Neither the cholesterol or arginine content of whole egg explains its beneficial effect on glucose homeostasis in BHE/cdb rats

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Male weanling BHE/cdb rats were fed either a whole-egg diet, a control diet, or a control diet that matched either the arginine or cholesterol content of the whole-egg diet. Glucose tolerance, renal function, cholesterol status, and urea cycle activity was assessed at 250 days of age. Neither the cholesterol content of the whole-egg diet nor its arginine content could explain the whole-egg diet effect on glucose tolerance, renal metabolism, or renal function. At 250 days of age BHE/cdb rats fed the whole-egg diet had a near normal glucose tolerance, whereas rats fed the other diets had impaired tolerance as well as impaired renal function. This diet difference might be attributable to either the vitamin A or lecithin content of the whole egg, but is not attributable to its arginine or cholesterol content. (J. Nutr. Biochem. 9:170–177, 1998) *© Elsevier Science Inc. 1998*

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Introduction

Recently, we reported that feeding a whole-egg diet to BHE/cdb rats delayed the onset of impaired glucose tolerance, while having little effect on the age-related renal lesion development.[1] This was an unexpected finding because previously we had found that the delay in impaired glucose tolerance was accompanied by a delay in the development of diabetic nephropathy when the diets compared contained beef tallow or corn oil or menhaden oil.[2] An additional study showed that the pivotal age at which diet-related differences in both glucose tolerance and renal disease could be observed was 250 days.[3] In the whole-egg longevity study[1] we made observations at 100-day intervals and found large diet differences between the 200- and 300-day interval with respect to glucose tolerance, urine glucose spillage, urine protein, and urinary electrolytes. The results of this longevity study suggested that the effects of the whole-egg diet on renal function should be revisited with observations made at the pivotal age of 250 days. Further, Reyes et al.[4,5] have suggested that the diet cholesterol content[4] and the arginine content[5] could influence the progression of renal dysfunction in streptozotocin diabetic animals. Thus, we hypothesized that the whole-egg diet effect was attributable to these components. We constructed diets that matched the whole-egg diet vis a vis its cholesterol content and its arginine content. We found that neither addition explained the beneficial effect of the whole-egg diet on the glucose tolerance of the BHE/cdb rat. Nonetheless, these additions did influence other aspects of renal function.

Methods and materials

Four groups of 60 male weanling BHE/cdb (UGA colony) rats were used. Animals were housed individually in hanging wire mesh cages in a room controlled for temperature (21 ± 1°C), humidity (40 to 50%), and light (lights on 06:00 to 18:00). The animals were fed an unrefined diet (Purina laboratory chow, Ralston Purina Co., St. Louis, MO USA) for 10 days. The rats were then randomly allocated to one of four diet groups. The composition of these four isocaloric diets are shown in *Table 1*. The diets contained the same amount of fat (19 g/100 g) and protein (20 g/100 g). Diet 1 contained dehydrated whole egg as the source of fat and protein, whereas Diet 2 (the control diet) contained the same amount of fat as beef tallow and corn oil and the same amount of protein as a 1:1 mixture of casein and

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Table 1 Diet composition

Ingredients (g/100 g diet)	Diet 1 Whole egg	Diet 2 Control	Diet 3 Arginine	Diet 4 Cholesterol
Whole egg	40	0	0	0
Casein	0	10	10	10
Lactalbumin	0	10	10	10
Corn Oil[1]	0	2	2	2
Beef tallow[1]	0	17	17	17
Cholesterol[2]	0	0	0	0.2
Mineral mix[3] (AIN 93)	4.9	4.9	4.9	4.9
Vitamin mix[3] (AIN 93)	1.1	1.1	1.1	1.1
Celufil	4	4	4	4
Sucrose	50	51	50.59	50.8
Arginine[4]	0	0	0.41	0
TOTAL	100	100	100	100

[1] The fatty acid profile of this fat mix matched that of whole egg.
[2] The cholesterol content in diet 4 matched that of the whole egg.
[3] Composition of Vitamin and Mineral mix may be found in *J. Nutrition*. 123, 1942, 1993.
[4] Calculated based on the arginine content of whole egg.

lactalbumin. The mixture of beef tallow and corn oil duplicated the fatty acid profile of the whole-egg lipid. The amino acid composition of the whole egg was calculated using data obtained from the USDA Home Economics Research Report, No. 4. The arginine content of Diet 3 matched that of the whole-egg diet. In Diet 4, the cholesterol content matched that of the whole-egg diet. Rats were supplied diet and water ad libitum. Food intakes and body weights were determined weekly. The care of the animals followed American Association for Laboratory Animal Care (ALAC) guidelines as outlined in NIH Publication 88-23, NIH Guide for the Care and Use of Laboratory Animals.

At 240 days of age, the animals were tested for glucose tolerance. Animals were starved overnight (about 16 hr), and initial blood samples were collected from the cut tip of the tail. The rats were then given a glucose challenge (25% glucose solution at 0.1 cc/100 g body weight per os), and blood samples were obtained at 30, 60, and 120 min post challenge. Serum was collected after centrifugation (3,500 rpm, 4°C, for 20 min), and used for the determination of glucose using glucose oxidase (Sigma kit No. 510, Sigma, St. Louis, MO USA). The serum was also used for creatinine analysis (Sigma Kit No. 555).

