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Effects of Dietary Fats on Blood Cholesterol and Other Measures Related to Chd Risk in Male and Female Laboratory Mice

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Abstract
Juvenile and adult male and female Swiss mice were fed one of four specially-formulated, pelleted diets containing respectively 8 percent saturated vegetable fat, 8 percent soya oil, 8 percent olive oil and 2 percent soya oil (with identities hidden from the experimenter) or a local commercial rodent food. It was intended to assess their impact on some blood indices linked to risk of coronary heart disease (CHD). Subjects were individually housed and their blood concentrations of cholesterol, high-density lipoprotein cholesterol (HDLC) and triglycerides were assessed. Clearly, these non-isocaloric diets differed in palatability, producing complex effects on growth as well as physiological measures. Many indices were influenced by age, sex, and the duration of dietary exposure. Interactions between factors were common but it appeared that males showed the greater increase in risk factors in response to some diets.

Introduction

Dietary fatty acids can be classified as saturated, monounsaturated, and (n-3) polyunsaturated and (n-6) polyunsaturated compounds. Grundy and Denke (1990), suggested that the different classes of fatty acids have varied effects on lipid metabolism and the fatty acid portion of the lipid molecule is responsible for its characteristics. Fatty acids occurring in food stuffs e.g. lauric or dodecanoic acid contain even numbers of carbon atoms in unbranched chains. Unsaturated fatty acids have one, two or sometimes up to six double bonds. A fatty acid with one double bond is referred to as a monounsaturated fatty acid (e.g., olive oil), whereas a fatty acid containing two or more double bonds is considered a polyunsaturated fatty acid (e.g., soybean oil). N-3 and n-6 fatty acids are widespread in food sources. They are considered essential fatty acids (EFAs) because they must be supplied in the diet (the body cannot synthesize them in significant amounts) to humans (Bjerve, 1989) as well as rodents (Burr and Burr, 1929).

Cholesterol is an important component of all animal tissues and plays essential roles in the body. However, high levels of cholesterol in the blood are associated in many countries with an increased risk of CHD (Benton, 1996). Conversely, Muldoon *et al*. (1990, 1992, 1993), shown that cholesterol reduction is associated with a lower mortality rate from CHD but with a higher mortality rate from other causes (including suicide and violence). This applied whether the lipid lowering was achieved by dietary regulation or pharmacological treatment. It suggests that lowered cholesterol values may be associated with behavioural changes that predispose the individual towards risk-taking. Engelberg (1992) has suggested that the increased rate of suicide and other violent death observed in cholesterol-lowering trials may be explained by alteration in mood or behaviour arising from a reduced expression of serotonin receptors on cell membranes. This whole area is still contentious (several authors challenge the view that a low cholesterol-high risk association exists). The area is substantially reviewed by LaRosa (1997) and the current evidence seems to substantially favour the view that low cholesterol carries a risk.

The type of dietary fat is a crucial determinant of plasma cholesterol concentration. Pioneering studies clearly pointed out the importance of fatty acids. More recent information (Bonanome and Grundy, 1988; Grundy, 1986; Hayes *et al*., 1991; Khosla and Hayes, 1991) suggests, however, that saturated fatty acids cannot be regarded as a single factor. In deed, the various saturated fatty acids have variable influences on plasma cholesterol concentrations. Kris-Etherton *et al*. (1993) found that linoleic acid (a component of soya oil diet) has a more potent hypocholesterolemic effect than oleic acid (a component of olive oil). Bourre *et al*. (1993) showed that soya oil contains n-3 fatty acids and it was suggested that these substances reduce the plasma cholesterol level in rats by increasing the transfer of cholesterol into bile. Bonanome and Grundy (1988), Hegsted *et al*. (1965), Keys *et al*. (1965) and Thomasson *et al*. (1967) essentially concluded that 16:0 fatty acids elevate total cholesterol and low-density lipoprotein cholesterol in men and women who are hospitalized or otherwise institutionalized. This may lead one to conclude that diets with a low percentage of polyunsaturated fatty acids decrease the level of blood cholesterol. Grundy (1987) showed that olive oil diet lowered the risk of heart disease by suppressing blood cholesterol level. More recently, workers have stressed the importance of fish oils in controlling cholesterol values (largely stemming from the observation that humans with a diet rich in fish have a low incidence of CHD). For example, Yaquoob *et al*. (1995) showed that serum cholesterol concentrations in rats were higher after ten weeks of feeding 20 percent by weight of olive oil, safflower oil, or Evening primrose oil diets than in counterparts given a 20 percent Menhaden (fish) oil or a low fat diet.

The present study analyzed whether diets differing in their type and/or amount of fat produced systematic changes in a range of blood incidences linked to clinical CHD risk in a mouse model. The effects of different durations of exposure were assessed in mice of different sex.

Materials and Methods

Animal Husbandry: Swiss albino mice, bred and housed in the Animal Facility of the King Fahad Research Medical Centre (Jeddah, Kingdom of Saudi Arabia) were used in this study. The subjects were kept with white lights on from

08:00 to 20:00 h (local time). Temperature was maintained between 20-25°C and relative humidity ranged from 45 to 55 percent. Animals were paired and the litters were not culled at birth i.e. there was a certain variation in rates of development in litters of different size. Post-weaning (at 21 days of age), the mice were housed in groups of 5 in transparent cages (type M II m. DO II. F30, E. Becker and Co, Germany) measuring 26×20×14 cm with stainless steel wire tops. Laboratory rodent pellets (Grain Silos and Flourmills Organization, Western Region, P.O. Box 5529, Jeddah 21432, Saudi Arabia. This diet was constituted from a mixture of wheat, soybean, corn, powdered alfalfa and palmetic oil as well as a vitamin/mineral mixture. It contained 200 g/Kg crude protein, 630 g/Kg crude carbohydrate, 30 g/Kg crude fat, 55 g/Kg crude fibre and 85 g/Kg of the vitamin/mineral mixture with an energy value of 2.85 kcal/g) and water were provided ad libitum to the mice. The sawdust substrate was supplied by a commercial carpenter and changed every two days.

