed a Control or Whole-Egg Diet

Age Days	n C[1]	n WE	Fasting glucose (mmol/l) C	Fasting glucose (mmol/l) WE	Urine glucose (mmol/day) C	Urine glucose (mmol/day) WE
100	5	5	5.44 ± 0.17[2]	5.43 ± 0.31	6.3 ± 0.7	6.2 ± 1.5
200	5	5	4.97 ± 0.30	5.19 ± 0.36	11.7 ± 1.0	8.6 ± 2.2
300	7	6	5.85 ± 1.38	4.84 ± 1.26	31.6 ± 3.5	26.5 ± 1.8
400	5	6	5.65 ± 0.09	5.74 ± 0.26	25.0 ± 3.8	23.0 ± 4.8
480	7	7	7.21 ± 0.37	6.51 ± 0.29[a]	38.3 ± 6.1	35.1 ± 7.1
560	6	5	6.31 ± 0.22	7.20 ± 1.61	88.8 ± 4.9	26.8 ± 3.5[a]
ANOVA						
Age			0.05		0.05	
Diet			0.05		ns	
Age-diet interaction			ns		ns	

[1] Abbreviation used: C, control diet; WE, whole-egg diet.
[2] Mean ± SEM; [a]Effect of diet at this age is significant ($P < 0.05$).

Table IV. Renal Lesion Scores in Aging BHE/Cdb Rats Fed a Control or Whole-Egg Diet

Age Days	n C[1]	n WE	Overall C	Overall WE	Glomerulus C	Glomerulus WE	Tubule Atrophy C	Tubule Atrophy WE	Casts C	Casts WE	Inflammatory Cells C	Inflammatory Cells WE	Fibrosis C	Fibrosis WE
100	5	5	1.0 ± 0.0[2,3]	1.0 ± 0.0	1.1 ± 0.1	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0	1.1 ± 0.1	1.2 ± 0.1	1.0 ± 0.0	1.0 ± 0.0
200	5	5	1.0 ± 0.0	1.8 ± 0.4	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0	1.6 ± 0.2	1.0 ± 0.0	2.1 ± 0.3	1.2 ± 0.1	1.3 ± 0.2	1.0 ± 0.0	1.3 ± 0.1
300	7	6	2.3 ± 0.3	1.5 ± 0.3	1.4 ± 0.2	1.3 ± 0.3	1.7 ± 0.3	1.4 ± 0.2	2.4 ± 0.3	1.9 ± 0.4	1.9 ± 0.2	1.1 ± 0.1	1.3 ± 0.2	1.2 ± 0.1
400	5	6	2.8 ± 0.5	3.1 ± 0.4	1.6 ± 0.3	1.4 ± 0.4	2.4 ± 0.4	2.8 ± 0.3	2.9 ± 0.5	3.1 ± 0.4	2.0 ± 0.5	2.8 ± 0.3	2.3 ± 0.5	3.1 ± 0.4
480	7	7	2.9 ± 0.4	3.5 ± 0.2	1.7 ± 0.5	1.8 ± 0.4	2.6 ± 0.4	3.1 ± 0.2	3.1 ± 0.3	3.6 ± 0.2	2.2 ± 0.3	2.7 ± 0.3	2.6 ± 0.3	3.3 ± 0.3
560	6	5	3.3 ± 0.1	3.1 ± 0.1	3.2 ± 0.5	2.7 ± 0.2	2.4 ± 0.4	2.5 ± 0.1	3.3 ± 0.5	3.1 ± 0.1	2.8 ± 0.3	2.7 ± 0.2	2.7 ± 0.3	2.9 ± 0.1
ANOVA														
Age			0.05		0.05		0.05		0.05		0.05		0.05	
Diet			ns		ns		ns		ns		ns		ns	
Age-diet interaction			ns		ns		ns		ns		ns		ns	

[1] Abbreviation used: C, control diet; WE, whole egg diet.
[2] Mean ± SEM.
[3] Scores range from 1, no lesions to 5, severe lesions.