After the glucose tolerance test, the rats were transferred to

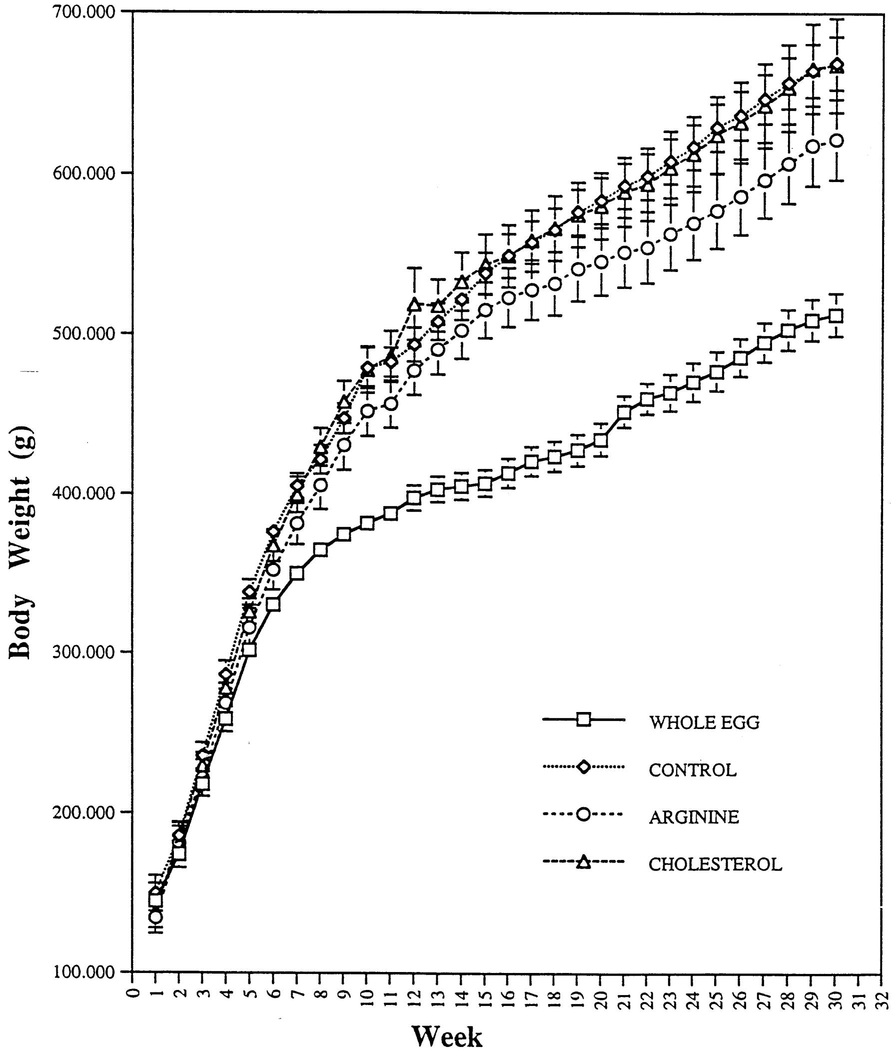

Figure 1 Body weights of male BHE/cdb rats fed either a whole-egg diet or a control diet or an arginine or cholesterol enriched diet from 4.5 weeks of age until 250 days of age (values are means ± SEM).