The mice were transferred shortly before being used in experiments to a separate room in the Medical Centre (their age of transfer depended on whether they were in the juvenile or adult category). In this new location, they were maintained under a reversed lighting schedule [white fluorescent lights on from 21:30 to 09:30 h (local time)]. Ambient temperature was maintained between 20 and 23°C. Each mouse was housed in a transparent plastic cage (type THF/2152/Aa, EHRET, Germany) measuring 26×20×14 cm with a stainless steel wire top.

Food Intake: Forty-eight mice (24 of each sex) were assigned for each diet. Half the animals of each sex were young (around three weeks at the commencement of the experiment) and half were adult (5-6 weeks). The mice were fed one of four experimental, pelleted diets (These non-isocaloric diets were supplied by SDS, PO Box 705, Witham, Essex, CM8 3AB, UK. All contained 270 g/Kg casein, 402 g/Kg cornstarch, 50 g/Kg Alphacel fibre and 76 g/Kg of a vitamin/mineral mix. They differed in respect to the source and percentage of fat with diet A containing 8 percent saturated vegetable fat (vegetarian lard), B 8 percent soya oil, c 8 percent olive oil and D 2 percent soya oil. The fats influenced the integrity of the pellets but the diets were coded such that the experiment could be conducted 'blind' without inadvertent bias) or the control commercial diet with which they were familiar. The experiments with the diets were run in three replications of four mice for each group. The duration of these experiments was three or six weeks. The daily amounts of food between 10.10 and 10.99 g were weighed on a digital balance (Model PB302, Mettler, Switzerland) and were provided to each mouse in their food hoppers. Unconsumed food (that remaining in the hoppers or collected as spillage from the sawdust substrate) was weighed after 24 hours using the same balance to calculate the quantities eaten.

Blood Sample Collecting: Ten mice were fasted for three hours (to standardize feeding in relation to blood measurements) before blood sampling. Mice were anaesthetized using ether (anaesthetic grade, May and Baker Ltd., England). The blood sample was rapidly taken into a size 0.65×10.25 cm evacuated blood collecting tube (VenoJect, Terumo Corporation, Tokyo, Japan) coated with 0.6 ml of EDTA solution by piercing the heart. It is appreciated that the ether is stressful but alternative anesthesia systems were not available and the duration of exposure was quite short (around 1 minute). Between 1.5-2.0 ml of blood were collected for each mouse and samples were obtained between 10:00 and 13:00 h (local time). The other ten mice of the group were sampled on the next day over the same time interval to reduce the impact of circadian rhythms. Subjects were killed as soon as the blood samples had been taken and the biochemical determinations wera carried out immediately on the fresh samples.

Quantification of Biochemical Parameters in Blood or Plasma: The Reflotron 2000 System (Boehringer Mannheim, Germany) has been designed for the quantitative determination of clinical chemistry parameters on whole blood, serum or plasma. It was used to measure three parameters, namely triglycerides and cholesterol in EDTA blood and HDLC in plasma. Plasma was separated from red blood cells using a centrifuge (Model EBA3S, Hettich, Germany) at around 3500 rpm for ten minutes.

The sample material was drawn up using the Reflotron pipette avoiding the inclusion of air. It was applied as a drop to the centre of the red application zone of the reagent carrier (strip) without allowing the pipette to touch the zone. The strip was inserted horizontally to the instrument. The concentration of the tested parameter was displayed in mg/dl on the screen of the instrument.

Statistical Analyses: Means and standard errors were calculated for all measures using the SPSS package for Windows version 6.0 (Copyright SPSS Inc., 1989-1993). General factorial ANOVA was completed by SPSS. The results were looked at starting with the interaction terms, as they may explain spurious main effects. Post hoc Duncan's tests were carried out using the SAS program (Proprietary Software 6.02 Copyright 1985, SAS Institute Inc., Cary, NC 27511, USA).

Results

The means and standard errors for the consumption values and the blood measures recorded in this study are provided separately.

Food Consumption: Table 1 shows the mean quantity of food consumed by mice of different ages and sexes given one of the five different diets for three or six weeks. General factorial ANOVA demonstrated significant effects for the diet ($DF = 4$ and 440, $F = 222.35$, $p<0.0001$), age ($DF = 1$ and 440, $F = 45.8$, $p<0.0001$), sex ($DF = 1$ and 440, $F = 210.95$, $p<0.0001$) and duration ($DF = 1$ and 440, $F = 68.66$, $p<0.0001$). The age x sex ($DF = 1$ and 440, $F = 5.97$, $p<0.02$), age x diet ($DF = 4$ and 440, $F = 8.94$, $p<0.0001$), sex x diet ($DF = 4$ and 440, $F = 2.99$, $p<0.02$), sex x duration ($DF = 1$ and 440, $F = 5.62$, $p<0.02$) and diet x duration ($DF = 4$ and 440, $F = 11.94$, $p<0.0001$) interactions were also all significant. There were also, age x sex x duration ($DF = 1$ and 440, $F = 12.04$, $p<0.0006$) and sex x diet x duration ($DF= 4$ and 440, $F = 2.94$, $p<0.02$) interactions.