Table V. Renal Function in Aging BHE/Cdb Rats Fed a Control or Whole-Egg Diet

Age Days	n C[1]	n WE	Urine volume ml/day C	Urine volume ml/day WE	Urine protein mg/day C	Urine protein mg/day WE	Creatinine clearance ml/min C	Creatinine clearance ml/min WE	Urea clearance ml/min C	Urea clearance ml/min WE	GFR ml/min C	GFR ml/min WE
100	5	5	10 ± 2[2b]	7 ± 2[b]	14 ± 5[b]	23 ± 3[b]	1.46 ± 0.08[b]	1.34 ± 0.29[b]	1.38 ± 0.21[b]	0.91 ± 0.19[b]	—	—
200	5	5	13 ± 2[b]	19 ± 5[c]	53 ± 34[b,c]	74 ± 40[c]	2.01 ± 0.14[c]	1.63 ± 0.35[b]	1.51 ± 0.42[b]	0.29 ± 0.16[a,c]	—	—
300	7	6	13 ± 3[b]	19 ± 4[c]	72 ± 28[c]	118 ± 31[d]	1.80 ± 0.32[b,c]	1.33 ± 0.21[b]	1.42 ± 0.12[b]	1.41 ± 0.09[b]	0.86 ± 0.41[b,c]	0.76 ± 0.19[b]
400	5	6	31 ± 8	27 ± 5[c,d]	215 ± 76[d]	367 ± 122[e]	1.69 ± 0.32[b]	1.49 ± 0.19[b]	1.84 ± 0.88[b]	0.95 ± 0.21[b]	0.50 ± 0.08[c]	0.62 ± 0.09[b,c]
480	7	7	36 ± 6[c]	32 ± 7[d]	354 ± 113[d,e]	430 ± 94[e]	3.65 ± 0.63[d]	2.25 ± 0.47[a,e]	0.82 ± 0.12[d]	1.03 ± 0.11[b]	0.91 ± 0.12[b]	1.19 ± 0.26[c]
560	6	5	36 ± 4[c]	17 ± 3[a,c]	472 ± 94[e]	302 ± 65[e]	0.42 ± 0.10[e]	0.53 ± 0.10[d]	0.25 ± 0.11[e]	0.35 ± 0.10[c]	0.90 ± 0.41[bc]	1.33 ± 0.25[c]
ANOVA												
Age			0.05		0.05		0.05		0.05		0.05	
Diet			ns		ns		ns		ns		ns	
Age-diet interaction			ns		ns		ns		ns		ns	

[1] Abbreviations used: C, control diet; WE, whole egg diet; GFR, glomerular filtration rate.
[2] Mean ± SEM; [a] Effect of diet is significant ($P < 0.05$); [b,c,d,e] means with unlike superscripts within this diet group are significantly different ($P < 0.05$).

containing beef tallow, menhaden oil, or corn oil, we did measure insulin binding and peripheral glucose uptake and metabolism using adipocytes isolated from aging rats (17). We found that those rats fed beef tallow had more active glucose transport (mobile glucose transporters), glucose oxidation, and glucose conversion to fatty acids than rats fed corn oil or menhaden oil. In this study (17) there were no diet-related differences in insulin receptor number or affinity but, just as in the present study, there was an age-related impairment in glucose tolerance and this was related to the composition of the diet. Those rats fed beef tallow lived longer, and their diet fat type influenced their age-related impaired glucose tolerance. Whereas the present study used a much higher fat level (18.5%), there is nonetheless, reason to assume that the fatty acids contributed by the egg would be similar to those contributed by the beef tallow used in the earlier study and that there would be some similarity between the previous and present work. Analysis of the fatty

Table VI. Electrolyte Levels in Plasma and Urine of Aging BHE/Cdb Rats Fed a Control or Whole-Egg Diet

Age	n		Sodium mEq				Potassium mEq	
			Urine		Plasma		Urine	
Days	C[1]	WE	C	WE	C	WE	C	WE
100	5	5	0.52 ± 0.05[2b]	0.52 ± 0.13[b]	138 ± 9[b]	143 ± 11[b,d]	0.96 ± 0.09[b]	0.96 ± 0.16[b]
200	5	5	0.60 ± 0.11[b]	1.24 ± 0.23[a,c]	150 ± 10[b]	156 ± 9[b]	1.27 ± 0.14[c]	1.51 ± 0.30[c]
300	7	6	0.32 ± 0.09[c]	2.11 ± 0.51[a,d]	121 ± 4[c]	121 ± 3[c]	0.70 ± 0.18[b]	2.07 ± 0.38[a,d]
400	5	6	0.71 ± 0.10[b]	2.05 ± 0.27[a,d]	121 ± 1[c]	119 ± 2[c]	1.36 ± 0.08[c]	2.11 ± 0.28[a,d]
460	7	7	1.04 ± 0.30[b,c]	2.32 ± 0.35[a,d]	114 ± 4[c]	119 ± 3[c]	2.20 ± 0.51[d]	2.28 ± 0.23[d]
580	6	5	0.90 ± 0.16[b]	1.52 ± 0.11[a,c]	130 ± 1[d]	130 ± 2[d]	1.73 ± 0.20[e]	1.39 ± 0.38[c]
ANOVA								
Age			0.05		ns		0.05	
Diet			0.05		ns		0.05	
Age-diet interaction			ns		ns		ns	

