

Effects of Canola oil-based high fat diets on growth, fat deposition and serum triglyceride and cholesterol levels in lines of mice selected for high and low fat percentage

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ABSTRACT

This study was conducted to determine if there are biologically important interactions of genotypes of mice, differing in polygenically controlled body fat percentage, with levels of dietary fat from Canola oil which is rich in monounsaturated fat and low in saturated fat. Four levels of fat (12.5, 25, 37.5 and 50% of dietary energy) were provided *ad libitum* from four to seven, or four to 10 weeks of age to three lines of mice (HF, high body fat content; LF, low body fat content, and RC, random control with intermediate fat content). The lines were developed by directional selection for epididymal fat pad weight as a percentage of body weight (EFP%) which is highly correlated with body fat percentage. The HF line exceeded ($P < 0.05$) LF in weight gain, feed intake, feed efficiency, body weight, EFP% and serum cholesterol and triglyceride levels. Body water percentage, a measure of lean tissue percentage, and liver weight as a percentage of body weight were greater ($P < 0.05$) in the LF line compared to HF. The 50% fat diet resulted in a reduction ($P < 0.05$) in energy intake, suggesting low palatability. Mice consuming 12.5, 25 and 37.5% fat did not differ in growth rate ($P > 0.05$), but feed efficiency at 25 and 37.5% was greater ($P < 0.05$) than at 12.5% since feed intake on a weight basis was reduced. There were no important biological effects of diet on EFP% or serum cholesterol level. At seven weeks of age serum triglyceride levels increased as dietary fat increased from 12.5 to 37.5% fat, but no clear trend was apparent. However, the LF line displayed a resistance to change in triglyceride levels as fat level increased. In general, while statistically significant genotype by dietary level interactions were detected for some traits, there were no biologically important interactions since the ranking of lines was not affected when compared across different levels of fat. The effects of dietary monounsaturated fat levels were generally smaller than the genetic line differences established by selection for high and low body fat percentage.

INTRODUCTION

Genotype and diet play important roles in human obesity (Meyer and Stunkard, 1994). High dietary saturated fat consumption may lead to obesity

and high serum triglyceride levels, which are both linked to an increased risk of cardiovascular disease (Dreon *et al.*, 1988; Grundy and Denke, 1990). While some studies indicate that replacement of saturated fat with polyunsaturated fat lowers serum cholesterol level and fat deposition (Keys *et al.*, 1965; Beynen and Katan, 1984), others indicate little effect on these variables (Huang *et al.*, 1984; Beynen, 1987). These apparent

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discrepancies may be the result of genotype by environmental interactions. For example, West *et al.* (1992) reported that feeding a high saturated fat diet caused some inbred strains of mice to be susceptible to dietary obesity whereas others were resistant. This type of study illustrates the value of comparing dietary fat treatments across different genotypes to determine the generality of results.

Although dietary fat is an important factor contributing to the onset of obesity and associated cardiovascular diseases, genetic predisposition may be a more potent risk factor (Weibust, 1973; Nichols *et al.*, 1976; Sing and Orr, 1978; Dunnington *et al.*, 1977; van Zutphen and den Bieman, 1983; Bouchard and Pérusse, 1994). Target genes for obesity are beginning to be identified (Lusis and Sparkes, 1989; Paigen *et al.*, 1989; Johnson and Gregoire, 1994). Many of these loci were discovered through the appearance of a mutant phenotype in mice causing extreme obesity. The recent elucidation of three single genes with major effects on obesity (*A^y*, Michaud *et al.*, 1994; *ob*, Zhang *et al.*, 1994; *fat*, Naggert *et al.*, 1995) has advanced the understanding of the genetics of obesity. However, fat deposition varies greatly and is approximately normally distributed in humans and mammalian models that have been examined (Festing, 1979), indicating that genetic variation in body fat content is determined by a multifactorial combination of genes with small to moderate effects on lipid metabolism, hormonal regulation and appetite control. Obesity in the mouse is well studied (Bray and York, 1979) and provides a useful mammalian model system for studying the interaction of dietary fat and genetic factors where, contrary to humans, environmental influences can be closely controlled and specific inbred or selected strains are available (Eisen *et al.*, 1996).

The present experiment was designed to study the effect of feeding low and high levels of Canola oil, a rich source of monounsaturated fat and low in saturated fat (Ackman, 1983), on growth, fat deposition, and serum lipid levels in three lines of mice that differ in body fat percentage as a result of directional selection (Eisen, 1987a).

MATERIAL AND METHODS

Origin of genetic lines

The lines of mice used in this study were developed by within full-sib family selection in two replicates for high (HF1, HF2), low (LF1, LF2) or random (RC1, RC2) right epididymal fat pad weight as

a percentage of body weight at 12 weeks of age (Eisen, 1987a). The epididymal fat pad was used as an indicator trait of body fat percentage because of the high phenotypic correlation ($r = 0.84$) between these two traits (Eisen and Leatherwood, 1981). Selection was maintained for 12 generations and then was relaxed for the following seven generations. At generation 19 the two replicates of each selection treatment were crossed producing the final lines, HF, LF and RC. These lines have been maintained by random mating, and in this experiment mice from generation 39 were used.

