EFFECTS OF DIETARY FAT AND CARBOHYDRATE ON WEIGHT GAIN AND SERUM LIPIDS IN RATS

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INTRODUCTION

In the United States (U.S.) 205.3 out of 100,000 persons died of coronary heart disease (CHD) in 1980 (1). Thirty-five percent of all deaths for that year were attributed to CHD (1, 2). Wide publicity on the management of CHD contributed to a decline in mortality rates from CHD from 1960 to 1979. However, it still remains the number one killer in the U.S. and the mortality rates rank the second highest in the world. Investigation into the etiology of the disease remains an important area for research.

Several dietary and non-dietary factors have been associated with CHD. The major non-dietary risk factors include elevated serum lipids, particularly cholesterol and triglycerides, obesity, hypertension, cigarette smoking and elevated blood sugar, while the dietary risk factors are high levels of consumption of saturated fat, cholesterol and sucrose (3, 4).

The pathology of CHD is atherosclerosis of the major arteries. These atherosclerotic lesions contain large amounts of fatty material, a large portion of which is cholesterol (3, 4). Cholesterol was discovered in 1812 by Michael Eugene of France, but it was not until about 1910 that Adolf Windaus found that the atheromatous aorta contained as much as twenty times the amount of cholesterol and cholesterol esters as found in the normal aorta (5). This finding led to concern about the relationship between cholesterol concentration in the serum and diet and CHD. Progressive research into the etiology of the disease has led to the hypothesis that a decrease in the level of saturated fat and cholesterol in the diet would lead to a decrease in the level of cholesterol in the serum and thus the incidence of heart disease. The relationship of dietary fat and

cholesterol to serum cholesterol levels and to atherosclerosis has been coined by some as the "lipid hypothesis" or the "fat hypothesis," though a direct link has not been established (3, 5).

McGill's literature review (5) of epidemiological evidence into the relationship of dietary cholesterol and fat to atherosclerosis in man showed that during World War II both the consumption of fat and the incidence of atherosclerosis decreased. Other factors such as change in life styles, increased consumption of fiber and a decrease in caloric intake also could have been related to this decline. Population studies of vegetarians and of people who move from areas of low risk to areas of high risk where the consumption of fat is higher, do show a relationship between atherosclerosis and dietary fat and cholesterol consumption (4, 5). The relationship of dietary fat to CHD and evidence that increased dietary polyunsaturated fat decreases serum cholesterol levels (5-7) prompted the Goals Committee of the U.S. Senate Select Committee on Nutrition and Human Needs (8) to propose a modification of the dietary patterns for the U.S. population. One of the proposed goals was aimed at a reduction in the level of fat in the diet from its present level of 40% of the total calories (based on the 1977 Food Consumption Survey) to a proposed level of 30%. Included in this change was an increase in the level of polyunsaturated fat in the diet from its level of 5% to 10% of the total calories and an increase in the P:S ratio from 0.4 to 1.0.

This proposed change in fat intake will result in a proportionate increase in the level of carbohydrate (both complex and simple) in the diet. Some researchers have become concerned about the effect an increase in the consumption of sugar will have on the incidence of CHD, in view of the hyperlipidemic effect of sugars which has been known for some time

(9, 10). The long term effect of dietary sugars on serum lipids have been called by some the "carbohydrate induced hyperlipidemia" (9, 11). Today there is an increasing trend in the consumption of fructose as high-fructose corn syrups and in reduced calorie foods. Some researchers (12) have indicated that it is the fructose component of the sucrose molecule that is hyperlipidemic.

This study was carried out with rats to investigate the impact on weight gain, serum lipids and epididymal fat pad weight of:

a) Modification of a dietary fat from 40% (P:S, 0.4) to 30% (P:S, 1.0) of the calories.

b) Substitution of fructose for sucrose in the diets.

REVIEW OF LITERATURE

LIPOPROTEINS AND CORONARY HEART DISEASE (CHD)

Lipoproteins are carriers of insoluble lipids including cholesterol and triglycerides in the blood (3, 4, 13). Since studies like the one conducted by Glynn et al. (14) have indicated that longitudinal changes in residual cholesterol and triglyceride levels in man are strong predictors of CHD, a study of the nature and properties of lipoproteins is important in understanding their role in the development of atherosclerosis. Macheboeuf first described the lipoproteins in 1929, and in the past decade much has been learned about their structure, properties and synthesis in the body (13).

Lipoproteins are classified according to their densities (15-20). Chylomicrons are the largest and lightest, they contain 80-95% exogenous triglyceride, 2 to 7% cholesterol, 3 to 6% phospholipid and 1 to 2% protein. Very low density lipoprotein (VLDL) contain 60 to 80% endogenous triglycerides and 6 to 12% cholesterol in the body. Low density lipoprotein (LDL) are the main carriers of cholesterol in the body, they contain 50 to 75% cholesterol and 25% protein. High density lipoproteins (HDL) are protective as they transport cholesterol away from the tissues, they contain 45 to 50% protein, 30% phospholipid, 20% cholesterol and 5% triglycerides.

Hyperlipoproteinemia

Hyperlipoproteinemia is a condition characterized by elevated serum lipids and is classified into five types depending on the type of lipid that is elevated in the serum (15, 19). Type I is characterized by elevated chylomicron levels; type IIa, elevated LDL levels, type IIb, elevated

LDL and VLDL levels; type III, intermediate low density lipoprotein (ILDL) levels; type IV, elevated VLDL levels and type V, elevated chylomicron and VLDL levels. The hyperlipoproteinemic conditions are generally familialy transmitted either as autosomal dominant or recessive traits, except for type IV which is brought about largely by the "American way of life" diet; though it can be transmitted as a Mendelian dominant trait.

High density lipoprotein (HDL)

Negative correlation between HDL levels and CHD as observed from findings of the Lipid Research Clinics (LRC) Program Prevalence Study aroused interest in the study of HDL (20). Using ultracentrifugation techniques HDL separates into two main fractions HDL_2, which contains 60% lipid and 40% protein and HDL_3, which contains 45% lipid and 55% protein (20-22). HDL_1 is precipitated by heparin and manganese.

High density lipoprotein is spherical in shape (20). The two main apolipoprotein (apo) components make up 90% of the protein, apo A-I and apo A-II and are in the ratio 3:1. An important property of this apolipoprotein is its high affinity for lipids. Apo A-I can bind phospholipid as much as 2.5 times its own weight. Apolipoprotein A solubilizes cholesterol by forming a lecithin-cholesterol-apo complex. Apolipoprotein C (C-I, C-II, C-III) make up 5% of the HDL particle and is the major protein found in VLDL.

