Genotype and Diet Effects in Lean and Obese Zucker Rats Fed Either Safflower or Coconut Oil Diets (44358)

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Abstract. **Previously we reported that suckling lean heterozygous (FA/fa) Zucker rats had a number of adipose tissue measurements intermediate between those of homozygous lean (FA/FA) and obese (fa/fa) rats. However, in young adult male rats maintained on a low-fat diet, these differences were no longer apparent (i.e., values for the two lean genotypes were similar). In the present study we determined whether the heterozygous effect of the "fa" gene was dependent on the consumption of a high-fat diet. Mother rats were fed high-fat diets containing either safflower (SOD) or coconut (COD) oil throughout mating and lactation. Homozygous lean male and female rats were bred, as well as obese male and lean heterozygous female rats. Suckling rats were studied at 17 days of age. Additional male rats were maintained on the same diet as their mothers until 11–12 weeks of age. Obese suckling rats had higher body weights than lean pups. Inguinal fat pad weights and pad-to-body weight ratios followed the pattern of obese greater than lean (FA/fa) pups that were greater than lean (FA/FA) pups. A similar relationship was found for adipose tissue lipogenic enzyme activities. At 11–12 weeks of age, measurements followed the general pattern of obese rats having greater values than lean rats (i.e., FA/fa = FA/FA). SOD-fa/fa rats had higher hepatic lipogenic enzyme activities than COD-fa/fa rats. In addition, SOD rats had higher fat cell numbers than COD rats. These results suggest that specific fatty acids can alter adipocyte proliferation and/or differentiation *in vivo*. In addition, there appears to be a defect of fatty acid regulation in livers of genetically obese rats. The heterozygous effect of the "fa" gene in suckling Zucker rats was confirmed. However, high-fat feeding did not result in a heterozygous effect in young adult lean male rats. We will next evaluate the role of sex on this effect.** [P.S.E.B.M. 1999, Vol 220]

It has been reported that the presence of the "*fa*" gene in suckling heterozygous (*FA/fa*) lean rats resulted in intermediate values for some factors in comparison to the two homozygous groups (1). Measurements included inguinal fat pad weight, fat pad-to-body weight ratio and fat cell size, as well as activities of adipose tissue lipid metabolizing enzymes. These findings led us to speculate that the lean heterozygous rat might provide an interesting model for overweight if the presence of the "*fa*" gene resulted in a tendency toward increasing adiposity as they matured. However, when we repeated these measurements using 10-week-old male rats, there were no differences between the two lean genotypes (2).

In these two studies, mother rats, as well as the weaned offspring, were fed a low-fat diet throughout the protocol (1, 2). However, during the suckling period, when the effects of the "*fa*" gene were observed in lean heterozygous (*FA/fa*) rats, the pups were consuming mothers' milk that contained 12.9 g/dl of fat (3). This was a substantially higher fat content than that of the diet consumed postweaning (i.e., 2 g/100 g). Furthermore, the fatty acid composition of the rat milk produced by mothers fed the low-fat diet was substantially different from that found in either the low-fat diet we used or that of commercially available rodent diets that usually contain polyunsaturated oils (3) (Cleary and Phillips, unpublished data). The present study was thus designed

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to address both the high-fat diet issue as well as the composition of the fat used. We fed the mother rats, as well as the weaned offspring, one of two high-fat diets. We chose coconut oil because its fatty acid composition resembles that of milk fat more closely than most other commercially available fat sources. Safflower oil was chosen due to its high level of polyunsaturated fatty acids. Two different fatty acid sources were chosen as there have been reports of different effects of polyunsaturated and saturated fatty acids on the metabolic pathways that we are studying (4–7). In the present study, measurements made included fat depot weights and cellularity and liver and adipose tissue lipogenic enzyme activities. In addition, serum insulin, glucose, cholesterol and triacylglycerol concentrations were measured.

Materials and Methods

Animals. Rats were obtained from a colony maintained at the Hormel Institute. Rooms were temperature(21°C) and humidity(50%) controlled and maintained with 12-hr light/dark cycles. The University of Minnesota Animal Care and Use Committee approved this study. The Hormel Institute Animal Facility is accredited by the American Association for Accreditation of Laboratory Animal Care.

The female rats designated to be mothers were fed diets containing 18% safflower oil (SOD) or coconut oil (COD) from 5 weeks of age. Composition of the diets is given in Table I, and fatty acid composition of the two diets is presented in Table II. Upon reaching 200 g body weight and/or 10 weeks of age, female rats were bred to male rats. The female rats were housed in plastic cages with wood shavings throughout mating, pregnancy, and lactation. Specially designed holders for the food containers were used to prevent the pups from gaining access to their mothers' food. Two breeding strategies were used: homozygous (*FA/FA*) lean male and female rats were mated to provide homozygous (*FA/FA*) lean offspring. In addition, homozygous (*fa/fa*) obese male rats were mated with heterozygous (*FA/fa*) lean female rats to provide heterozygous (*FA/fa*) lean and homozygous (*fa/fa*) obese offspring. All litters were maintained at 9–10 pups.