Diets

Canola oil has the lowest saturated fatty acid content of major vegetable oils (Ackman, 1983). Canola oil has an estimated fatty acid content as a percentage of total fatty acids of 1% palmitic acid (16:0), 32% oleic acid (18:1), 15% linoleic acid (18:2), 1% linolenic acid (18:3) and ~50% monounsaturated fatty acids (Steppanen-Laakso *et al.*, 1992). The particular Canola oil used for this study contained 6% saturated fat, 26% linoleic acid, 10% α -linoleic acid and 58% monounsaturated fat (Duke's[®], C.F. Sauer Co., Richmond, VA).

A total of four diets was fed *ad libitum* (Table I). Canola oil was the only source of fat used in these diets. All fat percentages listed for these diets are based on total dietary energy. The diets were based on a modification of the standard NIH AIN-76 diet. Both replicates used dietary fat levels of 12.5% and 25%. In replicate one the high fat diet was 50% fat, but due to its

Table I - Composition of experimental diets.

Ingredients (g)	Diets (fat %) ^a			
	12.5	25.0	37.5	50.0
Casein	20.0	20.0	20.0	20.0
DL-methionine	0.3	0.3	0.3	0.3
Cornstarch	15.0	15.0	15.0	15.0
Sucrose	49.2	37.2	25.2	13.1
Cellulose	5.0	5.0	5.0	5.0
Fat (Canola oil)	5.3	10.7	16.0	21.4
Mineral mix ^b	3.5	3.5	3.5	3.5
Vitamin mix ^c	1.0	1.0	1.0	1.0
Choline bitartrate	0.2	0.2	0.2	0.2

^aKilojoules of fat as a percentage of total kilojoules.

^bPercent of mineral mix: CaHPO₄, 50.0; NaCl, 7.4; K citrate.H₂O, 22.0; K₂SO₄, 5.2; MgO, 2.4; manganous CO₃, 0.001; Na₂SeO₃.5 H₂O, 0.001; CrK(SO₄)₂.12 H₂O, 0.055; powdered sucrose, 11.8.

^cPer kg of vitamin mixture (g): thiamin.HCl, 0.6; riboflavin, 0.6; pyridoxine.HCl, 0.7; niacin, 3.0; Ca pantothenate, 1.6; folic acid, 0.2; biotin, 0.020; vitamin B₁₂, 1 mg; vitamin A, 400,000 IU; vitamin E, 5,000 IU; vitamin D₃, 2.5 mg; vitamin K, 5.0 mg; powdered sucrose, to make 1,000 g.

seeming lack of palatability to the mice, it was replaced in replicate two with a diet which had a 37.5% fat level. Energy values were calculated from the nutrient composition of the diets using values of 16.74, 16.74 and 37.66 kJ/g of carbohydrate, protein and fat, respectively. The energy values of the diets were 16.23, 17.41, 18.74 and 20.33 kJ/g for the 12.5, 25.0, 37.5 and 50.0% fat diets, respectively. These diets were made available in glass jars with fitted twist-on lids that had a 20-mm diameter opening, and a wire screen to prevent spillage. Jars were weighed, refilled and reweighed every other day for the period of this experiment in order to record feed intake per cage.

Experimental procedure

The laboratory animal protocol was approved by the North Carolina State University Institutional Animal Care and Use Committee. Mice were housed in a controlled environment at approximately 21°C, and 55% relative humidity with a light:dark cycle of 12:12 h beginning at 7:00 h. The experiment was a randomized design twice replicated. The day after birth, all litters were standardized to 10 pups in order to minimize variation in preweaning growth that may occur due to the number of pups being suckled. Male pups were preferentially selected in order that enough male progeny were available for the purposes of the study. Female mice were not used in order to eliminate any complicating gender interactions. During the three weeks prior to weaning, dams and their litters were fed Purina 5015 Mouse Chow (Purina Mills, Richmond, IN) *ad libitum*. At three weeks of age, only male mice were weaned, housed four per cage, and fed Purina 5001 Lab Chow *ad libitum* for the first week following weaning.

At four weeks of age, a total of 60 mice were randomly selected from each of the three mouse lines, HF, LF and RC. Two mice of the same line were caged together. The 60 mice of each line were then randomly divided into three groups of 20 mice. Each group of 20 mice received one of the three dietary regimens. This was done for both replicates, but with the first replicate including the 50% fat diet and the second replicate replacing this diet with the 37.5% fat diet. Of the experimental groups of 20, half were fed the diets from four to seven weeks of age and the remaining half were fed from four to 10 weeks of age. The end points of seven and 10 weeks were used to represent periods when mice experience rapid postweaning growth rate and deceleration of growth rate, respectively.

Individual body weights were recorded at four weeks of age, and a final seven or 10 weeks postfast body weight (FINAL WT) was also recorded. Mean

metabolic body weights ($W^{0.75}$ kg) for four to seven weeks of age and for four to 10 weeks of age (MET 4-7 and MET 4-10) were calculated. Weight gains from week four to seven (WG 4-7), and from week four to 10 (WG 4-10) were calculated as were feed intakes and energy intakes from week four to seven (FI 4-7, EI 4-7) and week four to 10 (FI 4-10, EI 4-10). Energy intake was calculated by multiplying feed intake by the energy value of the diet. Gross feed efficiency (weight gain/feed intake) was calculated for the four to seven week period and the four to 10 week period (EFF 4-7 and EFF 4-10, respectively). Adjusted feed intake (feed intake/metabolic body weight) was calculated for the same periods (F/M 4-7 and F/M 4-10).