Several precurser particles to HDL are secreted by the liver and intestine, while catabolism of HDL is believed to occur in the kidney and liver lysosomes (20, 21). In normal subjects, plasma HDL has a half life of four days (20). The rate of catabolism is increased with high carbohydrate diets (20) and these diets are associated with lower HDL levels (10).

What functions HDL performs in the body are not completely understood but its levels in plasma are increased by moderate alcohol consumption, female sex hormones (estrogens) and physical activity (10, 20, 21, 23-25).

Lipid transport

Blood lipids originate from two sources, the exogenous lipids are from dietary sources and the endogenous lipids are synthesized by the body (26). Fatty acids from dietary fat and cholesterol are reesterified in the endoplasmic reticulum of the mucosal cells lining the small intestine to form non-polar triglycerides and cholesterol esters. These are then packed and concentrated in the secretory vesicles of the golgi apparatus with intestinal apo B-48, several apo A and polar lipids which form a monomolecular film around the non-polar lipids. These chylomicrons then leave the mucosal cells in this form; apo B-48 is a vital factor for its release. They are then transported through the thoracic duct into the blood via the lacteals in the intestinal villi. The chylomicrons acquire additional apolipoproteins, mainly E and C from HDL during its transport in the blood. Triglycerides are hydrolyzed from these modified chylomicrons by lipoprotein lipase of the endothelial surface of blood capillaries in extrahepatic tissue. The apolipoproteins are transformed to HDL while the chylomicron "remnants" are taken up by hepatic parenchymal cells and transported to the bile canaliculus. It is here that lysosomal catabolism releases cholesterol which is either excreted in the bile or incorporated with hepatogenous lipoprotein. Through this efficient process, blood cholesterol levels under normal conditions are not affected by a cholesterol rich meal.

Excess triglycerides synthesized by the liver are transported by VLDL to extrahepatic tissues to prevent steatosis. The LDL pathway provides

cholesterol needed for synthesis of steroid hormones and membrane synthesis. These cholesterol esters, including those found in HDL and VLDL are produced by the action of lecithin cholesterol acyl transferase (LCAT), secreted by the liver.

Lipoprotein profile as determined by age, sex and hormones

Several surveys have been carried out in recent years to determine the effect of age, sex and hormones on serum cholesterol and triglyceride levels and their distribution in the various lipoprotein fractions. The LRC Program Prevalence Study in 13 locations in the U.S., Canada, Israel and the Union of Soviet Socialist Republic (U.S.S.R.) is among the best known of these surveys conducted in recent years (27-29).

In North American white populations from ages 20 to 50 years, total plasma cholesterol and triglycerides were higher in males than in females (27, 29, 30) (figure 1). These levels reached their peak for males around age 45 and then gradually fell. In females, these levels increased with age; plasma cholesterol levels exceeded those for males after age 50. Women who took exogenous sex hormones had higher plasma cholesterol and triglyceride levels than those who did not take hormones. However levels for hormone users did not increase as sharply with age as those in non-hormone users. Cholesterol levels were lower in hormone users than in nonhormone users after age 50. With the various lipoproteins, HDL cholesterol levels were highest in female hormone users and lowest for males from ages 30 to 60 years (figure 2). The LDL-cholesterol levels showed a similar pattern to total cholesterol levels, female hormone users, had on an average, lower values than males or females not on hormones. The cholesterol content of VLDL, LDL and HDL increased in female hormone users from ages 20 to 60 years. It is not surprising that

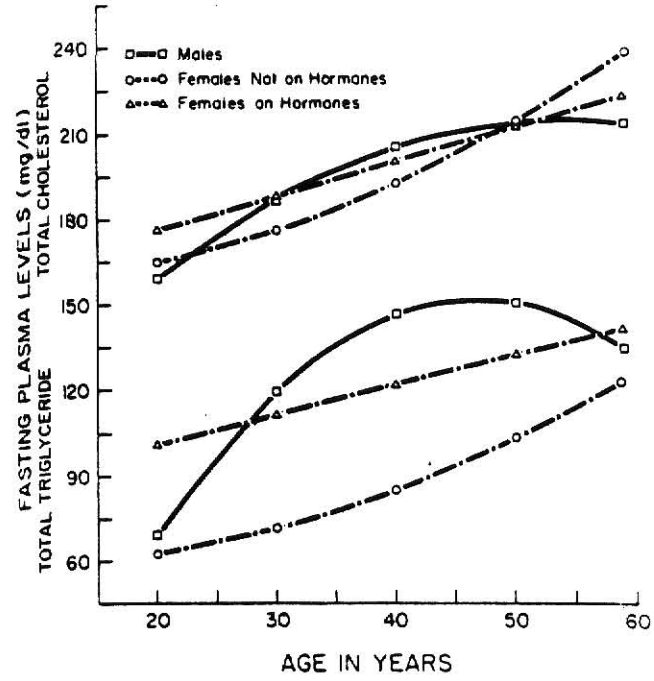

Fig. 1 Regression estimates of mean plasma lipid values by age for males, females not taking sex hormones and females taking sex hormone preparations. Lipid Research Clinics Prevalence Study, Visit 2, random sample (27).

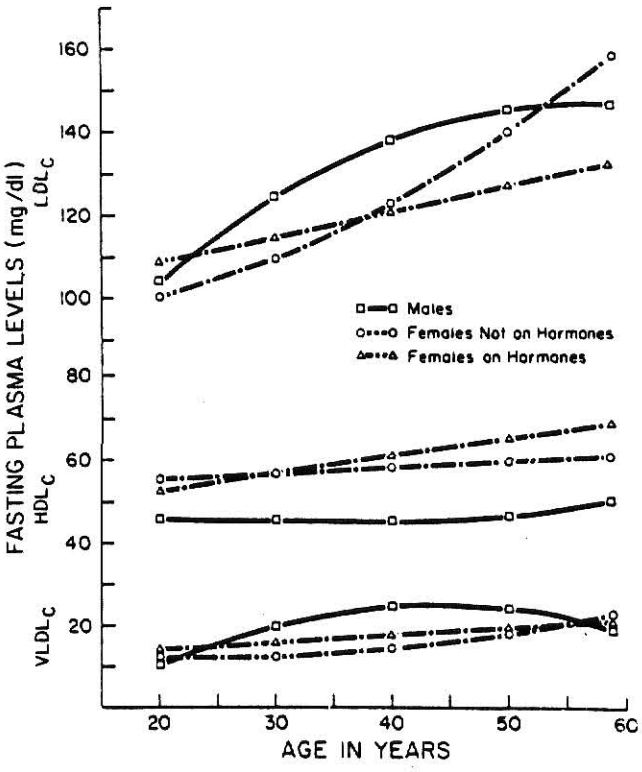

Fig. 2 Regression estimates of mean plasma lipoprotein-cholesterol values by age for males, females not taking sex hormones and females on sex hormone preparations. Lipid Research Clinics Prevalence Study, Visit 2, random sample (27).

men around 40 to 45 years are most prone to CHD, as plasma cholesterol levels in the LDL and VLDL fractions are the highest and in the HDL fraction are the lowest, and triglyceride levels are also the highest at this age.