Duncan's test revealed that mice given diet D (2 percent soya oil) consumed significantly ($p<0.05$)

Table 1: Mean (+SE) daily amounts (g) of different diets consumed by male and female mice of different ages exposed for variable periods of time (N=12)

Sex	Age	Duration (weeks)	Diet				
			A	B	C	D	Control
Male	Young	3	5.11±0.17	3.20±0.12	4.87±0.12	5.86±0.11	4.69±0.08
		6	5.26±0.07	4.42±0.16	5.58±0.12	6.27±0.90	5.18±0.12
	Adult	3	5.49±0.07	4.11±0.15	5.59±0.10	6.39±0.09	4.83±0.11
		6	5.28±0.11	5.01±0.20	5.87±0.13	6.43±0.08	5.20±0.16
Female	Young	3	4.94±0.11	3.26±0.16	7.71±0.08	5.42±0.16	4.15±0.08
		6	4.74±0.1 1	3.78±0.16	4.71±0.09	5.62±0.17	4.21±0.10
	Adult	3	4.66±0.13	3.80±0.10	4.90±0.12	4.93±0.08	4.44±0.22
		6	4.65±0.17	4.40±0.12	5.27±0.18	5.76±0.13	4.47±0.11

A = 8% saturated vegetable fat diet, B = 8% soya oil diet, C = 8% olive oil diet, D = 2% soya oil diet
Control = Local commercial diet

Table 2: Mean (±SE) body weight changes (g) of male and female mice of different ages given different diets for variable periods of time (N =12)

Sex	Age	Duration (weeks)	Diet				
			A	B	C	D	Control
Male	Young	3	13.3±0.18	7.7±1.06	12.5±0.86	14.6±1.1	11.6±1.42
		6	14.5±1.82	8.9±1.29	12.1-11.18	16.6±0.4	11.7±0.86
	Adult	3	-0.11±0.67	-0.8±0.34	1.4±0.68	1.93±0.4	-0.02±0.92
		6	-2.6±0.47	-1.67±0.5	-1.3±0.76	-0.72±0.74	-1.7±0.50
Female	Young	3	7.6±0.93	4.3±0.76	7.0±0.77	8.6±1.10	7.3±1.14
		6	10.9±0.59	8.4±1.18	12.1 t0.74	13.6±0.8	8.9±0.67
	Adult	3	0.45±0.52	0.3±0.42	0.55±0.56	0.68±0.4	-0.04±0.91
		6	-1.1±1.17	-0.81±0.42	-0.54±0.39	-0.86±0.43	-2.8±0.57

- = loss of weight A = 8% saturated vegetable fat diet, B = 8% soya oil diet, C = 8% olive oil diet, D = 2% soya oil diet, Control = Local commercial diet

Table 3: Mean (±SE) blood cholesterol values (mg/dl) in male and female mice of different ages given different diets for variable periods of time (N =12)

Sex	Age	Duration (weeks)	Diet				
			A	B	C	D	Control
Male	Young	3	118.6±2.5	116.9±4.9	115.0±2.9	110.4±1.9	109.8±2.1
		6	121.2±4.6	115.6±3.1	113.1±3.0	109.6±2.9	110.0±2.3
	Adult	3	115.0±4.0	112.3±2.1	120.8±5.3	111.4±2.2	107.9±21
		6	127.4±5.6	123.8±5.9	128.5±6.9	114.3±3.1	112.1±2.5
Female	Young	3	109.3±1.9	106.9±1.9	105.3±1.6	110.0±2.7	106.7±22
		6	106.9±2.8	113.7±2.9	102.3±1.3	102.9±2.2	103.9±1.8
	Adult	3	118.1±2.7	110.2±1.1	111.6±2.3	111.2±2.3	109.7±22
		6	116.5±3.3	108.4±2.1	107.8±2.6	108.3±2.8	107.6±2.5

A=8% saturated vegetable fat diet, B=8% soya oil diet, C=8% olive oil diet, D=2% soya oil diet
Control = Local commercial diet

Table 4: Mean (±SE) plasma HDLC values (mg/dl) in male and female mice of different ages given different diets for variable periods time (N = 12)

Sex	Age	Duration (weeks)	Diet				
			A	B	C	D	Control
Male	Young	3	89.2±3.08	83.5±6.23	66.8±8.19	63.3±7.55	52.1±4.40
		6	90.2±3.43	82.3±3.78	82.7±3.99	58.4±6.82	46.0±4.37
	Adult	3	86.8 t 4.71	62.6±4.94	75.2±5.61	73.5±3.98	59.7±4.40
		6	89.3±2.54	85.2±3.53	92.0±2.78	70.9±4.96	55.4±4.20
Female	Young	3	56.9±4.80	64.7±5.12	48.9 -±6.07	40.2±3.78	38.6±3.31
		6	66.4±3.16	75.7±4.38	54.3±3.48	42.0±3.66	29.2±2.35
	Adult	3	68.5±6.44	61.9±6.55	50.8±5.79	35.8±4.12	35.9±3.09
		6	70.1±3.37	49.3±4.77	61.9±6.4	37.3±4.84	35.2±2.89

A-8% saturated vegetable fat diet, B-8% soya oil diet, C-8% olive oil diet, D-2% soya oil diet
Control- local commercial diet

more than subjects fed on any other material. Mice fed diet C (8% olive oil) also consumed more (all $p<0.05$) than counterparts on diets A (8% saturated vegetable fat), B (8% soya oil) and control chow. Subjects fed on diet A consumed more (both $p<0.05$) food than those given diet B or control chow. Mice fed the control diet consumed more ($p<0.05$) food than subjects given diet B. It appears that the diet containing 2 percent soya oil is the most palatable diet whereas the 8 percent soya oil diet is the least palatable.

Table 5: Mean (±SE) blood triglyceride values (mg/dl) in male and female mice of different ages given different diets for variable periods of time (N = 12)

Sex	Age	Duration (weeks)	Diet				
			A	B	C	D	Control
Male	Young	3	104.8±4.7	95.2±4.7	100.5±4.4	095.7±2.3	099.3±3.5
		6	084.0±4.4	86.8±4.7	086.6±4.4	103.2±4.9	094.6±3.8
	Adult	3	100.0±6.6	95.3±4.0	100.1±3.7	100.6±4.5	092.9±8.0
		6	091.1±3.6	89.9±3.5	095.8±3.9	098.6±3.0	101.0±5.7
Female	Young	3	109.9±4.0	95.0±4.8	103.4±2.1	100.9±3.7	102.7±2.8
		6	084.7±2.7	82.3±3.7	080.9±-2.0	088.4±3.4	081.0±4.0
	Adult	3	103.7±4.4	90.9±3.8	104.3±4.4	098.0±3.1	089.7±3.7
		6	101.6±6.8	87.4±3.3	091.4±4.0	093.3±3.6	094.8±4.6