TABLE I. Components[a] of Safflower (SOD) Oil and Coconut (COD) Oil Diets (per 100 g diet)

	SOD	COD
Corn oil	2	2
Safflower seed oil	18	
Coconut seed oil		18
Casein	20.5	20.5
Methionine	0.5	0.5
Cornstarch	32	32
Celufil	17	17
Sucrose	5	5
Mineral Mix[b]	4	4
Vitamin Mix[c]	1	1

[a] All components from U.S. Biochemical (USB), Cleveland, OH.
[b] USP XXII, USB 21416. Composition of salt mixture (g/kg): calcium carbonate, 15.25; cobalt chloride, 0.00092; copper sulfate, 0.000191; ferrous sulfate, 1.08; magnesium sulfate 2.29; manganese sulfate, 0.160; potassium iodide, 0.0284; potassium phosphate monobasic, 15.56; sodium chloride, 5.56; zinc sulfate, 0.00212.
[c] Vitamin diet fortification mixture, USB 23431. Composition of vitamin mix (g/kg): RRR-α-tocopherol, 0.05; L-ascorbic acid, 0.45; choline chloride, 0.75; D-calcium pantothenate, 0.03; inositol, 0.05; menadione, 0.0225; niacin, 0.045; p-aminobenzoic acid, 0.050; pyridoxine-HCl, 0.01; riboflavin, 0.010; thiamin-HCl, 0.010; retinol acetate, 0.003; cholecalciferal, 0.001; in mg/kg: biotin, 0.20; folic acid, 0.90; vitamin B-12, 0.0135.

TABLE II. Diet Fatty Acid Composition of Safflower and Coconut Oil Diets

Fatty acid	Safflower oil diet	Coconut oil diet
8:0		0.72 ± 0.3
10:0		4.12 ± 0.06
12:0	0.09 ± 0.01*	43.61 ± 0.32
14:0	0.17 ± 0.10	18.50 ± 0.01
14:1ω5		
16:0	6.99 ± 0.21	10.93 ± 0.09
16:1ω7	0.08 ± 0.01	
17:0		
18:0	2.25 ± 0.02	3.18 ± 0.05
18:1ω9	14.11 ± 0.06	9.80 ± 0.15
18:2ω6	74.93 ± 0.12	9.00 ± 0.12
18:3ω3	0.38 ± 0.01	0.15 ± 0.01
20:0	0.34 ± 0.01	
21:0		
22:0	0.44 ± 0.01	
24:0	0.11 ± 0.01	
24:1ω9	0.14 ± 0.01	

* Area %, Mean ± SEM (n = 3).

Suckling Rats. At 17 days of age, pups were sacrificed by decapitation, and blood was collected for serum preparation. Livers and left and right inguinal fat depots were removed and weighed. When pups were obtained from the homozygous obese male/heterozygous lean female crosses, the fat pad-to-body weight ratio was calculated, and pups were assigned to either a lean or obese group. This technique has previously been shown to accurately identify the two genotypes (1, 8). In addition, a preliminary study was conducted using pups obtained from heterozygous female rats fed COD that were mated with obese male rats. Pups were lipectomized at 17 days of age, and their lean or obese status assessed at 6–8 weeks of age. Based on inguinal fat pad weight and fat pad-to-body weight ratio, all the pups were correctly genotyped. Tissues within a litter and genotype were pooled as needed to obtain sufficient sample sizes. A total of 4 SOD and 6 COD homozygous (*FA/FA*) lean litters were used. In addition, there were 6 SOD and 10 COD litters from obese male and heterozygous (*FA/fa*) lean female breedings providing 30 SOD-*fa/fa* pups from a total of 60 SOD offspring and 52 COD-*fa/fa* pups from 99 COD offspring. Numbers used for specific determinations are indicated in the table and figure legends.

Young Adult Rats. Rats were obtained from the mating strategies described above and kept with their moth-

ers until 28 days of age. At this time, male rats of the three genotypes were weaned onto the same diets that their mothers had consumed. They were maintained on these diets until 11–12 weeks of age. Following an overnight fast, the rats were sacrificed by decapitation, and blood was collected for serum preparation. Liver and inguinal and combined epididymal/retroperitoneal fat pads were removed and weighed. The epididymal and retroperitoneal fat pads were combined to provide adequate tissue to make the desired measurements.

Tissue Processing. Adipose tissue was sampled for fat cell number and size determination (9). A Coulter Counter Model ZM was used for fat cell counting. Livers and inguinal and epididymal/retroperitoneal fat tissues were homogenized in 0.25 *M* sucrose + 1 m*M* EDTA, pH 7.4, and supernatants were prepared for determination of activities of glucose-6-phosphate dehydrogenase (E.C. 1.1.1.49) (10), 6-phosphogluconate dehydrogenase (E.C. 1.1.1.44) (10), fatty acid synthetase (E.C. 2.3.1.85) (11), and malic enzyme (E.C. 1.1.1.40) (12). In fat tissue supernatants, lipoprotein lipase activity (E.C. 3.1.1.3) (13, 14) was also determined. Enzyme activities in liver were expressed per mg protein, and those for inguinal fat tissue were expressed on a per cell basis.

Serum samples were analyzed for insulin, glucose, cholesterol, and triacylglycerol using commercially available kits. Insulin was assayed using Micromedic Insulin Kit #D-1804 (ICN Micromedic Systems, Inc., Horsham, PA.) Glucose, cholesterol, and triacylglycerol were analyzed using Sigma Diagnostics Procedures #115, #352, and #336, respectively (Sigma Biochemicals, St. Louis, MO).

Statistical Analyses. Data have been expressed as means ± standard errors of the mean. To correct for potential effects from the litter, body weight, and liver weights from a litter and/or genotype were considered as one measurement. Results were analyzed by 2 × 3 analysis of variance followed by *F*-test to determine differences between specific groups if the effect for interaction was significant (15). A "p" value of less than 0.05 was considered significant.

Results

Suckling Rats. Within the SOD group, body weights of the lean homozygous pups were lighter than those of the lean heterozygous and obese pups (Table III). In the COD group, the two lean groups were lighter than the obese group. Liver weights of all the pups were similar.