At the end of the designated time periods (seven or 10 weeks of age), mice were anesthetized with sodium pentobarbital and blood was collected by heart puncture between 8:00 and 10:00 h. Mice were fasted overnight prior to blood sampling. Blood was left to coagulate for 4-6 h and then refrigerated overnight. Serum was then removed from the sample, centrifuged, and frozen at 0°C for determination within seven days of serum cholesterol and triglyceride levels. After the collection of blood samples, mice were killed by cervical dislocation and the liver and right epididymal fat pad were removed, weighed and returned to the carcass. The gastrointestinal tract was also removed, flushed and returned to the carcass to obtain an ingesta-free empty body weight. Carcasses were frozen at -6°C until both replicates were completed and then all were lyophilized for 48 h and weighed to obtain dry body weights.

Liver weight was analyzed as a percentage of final body weight (LIVER%), as was epididymal fat pad weight (EFP%). Percentage body water (WAT%) was calculated as $100 \times (\text{empty body weight} - \text{dry body weight}) / \text{empty body weight}$. The EFP% is an indirect measure of body fat percentage while WAT% is an indirect measure of lean tissue percentage (Eisen and Leatherwood, 1981; Eisen and Coffey, 1990).

Assays

Adaptations were made to the methods described in the procedure manual accompanying the reagents (Sigma Diagnostics, St. Louis, MO) used for the determination of serum cholesterol and triglyceride concentrations. Concentration curves established prior to the experiment with cholesterol and triglyceride calibrators (Sigma Diagnostics, St. Louis, MO) indicated that a sample size of 0.06 ml of serum was more desirable than the recommended 1 ml. The reagent amount was decreased to 0.94 ml for a final volume of 1.0 ml.

The enzymatic reactions for the analysis of triglyceride levels begin with the hydrolysis of triglycerides to glycerol and free fatty acids by lipoprotein lipase. Glycerol is then phosphorylated with ATP and glycerol kinase to form glycerol-3-phosphate and ADP. Glycerol-3-phosphate is then oxidized by glycerol phosphate oxidase to dihydroxyacetone phosphate and hydrogen peroxide. The peroxidase-catalyzed coupling of 4-aminoantipyrine and sodium N-ethyl-N-(3-sulfo-propyl)m-anisidine with the hydrogen peroxide produces a quinoneimine, which can be measured spectrophotometrically at 540 nm.

The enzymatic reactions for the analysis of total cholesterol begin with the hydrolysis of cholesterol esters by use of cholesterol esterase. Cholesterol is oxidized by cholesterol oxidase to form cholest-4-ene-3-one and hydrogen peroxide. The peroxidase-catalyzed coupling of 4-aminoantipyrine and p-hydroxybenzene-sulfonate with the hydrogen peroxide produces a quinoneimine dye, which is quantitated spectrophotometrically at 540 nm.

Statistical analysis

Least-squares analysis of variance procedures were used to estimate means of each trait based on the following statistical model:

$$Y_{ijklm} = m + R_i + L_j + D_k + LD_{jk} + A_l + LA_{jl} + DA_{kl} + LDA_{jkl} + e_{ijklm}$$

where m is the overall mean, R_i is the random replicate effect, L_j is the fixed line effect, D_k is the fixed dietary effect, A_l is the fixed age effect and e_{ijklm} is the random residual effect. The remaining terms are the corresponding interaction effects. The age effect and associated interactions were eliminated from the model when traits such as weight gain were analyzed on an age interval basis. Feed intake and feed efficiency were analyzed using the cage with two mice as the experimental unit. Contrasts among treatment means were based on least significant difference tests.

RESULTS

Weight gains, feed intake and feed efficiency

Weight gains, whether over the four- to seven-week or four- to 10-week period, showed no line by diet interactions ($P > 0.05$). At each period, the HF line gained more ($P < 0.05$) weight than either the LF or RC lines while LF gained more ($P < 0.05$) than RC from four to seven weeks (Table II). For the four- to seven-week age period, weight gains on the 37.5% and 50% high fat

diets were lower ($P < 0.05$) than on the 12.5% and 25% fat diets (Table III); a similar trend occurred from four to 10 weeks.

Feed intake on a weight and an energy basis from four to seven weeks and four to 10 weeks of age

Table II - Line least squares means and SE averaged over diets^a.

Traits ^b	Lines			SE ^c
	HF	LF	RC	
WG, g/d				
4-7 weeks	0.848 ^x	0.625 ^y	0.574 ^z	0.014
4-10 weeks	0.631 ^x	0.496 ^y	0.459 ^y	0.016
FI, g/d				
4-7 weeks	4.96 ^x	4.15 ^y	3.85 ^z	0.03
4-10 weeks	4.78 ^x	4.47 ^y	4.07 ^z	0.04
EI, kJ/d				
4-7 weeks	89.04 ^x	74.48 ^y	69.02 ^z	0.54
4-10 weeks	85.81 ^x	80.25 ^y	73.05 ^z	0.71
F/M, kg/d/kg ^{0.75}				
4-7 weeks	0.064 ^x	0.062 ^y	0.057 ^z	0.001
4-10 weeks	0.057 ^x	0.061 ^x	0.056 ^z	0.001
EFF, g/g				
4-7 weeks	0.173 ^x	0.154 ^y	0.147 ^y	0.003
4-10 weeks	0.132 ^x	0.111 ^y	0.113 ^y	0.003

^{xyz}Row means with no common superscripts denote statistical differences of $P < 0.05$.

^aPooled over replicates.

^bWG = Weight gain; FI (EI) = feed intake on a weight (energy) basis; F/M = feed intake/(mean body weight)^{0.75}; EFF = feed efficiency (WG/FI).