Evidence shows that female hormones (estrogen in particular) are protective against CHD as they increased HDL cholesterol concentrations while, in contrast, the androgens, or male hormones, decreased HDL cholesterol levels (27, 28, 30). Plasma HDL cholesterol concentrations in children and young adults show further evidence of the effect of sex hormones (28). In 6 to 10 year old children, HDL cholesterol levels were higher in males than in females (figure 3). From ages 11 to 17, these levels declined sharply in males while there was a gradual increase in levels in females. Total plasma cholesterol level fell between 10 to 16 years in males and females, after which it gradually increased and continued to increase with age.

DIET, BLOOD LIPIDS AND CORONARY HEART DISEASE (CHD)

Cholesterol

Studies on free-living people fail to show convincing evidence of the relationship between dietary cholesterol intakes and serum cholesterol levels. Interest in the possible role of dietary cholesterol in the etiology of CHD was aroused by reports in the 50's which revealed significant relationships between the decline in mortality rates from CHD in Western Europe during the world wars and fall in the intake of dietary cholesterol (5). However this decline could have been as a result of several other factors such as a decrease in the intake of saturated fat and sugars. Keys et al. (31), Paul et al. (32) and Nicholas et al. (33) in

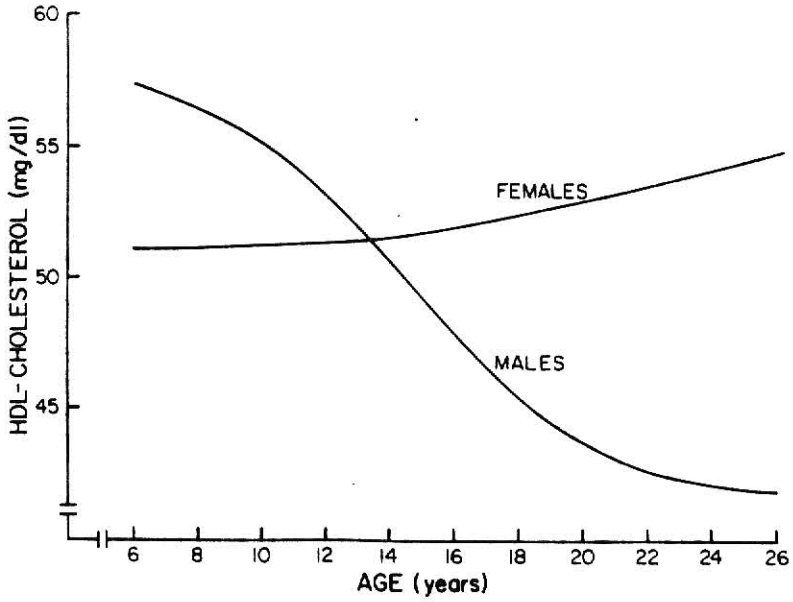

Fig. 3 The relationship of mean high-density lipoprotein (HDL) cholesterol and age estimated by a nonlinear model. Source: Lipid Research Clinics, Visit 2, survey examination (28).

their population studies of Americans found no relationship between dietary cholesterol and serum cholesterol concentrations.

High cholesterol foods are also high in saturated fats, except for liver and eggs. Those cholesterol rich, highly saturated fat foods are generally diets of the affluent populations who lead largely sedentary lives and have other characteristics such as obesity, diabetes mellitus and cigarette smoking (5).

Several well controlled human studies have indicated that dietary cholesterol does elevate serum cholesterol levels (11, 34, 35). The National Diet Heart study indicated that the average American diet, which provides 2,400 kcal and 600 mg cholesterol a day could account for 14 mg/dl of the total serum cholesterol concentration (35). Mattson et al. (36) fed 56 males formula-type diets with dietary fat composition similar to that in the American diet (40% of calories and a P:S of 0.3) and four levels of cholesterol (0, 106, 212 or 317 mg/1000 kcal) for 42 days. They concluded that the American diet with 317 mg cholesterol per 1000 kcal contributed 40 mg/dl to the total serum cholesterol concentration.

Recent investigations into the pathogenesis of atherosclerosis revealed that the absolute level of serum cholesterol may not be as important as how cholesterol is transported and by which lipoprotein. Diet induced hypercholesterolemia produced by high cholesterol diets increased the level of HDL with apo E (37). These normally represent 5 to 10% of the total serum HDL, have a lower density than HDL without apo E and resemble LDL by their interaction with apo B and E receptor sites.

Fat

A review by Kritchevsky (38, 39) on the relationship of diet to heart disease indicated that trends in fat consumption do not reflect the

incidence of CHD in America. Between 1909 and 1974, fat in the diet increased from 125 to 157 g/person/day, the ratio of animal fat to vegetable fat fell from 4.9 to 1.6 and the P:S ratio rose from 0.21 to 0.43. From 1940 to 1960, when the incidence of CHD increased, dietary fat increased from 135 to 143 g/person/day and the animal to vegetable fat ratio fell from 2.85 to 2.41. Since 1970 available fat has increased to 160 g/person/day while mortality rates from CHD have decreased.

The effect of dietary fat on plasma cholesterol levels depends on the nature of fat consumed. Fats are divided into three major groups depending on the degree of unsaturation (11). Saturated fats can be synthesized by the body from acetate and are not essential, they are hypercholesterolemic and increase the concentration of LDL. Monounsaturated fats have one double bond and generally have no effect on serum lipids. Oleic acid is the most common fat in this group; it has one double bond at W-9. Polyunsaturated fats (PUFA) are important constituents of cell membranes and are prostaglandin precursers. Linoleic acid (W-6) is considered essential in the diet as it cannot be synthesized by the body. The PUFA that are of dietary importance are with double bonds at W-3 and W-6.