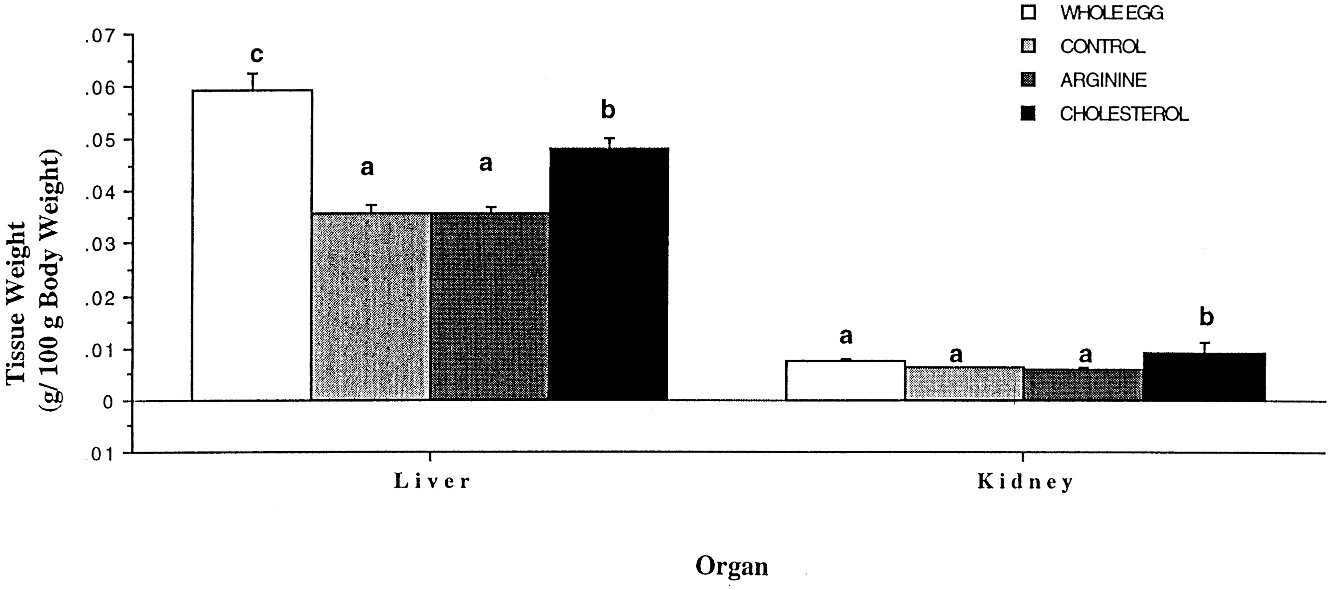

Figure 2 Liver and kidney weights of male BHE/cdb rats fed either a whole-egg diet or a control diet or an arginine or cholesterol enriched diet from 4.5 weeks of age until 250 days of age (values are means ± SEM; different letters indicate significant difference, $P < 0.05$).

metabolic cages. Food and water ad libitum were given at this time. Urine was collected over 24 hr into 50-mL polypropylene tubes that contained 2 mL glycerol as a preservative. Urine was analyzed for protein[6] and creatinine (Sigma kit No. 555). The values of plasma creatinine and urine creatinine were used to determine the creatinine clearance rate. The equation used for calculation of creatinine clearance rate was as follows:

creatinine clearance rate (mL/sec · m^2) =

$$\frac{\text{urine creatinine } (\mu\text{mol})}{\text{plasma creatinine } (\mu\text{mol})} \times \frac{1}{86400} \times \text{mL urine} \times \frac{1.73}{\text{A*}}$$

*A: body surface in m^2 = 12.54 × body weight (g)$^{0.6}$

The rats were killed at 250 days of age.

Cholesterol status

Six animals from diet groups 1, 2, and 4 were anesthetized with sodium pentobarbital (0.1 mg/100 g body weight), the thoracic cavity was opened and blood was withdrawn into EDTA treated vacutainers via heart puncture. After centrifugation, the plasma was stored and subsequently used for the determination of cholesterol (Sigma kit No. 352). At the same time, animal kidneys and livers were excised, weighed, and used for the determination of cholesterol content.[7,8]

The second subgroup of five rats from these same diet groups were also anesthetized with sodium pentobarbital (10 mg/kg). The femoral vein of rats was exposed and isolated from the artery and surrounding tissues. Radiolabelled cholesterol (37 Kbq [4-^{14}C]/100 g body weight) was infused into the femoral vein. The radiolabelled cholesterol was used to measure cholesterol uptake by liver and kidneys. After 1 hr, the thoracic cavity was opened and blood was withdrawn via heart puncture. The plasma, liver, and kidney were used to determine radiolabelled cholesterol content. The lipids were extracted from a 1 mL homogenized tissue sample using the Dole and Meinertz procedure.[9] After saponification with ethanolic KOH the saponified fraction was discarded, and the remaining fraction was extracted with heptane, the solvent evaporated off and the residue redissolved in scintillation fluid and counted.

Arginine effects

Six rats from groups 1, 2, and 3 were killed by decapitation and their renal and hepatic tissues excised for use in the determination of urea cycle enzymes.[10]

Data analysis

The data from these experiments were analyzed using one way analysis of variance (ANOVA) to identify significantly different means. A probability of less than 0.05 was accepted as significant. Statistical Analysis Software (SAS) and SuperANOVA were used.

Results

Food intake, body weight, and tissue weight

As the animals grew they ate less food per 100 g body weight and diet composition had no effect on this food intake (data not shown). The daily food intake stabilized after week 22.

The animals grew at a similar rate until week 6 (*Figure 1*). At this point the animals fed the whole-egg diet (Diet 1) began to gain weight at a slower rate than did rats in the other three groups. By week 30, rats fed Diet 1 reached a body weight of about 500 g, whereas rats from the other three diet groups were between 600 and 700 g. This diet difference in weight gain is consistent with our previous report.[1] These differences were significant ($P < 0.05$). Of the 60 rats used for this study three died from unknown causes. This resulted in unequal group sizes.

Diet 1 animals had greater relative liver weights than animals in the other three diet groups ($P < 0.05$) (*Figure 2*). The rats fed the cholesterol diet (Diet 4) had significantly smaller relative liver weights than the rats fed Diet 1, but larger relative liver weights than rats fed the control diet or the arginine enriched diet. Diet 4 animals had the greatest relative kidney weights when compared with the rats of the other three diet groups.