^cStandard error.

Table III - Diet least squares means and SE averaged over lines^a.

Traits ^b	Diets (fat %) ^a				SE ^c
	12.5	25.0	37.5	50.0	
WG, g/d					
4-7 weeks	0.750 ^w	0.756 ^w	0.701 ^x	0.521 ^y	0.013 0.021
4-10 weeks	0.537 ^w	0.548 ^w	0.509 ^{wx}	0.480 ^x	0.014 0.023
FI, g/d					
4-7 weeks	5.12 ^w	4.73 ^x	4.27 ^y	3.15 ^z	0.03 0.05
4-10 weeks	5.16 ^w	4.85 ^x	4.32 ^y	3.43 ^z	0.04 0.05
EI, kJ/d					
4-7 weeks	83.13 ^w	82.29 ^w	80.03 ^x	64.06 ^z	0.50 0.96
4-10 weeks	83.76 ^w	84.43 ^w	80.96 ^x	69.75 ^z	0.66 0.96
F/M, kg/d/kg ^{0.75}					
4-7 weeks	0.072 ^w	0.066 ^x	0.060 ^y	0.046 ^z	0.001 0.001
4-10 weeks	0.067 ^w	0.062 ^x	0.056 ^y	0.047 ^z	0.001 0.001
EFF, g/g					
4-7 weeks	0.146 ^w	0.160 ^x	0.162 ^x	0.165 ^x	0.003 0.004
4-10 weeks	0.104 ^w	0.113 ^x	0.118 ^x	0.140 ^y	0.002 0.004

^{wxyz}Row means with no common superscripts denote statistical differences of $P < 0.05$.

^aPooled over replicates.

^bSee Table II for definition of traits.

^cStandard errors for 12.5% and 25% fat diets, 37.5% and 50% fat diets, respectively.

showed a line by diet interaction ($P < 0.05$) which did not affect the ranking of the lines (data not shown). For all diets the HF line consumed the most ($P < 0.05$) feed on both a weight and energy basis followed by the LF line and then RC (Table II). Mice on the 12.5% fat diet had the highest ($P < 0.05$) feed intake on a weight basis and those on the 50% fat diet the lowest ($P < 0.05$) for all lines (Table III). On an energy basis, consumption did not differ between the 12.5 and 25% diets, and there was a small but significant reduction on the 37.5% fat diet. In contrast, the energy consumption of mice on the 50% fat diet was considerably lower than mice on the other three diets, suggesting that this diet was not palatable to the mice. The line and diet trends for feed intake per unit metabolic body size were essentially the same as for feed intake, although the relative differences were reduced (Tables II, III).

Feed efficiency was also calculated for the four- to seven- and four- to 10-week periods. For the earlier time period, there was a line by diet interaction ($P < 0.05$), although no change in ranking of lines across diets or vice versa could be discerned (data not shown). When averaged over diets, the HF mice were more ($P < 0.05$) efficient than either the RC or LF mice, while the latter two lines did not differ ($P > 0.05$) (Table II). From four to seven weeks of age, there were no differences ($P > 0.05$) in feed efficiency among mice on any of the high fat diets (25%, 37.5%, and 50%), and all three diets were more ($P > 0.05$) efficient than the 12.5% fat diet (Table III). From four to 10 weeks, mice on the 50% fat diet were more ($P < 0.05$) efficient than mice on both the 25% and 37.5% fat diets. Feed efficiency generally was reduced when measured through the older age interval.

Body weights, fat pads, percentage water and liver

There were no significant line by diet interactions for final or dry body weights at seven or 10 weeks of age, nor were there any significant line by age or diet by age interactions. The final and dry body weights of the HF line were larger ($P < 0.05$) than those for both the LF and RC lines, but there was no difference ($P > 0.05$) between LF and RC except for 10-week dry body weight where RC exceeded ($P < 0.05$) LF (Table IV). Across all lines, mice on the 50% fat diet weighed less ($P < 0.05$) than those on the three other fat levels (Table V). The lower energy intake of mice on the 50% fat diet may have led to lower weight gains and smaller final body weights at seven and 10 weeks of age.

Epididymal fat pad weight as a percentage of body weight (EFP%) is highly correlated with total body fat content. No line by diet or line by age interactions were found ($P > 0.05$) for EFP%. Epididymal fat percentage in the HF line was greater ($P < 0.05$) than in the RC line whereas the LF line had a lower EFP% ($P < 0.05$) than RC (Table IV). Also, 10-week old mice were fatter ($P < 0.05$) than those at seven weeks. The overall diet effect on EFP% in the analysis of variance was not

Table IV - Line by age least squares means averaged over diets^a.

Trait ^b	Age, weeks	Line			SE ^c
		HF	LF	RC	
FINAL WT, g	7	39.43 ^x	31.24 ^y	30.71 ^y	0.61
	10	46.71 ^x	38.08 ^y	37.19 ^y	0.56
DRY WT, g	7	17.56 ^x	10.35 ^y	11.11 ^y	0.37
	10	22.52 ^x	13.90 ^y	14.88 ^z	0.34
EFP%	7	2.87 ^x	0.95 ^y	1.38 ^z	0.075
	10	3.56 ^x	1.57 ^y	2.13 ^z	0.069
LIVER%	7	4.66 ^x	5.02 ^y	4.58 ^x	0.066
	10	4.03 ^x	4.44 ^y	4.06 ^x	0.060
WAT%	7	53.97 ^x	65.53 ^y	62.29 ^z	0.48
	10	50.33 ^x	62.27 ^y	58.29 ^z	0.44

^{xyz}Row means with no common superscripts denote statistical differences of $P < 0.05$.