Grundy and Ahrens (40) and Goodnight et al. (41) hypothesized that PUFA exerts its hypocholesterolemic action by either: a) decreasing endogenous cholesterol synthesis, b) decreasing cholesterol absorption, c) increasing fecal steroid excretion, or d) redistributing circulating cholesterol between the plasma and tissue pools. Jackson et al. (7) said that there was no significant research evidence to show that PUFA reduced cholesterol absorption or synthesis. Moore et al. (42) and Connor et al. (43) used diets containing saturated or unsaturated fats comprising 40% of the calories to show increased fecal steroid excretion in their human

subjects. Nestel et al. (44) observed greater endogenous excretion of cholesterol by male subjects fed a high PUFA diet. Grundy and Ahrens (40) fed hyperlipoproteinemic subjects liquid formula diets with 40% of the calories from fat. Their subjects failed to show an increased excretion of steroids. They hypothesized that the fall in serum cholesterol levels of subjects fed the high PUFA diet was a result of a transfer of cholesterol from serum to tissues.

Alterations in plasma lipoprotein composition has been suggested as another possible mechanism by which PUFA exerts its hypocholesterolemic action (41, 45). High PUFA diets cause a significant decrease in the palmitate and stearate content of HDL triglycerides, phospholipids and cholesterol esters and an increase in the linoleate content (7, 45). These changes bring about increased fluidity of the lipoprotein which may account for the hypocholesterolemic effect of PUFA. Alterations in lipoprotein composition leads to changes in membrane composition increasing membrane fluidity; this could affect the rate of LDL uptake by altering LDL receptors (7).

Research has convincingly shown that a high PUFA diet decreases total serum cholesterol levels but its role in decreasing triglyceride levels are conflicting (46-53). Birchwood et al. (46) studied hyperlipoproteinemic patients in part 2 of a three part study conducted at the University of Toronto. In part 2 of the study with dietary fat at 35% of the calories, (P:S, 1.4) and sucrose or starch at 40% of the calories, serum cholesterol and triglyceride levels tended to fall. The decrease in serum lipids were similar when comparing starch and sucrose diets, and the final lipid levels were also. In part 1 of the study, Little et al. (47) observed that in type II patients fed diets with 20% of the calories as sucrose or starch

and PUFA at 33% of the calories serum cholesterol levels fell ($P < 0.05$) while serum triglyceride levels were not significantly altered. In type III and IV patients fed 14% starch or sucrose diets and PUFA at 36% of the calories, serum cholesterol levels were similar while triglyceride levels were higher on the sucrose diet. In type V patients fed 54% sucrose or 56.5% starch diets and with PUFA at 2.8% or 2.5% of the calories, inconsistent differences in serum cholesterol levels were observed, although triglyceride levels were higher on the sucrose diet.

To further support the hypothesis of a hypolipidemic effect of a high PUFA diet, particularly when the level of sucrose in the diet is high, Mann et al. (53) fed 9 male volunteers three dietary regimes for 2 weeks each. Diet 1 was patterned after the "western" diet with carbohydrate at 54% of the calories and fat at 30%. In diet 2, the level of sucrose in diet 1 was increased from 17 to 34% while the level of fat remained the same. In diet 3, sucrose was kept at 34% while PUFA was increased to 75% of the total fat. Increases ($P < 0.05$) were observed in serum cholesterol and triglyceride levels with diet 2, and cholesterol and triglyceride levels were lower ($P < 0.01$) with diet 3 than those observed with diet 2.

Harris et al. (50) and Engleberg (51) indicated that vegetable oils which predominantly contain W-6 fatty acids are effective only in decreasing serum cholesterol levels, while the fish oils with W-3 fatty acids decrease triglyceride levels as well. Engleberg (51) used fish liver oil to demonstrate the hypotriglyceridemic effect of a high PUFA diet in humans. A literature review by Goodnight et al. (41) concluded that studies concerned with the effects of W-3 PUFA on plasma lipids have consistently shown the ability of those fats to decrease plasma cholesterol, triglyceride and VLDL levels, while there was little evidence to prove the

hypotriglyceridemic effects of vegetable oils. Harris et al. (50) have shown that this hypotriglyceridemic mechanism was not due to a difference in the degree of unsaturation of the fats. The principal fish oils contain, on an average, 5.5 double bonds per molecule which is 2.75 times the unsaturation of the W-6 fats. Harris et al. (50) hypothesized that the hypotriglyceridemic property of the W-3 fatty acids could relate to their structure or the presence of high amounts of C_{20} and C_{22} fatty acids.

Bruckdorfer et al. (54) did not find a hypocholesterolemic effect of a high PUFA diet. When Sprague Dawley rats were fed diets with 50% (by weight) starch or sucrose and with arachis oil at 15% or hydrogenated coconut oil at 20% for 150 days there was no significant differences in plasma cholesterol levels while there was a difference ($P < 0.01$) in plasma triglyceride levels between sucrose and starch, and saturated fat and unsaturated fat in the diets.

The ability of dietary PUFA to decrease serum cholesterol levels is dependent on its ratio to saturated fat in the diet. To demonstrate the hypocholesterolemic action of a high PUFA diet, Chait et al. (55) fed hypolipidemic and normal men diets high in saturated fat (P:S, 0.2) and high in PUFA (P:S, 2.4) for 20 days; fat in these diets provided 40% of the calories and carbohydrate 45%. They observed a 16% reduction ($P < 0.001$) in serum cholesterol levels with the high PUFA diet, as well as a 35% reduction ($P < 0.001$) in triglyceride levels. Vessby et al. (56) also observed a reduction ($P < 0.05$) in cholesterol lipoprotein carriers in hyperlipoproteinemic patients fed a high PUFA (P:S, 2.0) diet. In another study, Vessby et al. (57) observed reductions ($P < 0.05$) in serum cholesterol and triglyceride levels in hyperlipoproteinemic patients fed diets with fat at 44% of the calories and high PUFA (P:S, 2.0)

as compared with a low PUFA (P:S, 0.2) diet. Shepherd et al. (45) using fat at 40% of the calories which was high in PUFA (P:S, 4.0) and a low PUFA fat (P:S, 0.25) observed a 25% reduction ($P < 0.01$) in plasma cholesterol and a 13% reduction ($P < 0.01$) in triglyceride levels with the high PUFA diet in 4 healthy males. Anderson et al. (58) fed diets containing either safflower oil or 1 part coconut oil and 2 parts palm oil to 12 male university students. Levels of serum cholesterol and triglyceride were lower in students fed the high PUFA diet. Keys et al. (49) in the 1950's and Hegstead et al. (52) in 1965 derived equations which demonstrated the change in total serum cholesterol levels achievable by alterations in the level of PUFA and saturated fats in the diet.