A-8% saturated vegetable fat diet, B-8% soya oil diet, C-8% olive oil diet, D-2% soya oil diet
Control-local commercial diet

Unremarkably, adults generally consumed more food than younger counterparts; males more food than females and animals in the categories exposed to particular diets longest increased their consumption (suggesting that the mice became attuned to the diets). On maturing, the increased intake of males was greater than in female counterparts. Increasing duration of exposure to the diet-produced a more dramatic augmentation in male than in female subjects. This effect was stronger in younger males than in adult males but in adult females rather than younger counterparts. These results are important as they stress that the intakes of the different diets are varied. They are influenced by the nature of the fat; the nature of the subjects as well as their familiarity with individual diets.

Body Weight Changes: Table 2 shows the mean of body weight changes (the gains or losses) in mice of different ages and sexes given one of 5 different diets for three and six weeks. General factorial ANOVA demonstrated significant effects for treatment (df = 4 and 440, F = 16.03, $p<0.0001$), age (df = 1 and 440, F = 1206.26, $p<0.0001$) and sex (df = 1 and 440, F = 35.44, $p<0.0001$). The age x sex (df = 1 and 440, F = 30.51, $p<0.0001$), age x treatment (df = 4 and 440, F = 6.32, $p<0.0001$), age x duration (df = 1 and 440, F = 52.86, $p<0.0001$) and duration x sex (df = 1 and 440, F = 8.55, $p<0.0001$) interactions were also all significant.

Duncan's test revealed that mice given diet D (2% soya oil) were significantly ($p<0.05$) heavier than all other categories. Where mice were fed on diets A (8% saturated vegetable fat) and C (8% olive oil) their body weight increases were significantly greater (all $p<0.05$) than counterparts given B (8% soya oil) or control chow. Subjectsfedon control diet were heavier ($p<0.05$) than animals fed on diet B.

Mice fed on the 2 percent soya oil diet (the most palatable) evidenced the greatest increase ($p<0.05$) in body weight. Mice on the 8 percent soya oil diet (the least palatable) produced a lower body weight gain than all counterparts ($p<0.05$). Males (who generally consumed more food than females) showed greater ($p<0.05$) increases in body weight. Although the adults consumed more food than juveniles did, the latter showed an increase in body weights whereas the adults generally showed a decline in body weights. Longer exposure to the diets produced a greater increase in the body weights of adult mice whereas the weights of younger subjects actually decreased. It thus appears likely that all mice in the growing phase show increases in body weight but that the rate is influenced by the fat content of the diet, palatability and the sex of the subjects. Mature animals (those who started treatment as adults or were maintained on the diets longer) showed more modest growth or even, in some cases, declines in weight. Again, there is clear evidence that the different diets had varied effects on these mice.

Blood Cholesterol: Table 3 shows mean blood cholesterol values in mice of different ages and sexes given one of five different diets for three or six weeks. General factorial ANOVA demonstrates significant effects of diet (DF = 4 and 440, F = 8.27, $p<0.0001$), age (DF = 1 and 440, F = 15.65, $p<0.0001$) and sex (DF = 1 and 440, F = 44.39, $p<0.0001$). There were also interactions between diet x sex (DF = 4 and 440, F = 3.11, $p<0.02$), age x duration (DF = 1 and 440, F = 4.08, $p<0.04$) and sex x duration (DF = 1 and 440, F = 7.37, $p<0.007$). There was also an age x sex x duration interaction (DF = 1 and 440, F = 5.74, $p<0.02$). Further analysis with Duncan's test revealed that mice given diet A (8% saturated vegetable fat) had significantly ($p<0.05$) higher blood cholesterol values than all other counterparts. Moreover, mice fed diets C (8% olive oil) and B (8% soya oil) had significantly ($p<0.05$) higher mean blood cholesterol values than mice given D (2% soya oil) or the control diets.

There was also evidence that older mice and males generated higher (both $p<0.05$) cholesterol values than their respective counterparts. The significant effects (all $p<0.05$) of the 8 percent saturated vegetable fat, 8 percent soya oil and 8 percent olive oil diets on cholesterol values are largely due to their impact on the adult male (see the age x sex x duration interaction). This finding is of considerable interest given the fact that this category presents the higher risk in terms of CHD in most human populations.

HDLC: Mean HDLC concentrations in mice of differing sex and ages maintained on different diets for varying periods of are shown in Table 4. General factorial ANOVA produced significant effects of diet (DF = 4 and 440, F = 64.87, $p<0.0001$), sex (DF = 1 and 440, F = 214.46, $p<0.0001$) and duration (DF = 1 and 440, F = 3.88, $p<0.049$). There were also significant age x diet (DF = 4 and 440, F = 4.92, $p<0.001$) and diet x duration (DF = 4 and 440, F = 3.81, $p<0.005$) interactions. Duncan's test revealed that mice given diet A (8% saturated vegetable fat) had significantly ($p<0.05$) higher mean HDLC values than all other counterparts. Mice fed on diets B (8% soya oil) and C (8% olive oil) had significantly (all $p<0.05$) higher mean HDLC

values than counterparts given diet D (2% soya oil) or control. Mice given diet D had significantly ($p<0.05$) higher mean HDLC values than subjects given control diet. HDLC levels were higher (both $p<0.05$) in males and in subjects exposed to diets for the longer period. In general, older animals and subjects exposed to the diets for 6 weeks showed greater elevations of HDLC except for mice fed on 8 percent soya oil where the younger animals showed significantly ($p<0.05$) higher values than their older counterparts.