^aPooled over replicates.

^bFINAL WT = Body weight following fast; DRY WT = body weight following lyophilization; EFP% = epididymal fat pad weight as a percentage of FINAL WT; LIVER% = liver weight as a percentage of FINAL WT; WAT% = body water weight as a percentage of empty body weight.

^cStandard error of line by age mean.

Table V - Diet by age least square means averaged over lines^a.

Trait ^b	Age, weeks	Diet (fat %)				SE ^c	
		12.5	25	37.5	50		
FINAL WT, g	7	34.51 ^w	34.39 ^w	34.94 ^w	31.30 ^x	0.58	0.86
	10	41.23 ^w	42.52 ^w	40.25 ^{wx}	38.59 ^x	0.53	0.80
DRY WT, g	7	13.17 ^w	13.30 ^w	14.06 ^w	11.51 ^x	0.35	0.52
	10	17.51 ^w	17.94 ^w	16.67 ^{wx}	16.27 ^x	0.32	0.48
EFP%	7	1.59 ^w	1.75 ^w	2.00 ^x	1.60 ^w	0.071	0.106
	10	2.35 ^w	2.46 ^w	2.36 ^w	2.50 ^w	0.065	0.097
LIVER%	7	4.79 ^w	4.63 ^w	4.39 ^x	5.19 ^y	0.062	0.092
	10	4.05 ^w	4.06 ^w	4.27 ^x	4.31 ^x	0.057	0.085
WAT%	7	60.89 ^w	60.21 ^{wx}	58.95 ^x	62.33 ^{wx}	0.45	0.67
	10	56.50 ^w	56.64 ^w	57.93 ^w	56.79 ^w	0.41	0.62

^{wxy}Row means with no common superscripts denote statistical differences of $P < 0.05$.

^aPooled over replicates.

^bSee Table IV for definition of traits.

^cStandard errors for 12.5% and 25% fat diets, 37.50% and 50% fat diets, respectively.

significant ($P = 0.086$), but there was a significant diet by age interaction ($P = 0.043$). Examination of the EFP% means in Table V indicates that the interaction was caused because the seven-week old mice fed the 37.5% fat diet were fatter ($P < 0.05$) than mice on the other diets, while there were no diet differences ($P > 0.05$) at 10 weeks of age. Percentage body water is highly negatively correlated with EFP% and reflects the percentage of lean tissue. As such, water percentage means generally were inversely related to EFP% for line, diet and age effects.

Adjusted liver weight was measured since the liver plays a central role in lipid metabolism. Although there was a significant line by diet interaction of liver weight as a percentage of body weight (LIVER%), the ranking of lines remained unchanged. The LIVER% was greater ($P < 0.05$) in the LF line compared to the RC controls whereas LIVER% in the HF line did not differ from RC. Older mice had smaller livers on a body weight basis ($P < 0.05$), and there was no significant line by age interaction; there was a significant diet by age interaction ($P < 0.01$). There was no difference in LIVER% between the 12.5 and 25% fat diets at both seven and 10 weeks of age. On the 37.5% fat diet, proportional liver weight decreased at seven weeks and increased at 10 weeks compared to the 12.5 and 25% fat diets. Mice fed the 50% fat diet showed an increase in LIVER% above the mice fed 12.5 and 25% fat at both seven and 10 weeks of age.

Serum cholesterol and triglyceride levels

Line, diet and line by diet effects were significant ($P < 0.01$) for serum cholesterol level. At all levels of dietary fat, HF mice had higher cholesterol levels than LF mice, and were significant ($P < 0.05$) at all but the 37.5% fat diet (Figure 1). There was no clear trend of dietary fat level affecting serum cholesterol level. The major cause of the line by diet interaction was the LF versus RC comparison; at 12.5 and 50% fat there was no difference ($P > 0.05$), at 25% fat RC > LF ($P < 0.05$) and at 37.5% fat RC < LF ($P < 0.05$). Serum cholesterol level also showed line by age ($P < 0.01$) and diet by age interactions. At seven weeks of age, line ranking for serum cholesterol level was HF > RC > LF while at 10 weeks the ranking was HF > RC, LF (Figure 2). Thus, at both ages there was a clear divergence in serum cholesterol level between the high fat and low fat genotypes. The diet by age interaction was due to a distinct difference in the diet response at seven vs. 10 weeks of age (Figure 3). At seven weeks there was a quadratic trend in serum cholesterol level with a peak response at 25% fat while at 10 weeks serum cholesterol

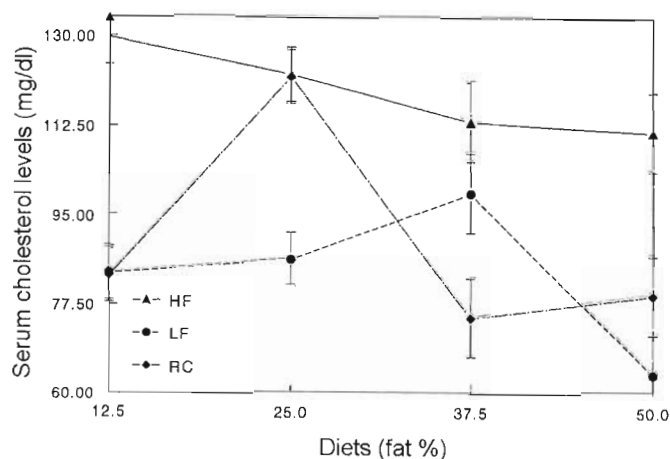


Figure 1 - Line by diet least squares means \pm SE for fasting serum cholesterol levels averaged over ages.