Despite the much publicized benefits derived from the intake of PUFA, the Committee on Dietary Allowances of the Food and Nutrition Board warns that in view of the possible hazards of high intake of PUFA, an upper limit of 10% of dietary energy as PUFA is advisable (59). Some of the possible hazards are: a) reduction in HDL cholesterol and apo A-I concentrations (45, 56), b) as possible carcinogens or cocarcinogens (11, 60), c) increased body requirements for antioxidants like vitamin E (11, 60-63), d) formation of cholesterol gallstones (60) and e) suppression of the immune response of the body (64-66).

Carbohydrates

It is estimated that 9 to 17% of the adult population in the U.S. have type IV hyperlipoproteinemia (67, 68). This lipid pattern characterized by elevated triglyceride levels is believed to be induced by high carbohydrate diets, and dietary treatment is accomplished by a reduction in the intake of simple sugars (19, 67).

Epidemiological evidence indicates that refined carbohydrates, high sucrose and fat diets are associated with a number of "western risk" diseases (69, 70). This finding and current research have caused several to question the beneficial impact of the proposed dietary goal. Controlled human and animal feeding studies have shown that fructose and sucrose increase plasma cholesterol and triglyceride levels.

Using diets containing either starch or sucrose at 33% and fat at 42% of the calories (P:S, 0.26), Reiser et al. (68) observed a 33% increase in serum triglyceride and a 7.4% increase in cholesterol levels with the sucrose diet in 10 male and 9 female subjects. Similar results were observed by Reiser et al. (67) two years later when they fed human subjects diets containing sucrose either at 5%, 18% or 33% of the calories and fat at 42% (P:S, 0.29) for 6 weeks. Plasma triglyceride levels in male subjects were 31% higher on the 18% sucrose diet as compared with the 5% sucrose diet. These levels increased by an additional 40% with the 33% sucrose diet. There were no significant changes in plasma triglyceride levels in female subjects on the same diets. Cholesterol levels increased significantly in males (31%) and females (13%) from the low to the high sucrose diets. HDL cholesterol levels also increased by about 14% in male and female subjects on the 18% sucrose diet, with no further change on the 33% sucrose diet. These results are consistent with the findings of Naismith et al. (71) which indicated an increase ($P < 0.01$) in plasma cholesterol and triglyceride levels in male subjects fed 300 versus 105 g/day sucrose with their diets. Naismith and Rana (72) also noted an increase ($P < 0.01$) in plasma triglyceride levels in 7 day old Sprague Dawley rats fed 68.2% (by weight) sucrose, maltose, glucose or fructose diets versus starch diets for 50 days. In part 3 of the study conducted

at the University of Toronto, Antar et al. (73) also observed increases ($P < 0.01$) in serum cholesterol and triglyceride levels in hyperlipoproteinemic patients fed a 40% (of the calories) sucrose diet as compared with a 40% starch diet, with fat at 35% (P:S, 0.1).

Contrary to these findings of a hyperlipidemic response to diets high in sugars, some researchers have reported no change in cholesterol levels with high carbohydrate diets. Coulston et al. (9) reported no change in plasma cholesterol levels in 5 males and 6 females fed a carbohydrate diet at 60% of the calories with sucrose at 25% and fat at 21% (P:S, 1.2) and carbohydrate at 41% with sucrose at 22% and fat at 40% (P:S, 1.3). Coulston et al. (9) did observe increases in plasma triglyceride concentrations ($P < 0.001$) and a decrease in plasma HDL cholesterol ($P < 0.05$) with the high carbohydrate diet. Laube et al. (12) fed Wistar rats diets containing either starch, sucrose or an equal mixture of fructose and glucose at 68% of the calories for 15 weeks, reported increased serum triglyceride levels ($P < 0.05$) with no change in cholesterol levels with the sugars as compared with the starch diet. Hallfrisch et al. (74) reported no significant change in serum cholesterol or triglyceride levels in Wistar rats fed sucrose or starch diets at 30% of the calories, with fat at 40% (P:S, 0.3) for 9 weeks.

A comparison of the various sugars indicates that the greatest increases in triglyceride levels are observed with fructose feeding. Zavaroni et al. (75) observed about 30% higher plasma triglyceride levels in Sprague Dawley rats fed for 1 week a 66% (of the calories) fructose diet in comparison with a 66% glucose diet. Kanerak and Orthen-Gambill (76) fed their rats ad libitum 32% fructose/glucose/sucrose solutions or granulated sucrose with the stock diet. Even though rats on the 32%

fructose solution derived only 38% of their calories from this sugar, as compared with 43% from granulated sucrose, 52% from glucose solution and 58% from sucrose solution with rats on these diets, serum triglyceride levels were highest with the fructose solution, 108 mg/dl as compared with 89.2 mg/dl with the granulated sucrose, 74.0 mg/dl with the sucrose solution, 60.7 mg/dl on the glucose solution and 58.7 mg/dl on the stock diet alone (control). Selder et al. (77) observed Sprague Dawley rats on a 66% of the calories fructose diet had plasma triglyceride levels averaging 380 mg/dl as compared with 142 mg/dl on a 66% dextrose diet after 1 week of feeding. A comparison of triglyceride levels as affected by feeding 11 hemodialysis patients 85% of the calories dextrimaltose or sucrose in fat free diets revealed no significant differences between the sugars as indicated in the study by Goldberg et al. (78).

Despite the controversy over the carbohydrate induced hyperlipidemia thought to be brought about by short term feeding of high carbohydrate diets (9, 11, 74, 79), the LRC population study which revealed a relationship between dietary sucrose and starch and HDL cholesterol levels, found no relationship between saturated dietary fat and HDL cholesterol levels (10). The hyperlipidemic effects of sucrose have been attributed mainly to the fructose component of the molecule. When sucrose is hydrolyzed, it yields fructose and glucose, while starch yields only glucose (12).