Results of the serum analyses for the 17-day-old rats are also presented in Table III. Serum insulin concentrations were similar for *SOD-FA/fa*, SOD-*fa/fa*, COD-*FA/fa* and COD-*fa/fa* pups, and these values were significantly higher than those of the two groups of lean homozygous pups, SOD-*FA/FA* and COD-*FA/FA*. Serum glucose levels were significantly higher in the SOD pups as a group compared to COD pups. COD-*fa/fa*, COD-*FA/fa*, and SOD-*fa/fa* pups had similar serum cholesterol concentrations, but only the COD-*fa/fa* and COD-*FA/fa* pups had significantly higher levels compared to the other three groups, COD-*FA/FA*, SOD-*FA/fa*, and SOD-*FA/FA*. Serum triacylglycerol levels followed a similar pattern.

Inguinal fat pad weights (Fig. 1A) and fat pad-to-body weight ratios (Fig. 1B) were similar in the SOD and COD groups. Within each diet, fat pad weights and pad-to-body weight ratios were highest in obese pups, intermediate in heterozygous (*FA/fa*) lean rats, and lowest in homozygous lean rats. Fat cell numbers (Fig. 1C) were significantly higher in SOD pups than in COD pups. Fat cell sizes (Fig. 1D) were significantly greater as a group in COD pups compared to SOD pups. Within diet groups, fat cell sizes of obese pups were larger than those of the two lean groups (*FA/fa* = *FA/FA*).

Results for hepatic lipogenic enzyme activities of the 17-day-old pups are presented in Table IV. Liver glucose-6-phosphate dehydrogenase activity was higher in COD compared to SOD pups. Hepatic 6-phosphogluconate dehydrogenase activity was also higher in COD compared to SOD pups. However, within each diet, 6-phosphogluconate dehydrogenase activity was significantly higher in obese pups compared to the homozygous (*FA/FA*) lean pups, and heterozygous (*FA/fa*) lean pups had an intermediate value between but not significantly different from the two homo-

TABLE III. Body and Liver Weights and Serum Insulin, Glucose, Cholesterol, and Triacylglycerol Concentrations in 17-day-old Zucker Rats Obtained from Mothers Fed Either Safflower or Coconut Oil Diets

	Safflower oil			Coconut oil		
	FA/FA	*FA/fa*	*fa/fa*	*FA/FA*	*FA/fa*	*fa/fa*
Body weight (g)	$28.0^{a} \pm 4$ ($n = 4$)	$29.2^{b} \pm 0.9$ ($n = 6$)	$30.8^{b} \pm 1.0$ ($n = 6$)	$28.8^{a} \pm 0.9$ ($n = 6$)	$27.8^{a} \pm 1.0$ ($n = 10$)	$29.6^{b} \pm 1.0$ ($n = 10$)
Liver weight (g)	0.84 ± 0.01 ($n = 4$)	0.88 ± 0.03 ($n = 6$)	0.92 ± 0.03 ($n = 6$)	0.91 ± 0.04 ($n = 6$)	0.89 ± 0.04 ($n = 10$)	0.92 ± 0.05 ($n = 10$)
Serum insulin (μU/ml)	$39^{a} \pm 2$	$45^{b} \pm 2$	$45^{b} \pm 2$	$39^{a} \pm 2$	$47^{b} \pm 1$	$48^{b} \pm 2$
Serum glucose (mg/dl)	$155^{*} \pm 1$	162 ± 1	156 ± 5	148 ± 2	153 ± 1	148 ± 3
Serum cholesterol (mg/dl)	$211^{a} \pm 1$	$195^{a} \pm 4$	$218^{a,b} \pm 6$	$206^{a} \pm 3$	$231^{b} \pm 9$	$252^{b} \pm 10$
Serum triacylglycerol (mg/dl)	$273^{a} \pm 10$	$287^{a} \pm 20$	$319^{a,b} \pm 26$	$263^{a} \pm 11$	$375^{b} \pm 15$	$366^{b} \pm 33$

Note. Body weight and liver data based on mean per litter/genotype. Other values are means of pooled samples (by litter/genotype) ± SEM ($n = 5$). Values within a row with different letter superscripts are significantly different. *Indicates diets are significantly different.