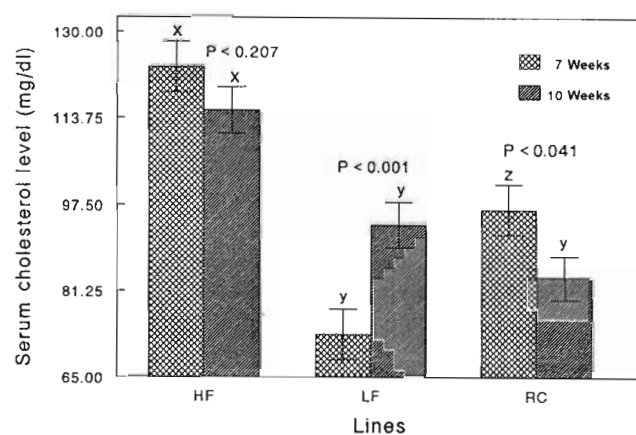


Figure 2 - Line by age least squares means \pm SE for fasting serum cholesterol level averaged over diets. ^{xy} Within each age, line means with no common letter denote statistical significance ($P < 0.05$). P value above each pair of means refers to significance of age difference within lines.

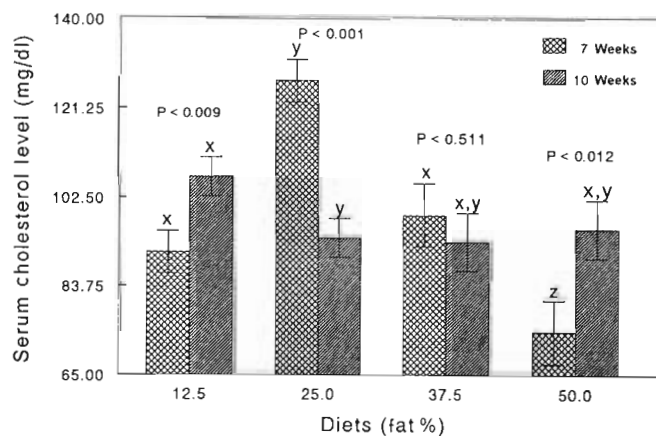


Figure 3 - Diet by age least squares means \pm SE for fasting serum cholesterol levels averaged over lines. ^{xy} Within each age, line means with no common letter denote statistical significance ($P < 0.05$). P value above each pair of means refers to significance of age differences with diets.

level was highest at 12.5% fat with no differences among the three highest fat levels.

Serum triglyceride level did not show a significant interaction of line by diet ($P = 0.145$) or line by age ($P = 0.136$). The HF line had higher triglyceride levels than LF at each level of fat percentage, the difference being significant ($P < 0.05$) overall and at each level of fat except 50% (Figure 4). The RC line tended to be intermediate in triglyceride level. While there was a great deal of variation among the triglyceride level means across dietary fat levels, the LF means when plotted against the dietary fat levels formed a zero slope. Thus, the LF line displayed a resistance to change in its triglyceride levels when fed increments of 12.5 to 50% fat from a source containing a high level of monounsaturated fats. Serum triglyceride levels exhibited a diet by age interaction ($P < 0.01$). At seven weeks of age, triglyceride level rose linearly from 12.5 to 37.5% fat and then was reduced sharply at 50% fat (Figure 5). In contrast, triglyceride levels at 10 weeks showed no clear trend.

DISCUSSION

The feeding of four levels of monounsaturated fat diets derived from Canola oil to lines of mice that had been selected for high (HF), intermediate (RC, control) and low (LF) fat content was examined in order to determine if lines differing in polygenetically controlled fat content would show similar responses to these diets. Generally, responses of the lines on the diet containing 12.5% of energy from Canola oil were similar to the commercial mouse diet used during selection (Eisen, 1987a,b). As in previous studies, body weights, fat content, feed efficiency, feed intake and epididymal fat pad weight as a percentage of body weight were greater in the fat (HF) than in the lean (LF) line while percentage body water and liver weight as a percentage of body weight were lower in the fat line (Eisen, 1987a; Prasetyo and Eisen, 1989; Eisen and Coffey, 1990; Eisen *et al.*, 1996). The divergence in fat content between the HF and LF lines increased as the mice became older which is typical of the fat pattern seen in mice and other mammals (Prasetyo and Eisen, 1989). In addition, the ranking of the lines for weight gain, feed intake and feed efficiency were unaffected by feeding different levels of monounsaturated fat, indicating that genotype by monounsaturated dietary fat level interactions were not biologically important for these traits.