Glucose tolerance is shown in *Figure 3*. Animals fed the

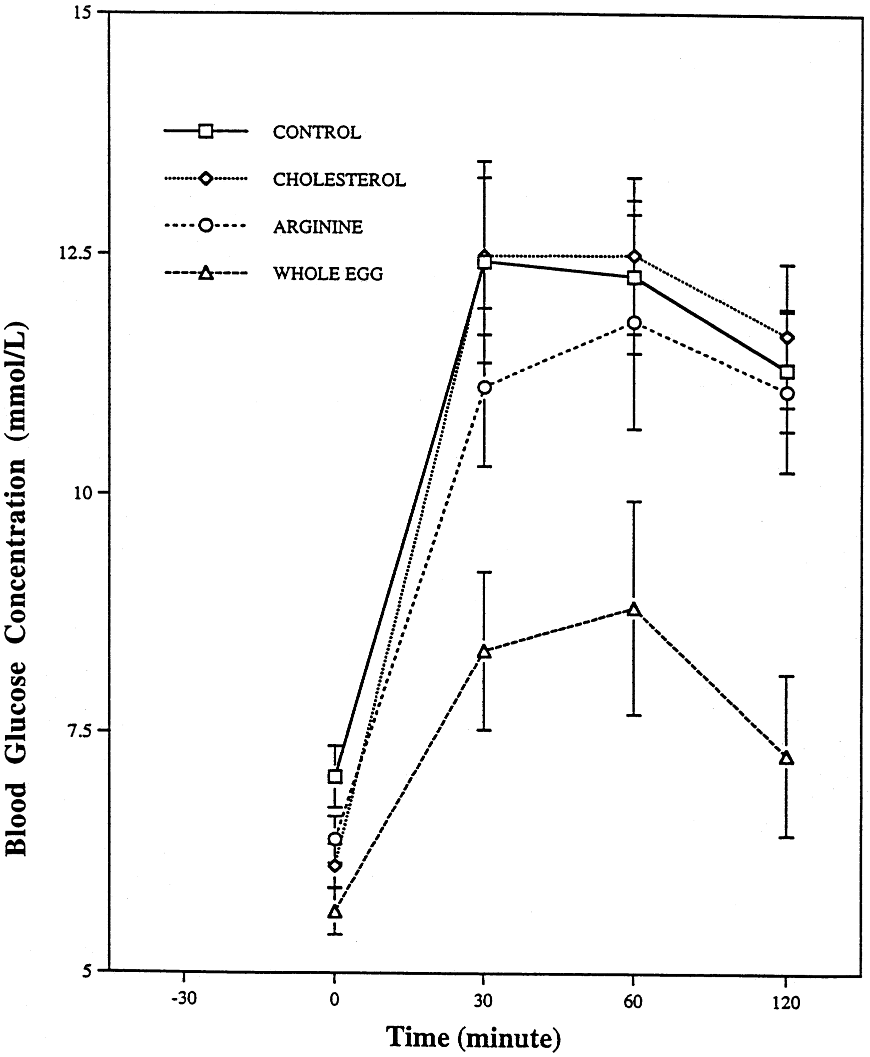

Figure 3 The effect of diet on glucose tolerance in male BHE/cdb rats fed either a whole-egg diet or a control diet or an arginine or cholesterol enriched diet from 4.5 weeks of age until 250 days of age (values are means ± SEM).

whole-egg diet had nearly normal glucose tolerance, whereas rats fed either the control diet or the cholesterol or arginine enriched diets did not. The glucose level in the blood failed to return to the prechallenge level by 120 min post challenge. This is consistent with our previous report.[1]

Cholesterol status

Animals fed Diet 4 had the highest fasting plasma cholesterol level (*Figure 4*). No differences were seen between Diet 1 and Diet 2 groups. Animals fed Diet 3 were not used for these comparisons.

No differences attributable to diet were observed in the total cholesterol content of the renal tissue (*Figure 5*). However, significant differences existed in total hepatic cholesterol content between animals fed Diet 1, 2, and 4. The animals fed Diet 2 had 0.017 ± 0.002 mmol/g, whereas Diet 1 had 0.077 ± 0.003 mmol/g, and Diet 4 had 0.121 ± 0.009 mmol/g.

Animals fed Diet 1 had significantly more labeled cholesterol in their renal tissue (*Figure 6*). The labeled cholesterol value was almost five fold higher than that of the renal tissues from Diet 2 and Diet 4 rats. No significant differences attributable to diet were seen in the hepatic radiolabeled cholesterol.

Urea cycle enzymes activity in hepatic and renal tissue

No diet differences in the urea cycle enzymes (ornithine transcarbamoylase, argininosuccinate synthetase, argininosuccinase, and arginase) were observed either in liver or kidney. The enzyme activity values are reported in *Table 1*. Animals fed Diet 4 were not used in this comparison.

Urine metabolites

No significant differences attributable to diet were seen in urine volume. Urine protein level was higher in rats fed Diet 4 than in rats fed the other three diets, but the difference was not significant. However, significant diet differences were identified in creatinine clearance rate (*Table 2*). The animals fed Diet 1 had the highest creatinine clearance rate among the four diet groups.