[1] Abbreviations used: C, control diet; WE, whole egg diet.
[2] Mean ± SEM; [a] Effect of diet at this age is significant ($P < 0.05$); [b,c,d,e] Means within a diet group are significantly different ($P < 0.05$).

acids in the two diets (data not shown) revealed that the egg diet profile was quite similar to that of the tallow diet used previously.

Earlier, we reported (18) that feeding this whole-egg diet resulted in a downregulation of gluconeogenesis by hepatocytes isolated from 48-hr starved rats. Increased gluconeogenesis is a characteristic of the BHE/Cdb rat (6) as well as the diabetic human (19, 20). It is possible that the delay in the development of impaired glucose tolerance in the whole-egg-fed rats could be explained not only by a diet effect on body weight as discussed above, but also by a diet effect on hepatic gluconeogenesis. A reduction in gluconeogenesis would result in lower blood glucose levels due to a reduction in the hepatic contribution to this glucose pool.

Despite the above effects of diet on glucose tolerance, diet had few effects on the development of renal lesions and renal function. Although small diet differences did occur in the various measurements reported in Tables V and VI, these differences probably were not biologically significant with respect to the sequence of changes observed in these aging rats. Diet had no effect on renal pathology. Others (21) have reported that diet restriction can attenuate the time course of renal disease development. We did not restrict the amount of food available to the rats but allowed them free access to the food. On a body weight basis this meant that both groups of rats consumed the same amount of food. However, if one does not take into account the differences in body weight, it would appear that the rats provided the egg diet consumed less food per day than the rats fed the control diet. Despite this difference in unadjusted daily food intake, there were no differences in renal disease development. Thus we must conclude that diet composition or diet intake did not affect the time course of lesion development, and we must examine the data for other explanations of this age-related phenomenon.

Renal function is highly dependent on adequate supplies of ATP. Na^+K^+ATPase, for example, has a high requirement for ATP and may consume a substantial amount that must be regenerated *via* oxidative phosphorylation. Many years ago, Kleiber (22) reported that Borsook and Winegarden (23) calculated the work of the kidney and found that 860 kcal (3598 kJ) per day were required for urea excretion. Depending on how one calculates the energy value of a mole of ATP, 98–113 moles of ATP would be needed to support this work. Additional ATP is, of course, needed to sustain renal metabolism (i.e., urea synthesis, glycolysis, and so forth). We previously reported (3) the existence of a mutation in F_0ATPase in the BHE/Cdb rat that results in a decreased efficiency of ATP production. Although the decrease in efficiency is only a matter of 10%–15%, over a lifetime this decrease could have cumulative effects on tissues that have a high ATP need. We reported that there is an age-related impaired insulin release by the islet cell (24). β cells constantly synthesize insulin and release it. These are ATP-requiring processes. Both renal and islet tissue function could be impaired due to the cumulative effects of a decline in ATP synthesis efficiency. Whereas the feeding of whole egg appeared to rescue the islet cells by reducing gluconeogenesis and thus the glucose mediated signal for insulin release, this diet had no effect *vis-à-vis* rescue of the kidney. There was no diet difference in the age-related lesion development in the two groups of rats studied over their lifetime. The linkage between renal ATP production and need and lesion development is far from clear. More research is needed to determine whether such lesions could result from an ATP shortfall. In humans, renal as well as heart lesions have been reported in people having a mitochondrial genomic error in F_0ATPase (25).