Blood Triglycerides: Table 5 shows mean blood triglyceride values in mice of different ages and sexes given five different diets for three or six weeks. General factorial ANOVA demonstrated significant effects of diet ($DF = 4$ and 440, $F = 3.72$, $p<0.006$) and duration ($DF = 1$ and 440, $F = 38.18$, $p<0.0001$). There were also significant diet x duration ($DF = 4$ and 440, $F = 3.23$, $p<0.01$), sex x duration ($DF = 1$ and 440, $F = 4.96$, $p<0.03$) and age x duration ($DF = 1$ and 440, $F = 15.22$, $p<0.0001$) interactions.

Duncan's test revealed that mice given diets A (8% saturated vegetable fat), C (8% olive oil), or D (2% soya oil) had significantly (all $p<0.05$) higher mean blood triglyceride values than counterparts given diet B (8% soya oil).

Animals receiving a diet with 8 percent of soya oil had lower triglyceride levels than counterparts given diets containing 8 percent saturated vegetable fat, 8 percent olive oil or 2 percent soya oil. Although this essentially contradictory result appears difficult to explain (especially the effects of different concentrations of soya oil)-it may reflect changes in the uptake and metabolism of lipids from the diet. Animals on diet D (2% soya oil) had relatively low cholesterol and HDLC levels but these subjects also consumed more food than other categories (their intake of fats could actually be high). The 8% soya oil diet was the least preferred food item such that the mice probably took in less fat from this source.

Discussion

Variations in fatty acid content of the diet change tissue compositions in animals and humans. These substances have been also shown to affect several metabolic processes. These include metabolic rate (Friedman, 1990); variables relating to CHD (Berry *et al*., 1986; Flaten *et al*., 1990; Phillipson *et al*., 1985; Sanders and Roshanai, 1983) and breast, prostate, colon and lung cancers in humans (Kuller, 1997). This paper continues the tradition of looking for evidence of the impact of dietary factors on physiology by using an animal model. It attempts to be more wide-ranging than earlier. It also looks at the effects of age, sex and duration of treatment in some detail. The evidence generated from these studies is best sub-divided into sections.

Dietary fat content reportedly has little significant effect on daily energy expenditure (Liebel *et al*., 1992; Saltzman *et al*., 1997). Several types of epidemiological study have been employed to evaluate the relationship between dietary composition and body weight (Lissner and Heitmann, 1995). Lichtenstein *et al*. (1998) has pointed out, however, that exercise has an important effect on body weight gain. They concluded that the prevalence of human obesity has increased in several countries during that period when dietary fat intake (both in absolute terms and as a percentage of total dietary energy) has decreased. This must presumably reflect the adoption of a more sedentary life style in these world locations. Sex and age also reportedly affect an animal's energy intake (Prosser and DeVillez, 1991). For example, Bell and Zucker (1971) found that male rats in their home cages show a greater food intake than females.

In general, in the present studies, body weights were affected by total food intake, age, sex, duration of exposure to diets, the nature of caging and dietary fat composition. Food consumption clearly generally stimulated body weight gain. The only exception in the present studies was that the body weights of the mice fed on the 2 percent soya oil diet showed the lowest increase despite the animals consuming the highest quantities of food. The modest body weight gain in these subjects may be due to low fat content of that diet (which would reduce its calorific content). There were also sex and age differences in the present data. Male mice generally gained weight more quickly than did females. Age also had an impact as food intake in juveniles was positively related to weight gain but adult mice (especially males) tended to show an inverse relationship between food consumption and body weight. The fact that the juveniles are developing presumably accounts for this difference. Duration of dietary treatment is also critical. Longer exposures to dietary factors increase the body weights of adult mice whereas body weights of juvenile animals are decreased by prolonged exposure to the diets.

Diet and Blood Cholesterol Level: The present studies show that adult mice have higher levels of blood cholesterol than younger subjects do. This broadly agrees with the basal and net rates of lipolysis are generally unaffected by diet but increase with the age of the animal. Male mice had higher cholesterol levels than females especially when subjects were fed on the high fat diets (8% saturated vegetable fat, 8% soya oil or 8% olive oil). Cholesterol levels increased in young and male mice but declined in adult and female subjects after longer exposures to diets. In spite of the high quantity of food consumed by mice on the 2 percent soya oil diet, their blood levels of cholesterol were low in comparison with subjects on the other diets. The elevated levels of blood cholesterol obtained from the mice fed on saturated vegetable fat diet in the present data broadly support the findings of Bonanome and Grundy (1988) and the others. Despite their differences in food intake, using the 8 percent soya oil (low intake) and 8 percent olive oil (high intake) diets produced similar levels of blood cholesterol. This supports Grundy (1987) in and study specifically looking at olive oil. It was intriguing to find that the highest cholesterol levels were evident in mature males (reflecting the high-risk group in human populations).

Diet and Plasma HDLC Levels: In most human societies today, there is a well-known inverse correlation between CHD risk and HDLC levels (Castelli *et al*., 1986; Gordon *et al*., 1986; Miller, 1987). Paradoxically, however, diets high in saturated fat and cholesterol (which increase

arteriosclerosis risk) raise HDLC levels (Blum *et al*., 1977; Brinton *et al*., 1990; Gordon and Rifkind, 1989; Schaefer *et al*., 1981; Shepherd *et al*., 1978; Zanni *et al*., 1987). Polyunsaturated vegetable oils have been found to lower HDLC levels in some studies (Nestel *et al*., 1974; Shepherd *et al*., 1978). Other studies indicate that feeding with vegetable oil, does not affect plasma HDLC levels (Chait *et al*., 1974; Nestel *et al*., 1975). On the other hand, feeding fish oil has been found to have a slight elevating effect (Sanders and Roshanai, 1983) or no effect upon HDLC levels (Bronsgeest-Schoute *et al*., 1981; Harris *et al*., 1983). Hayek *et al*. (1993) explained that dietary fat increases HDLC levels by both increasing the transport rates and decreasing the fractional catabolic rates of HDLC esters and apolipoprotein (APO). As noted earlier, Catapano (1987) suggested that HDLC plays an essential role in "reverse cholesterol" transport. Polyunsaturated fat diets have been shown to decrease plasma HDLC levels (Mattson and Grundy, 1985; Sirtori *et al*., 1986). Kris-Etherton *et al*. (1993) showed that although linoleic acid (soya oil) has a more potent hypocholesterolemic effect than oleic acid (olive oil), it does not affect HDLC levels.