No diet differences were observed in the urea content of hepatic and renal tissue. Furthermore, no diet differences of

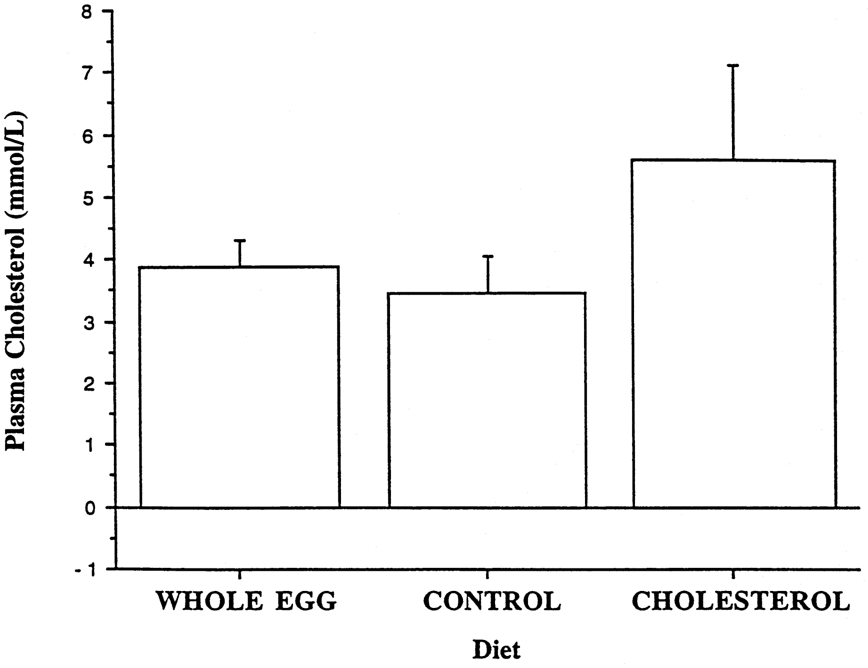

Figure 4 The effect of diet on fasting plasma cholesterol in male BHE/cdb rats fed a whole-egg diet, a control diet, or a cholesterol-enriched diet (values are means ± SEM).

urease activity in hepatic and renal tissue were found. The urea content and urease activity rate are reported in *Table 3.*

Discussion

In this study, animals fed a whole-egg diet had less of an impaired glucose tolerance and a greater creatinine clearance rate than rats fed the control diet. Matching the arginine content (Diet 3) or cholesterol content (Diet 4) did not explain the egg effect.

Furthermore, there was no correlation between the cholesterol content of the diet and renal function. Animals fed the cholesterol enriched diet had high plasma cholesterol levels, but these levels were not significantly different from those in animals fed other diets. The relationship of dietary cholesterol to renal function is controversial. Dietary cholesterol was shown to ameliorate the development of renal tubule lesions in streptozotocin diabetic rats[4] and in BHE/cdb rats fed a 10% beef tallow diet.[3] The diet used for the present work contained substantially more fat (18%) and

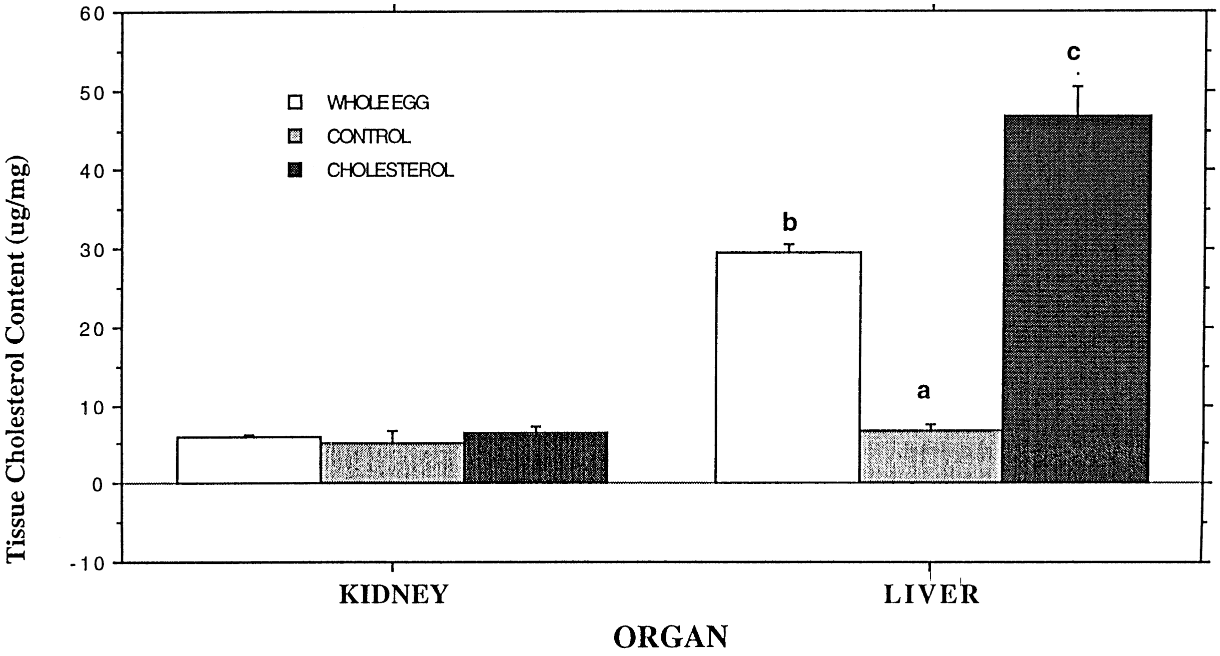

Figure 5 The effects of diet on renal and hepatic cholesterol in male BHE/cdb rats fed either a whole-egg diet or a control diet or a cholesterol enriched diet until 250 days of age (values are means ± SEM; different letters indicate significant difference, $P < 0.05$).