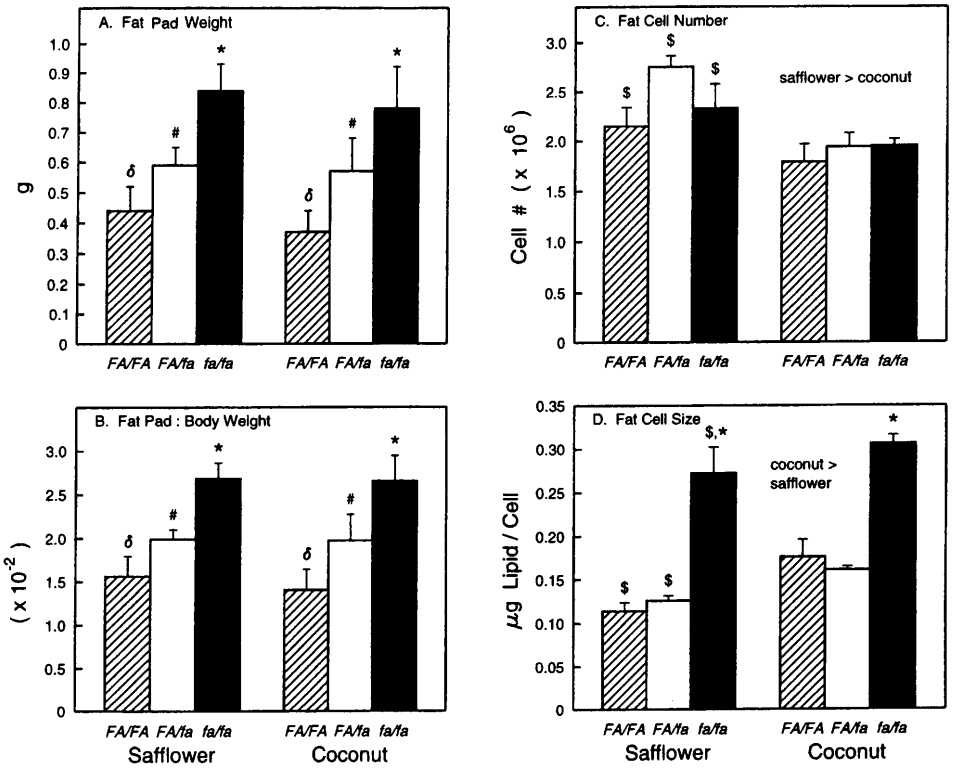

Figure 1. Inguinal fat pad weight, cell size, and number for 17-day-old Zucker rats obtained from mothers fed safflower (SOD) or coconut oil (COD). (A) Inguinal fat pad weight. (B) Inguinal fat pad-to-body weight ratio. (C) Inguinal fat cell number. (D) Inguinal fat cell size. Data are means ± SEM ($n = 8$). Columns with different superscript symbols are significantly different from each other. $ Indicates SOD rats significantly different from COD rats.

TABLE IV. Liver Lipogenic Enzyme Activities in 17-day-old Zucker Rats from Dams Fed Either Safflower or Coconut Oil Diets

	Safflower oil			Coconut oil		
	FA/FA	*FA/fa*	*fa/fa*	*FA/FA*	*FA/fa*	*fa/fa*
Per mg protein						
Fatty acid synthetase[1]	$5.25^{d} \pm 0.46$	$3.93^{a,b} \pm 0.25$	$4.56^{b,c} \pm 0.27$	$3.22^{a} \pm 0.41$	$3.99^{a,b} \pm 0.21$	$5.18^{c,d} \pm 0.48$
Glucose-6 PO_4[2] dehydrogenase	$10.2^{*} \pm 1.0$	12.1 ± 0.6	10.9 ± 1.1	13.1 ± 1.1	13.6 ± 1.0	13.6 ± 1.0
6-Phosphogluconate[2] dehydrogenase	$19.3^{*,a} \pm 2.0$	$22.4^{a,b} \pm 0.8$	$23.7^{b} \pm 0.8$	$21.6^{a} \pm 2.3$	$25.5^{a,b} \pm 1.5$	$27.4^{b} \pm 1.8$
Malic enzyme[2]	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.

Note. Values are means of pooled samples (litter/genotype) ± SEM ($n = 8$). Values within a row with different superscripts are significantly different. *Indicates a significant diet effect. N.D. = Not detected.
[1] Activity = nmol fatty acid produced/min.
[2] Activity = nmol $NADP^{+}$ reduced/min.

zygous groups. There were a number of significant differences in fatty acid synthetase activity among the six groups of 17-day-old rat pups. However, there was not a consistent pattern with respect to either genotype or diet. As previously reported (1, 16, 17), hepatic malic enzyme activity was very low in suckling rats.

Activities for the five enzymes measured in inguinal adipose tissue are presented in Figure 2. For fatty acid synthetase (Fig. 2A), COD-*fa/fa* pups had the highest activity followed by SOD-*fa/fa* > COD-*FA/fa* > SOD-*FA/fa* > COD-*FA/FA* > SOD-*FA/FA*. Malic enzyme (Fig. 2C) activity pattern was similar to that of fatty acid synthetase except that SOD-*FA/fa* and COD-*FA/FA* pups had similar activities. Activities for both 6-phosphogluconate (Fig. 2D) and glucose-6-phosphate (Fig. 2E) dehydrogenases followed the pattern of COD-*fa/fa* > SOD-*fa/fa* > COD-*FA/fa* > SOD-*FA/fa* = COD-*FA/FA* with SOD-*FA/fa* also having similar activity as SOD-*FA/FA* rats. Lipoprotein lipase activity (Fig. 2B) was higher in obese compared to lean rats independent of the diet consumed by the mother rats.

Young Adult Rats. At 11–12 weeks of age, COD-*fa/fa* rats had the highest body weights followed by SOD-*fa/fa* rats (Table V). The body weights of all four groups of lean rats were similar (SOD-*FA/fa* = COD-*FA/fa* = SOD-*FA/FA* = COD-*FA/FA*). Liver weights followed this pattern (Table V). Serum measurements are also presented in Table V. Serum insulin concentrations were higher in SOD rats as a group than in COD rats with obese rats having