In the present studies, male mice had higher levels of HDLC than females. Such levels increased with longer exposure to the different diets (especially with the 8 percent olive oil diet that seems to contrast with the Kris-Etherton *et al*. (1993). Younger mice given the 8 percent soya oil diet showed significantly higher values on this measure than adults fed on the same diet. The HDLC level in plasma of mice was very significantly influenced by diet. Mice fed on the 2 percent soya oil, had lower values than counterparts given one of the high fat (8% saturated vegetable fat, 8% soya oil or 8% olive oil) diets. In addition, mice fed on the 8 percent soya or olive oil diets showed lower levels of HDLC than counterparts fed on the 8 percent saturated vegetable fat diet. This suggests either that low concentrations of dietary polyunsaturated fatty acids decrease the plasma HDLC level or that high concentrations increase it. How precisely this is achieved is highly speculative but the thesis consistently generated support for the view that mice could 'adjust' their patterns of lipids in response to particular fat-containing diets.

Diet and Blood Triglyceride Level: Several investigators (who have reached divergent conclusions) have studied the effects of polyunsaturated vegetable oils upon plasma triglyceride levels. Some workers have noted that vegetable oils decrease triglyceride levels i.e. are hypotriglyceridemic (Chait *et al*., 1974; Nestel *et al*., 1974; Shepherd *et al*., 1980). Others have found no effect (Connor *et al*., 1969; Harris *et al*., 1983; Nestel *et al*., 1973, 1975). Among those reporting a hypotriglyceridemic effect of vegetable oils, was a study in which normal and hypertriglyceridemic subjects were examined for a 10 day dietary period (Chait *et al*., 1974). In contrast, a study using much longer dietary periods (7-9 weeks), came to the conclusion that the vegetable oil had no effect upon plasma triglyceride levels. It is likely that the plasma lipids stabilize after several days, so there is only an acute effect of vegetable oils (see also section 6.4.). In two other studies (Nestel *et al*., 1974; Shepherd *et al*., 1980), plasma triglyceride levels were depressed by only 14-15 percent during vegetable oil feeding. This is in broad agreement with Khosla and Hayes (1992). They showed that triglyceride concentrations in Rhesus monkeys eating palm oil (rich in saturated 16:0) fatty acids) were respectively 34 and 63 percent higher than counterparts given high-oleic acid olive oil (mono 18:1 fatty acids) or high-linoleic acid safflower oil (polyunsaturated 18:2 fatty acids). Dagnelie *et al*. (1994) suggested that reduced lipolysis and increased hepatic-oxidation/ketogenesis might contribute to the reduced trig'yceride levels seen after 3 fatty acid supplementation in humans.

Theorell and Emlund (1993) have suggested that negative life changes increase whereas positive life changes decrease triglyceride levels in working men tested on four occasions in and single year. This also suggests a connection between these lipids and 'mood'. Diet had a highly significant effect on blood triglyceride concentrations in mice in the present study. A relative lowering effect on the level of triglyceride in blood was produced with higher concentration of soya oil diet but not with the lower one. In general, longer exposure to the diets decreased the blood triglyceride levels. However, the most significant decreases occurred with 8 percent saturated vegetable fat, 8 percent soya and olive oil diets, suggesting that high levels of fats decrease blood triglycerides after long dietary exposures.

In conclusion, the impact of diets on different parameters indicated that saturated vegetable fat diet raised all measured blood parameters. The 8 percent soya oil diet generated a moderate level of blood cholesterol but it also increased the plasma HDLC level and concomitantly decreased blood triglyceride levels. The 8 percent olive oil diet produced moderate levels of measured blood parameters. Despite the beneficial effect of the 2 percent soya oil diet in terms of lowering blood cholesterol levels, it also lowered 'good' HDLC and increased blood triglyceride levels, which would increase the risk of CDH in humans.

Many interesting developments could follow if more studies were conducted with different kinds and concentrations of fats. More detail concerning the chemistry of body fat and that of the brain may provide a clearer view of the metabolic mechanisms involved in responding to dietary change. Another approach would be to look at a range of 'doses' of different fats in adult animals so that graded responses were obtained (like and dose-response curve). In an ideal world, it would be advantageous to be able to use isocaloric diets, to ensure that the quantities consumed were identical and to be able to precisely relate intake to fat absorbed into the system. The interesting finding that adult

males show some of the greatest rises in cholesterol given exposure to certain diets (as seems to be the case in humans) also seems worthy of being followed up.

References

Bell, D.D. and I. Zucker, 1971. Sex differences in body weight and eating: Organization and activation by gonadal hormones in the rat. Physiol. Behav., 7: 27-34.

Benton, D., 1996. Food for Thought. Penguin Books Ltd., London.

Berry, E.M., J. Hirsch, J. Most, D.J. McNamara and J. Thornton, 1986. The relationship of dietary fat to plasma lipid levels as studied by factor analysis of adipose tissue fatty acid composition in a free-living population of middle-aged American men. Am. J. Clin. Nutr., 44: 220-231.

Bjerve, K.S., 1989. n-3 fatty acid deficiency in man. J. Internal Med., 225: 171-175.

Blum, C.B., R.I. Levy, S. Eisenberg, M. Hall, R.H. Goebel and M. Berman, 1977. High density lipoprotein metabolism in man. J. Clin. Invest., 60: 795-807.

Bonanome, A. and S.M. Grundy, 1988. Effect of dietary stearic acid on plasma cholesterol and lipoprotein levels. New Eng. J. Med., 318: 1244-1248.

Bourre, J.M., M. Bonneil, M. Clement, O. Dumont and G. Durand *et al*., 1993. Function of dietary polyunsaturated fatty acids in the nervous system. Prostaglandins Leukotrienes Essential Fatty Acids, 48: 5-15.