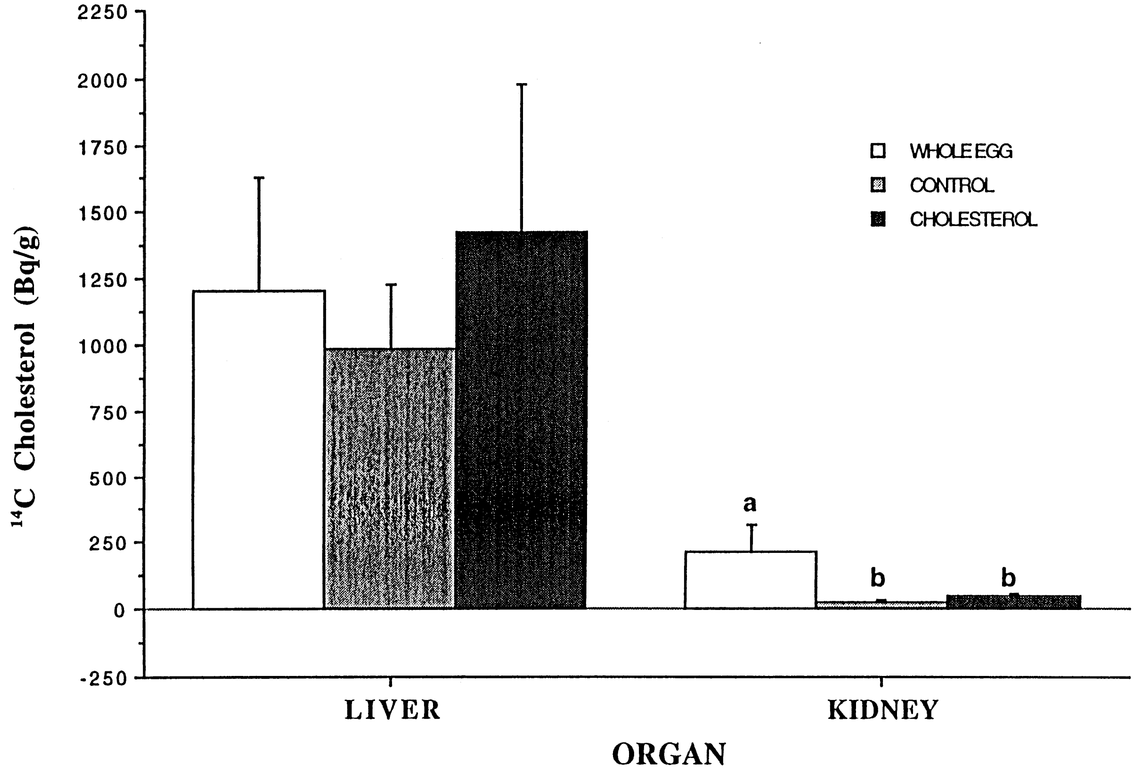

Figure 6 The effects of diet on radiolabeled cholesterol in hepatic and renal tissue of male BHE/cdb rats fed either a whole-egg diet or a control diet or a cholesterol enriched diet until 250 days of age (values are means ± SEM, $n = 6$; different letters indicate significant difference, $P < 0.05$).

cholesterol than did the 10% beef tallow diet used previously. Some studies have shown that high serum cholesterol levels were associated with impaired renal function,[11,12] whereas, other studies showed no relationship between serum cholesterol levels and renal function.[13] It would seem that the level of cholesterol in the diet determines whether an effect on renal function and renal disease will be observed. At high levels (as in the present study) no relationship was observed, whereas with more modest levels a relationship can be found.

Table 2 The effects of diet on urea cycle enzymes activity in hepatic and renal tissue of male BHE/cdb rats[1,2]

	Diet 1 Whole egg	Diet 2 Control	Diet 3 Arginine
Ornithine transcarbamoylase			
Liver	2.74 ± 0.09	2.81 ± 0.15	2.90 ± 0.09
Kidney	0.04 ± 0.01	0.04 ± 0.01	0.04 ± 0.01
Argininosuccinate synthetase			
Liver	0.62 ± 0.08	0.64 ± 0.05	0.65 ± 0.05
Kidney	0.17 ± 0.02	0.17 ± 0.01	0.16 ± 0.01
Argininosuccinase			
Liver	1.08 ± 0.31	1.42 ± 0.46	0.79 ± 0.19
Kidney	0.25 ± 0.06	0.33 ± 0.11	0.30 ± 0.06
Arginase			
Liver	9.59 ± 1.05	15.68 ± 3.79	11.22 ± 1.06
Kidney	0.58 ± 0.09	0.51 ± 0.09	0.49 ± 0.08

[1] Mean ± SEM.
[2] Unit: mmol product/hr/g tissue.