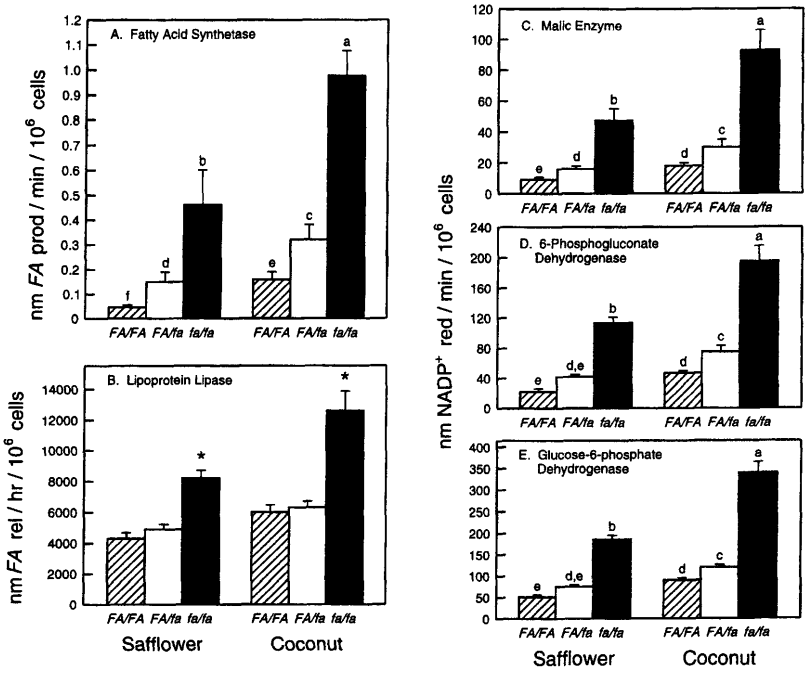

Figure 2. Inguinal adipose depot enzyme activity measurements from 17-day-old Zucker rats obtained from mothers fed safflower oil (SOD) or coconut oil (COD). (A) Fatty acid synthetase. (B) Lipoprotein lipase. (C) Malic enzyme. (D) 6-Phosphogluconate dehydrogenase. (E) Glucose-6-phosphate dehydrogenase. All activities are means ± SEM (n = 8). Columns with different letter superscripts are significantly different from each other. Stars indicate that *fa/fa* rats' values are significantly different from those of lean rats.

TABLE V. Body and Liver Weights and Serum Insulin, Glucose, Cholesterol, and Triacylglycerol Concentrations in 11–12-week-old Male Rats Fed Either Safflower or Coconut Oil Diets

	Safflower oil			Coconut oil		
	FA/FA	*FA/fa*	*fa/fa*	*FA/FA*	*FA/fa*	*fa/fa*
Body weight (g)	$259^{a} \pm 9$	$275^{a} \pm 3$	$351^{b} \pm 6$	$255^{a} \pm 4$	$257^{a} \pm 6$	$412^{c} \pm 7$
Liver weight (g)	$7.0^{a} \pm 0.2$	$7.3^{a} \pm 0.2$	$11.9^{b} \pm 0.4$	$6.5^{a} \pm 0.1$	$6.2^{a} \pm 0.1$	$13.3^{c} \pm 0.8$
Serum insulin (μU/ml)	$53^{*,a} \pm 5$	$73^{a} \pm 9$	$297^{b} \pm 23$	$33^{a} \pm 3$	$34^{a} \pm 3$	$233^{b} \pm 43$
Serum glucose (mg/dl)	$115^{a} \pm 2$	$121^{a} \pm 9$	$169^{b} \pm 8$	$120^{a} \pm 4$	$115^{a} \pm 11$	$135^{b} \pm 2$
Serum cholesterol (mg/dl)	$100^{a} \pm 5$	$103^{a} \pm 5$	$215^{c} \pm 8$	$112^{a} \pm 4$	$102^{a} \pm 8$	$195^{b} \pm 11$
Serum triacylglycerol (mg/dl)	68*,† ± 2	115 ± 20	525 ± 60	67 ± 4	86 ± 6	418 ± 34

Note. Values are means of pooled samples (litter/genotype) ± SEM (n = 8). Values within a row with different letter superscripts are significantly different. *Indicates significant diet effect. †Indicates genotypes are significantly different.

higher levels than lean rats. There were no significant differences among the lean rats. For serum glucose, obese rats had higher levels than did lean rats. Serum cholesterol levels were highest in SOD-*fa/fa* rats, followed by COD-*fa/fa* rats, and then the lean rats (SOD-*fa/fa* = COD-*FA/fa* = SOD-*FA/FA* = COD-*FA/FA*). Serum triacylglycerol values were higher in SOD than COD rats, and within each diet group, *fa/fa* rats had the highest levels followed by heterozygous lean rats, and the homozygous lean rats had the lowest values.

Adipose tissue weights and fat cell sizes and numbers are presented in Table VI. Inguinal fat pad weight and fat cell size were highest in COD-*fa/fa* rats followed by the SOD-*fa/fa* rats and then the lean rats (SOD-*FA/fa* = COD-*FA/fa* = SOD-*FA/FA* = COD-*FA/FA*). A different pattern was found for inguinal fat cell number. SOD-*fa/fa* rats had the highest fat cell number followed by COD-*fa/fa* rats, as well as by SOD-*FA/fa* and SOD-*FA/FA* rats. The two COD lean groups had the lowest fat cell number (COD-*FA/fa* = COD-*FA/FA*). The weight of the epididymal + retroperitoneal depot fat pad followed a similar pattern as that described above for the inguinal depot (i.e., COD-*fa/fa* > SOD-*fa/fa* > SOD-*FA/fa* = COD-*FA/fa* = SOD-*FA/FA* = COD-*FA/FA*). Fat cell size was also highest in COD-*fa/fa* rats followed by SOD-*fa/fa* rats. Values for the lean rats were all significantly lower than those of the obese rats. There were significant differences among values for the lean rats (i.e., COD-*FA/fa*, COD-*FA/FA* and SOD-*FA/fa* had similar values as did SOD-*FA/fa*, SOD-*FA/FA* and COD-*FA/fa*). COD-*FA/FA* rats had significantly greater epididymal + retroperitoneal fat cell size than did SOD-*FA/FA* rats. Fat cell number was highest in SOD-*fa/fa* rats compared to all other groups. The lean SOD rats of both genotypes had values similar to the two homozygous COD groups (i.e., COD-*fa/fa* and COD-*FA/FA* rats, but significantly greater than that of the heterozygous COD-*FA/fa* rats). The three COD groups had similar fat cell numbers.