Several explanations have been offered to explain the hyperlipidemic effects of fructose and sucrose feeding:

a) Carbohydrate metabolism is increased in the liver with the ingestion of diets high in fructose or sucrose, since fructokinase (an enzyme necessary for the initiation of fructose metabolism) is present only in the liver and is lacking in adipose tissue and muscle (72). This

hypothesis could not explain the increase in hepatic lipogenesis observed with maltose (which yields only glucose) and glucose feeding in rats studied by Naismith and Rana (72).

b) Rates of digestion and absorption may differ with the various carbohydrates. Naismith and Rana (72) plotted meal tolerance curves for the various sugars (sucrose, fructose, maltose and glucose) and could not support this hypothesis of differences in rates of digestion and absorption among sugars.

c) Glucose feeding leads to a hyperinsulinemic response which in turn increases the adipose tissue lipoprotein lipase (LPL) activity and accelerates removal of VLDL triglycerides from the blood. Fructose does not stimulate insulin secretion so LPL activity is not stimulated and removal rates for VLDL triglycerides from the blood are reduced. Goldberg et al. (78) used hypothesis "c" to explain the differences observed in post-prandial plasma triglyceride levels in their study with hemodialysis patients. They reported a sharp fall after dextrose feeding but little change after sucrose feeding. This explanation of increased removal rates of VLDL triglycerides from the blood stimulated by a hyperinsulinemic response to glucose feeding, has been disproved by Selder et al. (77) who observed that feeding rats 66% (of the calories) fructose diets for a week resulted in a three fold increase in insulin activity as compared to a two fold increase with a 66% glucose diet. Merkins et al. (80) who used adult Sprague Dawley rats and 60% (by weight) glucose, fructose or starch diets and Zavaroni et al. (75) also observed no difference in insulin release with fructose versus glucose feeding in rats.

d) An increased hepatic secretion without a decrease in the catabolic

rates of VLDL triglycerides. Metabolism of fructose leads to an increased formation of glycerol-3-phosphate, a precursor to lipid synthesis (75). Kannan et al. (81) observed the secretion and removal rates of VLDL triglycerides in Sprague Dawley rats to be 75% faster on a 58% (of the calories) fructose diet compared with a 58% glucose diet.

The hyperlipidemic response to diets high in sucrose or fructose has not been satisfactorily explained. Zavaroni et al. (75) indicated that exercise can control the hyperlipidemic response to high fructose diets, while Birchwood et al. (46) have shown that with high PUFA diets (P:S, 1.4) there was no significant difference in serum lipid levels with a 40% starch or sucrose diet in hyperlipidemic patients.

MATERIALS AND METHODS

Animals and their care

Thirty-six male weanling Sprague Dawley rats[1] weighing between 40-66 g at the start of the experiment, were housed individually in stainless steel cages in a temperature controlled room (21° ± 2) maintained on a 12:12 hour light-dark cycle (lights on: 0600-1800 hours). The animals were shipped in three batches of twelve rats each over a three week period. Three rats from each batch were assigned at random to one of four experimental diets, after a one day adjustment period. Feed and water were provided ad libitum for four weeks and weight gains and feed intake were measured[2] at weekly intervals.

Experimental design and diets

Two of the rat diets used were as outlined in the project proposal of the North Central Regional project, NC-167, "Dietary modifications designed to affect lipid metablism." The quantity of fat and distribution of fatty acids were designed to approximate a) the present United States (U.S.) diet based on the United States Department of Agriculture (USDA) 1977 Food Consumption Survey or b) the Dietary Goals for the U.S. proposed by the U.S. Senate Select Committee on Nutrition and Human Needs. The other two diets had the same composition as the first two except that fructose was substituted for sucrose. The composition of the diets are shown in table 1 and the energy distribution in table 2. The weighed ingredients[3] for the

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[1]Harlan Sprague Dawley Inc., Madison, Wisconsin.

[2]Toledo Scale.

[3]Mettler P5.

TABLE 1

Composition of diets by weight[2]

Ingredients[1]	USDA 1977		Dietary Goal	
Protein	223		223	
Casein-high protein		220		220
DL-methionine		3		3
Carbohydrate	472		536	
Corn starch		236		268
Sucrose/fructose		236		268
Fat	208		144	
Lard		187		---
High-oleate, safflower oil		10.5		---
Safflower oil		10.5		7.2
Beef tallow[2]		---		68.4
Corn oil		---		68.4
Fiber	50		50	
Non-nutritive cellulose		50		50
Mineral	35		35	
AIN-76 mineral mix[3]		35		35
Vitamin	12.011		12.011	
AIN-76 vitamin mix[4]		10		10
Choline bitartrate		2		2
Menadione		0.001		0.001
Ethoxyquin		0.01		0.01
		1000.011		1000.011

[1]ICN Nutritional Biochemicals, Cleveland, Ohio. [2]Oscar Mayer & Co., Madison, Wisconsin. [3]ICN #905455, amounts in g/kg mixture:calcium phosphate dibasic ($CaHPO_4$) 500.0; sodium chloride (NaCl) 74.0; potassium citrate monohydrate $(HOC(COOK)CH_4COOK)_4H_2O$ 220.0; potassium sulfate (K_2SO_4) 52.0; magnesium oxide (MgO) 24.0; manganous carbonate (43-48% Mn) 3.5; ferric citrate (16-17% Fe) 6.0; zinc carbonate (70% ZnO) 1.6; cupric carbonate (53-55% Cu) 0.3; potassium iodate (KIO_3) 0.01; sodium selenite ($Na_2SeO_3 \cdot 5H_2O$) 0.01; chromium potassium sulfate $(CrK(SO_4)_2 \cdot 12H_2O$ 0.55; sucrose, finely powdered 118.0. [4]ICN #905454, amounts per kg mixture: thiamine HCl 600 mg; riboflavin 600 mg; pyridoxine HCl 700 mg; nicotinic acid 3 g; D-calcium pantothenate 1.6 g; folic acid 200 mg; D-biotin 20 mg; cyanocabalamin 1 mg; retinyl palmitate (Vit. A), pre-mix 800 mg; dl-α-tocopherol acetate (Vit. #), pre-mix 20 mg; cholecaliferol (Vit. D_3) 2.5 mg; menaquinone (Vit. K) 5.0 mg; sucrose finely powdered 972.9 g.

[2]Diets as outlined in the project proposal of the North Central Regional project, NC-167, "Dietary modifications designed to affect lipid metabolism."

TABLE 2

Energy distribution[1]

	USDA 1977	Dietary Goal
Protein	19.2	20.6
Carbohydrate	40.6	49.5
Fat	40.2	29.9
Saturated	14.7	9.8
Monoene	19.9	10.2
Polyene	5.6	9.9
Kcal/g	4.652	4.332
g protein/100 Kcal	47.9	51.5

[1]Diets as outlined in the project proposal of the North Central Regional project, NC-167, "Dietary modifications designed to affect lipid metabolism."

diets were mixed in a Hobart mixer and stored in a refrigerator in plastic gallon jars.