The whole egg diet affected the body weight gain after maturity. Abnormal glucose tolerance was not found in the animals fed whole-egg diet, and renal function was improved in these animals. The four test diets contained the same level of lipids and had a similar fatty acid composition. All the diets contained the AIN recommended amounts of vitamins and minerals, however, the use of dried whole egg in these diets resulted in an increase (three fold) in vitamin A and also lecithin. In the longevity study we used additional diets that had dehydrated egg white as the protein source (unpublished data). This was essentially ovalbumin. The fat source was corn oil. Rats fed this diet grew poorly and many evidenced signs and symptoms (alopecia, greying hair, poor weight gain) of micronutrient deficiency. For this reason, we elected not to use this protein in the control diet.

As reported previously,[1] feeding the whole-egg diet affected glucose tolerance. Whether this was attributable to the above mentioned effect on body size or an effect of vitamin A or lecithin on glucose homeostasis has yet to be determined. We have reported that gluconeogenesis is less in BHE/cdb rats fed a whole-egg diet.[14] This reduction is not attributable to an effect on PEPCK[15] but probably to an effect on three carbon cycling as proposed by Rogstad.[16] Tappy et al.[17] has shown that cycling between glucose and three carbon intermediates accounts for a major contribution to hepatic glucose production in diabetic hyperglycemia.

Alternatively, we could suggest that the three fold

Table 3 The effects of diet on urine metabolites in male BHE/cdb rats[1,2]

	Diet 1 Whole egg	Diet 2 Control	Diet 3 Arginine	Diet 4 Cholesterol
Urine volume (mL/24 hr)	21.83 ± 5.09	26.17 ± 3.19	18.86 ± 3.51	17.00 ± 3.08
Urine protein (mg/mL)	7.99 ± 1.40	7.28 ± 1.35	6.67 ± 2.53	13.43 ± 4.00
Creatinine clearance (mL/sec.m2)	1.59 ± 4.23[a]	0.83 ± 0.15	0.64 ± 0.10	0.76 ± 0.13

[1] Mean ± SEM.
[2] Values in the same row with different superscript letters are significantly different; $P < 0.05$.

Table 4 The effects of diet on urea content and urease activity in hepatic and renal tissue of male BHE/cdb rats[1]

	Diet 1 Whole egg	Diet 2 Control	Diet 3 Arginine
Urea content (μmol/mg)			
Liver	0.28 ± 0.05	0.21 ± 0.04	0.24 ± 0.03
Kidney	0.002 ± 0.002[a]	1.11 ± 0.01[b]	2.32 ± 1.68[b]
Urease activity (mol product/min/g tissue)			
Liver	0.73 ± 0.12	0.68 ± 0.14	0.72 ± 0.09
Kidney	1.24 ± 0.06	1.33 ± 0.07	1.37 ± 0.08

[1] Mean ± SEM; means on the same line having unlike superscripts are significantly different, $P < 0.05$.

increase in dietary vitamin A could have influenced insulin release by the pancreatic β cell. Chertow et al.[18] has suggested such a role for this vitamin. Insulin release is impaired in the BHE/cdb rats as they age.[19] These rats have a mutation in the mtDNA F_1F_0ATPase 6 gene that affects the efficiency of ATP synthesis[20] upon which insulin secretion depends.[21,22] Vitamin A might stimulate ATPase 6 gene transcription with the net result of an increase in gene product that, in turn, might result in an increase ATPsynthesis and increased insulin release.

Although the percent lipid in the experimental diets was the same, as were the fatty acid profiles, the whole-egg diet was richer in lecithin (phosphatidylcholine) than were the other diets. Lecithin is an important component of cell membranes, plasma lipoproteins, and also serves as a pulmonary surfactant.[23] Lecithin, as a membrane phospholipid, provides diacylglycerol (DAG), fatty acids (e.g., arachidonate), and the phosphorylcholine needed for cell signaling.[24,25] These metabolites of lecithin can modulate the calcium-phospholipid dependent protein kinase C (PKC).[24] PKC has been identified in islets of Langerhans and its activation can lead to stimulation of insulin release when glucose is present.[26] This mechanism might explain the whole-egg diet effect on glucose tolerance. Although we did not document either an effect of vitamin A or lecithin on glucose homeostasis, future work will address these two issues.