Activities for the hepatic lipogenic enzymes are presented in Fig. 3. Malic enzyme (Fig. 3A), glucose-6-phosphate dehydrogenase (Fig. 3C) and fatty acid synthetase (Fig. 3D) activities were greatest in SOD-*fa/fa* rats followed by COD-*fa/fa* rats. Glucose-6-phosphate dehydro-

TABLE VI. Adipose Tissue Weights and Cellularity in 11–12-week-old Male Rats Fed Either Safflower or Coconut Oil Diets

	Safflower oil			Coconut oil		
	FA/FA	*FA/fa*	*fa/fa*	*FA/FA*	*FA/fa*	*fa/fa*
Inguinal pad						
Pad weight (g)	$4.0^{a} \pm 0.2$	$5.2^{a} \pm 0.4$	$26.7^{b} \pm 1.1$	$4.3^{a} \pm 0.4$	$3.8^{a} \pm 0.2$	$35.2^{c} \pm 1.5$
Cell size (μg lipid/cell)	$0.091^{a} \pm 0.005$	$0.126^{a} \pm 0.016$	$0.401^{b} \pm 0.017$	$0.150^{a} \pm 0.007$	$0.160^{a} \pm 0.014$	$0.851^{c} \pm 0.034$
Cell number ($\times 10^6$)	$24.8^{b,*} \pm 2.8$	$22.4^{b} \pm 0.4$	$50.6^{c} \pm 2.9$	$15.9^{a} \pm 2.0$	$12.8^{a} \pm 0.5$	$28.3^{b} \pm 1.1$
Retroperitoneal + epididymal pads						
Pad weight (g)	$3.7^{a} \pm 0.3$	$4.2^{a} \pm 0.4$	$16.6^{b} \pm 0.6$	$4.4^{a} \pm 0.4$	$3.2^{a} \pm 0.2$	$23.5^{b} \pm 1.2$
Cell size (μg lipid/cell)	$0.125^{a} \pm 0.013$	$0.160^{a,b} \pm 0.014$	$0.329^{c} \pm 0.020$	$0.222^{b} \pm 0.008$	$0.165^{a,b} \pm 0.009$	$0.963^{d} \pm 0.064$
Cell number ($\times 10^6$)	$21.5^{b,*} \pm 1.3$	$18.6^{b} \pm 1.0$	$38.2^{c} \pm 2.9$	$13.6^{a,b} \pm 1.3$	$12.7^{a} \pm 0.4$	$17.8^{a,b} \pm 1.6$

Note. Values are means of pooled samples (litter/genotype) ± SEM ($n = 8$). Values within a row with different superscripts are significantly different. *Indicates significant diet effect.

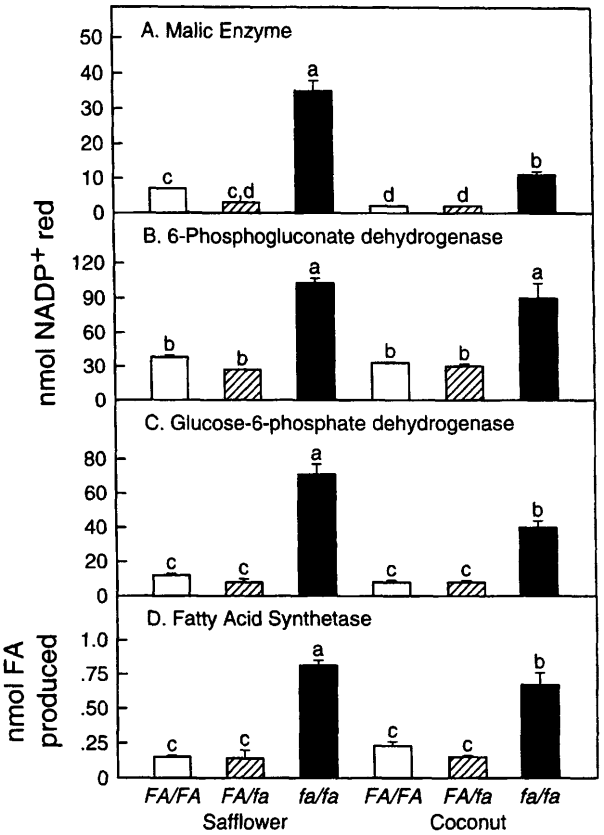

Figure 3. Liver lipogenic enzyme activity measurements from 11–12-week-old male rats fed either safflower oil (SOD) or coconut oil (COD). (A) Malic enzyme. (B) 6-Phosphogluconate dehydrogenase. (C) Glucose-6-phosphate dehydrogenase. (D) Fatty acid synthetase. All activities are means ± SEM ($n = 8$) and presented on a per min per mg of protein basis. Columns with different letter superscripts are significantly different from each other.

genase and fatty acid synthetase activities of all lean rats were similar. Malic enzyme activity had a different pattern in lean rats, SOD-*FA/fa* and SOD-*FA/FA* rats had similar activities, and SOD-*FA/FA* rats had greater activity than did the two COD lean groups. The activity of 6-phosphogluconate dehydrogenase (Fig. 3B) was similar in the two obese groups and their values were higher than those of the four lean groups independent of the diet consumed.

In the inguinal fat depot, malic enzyme (Fig. 4A) activity followed the pattern: COD-*fa/fa* > SOD-*fa/fa* = SOD-*FA/fa* = SOD-*FA/FA* > COD-*FA/fa* = COD-*FA/FA*. For 6-phosphogluconate dehydrogenase (Fig. 4B) activity in the inguinal fat the relationship was as follows: COD-*fa/fa* > SOD-*fa/fa* > SOD-*FA/fa* > SOD-*FA/FA* = COD-*FA/fa* = COD-*FA/FA*. Glucose-6-phosphate dehydrogenase (Fig. 4C) and lipoprotein lipase(4E) activities followed similar patterns (i.e., COD-*fa/fa* > SOD-*fa/fa* > SOD-*FA/fa* = COD-*FA/fa* = SOD-*FA/FA* = COD-*FA/FA*). Fatty acid synthetase (Fig. 4D) activity of the COD-*fa/fa* rats was higher than that of the other five groups that all had similar measurements. Similar results were obtained from the epididymal/retroperitoneal fat depot (results not shown).