Caloric distributions of fat and sugar in the four diets were:

USDA 1977	Diet 1:	40% fat, 5 (polyunsaturated):18 (monounsaturated) :12 (saturated)
		20% sucrose. Caloric yields.
	Diet 2:	40% fat, 5:18:12
		20% fructose
Dietary Goals	Diet 3:	30% fat, 10:10:10
		25% sucrose
	Diet 4:	30% fat, 10:10:10
		25% fructose

Autopsy procedure

At the end of the study (day 28), the rats were fasted overnight (15-19 hours) and anesthetized with an intraperitoneal injection of Barb-Euthol[1] (0.023 ml/100 g body weight) after final body weights were noted. Blood was drawn from the abdominal aorta, allowed to stand at room temperature to facilitate clotting, then centrifuged at 10° for 5 minutes at 2,000 rpm,[2] kept overnight in a refrigerator and centrifuged again at 2,000 rpm for 5 minutes to obtain serum. The serum was stored at -12°. Livers and epididymal fat pads were excised immediately, rinsed in normal saline solution, blotted and weighed.[3]

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[1]Barb-Euthol (nitrogenized), Haver Lockhart Laboratories, Shawnee, Kansas.

[2]Sorvall superspeed, automatic refrigerated centrifuge (model RC2-B).

[3]Ainsworth (Fisher model 250).

Analytical techniques

Total serum cholesterol was determined by the ferric chloride-sulfuric acid reaction (Leffler, modified) (17). HDL was fractioned by selective precipitation with heparin and manganous chloride and then analyzed for cholesterol using the ferric chloride-sulfuric acid method to determine HDL cholesterol (17). Serum triglycerides were determined with a Sigma Kit.[1] See appendix for description of methods.

Statistical analysis

A randomized complete block (RCB) design with two levels of fat and two sources of carbohydrate or four dietary treatments, and 3 replications to each diet and 3 rats to each replication was used. The data were subjected to a general linear analysis procedure (GLM). Two linear contrasts of the significance between means of the two levels of fat (diets 1,2 versus 3,4) and between means of the two types of sugar (diets 1,3 versus 2,4) also were tested using GLM. Duncan's multiple range test (DMRT) was used to separate the means for the four diets at $\alpha = 0.05$. To test the hypotheses for the significance among diet means, two linear contrasts and separation of means with DMRT, the mean square for the interaction between replication and diet (M.S. Rep × Diet) was used as the error term.

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[1]Sigma Kit No. 405, Sigma Chemical Company, Saint Louis, Missouri.

RESULTS AND DISCUSSION

Weight gain

There was a difference ($P < 0.05$) in weight gained among animals fed the four diets at the end of the 28 day feeding period (table 3). The type of sugar appeared to have a greater effect than the nature of fat consumed. Rats on the sucrose diets (diets 1,3) gained more weight ($P < 0.05$) than rats on the fructose diets (diets 2,4). When the means for the four diets (table 4) were separated at the 5% level with Duncan's multiple range test (DMRT) there was no significant difference between diets 2,3 and 4, but the mean weight gains for diets 2 and 4 were lower ($P < 0.05$) than that for diet 1.

Consistent with the findings of the present study of low weight gain in rats on the fructose diets, Selder et al. (77) reported less ($P < 0.001$) weight gain in fructose-lard fed Sprague Dawley rats in comparison to glucose-lard or chow fed rats. They attributed the lower weight gain in rats fed the fructose-lard diet to eating less the first few days of the 7-day study. Merkins et al. (80) also reported lower overall weight gains in Sprague Dawley rats fed fructose versus those fed glucose or starch at 60% of the calories for 3 days.

Several other groups of researchers (54, 72, 76) have indicated no significant differences in weight gained in Sprague Dawley rats fed various sugars or starch. Kanerak and Orthen-Gambill (76) reported no significant difference in weight gained in rats fed 32% sucrose/fructose/glucose solutions or granulated sucrose ad libitum with the stock diet for 50 days. Naismith and Rana (72) also indicated no difference in growth rates in rats fed sucrose, fructose, maltose, glucose or starch at 68.2% of the diet (by weight) for 50 days. Bruckdorfer et al. (54) demonstrated

TABLE 3

Analysis of variance of weight gain and feed intake

Source	d.f.[2]	Mean square and significance[1]		
			Feed intake	
		Weight gain	Grams	Calories
Replication	2	61.44	1076.86	22126
Diet	3	421.26*	1767.21*	5330.5 n.s.
Replication × diet	6	85.48 n.s.	233.27 n.s.	4992.9 n.s.
Contrasts; diets				
1,2 vs 3,4	1	81.00 n.s.	4601.36**	1021.40 n.s.
1,3 vs 2,4	1	821.78*	552.25 n.s.	11558.11 n.s.

[1]** $P < 0.01$, * $P < 0.05$, + $P < 0.10$, n.s. $P > 0.10$. [2]Degrees of freedom.

TABLE 4

Effect of diets on mean weight gain and feed intake

Mean[2]	40% Fat (P:S, 0.4)[1]		30% Fat (P:S, 1.0)[1]		M.S.E.[3]
	20% Sucrose	20% Fructose	25% Sucrose	25% Fructose	
Weight gain (g)	187.0[a]	171.11[b]	177.67[ab]	174.44[b]	85.48
Feed intake					
Grams	345.33[bc]	333.44[c]	363.89[a]	360.11[ab]	233.27
Calories	1606.5[a]	1551.2[a]	1576.4[a]	1560.0[a]	4992.9

[1]Polyunsaturated:saturated fat ratio. [2]For 28-day feeding period. [3]Mean square error. Means with different superscript alphabet are significantly different from each other as determined by Duncan's multiple range test ($P < 0.05$).

that altering the type of carbohydrate, starch versus sucrose, and the level of saturation of fat had no effect on weight gain or growth rates in Sprague Dawley rats.

In contrast to feeding studies using Sprague Dawley rats, weanling Wistar rats fed a sucrose versus starch diet at 30% of the calories with fat at 40% were heavier ($P < 0.05$) on the sucrose diet at the end of 12 weeks (74). In contrast Laube et al. (12) reported higher weight gain ($P < 0.05$) in rats fed corn starch diet in comparison to sucrose or an equal mixture of fructose-glucose at 68% of the calories for 15 weeks.

There was no significant difference in weight gains in human subjects fed diets to demonstrate the impact of feeding various levels and types of carbohydrate and fat on serum lipid levels (46, 47, 53, 73, 78).