References

1 Berdanier, C.D., Kras, K.M., Wickwire, K., and Hall, D.G. (1997). Whole egg diet delays the age related impaired glucose tolerance of BHE/cdb rats. *Proc. Soc. Exp. Biol. Med.* (in press)

2 Berdanier, C.D., Johnson, B., Hartle, D.K., and Crowell, W. (1992). Lifespan is shortened in BHE/cdb rats fed a diet containing 9% menhaden oil and 1% corn oil. *J. Nutr.* **122,** 1309–1317

3 Fowler, K., Crowell, W.A., and Berdanier, C.D. (1993). 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

4 Reyes, A.A., Kissane, J., and Klahr, S. (1990). A high cholesterol diet ameliorates renal tubular lesions in diabetic rats. *Proc. Soc. Exp. Biol. Med.* **194,** 177–185

5 Reyes, A.A., Purkerson, M.L., Karl, I., and Klahr, S. (1992). Dietary supplementation with L-arginine ameliorates the progression of renal disease in rats with subtotal nephrectomy. *Am. J. Kidney Dis.* **20,** 168–176

6 Bergmeyer, H.U., Bernt, E., Gawehn, K., and Michal, G. (1974). Handling of biochemical reagents and samples. In *Methods of Enzymatic Analysis* (H.U. Bergmeyer, ed.), pp. 174–175, Academic Press, New York, NY USA

7 DeHoff, J., Davidson, L., and Kritchevsky, D. (1978). An enzymatic assay for determining free and total cholesterol in tissue. *Clin Chemistry* **24,** 433–435

8 Cho, B.H. (1983). Improved enzymatic determination of total cholesterol in tissues. *Clin Chemistry* **29,** 166–168

9 Dole, V.P. and Meinertz, H. (1960). Microdetermination of long chain fatty acids in plasma and tissues. *J. Biol. Chem.* **235,** 2595–2599

10 Nuzum, T.C. and Snodgrass, P.J. (1976). Multiple assays of the five urea cycle enzymes in human liver homogenates. In *The Urea Cycle* (R.B. Grisola and F. Mayor, eds.) pp. 324–349, Academic Press, New York, NY USA

11 Mulec, H., Johnson, S.A., Wiklund, O., and Bjorck, S. (1993). Cholesterol: A renal risk factor in diabetic nephropathy? *Am. J. Kidney Disease* **22,** 196–201

12 Kasiske, B.L., O'Donnell, M.P., Schimtz, P.G., Kim, Y., and Keane, W.F. (1990). Renal injury of diet-induced hypercholeresterolemia in rats. *Kidney Int.* **35,** 40–47

13 Takahashi, H., Nakanishi, T., Nishimura, M., Fukumitsu, S., and Yoshimura, M. (1993). Role of insulin in the pathogenesis of hypertension associated with glucose intolerance. *Clin. Exper. Hypertension* **15,** 575–584

14 Kullen, M.J., Parente, J.A. Jr., and Berdanier, C.D. (1994). Effects of type of dietary fat and protein on gluconeogenesis in isolated hepatocytes from BHE/cdb rat. *J. Nutr. Biochem.* **5,** 227–231

15 Kullen, M.J., Berdanier, L.A., Dean, R., and Berdanier, C.D. (1997). Gluconeogensis is less active in BHE/cdb rats fed menhaden oil than in rats fed beef tallow. *Biochem. Arch.* **13,** 77–85

16 Rognstad, R. (1996). Futile cycling in carbohydrate metabolism I. background and current controversies on pyruvate cycling. *Biochem. Archives* **12,** 71–83

17 Tappy, L., Acheson, K., Curchod, B., Schneiter, P., Normand, S., Pachiaudi, C., Temler, E., Riou, J.P., and Jequier, E. (1994). Overnight glucose metabolism in obese non-insulin-dependent diabetic patients and in healthy lean individuals. *Clin. Physiol.* **14,** 251–265

18 Chertow, B.S., Blaner, W.S., Rajan, N., Picmerano, D.A., Meda, P., Cirulli, V., Krozowski, Z., Smith, R., and Cordle, M.S. (1993). Retinoic acid receptor, cytosolic retinol-binding and retinoic acid-binding protein mRNA transcripts and proteins in rat insulin-secreting cells. *Diabetes* **42,** 1109–1114

19 Liang, Y., Bonner-Weir, S., Wu, Y-J., Berdanier, C.D., Efrat, S., and Matschinsky, F. (1994). In situ glucose uptake and glucokinase activity of pancreatic islets in diabetic and obese rodents. *J. Clin. Invest.* **93,** 2473–2481

20 Mathews, C.E., McGraw, R.A., and Berdanier, C.D. (1995). A point mutation in the mitochondrial DNA of diabetes prone BHE/cdb rats. *FASEB J* **9,** 1638–1642

21 Matschinsky, F. (1996). A lesson in metabolic regulation inspired by the glucokinase sensor paradign. *Diabetes* **45,** 223–241

22 Ramirez, R., Rasschaert, J., Sener, A., and Malaisse, W.J. (1994). The coupling of metabolic to secretory events in pancreatic islets. Glucose-induced changes in mitochondrial redox state. *Biochim. Biophys. Acta.* **1273,** 163–167

23 Kuksis, A. and Mookerjea, S. (1984). Choline. In *Present Knowledge in Nutrition* (R.E. Olson, ed.), The Nutrition Foundation, Washington, D.C. USA

24 Nishizuka, Y. (1986). Studies and perspectives of protein kinase C. *Science* **233,** 305–312

25 Zeisel, S.H. (1993). Choline phospholipids: signal transduction and carcinogenesis. *FASEB J.* **7,** 551–557

26 Howell, S.L. (1995). Regulation of insulin secretion. In *Diabetes Clinical Science In Practice* (R.D.G. Leslie and D.C. Robbins, eds.) pp. 1–12, Cambridge University Press, UK