Brinton, E.A., S. Eisenberg and J.L. Breslow, 1990. A low-fat diet decreases High Density Lipoprotein (HDL) cholesterol levels by decreasing HDL apolipoprotein transport rates. J. Clin. Invest., 85: 144-151.

Bronsgeest-Schoute, H.C., C.M. van Gent, J.B. Luten and A. Ruiter, 1981. The effect of various intakes of omega 3 fatty acids on the blood lipid composition in healthy human subjects. Am. J. Clin. Nutr., 34: 1752-1757.

Burr, G.O. and M.M. Burr, 1929. A new deficiency disease produced by the rigid exclusion of fat from the diet. J. Biol. Chem., 82: 345-367.

Castelli, W.P., R.J. Garrison, P.W. Wilson, R.D. Abbott, S. Kalousdian and W.B. Kannel, 1986. Incidence of coronary heart disease and lipoprotein cholesterol levels. The framingham study. J. Am. Med. Assoc., 256: 2835-2838.

Catapano, A.L., 1987. Binding sites for HDL and HDL catabolism. Atherosclerosis Rev., 16: 109-120.

Chait, A., A. Onitiri, A. Nicoll, E. Rabaya, J. Davies and B. Lewis, 1974. Reduction of serum triglyceride levels by polyunsaturated fat Studies on the mode of action and on very low density lipoprotein composition. Atherosclerosis, 20: 347-364.

Connor, W.E., D.T. Witiak, D.B. Stone and M.L. Armstrong, 1969. Cholesterol balance and fecal neutral steroid and bile acid excretion in normal men fed dietary fats of different fatty acid composition. J. Clin. Invest., 48: 1363-1375.

Dagnelie, P.C., T. Rietveld, G.R. Swart and T. Stijnen, 1994. Effect of dietary fish oil on blood levels of free fatty acids, ketone bodies and triacylglycerol in humans. Lipids, 29: 41-45.

Engelberg, H., 1992. Low serum cholesterol and suicide. Lancet, 339: 727-729.

Flaten, H., A. Hostmark, P. Kierulf, E. Lystad, K. Trygg, T. Bjerkedal and A. Osland, 1990. Fish-oil concentrate: Effects on variables related to cardiovascular disease. Am. J. Clin. Nutr., 52: 300-306.

Friedman, M.I., 1990. Body fat and the metabolic control of food intake. Int. J. Obes., 3: 53-67.

Gordon, D.J. and B.M. Rifkind, 1989. High-density lipoprotein-the clinical implications of recent studies. New Engl. J. Med., 321: 1311-1316.

Gordon, D.J., J. Knoke, J.L. Probstfield, R. Superko and H.A. Tyroler, 1986. High-density lipoprotein cholesterol and coronary heart disease in hypercholesterolemic men: The lipid research clinics coronary primary prevention trial. Circulation, 74: 1217-1225.

Grundy, S.M., 1986. Comparison of monounsaturated fatty acids and carbohydrates for lowering plasma cholesterol. New Engl. J. Med., 314: 745-748.

Grundy, S.M., 1987. Monounsaturated fatty acids, plasma cholesterol and coronary heart disease. Am. J. Clin. Nutr., 45: 1168-1175.

Grundy, S.M. and M.A. Denke, 1990. Review-Dietary influences on serum lipids and lipoproteins. J. Lipid Res., 31: 1149-1172.

Harris, W.S., W.E. Connor and M.P. McMurry, 1983. The comparative reductions of the plasma lipids and lipoproteins by dietary polyunsaturated fats: Salmon oil versus vegetable oils. Metabolism, 32: 179-184.

Hayek, T., Y. Ito, N. Azrolan, R.B. Verdery, K. Aalto-Setala, A. Walsh and J.L. Breslow, 1993. Dietary fat increases high density lipoprotein (HDL) levels both by increasing the transport rates and decreasing the fractional catabolic rates of HDL cholesterol ester and apolipoprotein (Apo) A-I. Presentation of a new animal model and mechanistic studies in human Apo A-I transgenic and control mice. J. Clin. Invest., 91: 1665-1671.

Hayes, K.C., A. Pronezuk, S. Lindsey and D. Diersen-Schade, 1991. Dietary saturated fatty acids (12:0, 14:0, 16:0) differ in their impact on plasma cholesterol and lipoproteins in nonhuman primates. Am. J. Clin. Nutr., 53: 491-498.

Hegsted, D.M., R.B. McGandy, M.L. Myers and F.J. Stare, 1965. Quantitative effects of dietary fat on serum cholesterol in man. Am. J. Clin. Nutr., 17: 281-295.

Keys, A., J.T. Anderson and F. Grande, 1965. Serum cholesterol response to changes in the diet: IV. Particular saturated fatty acids in the diet. Metabolism, 14: 776-787.

Khosla, P. and K.C. Hayes, 1991. Dietary fat saturation in rhesus monkey affects LDL concentrations by modulating the independent production of LDL apolipoprotein B. Biochim. Biophys. Acta (BBA)-Lipids Lipid Metab., 1083: 46-56.

Kholsa, D. and K.C. Hayes, 1992. Comparison between the effects of dietary saturated (16:0), monounsaturated (18:1) and polyunsaturated (18:2) fatty acids on plasma lipoprotein metabolism in cebus and rhesus monkeys fed cholesterol-free diets. Am. J. Clin. Nutr., 55: 51-62.

Kris-Etherton, P.M., J. Derr, D.C. Mitchell, V.A. Mustad and M.E. Russell *et al*., 1993. The role of fatty acid saturation on plasma lipids, lipoproteins and apolipoproteins: I. Effects of whole food diets high in cocoa butter, olive oil, soybean oil, dairy butter and milk chocolate on the plasma lipids of young men. Metabolism, 42: 121-129.

Kuller, L.H., 1997. Dietary fat and chronic diseases: Epidemioloic overview. J. Am. Diet. Assoc., 97: S9-S15.