Lastly, we want to point out the relevance of these findings to humans with NIDDM. In this population, several mutations in the mitochondrial genome have been found (25–30). Five mutations (26–27) have been found in the tRNAs, and estimates of frequency vary from 1.4%–10%

depending on the population studied and the particular mutation. Four mutations (29–30) in the mtATPase 6 gene have been reported, and one of these (at bp 8860) is in the same position as the one (bp 8204) we have reported (3) for the BHE/Cdb rat. Another, at bp 8993,[4] is very close to a second BHE/Cdb mutation at bp 8289 (Herrnstadt *et al.* unpublished observations). Both mutations are reasonably close denoting a possible hot spot in the DNA. The frequency in BHE/Cdb rats is 100% whereas in NIDDM humans, the frequency of ATPase 6 mutation is about 10%. This figure may change as the population examined increases in number. Lastly Anderson *et al.* (31) reported that 80% of her NIDDM population had a series of 12 missense mutations in their mtATPase 8 gene. In all of these mutations a reduction in ATP synthesis efficiency has been reported in addition to reports of varying tissue function impairment. Renal tissue and islet tissue are frequently mentioned as being affected by these mutations.

What we have shown by the present work using a diet that mimics the approximate composition of the human diet, is that the source of fat and protein can modify the phenotypic expression (impaired glucose tolerance) of the genotype characterized by a base substitution in the mtATPase gene. The feeding of whole egg delayed the development of abnormal glucose tolerance in these rats. Humans could derive a similar benefit. However, one should be mindful of the limitations of this study; whole egg was the sole source of fat and protein. Humans would not consume such an egg-rich diet. Also, the whole-egg diet did not delay diabetic nephropathy in these rats and finally, the data reported were from the survivors. Whether benefits could be derived with a lower level of egg in the rat diet remains to be demonstrated.

Supported by the Georgia Agricultural Experiment Station Project H-611, The UGA Diabetes Research Fund and by a grant from the US Poultry and Egg Association.