Discussion

Four major findings resulted from these experiments. 1) Lean 17-day-old heterozygous (*FA/fa*) Zucker pups, consuming milk from mothers fed high-fat diets, demonstrated an effect of the "*fa*" gene in adipose tissue measurements; 2) this effect of the "*fa*" gene was no longer seen in young adult lean heterozygous male rats fed these high-fat diets; 3) livers of the 11–12-week-old SOD-*fa/fa* rats had higher lipogenic enzyme activities than did livers of COD-*fa/fa* rats; and 4) SOD rats at both ages had a tendency for increased fat cell number compared to COD rats. These findings will be discussed below.

In a previous study, we reported that inguinal adipose tissue metabolism was affected by the presence of the "*fa*" gene in suckling lean heterozygous (*FA/fa*) rats obtained from mother rats fed a low-fat diet (1). Values for inguinal fat pad weight, fat pad-to-body weight ratio, fat cell size, and 6-phosphogluconate dehydrogenase and lipoprotein li-

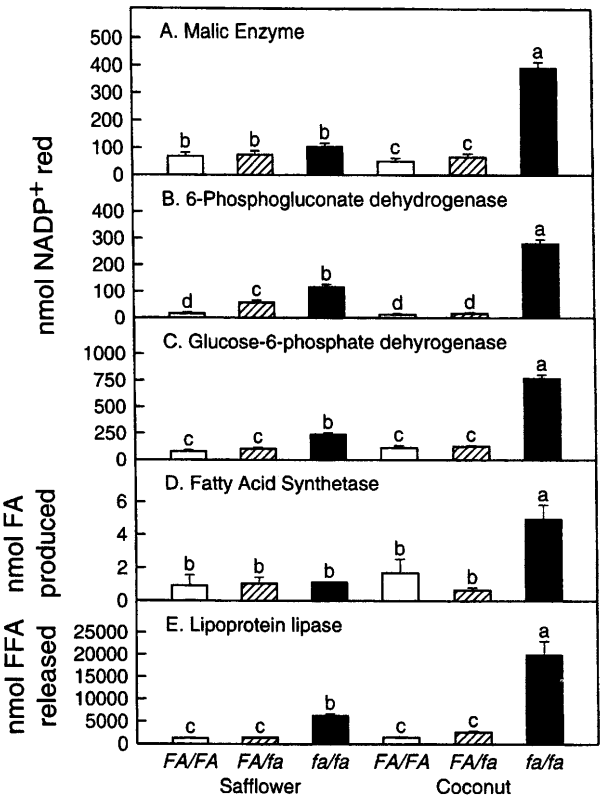

Figure 4. Inguinal adipose tissue enzyme activity measurements from 11–12-week-old male rats fed either safflower oil (SOD) or coconut oil (COD). (A) Malic enzyme. (B) 6-Phosphogluconate dehydrogenase. (C) Glucose-6-phosphate dehydrogenase. (D) Fatty acid synthetase. (E) Lipoprotein lipase. All activities are means ± SEM (n = 8) and are presented on a per min per ($\times 10^6$) cell basis, except for lipoprotein lipase which is presented on a per hr per ($\times 10^6$) cell basis. Columns with different letter superscripts are significantly different from each other.

pase activities were intermediate between homozygous lean (*FA/FA*) and obese (*fa/fa*) pups. A heterozygous effect of the "*fa*" gene was also present for CO2 and fatty acid production in isolated fat cells. In the present study, lean heterozygous (*FA/fa*) pups obtained from mothers fed high-fat diets also demonstrated an effect of the "*fa*" gene with respect to inguinal fat pad weight and fat pad-to-body weight ratio, as well as for most of the adipose tissue lipogenic enzymes activities measured. However, fat cell size and lipoprotein lipase activity did not follow this pattern in pups obtained from mothers fed high-fat diets, but rather these measurements were statistically greater in obese compared to lean rats. Several other studies have confirmed the finding of an impact of the "*fa*" gene when F2 offspring from BN/Crl × Crl:Zuc-*fa* F1 intercrosses were used. For example, Truett *et al.* (18) reported that at 7 days of age, the number of copies of the "*fa*" gene in these hybrid rats had a linear effect on inguinal fat pad weights. Zhang and coworkers (19) reported that fat mass and plasma leptin levels in 10-day-old pups from the same crosses also followed the pattern of *fa/fa* > *FA/fa* > *FA/FA*. In an additional study using these hybrid offspring, *FA/fa* pups had more body fat than the *FA/FA* pups at both 7 and 16 days of age (20).

As indicated in the introduction, young adult lean male rats maintained from weaning on a low- fat diet no longer appeared to demonstrate a heterozygous effect of the "*fa*" gene (i.e., homozygous (*FA/FA*) and heterozygous (*FA/fa*) lean rats had similar values (2)). We hypothesized that the heterozygous effect of the "*fa*" gene was dependent on the level of dietary fat, as we had initially observed this effect when rats were consuming high-fat mothers' milk. Since similar results were obtained in the present study in young lean adult rats fed high-fat diets, this does not support our hypothesis. However, in another study using lean male hybrid offspring of BN/Crl × Crl-*fa* and BNZ F2-*fa*/+ × ZUC +/+ matings that were fed either low- or high-fat diets from 6–13 weeks of age, it was reported that retroperitoneal and epididymal fat pad weights were heavier in heterozygous compared to homozygous rats fed either diet (21). We made similar comparisons using data from the lean COD rats in the present experiment, as a coconut oil diet was used by Maher and co-workers, and from the lean low-fat rats from our earlier study, to determine if significant differences were obtained when only lean rats were evaluated. However, no significant effects were found for either the low- or high-fat diet comparisons.