LaRosa, J.C., 1997. Cholesterol Lowering, Low Cholesterol and Non-Cardiovascular Disease. In: Lipids, Health and Behavior, Hillbrand, M. and R.T. Spitz (Eds.). American Psychological Association, Washington, DC., pp: 275-296.

Lichtenstein, A.H., E. Kennedy, P. Barrier, D. Danford and N.D. Ernst *et al*., 1998. Dietary fat consumption and health. Nutr. Rev., 56: 3-19.

Liebel, R.L., J. Hirsch, B.E. Appel and G.C. Checani, 1992. Energy intake required to maintain body weight is not affected by wide variation in diet composition. Am. J. Clin. Nutr., 55: 350-355.

Lissner, L. and B.L. Heitman, 1995. Dietary fat and obesity: Evidence from epidemiology. Eur. J. Clin. Nutr., 49: 79-90.

Mattson, F.H. and S.M. Grundy, 1985. Comparison of effects of dietary saturated, monounsaturated and polyunsaturated fatty acids on plasma lipids and lipoproteins in man. J. Lipid Res., 26: 194-202.

Miller, N.E., 1987. Associations of high-density lipoprotein subclasses and apolipoproteins with ischemic heart disease and coronary atherosclerosis. Am. Heart J., 113: 589-597.

Muldoon, M.F., S.B. Manuck and K.A. Matthews, 1990. Lowering cholesterol concentrations and mortality: A quantitative review of primary prevention trials. Br. Med. J., 301: 309-314.

Muldoon, M.F., J.R. Kaplan, S.B. Manuck and J.J. Mann, 1992. Effects of a low-fat diet on brain serotonergic responsivity in cynomolgus monkeys. Biol. Psychiatry, 31: 739-742.

Muldoon, M.F., J.E. Rossouw, S.B. Manuck, C.J. Glueck, J.R. Kaplan and P.G. Kaufmann, 1993. Low or lowered cholesterol and risk of death from suicide and trauma. Metabolism, 42: 45-56.

Nestel, P.J., N. Havenstein, H.M. Whyte, T.J. Scott and L.J. Cook, 1973. Lowering of plasma cholesterol and enhanced sterol excretion with the consumption of polyunsaturated ruminant fats. New Engl. J. Med., 288: 379-382.

Nestel, P.J., N. Havenstein, T.W. Scott and L.J. Cook, 1974. Polyunsaturated ruminant fats and cholesterol metabolism in man. Intern. Med. J., 4: 497-501.

Nestel, P.J., N. Havenstein, Y. Homma, T.W. Scott and L.J. Cook, 1975. Increased sterol excretion with polyunsaturated-fat high-cholesterol diets. Metabolism, 24: 189-198.

Phillipson, B.E., D.W. Rothrock, W.E. Connor, W.S. Harris and D.R. Illingworth, 1985. Reduction of plasma lipids, lipoproteins and apoproteins by dietary fish oils in patients with hypertriglyceridemia. N. Engl. J. Med., 312: 1210-1216.

Prosser, C.L. and E.J. DeVillez, 1991. Feeding and Digestion. In: Environmental and Metabolic Animal Physiology, Prosser, C.L. (Ed.). Willey-Liss Inc., New York, pp: 205-229.

Saltzman, E., G.E. Dallal and S.B. Roberts, 1997. Effect of high-fat and low-fat diets on voluntary energy intake and substrate oxidation: Studies in identical twins consuming diets matched for energy density, fiber and palatability. Am. J. Clin. Nutr., 66: 1332-1339.

Sanders, T.A.B. and F. Roshanai, 1983. The influence of different types of ω3 polyunsaturated fatty acids on blood lipids and platelet function in healthy volunteers. Clin. Sci., 64: 91-99.

Schaefer, E.J., R.I. Levy, N.D. Ernst, F.D. van Sant and H.B. Brewer, 1981. The effects of low cholesterol, high polyunsaturated fat and low fat diets on plasma lipid and lipoprotein cholesterol levels in normal and hypercholesterolemic subjects. Am. J. Clin. Nutr., 34: 1758-1763.

Shepherd, J., C.J. Packard, J.R. Patsch, A.M. Gotto Jr. and O.D. Taunton, 1978. Effects of dietary polyunsaturated and saturated fat on the properties of high density lipoproteins and the metabolism of apolipoprotein A-I. J. Clin. Invest., 61: 1582-1592.

Shepherd, J., C.J. Packard, S.M. Grundy, D. Yeshurun, A.M. Gotto and O.D. Taunton, 1980. Effects of saturated and polyunsaturated fat diets on the chemical composition and metabolism of low density lipoproteins in man. J. Lipid Res., 21: 91-99.

Sirtori, C.R., E. Tremoli, E. Gatti, G. Montanari and M. Sirtori *et al*., 1986. Controlled evaluation of fat intake in the Mediterranean diet: Comparative activities of olive oil and corn oil on plasma lipids and platelets in high-risk patients. Am. J. Clin. Nutr., 44: 635-642.

Theorell, T. and N. Emlund, 1993. On physiological effects of positive and negative life changes-a longitudinal study. J. Psychosomatic Res., 37: 653-659.

Thomasson, H.J., J. de Boer and H. de Iongh, 1967. Influence of dietary fats on plasma lipids. Pathobiology, 30: 629-647.

Yaquoob, P., E.J. Shirington, N. Jeffery, P. Sanderson, D.J. Harvey, E. Newsholme and P.C. Calder, 1995. Comparison of the effects of a range of dietary lipids upon serum and tissue lipid composition in the rat. Int. J. Biochem. Cell Biol., 27: 297-310.

Zanni, E.E., V.I. Zannis, C.B. Blum, P.N. Herbert and J.L. Breslow, 1987. Effect of egg cholesterol and dietary fats on plasma lipids, lipoproteins and apoproteins of normal women consuming natural diets. J. Lipid Res., 28: 518-527.