1. Berdanier CD. The BHE rat: An animal model for the study of noninsulin-dependent diabetes mellitus. FASEB J **5:**2139–2144, 1994.
2. Berdanier CD. NIDDM in the nonobese BHE/Cdb rat. In: Shafrir E, Ed. Lessons from Animal Diabetes. London: Smith Gordon, pp231–246, 1994.
3. Mathews C, McGraw RA, Berdanier CD. A point mutation in the mitochondrial DNA of diabetes-prone BHE/Cdb rats. FASEB J **9:**1638–1642, 1995.
4. Lakshmanan MK, Berdanier CD, Veech RL. Comparative studies on lipogenesis and cholesterogenesis in lipemic BHE and normal Wistar rats. Arch Biochem Biophys **183:**355–360, 1977.
5. Berdanier CD, Tobin RB, DeVore V. Studies on the control of lipogenesis: Strain differences in hepatic metabolism. J Nutr **109:**247–260, 1979.
6. Berdanier CD. Rat strain differences in gluconeogenesis by isolated hepatocytes. Proc Soc Exp Biol Med **169:**74–79, 1982.
7. Fowler K, Berdanier CD. Low-dose intakes of mitochondrial uncouples differentially affect hepatic lipogenesis in BHE/Cdb and Sprague-Dawley rats. Biochem Arch **7:**269–274, 1991.
8. Berdanier CD, Johnson B, Hartle DK, Crowell WA. Lifespan is shortened in BHE/Cdb rats fed a diet containing 9% menhaden oil and 1% corn oil. J Nutr **122:**1109–1131, 1992.
9. Fowler KA, Crowell WA, Berdanier CD. Early renal disease in BHE/cdb rats is less in rats fed beef tallow than in rats fed menhaden oil. Proc Soc Exp Biol Med **203:**163–171, 1993.
10. Berdanier CD, Kras K, Wickwire K, Hall DG, Gunnett C, Hartle D. Progressive glucose intolerance and renal disease in aging BHE/Cdb rats. Int J Diabetes **5:**27–38, 1997.
11. Wickwire K, Porter M, Berdanier CD. Menhaden oil decreases renal and hepatic cholesterol uptake by diabetes prone BHE/Cdb rats. Proc Soc Exp Biol Med **214:**346–351, 1997.
12. Wickwire K, Kras K, Gunnett C, Hartle D, Berdanier CD. Menhaden oil feeding increases potential for renal free radical production in BHE/Cdb rats. Proc Soc Exp Biol Med **209:**397–402, 1995.
13. Kullen MJ, Berdanier CD. Influence of fish oil feeding on the vitamin E requirement of BHE/Cdb rats. Biochem Arch **8:**247–257, 1992.
14. Brown HH. Determination of blood urea with p-dimethylaminobenzaldehyde. Anal Chem **31:**1844–1846, 1959.
15. Michels LD, Davidman M, Keane WF. Determinants of glomerular filtration and plasma flow in experimental diabetic rats. J Lab Clin Med **98:**869–885, 1994.
16. White RP, Samson FE. Determination of inulin in plasma and urine by anthrone. J Lab Clin Med **43:**475–478, 1954.
17. Pan J-S, Berdanier CD. Dietary fat saturation affects glucose metabolism without affecting insulin receptor number and affinity in adipocytes from BHE rats. J Nutr **121:**1811–1819, 1991.
18. Kullen MJ, Parente JA, Berdanier, CD. Effects of type of dietary fat and protein on gluconeogenesis in isolated hepatocytes from BHE/Cdb rats. J Nutr Biochem **5:**227–231, 1994.
19. Puhakainen I, Koivisto VA, Yki-Jarvinen H. Lipolysis and gluconeogenesis from glycerol are increased in patients with noninsulin-dependent diabetes. J Clin Endocrinol Metab **75:**789–794, 1992.
20. Consoli A, Nurijhan N, Capani F, Gerich J. Predominate role of gluconeogenesis in increased hepatic glucose production in NIDDM. Diabetes **38:**550–557, 1988.
21. Johnson PR, Stern JS, Horwitz BA, Harris RE, Greene SF. Longevity in obese and lean male and female rats of the Zucker strain: Prevention of hyperphagia. Am J Clin Nutr **66:**890–903.
22. Kleiber M. *Fire of Life: An Introduction to Animal Energetics.* Huntington, NY: Robert E. Krieger Publishing Company, pp. 391–393, 1975.
23. Borsook H, Winegarden HM. The energy cost of the excretion of urine. Proc Nat Acad Sci (USA) **17:**13–28, 1931.
24. Liang Y, Bonner-Weir S, Wu Y-J, Berdanier CD, Berner DK, Efrat S, Matschinsky FM. *In situ* glucose uptake and glucokinase activity of pancreatic islets in diabetic and obese rodents. J Clin Invest **93:**2473–2481, 1994.
25. Gerbitz K-D, van den Ouweland JMW, Maassen JA, Jakoch M. Mitochondrial diabetes mellitus: A review. Biochim Biophys Acta **1271:**253–260, 1995.
26. Odawara M, Sasaki K, Yamashita K. A G to A substitutes at nucleotide position 3316 in mitochondrial DNA is associated with Japanese noninsulin-dependent diabetes mellitus. Biochem Biophys Res Commun **227:**147–151, 1996.
27. Hirai M, Suzuki S, Onoda M, Hinokio Y, Ai L, Kirai A, Ohtomo M, Komatsu K, Kasuga S, Satoh Y, Akai H, Toyota T. Mitochondrial DNA 3394 mutation in the NADH dehydrogenase subunit 1 associated with noninsulin-dependent diabetes mellitus. Biochem Biophys Res Commun **219:**951–955, 1996.
28. Velho G, Byrne MM, Clement K, Sturio J, Pueyo ME, Blanche H, Vionnet N, Fiet J, Passa P, Robert JJ, Polansky KS, Froguel P.

[4] The bp numbers differ because of species differences in mtDNA size. The human has 16,569 bp whereas the rat has 16,248 bp.

Clinical phenotypes, insulin secretion, and insulin sensitivity in kindreds with maternally inherited diabetes and deafness due to mitochondrial $tRNA^{Leu(UUR)}$ gene mutation. Diabetes **45:**478–487, 1996.

29. Robinson BH. mtDNA and nuclear mutations affecting oxidative phosphorylation correlating the severity of clinical defect with the extent of bioenergetic compromise. J Bioenerg Biomembr **26:**311–316, 1994.
30. Houstek J, Klement P, Hermanska J, Houstkova H, Van den Bogert C, Zeman J. Altered properties of mitochondrial ATPase synthetase in patients with a T→G mutation in the ATPase 6 (subunit a) gene at position 8993 of mtDNA. Biochim Biophys Acta **1271:**349–357, 1995.
31. Anderson C, Herrnstadt C, Miller S, Olefsky J, Davis R. Mutations in mitochondrial ATPase 8 gene are associated with NIDDM. Diabetes **46:**174A, 1997.