Selection for high and low fat content led to positive divergence in serum triglyceride and cholesterol levels, confirming an earlier study with these lines

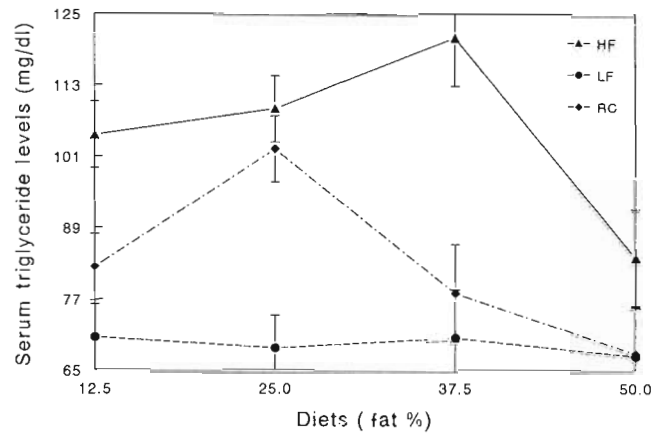


Figure 4 - Line by diet least squares means \pm SE for fasting serum triglyceride level averaged over ages.

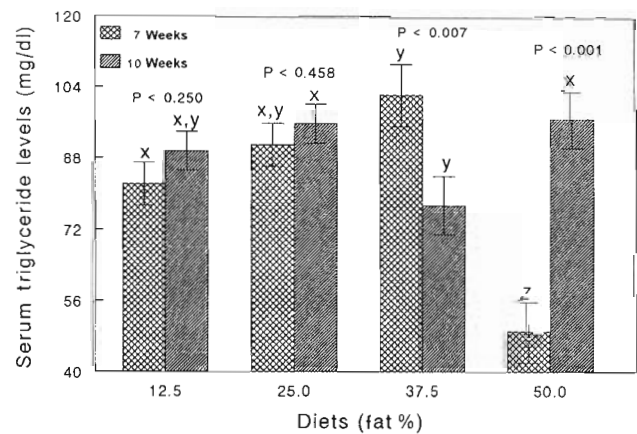


Figure 5 - Diet by age least squares means \pm SE for fasting serum triglyceride levels averaged over lines. ^{x,y,z} Within each age, diet means with no common letter denote statistical significance ($P < 0.05$). P value above each pair of means refers to significance of age differences within diets.

(Eisen *et al.*, 1996). These data suggest that there is a positive pleiotropic association between body fat content and blood cholesterol and triglyceride concentrations. Supporting the role of pleiotropy is a recent finding in backcross progeny of *Mus spretus* with C57BL/6J of significant linkage between a marker on mouse chromosome 7 and both plasma cholesterol and carcass lipid (Warden *et al.*, 1993). Robeson *et al.* (1981) reported that adult mice that became moderately obese after being selected for increased growth rate were characterized as hypercholesterolemic. Dunnington *et al.* (1981b) reported that selection for divergence in serum cholesterol level yielded positive divergence in body fat percentage in females but not males. Earlier studies have shown that blood cholesterol level has a moderate additive genetic component in mice with realized heritabilities ranging between 0.37 and 0.59 (Weibust, 1973; Dunnington *et al.*, 1981a). Other mammalian species have a similar range of heritabilities

for blood cholesterol level. In humans, the heritability estimates of total cholesterol range from 0.49 to 0.64 (Sing *et al.*, 1988). Divergent selection for serum cholesterol level in 56-day old pigs resulted in a realized heritability of 0.31 (Young *et al.*, 1993). Heritability of plasma cholesterol in squirrel monkeys was 0.46 (Clarkson *et al.*, 1971) while in baboons the heritability of serum cholesterol level averaged 0.45 (Flow *et al.*, 1981). Heritability estimates of triglyceride levels in mice have not been reported. However, the positive correlated responses in serum, liver and small intestine triglyceride levels as a result of selection for body fat content in mice point to the presence of moderate additive genetic variation in triglyceride levels and positive pleiotropy between triglyceride levels and body fat percentage (Eisen *et al.*, 1996). In humans, the fraction of additive genetic variation in blood triglyceride levels ranges from 0.19 to 0.52 (Sing *et al.*, 1988).

In general, mice consuming 12.5, 25 and 37.5% fat from the high monounsaturated fat source of Canola oil did not differ in growth rate, but feed efficiency at 25 and 37.5% fat was improved since feed intake on a weight basis was reduced. Also, the 37.5% fat diet increased fat deposition at seven weeks of age but not at 10 weeks. The 50% fat diet proved to have low palatability and, as a result, both feed intake and weight gain were reduced.

The failure of the distinct genotypes to show any biologically important genotype by dietary fat level interactions in body fat percentage is surprising considering the diverse nature of the genotypes (high, intermediate and low body fat percentage due to polygenic variation) and the extreme levels of the dietary fat. Previous studies have shown such interactions to be important (Fenton and Carr, 1951; Schemmel *et al.*, 1970; Paigen *et al.*, 1985; Fisler and Bray, 1990 and West *et al.*, 1992). These studies, however, all involved substituting a carbohydrate source for a highly saturated fat source whereas the present study used a fat source, Canola oil, high in monounsaturated and polyunsaturated fat and low in saturated fat. Further studies will be required to determine if this is a satisfactory explanation for the different type of response.

The present research was not the first to be unable to detect any substantial effect of fat in the diet on serum cholesterol level, especially on a diet low in or free of cholesterol. Beynen (1987) found that cholesterol-free diets show no effect of saturated, monounsaturated or polyunsaturated fat on cholesterol level which contradicts earlier work done by Kritchevsky *et al.* (1983) and Beynen and Katan (1984).