Another potential explanation for the observation that there was an effect of the "*fa*" gene during suckling but not in young adults was the sex of the rats. In both our previous and present experiments, tissue from male and female suckling rats was pooled, whereas in the adult experiments only tissue from male rats was used. As indicated above, Truett *et al.* (18) reported that at 7 days of age the number of copies of the "*fa*" gene had a linear effect on inguinal fat pad weights. However, when the sex of the rats was considered, it was found that the female pups were primarily responsible for this heterozygous effect. Several other papers that identified effects of the "*fa*" gene in relation to insulin metabolism used female Zucker rats (22–25). But, Maher *et al.* (21) reported little effect of diet and/or presence of the "*fa*" gene for heterozygous female hybrid Zucker lean rats, in contrast to the results they reported for male rats. Recently, we found that body weight, fat pad weight, fat cell size and serum leptin levels were higher in lean heterozygous female compared to lean homozygous female Zucker rats (Cleary and Phillips, submitted). It appears that during suckling the heterozygous effect of the "*fa*" gene can be consistently documented. However, postweaning it remains to be clarified what the interactions of genetic background, gender and environment play to elicit this response.

In the present investigation we found that activities of hepatic lipogenic enzyme of the young adult SOD-*fa/fa* rats were higher than those of COD-*fa/fa* rats. These findings are in agreement with an earlier study where it was reported that glucose-6-phosphate dehydrogenase and malic enzyme activities were highest in safflower oil-fed obese Zucker rats followed by coconut oil-fed, and then menhaden oil-fed obese rats (26). These findings disagree with the dogma that

polyunsaturated ω-6 fatty acids have a more powerful effect on inhibition of lipid synthesis than do saturated fats or carbohydrates. Most of the studies that have compared the effects of dietary fatty acid amounts and composition of lipogenic enzymes were of short-term duration and used either fasting/refeeding or meal-feeding protocols with "controls" fed either fat-free diets or diets high in either glucose or sucrose. However, several recent publications using other strains of genetically obese rats complement our findings. For example, Iritani *et al.* reported that 9–10-week-old Wistar fatty rats, fasted for 2 days and then refed with diets containing either 10% corn oil or hydrogenated beef tallow, had similar liver lipogenic enzyme activities and mRNA levels (27). As expected, the lean rats fed corn oil had enzyme activities and mRNA levels that were significantly lower than those of lean rats fed the hydrogenated fat. In an additional study, female corpulent rats fed a 45% safflower oil diet did not have different hepatic fatty acid synthetase mRNA in comparison to corpulent rats fed chow (28).

These studies suggest that livers of obese rats are "resistant" to the effects of ω-6 fatty acids with respect to regulation of fatty acid synthesis (29, 30). On the other hand, liver lipogenic enzymes of obese rats responded to fish oil feeding as expected with activities lower in comparison to feeding dietary oils with high ω-6 concentrations (26, 31); Additional studies should be done to confirm these findings perhaps by using corn oil or safflower oil with the corresponding hydrogenated oil to match for chain length. Effects on whole body lipogenesis could also be determined using ^3H_2O. It is also interesting to note that only hepatic lipogenic enzymes were affected, as adipose tissue lipogenic enzyme activities of the SOD-*fa/fa* rats were lower compared to those of the COD-*fa/fa* rats. We also found that conversion of uniformly labeled glucose to fatty acids in isolated adipocytes was substantially lower in the SOD-*fa/fa* compared to COD-*fa/fa* rats (Cleary and Phillips, unpublished data). Understanding the molecular events responsible for these differences is clearly of interest.

When safflower oil was the major source of dietary fat compared to coconut oil, there was an increase in fat cell number in both suckling and young adult rats. *In vitro* studies have suggested that long chain fatty acids may have important role(s) in fat cell proliferation and/or differentiation. For example, arachidonic acid(20:4ω6) enhanced differentiation of the precursor adipocyte cell lines, OB1771 and 3T3-F3442, as well as of preadipocytes obtained from rats (32). A possible mechanism through which fatty acids may mediate effects on adipose tissue differentiation is the peroxisome proliferator-activated receptor, PPARγ2. A chimera of rat PPAR and the human glucocorticoid receptor was shown to be activated by both linoleic(18:2ω6) and arachidonic(20:4ω6) acids (33). The addition of 5,8,11,14-eicosatetraynoic acid, a synthetic arachidonic acid analog, to 3T3 L1 preadipocytes also led to fat cell conversion (34). When fibroblasts were transiently infected with PPARγ2, they differentiated into fat cells, and the addition of either linoleic acid or 5,8,11,14-eicosatetraynoic acid enhanced this process (35). Whether such a mechanism occurs *in vivo* remains to be determined. Also, the significance of this pathway in the regulation of fat cell differentiation needs to be clarified, as recent studies have suggested that other tissues may also express PPARγ2 (36–38).

In conclusion, although these data did not support our original hypothesis that the presence of the "*fa*" gene results in an intermediate effect in heterozygous lean young adult rats exposed to high- fat feeding, two interesting observations were obtained. First, young obese Zucker rats did not downregulate hepatic lipogenesis in response to dietary ω-6 fatty acids. Second, feeding a high-fat diet composed primarily of ω-6 fatty acid resulted in an apparent increased fat cell number. The mechanisms by which these processes occur remain to be determined. In addition, we will pursue studies to investigate the role of gender in the clarification of the role of the "*fa*" gene in body weight and body fat regulation.

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