There has been little attention given to the influence of diet on serum triglyceride levels because

triglycerides have not been seen as a risk factor for coronary heart disease or atherosclerosis. Recent epidemiological data, however, point to a positive correlation between triglyceride levels and coronary heart disease (Grundy and Denke, 1990). A decrease in the dietary fat to carbohydrate ratio usually results in a significant elevation of serum triglycerides (Beynen and Katan, 1984). Thus, for our experiment, mice on the 50% fat diet, with a 1.6:1.0 ratio, and the 37.5% fat diet, with a 1.0:1.6 ratio of carbohydrate to fat, should have been equally high in their triglyceride levels. In fact, at seven weeks of age, the 37.5% fat diet had the highest triglyceride level while the 50% fat diet had the lowest level of the four diets. By 10 weeks of age, however, this situation had become completely reversed, with the 50% fat diet having the highest triglyceride content of all the diets and the 37.5% fat diet having the lowest. It has been argued that in humans the carbohydrate-induced hypertriglyceridemia is only a short-term phenomenon that reaches its maximum point after a few weeks and then dissipates (Mancini *et al.*, 1973). This could explain the results of our study, with the different diets reaching their maximum triglyceride levels on unique schedules.

The difference in serum triglyceride concentration between seven and 10 weeks with increasing dietary content of unsaturated fatty acid is difficult to explain. There were gradual, although not significant, increases in triglyceride concentration from seven to 10 weeks when mice were fed diets containing 12.5 and 25.0% fat. It is difficult to reconcile the decrease in serum triglyceride concentration that occurred between seven and 10 weeks with mice fed the 37.5% fat diet. The significant decrease could be explained by an age-related decrease in synthesis of triglyceride or an age-related increase in triglyceride metabolism in the control and low fat lines in contrast to the high fat line. At seven weeks, the animals fed the 50% fat diet had a significantly reduced triglyceride concentration in comparison to the three lower fat diets. It is possible that the decrease could be due to the dietary lipid intake, but there was also a significantly lower dietary intake throughout the entire 10-week period, and the 10-week plasma triglyceride level was elevated and not significantly different from the triglyceride concentrations of animals fed the 12.5 and 25% fat diets.

It is unlikely that changes in triglyceride content of membranes occur as a result of feeding unsaturated fatty acids. It is assumed that the unsaturated tail of the original 18:1 fatty acid is not esterified to yield triglycerides containing the unsaturated remnant. There is also a possibility that the remnant fatty acyl CoA acts as an inhibitor of triglyceride synthesis, but

there is no awareness of any enzymatic studies which have investigated the effects of short chain unsaturated fatty acyl CoA on triglyceride synthesis.

For the time being, the triglyceride profile as seen in Figure 5 is an anomaly and must await further investigation in order to explain the changes with respect to time as well as diet.

In conclusion, the feeding of high and low monounsaturated fat diets to lines of mice differing in polygenically controlled body fat content showed no biologically important genotype by dietary fat level interactions for fat content, growth rate, feed intake, feed efficiency and serum cholesterol and triglyceride levels. The effects of dietary monounsaturated fat levels on these traits were variable and generally were smaller in magnitude than the genetic line differences established by directional selection for high and low body fat.

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RESUMO

O objetivo do presente trabalho foi determinar se existem interações biologicamente importantes de genótipos de camundongo, diferindo em porcentagem poligenicamente controlada de gordura corporal, com níveis de gordura dietética do óleo de Canola, que é rico em gordura monoinsaturada e baixo em gordura saturada. Quatro níveis de gordura (12,5, 25, 37,5 e 50% da energia dietética) foram colocados à disposição, *ad libitum*, para os camundongos, da quarta à sétima ou da quarta à décima semanas de vida, para três linhagens de camundongos (HF, alto conteúdo de gordura corporal; LF, baixo conteúdo de gordura corporal; RC, controle com conteúdo intermediário de gordura corporal). As linhagens foram desenvolvidas por seleção direcional para peso do coxim gorduroso do epidídimo como porcentagem do peso corporal (EFP%), que é altamente correlacionado com porcentagem da gordura corporal. A linhagem HF excedeu ($P < 0,05$) a LF em ganho de peso, ingestão alimentar, eficiência alimentar, peso corporal, EFP% e níveis séricos de colesterol e triglicérides. A porcentagem de água corporal, que é uma medida da porcentagem de tecido magro, e o peso do fígado como porcentagem do peso corporal foram maiores ($P < 0,05$) na linhagem LF quando comparada com HF. A dieta com 50% de gordura resultou em

uma redução ($P < 0,05$) na ingestão calórica, sugerindo baixa palatabilidade. Os camundongos que consumiram 12,5, 25 e 37,5% de gordura não diferiram na taxa de crescimento ($P > 0,05$), mas a eficiência alimentar com 25 e 37,5% foi maior ($P < 0,05$) que com 12,5%, já que a ingestão alimentar em relação ao peso foi reduzida. Não houve efeitos biológicos importantes da dieta na EFP% ou no nível de colesterol plasmático. Em sete semanas de idade, os níveis séricos de triglicérides aumentaram à medida que a gordura passou de 12,5 para 37,5%, mas não se notou nenhuma tendência clara. Contudo, a linhagem LF mostrou uma resistência para alterações nos níveis de triglicérides à medida que o nível de gordura aumentava. Em geral, embora interações genótipo x nível dietético estatisticamente significantes tenham sido detectadas para alguns caracteres, não houve interações biologicamente importantes, já que a posição relativa das linhagens não foi afetada quando comparada em diferentes níveis de gordura. Os efeitos dos níveis de gordura dietética monoinsaturada foram geralmente menores que as diferenças de linhagens genéticas estabelecidas por seleção para alta e baixa porcentagem de gordura.

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