Feed intake

Feed intake expressed as grams of feed ingested by rats during the 28-day feeding period was significant ($P < 0.05$) among the four diet groups (table 3). The level of fat in the diets affected the amount of feed ingested rather than the carbohydrate source at either fat level (table 4). Rats on the 40% fat diets (diets 1,2) ate less ($P < 0.01$) than rats on the 30% fat diets (diets 3,4). The reason for this difference in feed intake was that the 40% fat diets had a higher caloric yield of approximately 7% per gram as compared to the 30% fat diets. Rats on the 30% fat diets consumed more diet compared to the 40% fat groups in order to obtain the calories they needed for growth and metabolic functions. To eliminate a bias in results due to this difference in caloric yields of diets, feed intake expressed as calories consumed was analyzed and was found not be significant among diets (tables 3, 4). Caloric values for feed intakes were calculated by multiplying grams of feed consumed .

by 4.652 for diets 1 and 2 and 4.332 for diets 3 and 4; these factors are the calorie yields per gram of diet (table 2).

Consistent with the findings of the present study of no significant influence of the source or level of carbohydrate on feed intake in rats, Naismith and Rana (72) observed no difference in feed intake in rats fed sucrose, fructose, maltose, glucose or starch at 68.2% (by weight) of the diet. Reiser et al. (67) also found no differences in caloric intake in human subjects fed diets with 3 levels of sucrose, 5%, 18% and 33% of the calories, with fat held constant at 42% (P:S, 0.29) for 6 weeks.

Feed efficiency

Feed efficiency was calculated as: a) weight gained (g)/feed intake (g) and as b) weight gained (g)/caloric intake × 100. When analyzed using grams of feed ingested, feed efficiency was significant among diets ($P < 0.01$) (table 5). A comparison of the two levels of fat (diets 1,2 versus 3,4) and the two types of sugar (diets 1,3 versus 2,4) indicated that both affected feed efficiency ratio "a" ($P < 0.01$). The significant effect of the level and type of fat on efficiency of the diet was a result of a significant difference in feed intake due to a difference in caloric yields between the 40% fat and 30% fat diets. As with feed intake, to correct for this difference in caloric yields, feed efficiency was recalculated in terms of caloric intake, ratio "b". When ratio "b" was analyzed there was a difference ($P < 0.01$) among diets, table 5, but the level of fat had no effect on the result. A comparison of the sugars indicated that this ratio was significantly ($P < 0.01$) affected by the type of sugar (table 5). When means among diets were separated using DMRT (table 6) the ratio for diet 1 was higher ($P < 0.05$) than the ratios obtained for the other three diets.

TABLE 5

Analysis of variance of feed efficiency

Source	d.f.[2]	Mean square and significance[1]	
		Feed efficiency	
		Feed in "a"[3]	Calorie in × 100 "b"[4]
Replication	2	0.000898	0.4465
Diet	3	0.00632**	0.609**
Replication × diet	6	0.000088n.s.	0.0440n.s.
Contrasts; diets			
1,2 vs 3,4	1	0.01527**	0.1147n.s.
1,3 vs 2,4	1	0.002402**	1.1317**

[1]** $P < 0.01$, n.s. $P > 0.10$. [2]Degrees of freedom. [3]Weight gain (g)/feed intake (g). [4]Weight gain (g)/caloric intake × 100.

TABLE 6

Effect of diets on feed efficiency

Mean[2]	40% Fat (P:S, 0.4)[1]		30% Fat (P:S, 1.0)[1]		M.S.E.[3]
	20% Sucrose	20% Fructose	25% Sucrose	25% Fructose	
Feed efficiency					
Feed intake[4]	0.542[a]	0.514[b]	0.489[c]	0.484[c]	.000088
Caloric in[5]	11.65[a]	11.04[b]	11.29[b]	11.18[b]	.044

[1]Polyunsaturated:saturated fat ratio. [2]For 28-day feeding period. [3]Mean square error. [4]Weight gain (g)/feed intake (g). [5]Weight gain (g)/caloric intake × 100. Means with different superscript alphabet are significantly different from each other as determined by Duncan's multiple range test ($P < 0.05$).

A higher feed efficiency with sucrose diets also was reported by Kanerak and Orthen-Gambill (76). Their Sprague Dawley rats fed a granulated sucrose gained the most weight (4.48 g/100 kcal) followed by rats on a 32% fructose solution (4.1 g/100 kcal). However, their values for feed efficiency were lower than those observed in the present study (table 6). The reason for this difference may be that in the present study the animals were younger and as a result utilized feed more efficiently for growth. Kanerak and Orthen-Gambill (76) calculated feed efficiency as weight gained in rats as a function of caloric intake.

Epididymal fat pad weights

Epididymal fat pad weights often are used as a yardstick for measuring obesity or fat deposition in rats. Differences ($P < 0.10$) were observed among diets in removable fat pad weights (table 7). Using DMRT to separate means for the various diets (table 8), rats fed the 40% fat, 20% sucrose diet had more removable fat than rats fed the 30% fat, 25% fructose diet. Diets 1,2 and 3 were similar and diets 2,3 and 4 were similar. The level and saturation of fat had no effect on fat deposition (table 7) while the type of sugar did. Rats on the sucrose diets deposited more ($P < 0.05$) fat than rats fed the fructose diets.

In agreement with the findings of the present study of more fat deposition with diet 1, Hallfrisch et al. (74) reported more removable fat ($P < 0.05$) on the 30% sucrose diet in comparison with the 30% starch diet. In contrast Naismith and Rana (72) reported no difference in epididymal fat pad weights in rats fed various dietary carbohydrates. Carlson and Arnrich (82) indicated an inhibition of epididymal lipogenesis in Wistar rats fed a 20% by weight safflower oil diet and a more efficient conversion to body fat of rats fed a 20% beef tallow diet. A difference

TABLE 7

Analysis of variance of epididymal fat pad and liver weights

Source	d.f.[2]	Mean square and significance[1]	
		Fat pad	Liver
Replication	2	0.251	2.587
Diet	3	0.259^{+}	$0.843^{n.s.}$
Replication × diet	6	$0.07^{n.s.}$	$0.388^{n.s.}$
Contrasts; diets			
1,2 vs 3,4	1	$0.047^{n.s.}$	$0.840^{n.s.}$
1,3 vs 2,4	1	0.723^{*}	$0.967^{n.s.}$

[1] $^{*}P < 0.05$, $^{+}P < 0.10$, $^{n.s.}P > 0.10$. [2] Degrees of freedom.

TABLE 8

Effect of diets on mean epididymal fat pad and liver weights

Mean[2]	40% Fat (P:S, 0.4)[1]		30% Fat (P:S, 1.0)[1]		M.S.E.[3]
	20% Sucrose	20% Fructose	25% Sucrose	25% Fructose	
Fat pad	2.19^{a}	1.93^{ab}	2.14^{ab}	1.83^{b}	0.07
Liver	7.99^{a}	7.38^{a}	7.40^{a}	7.36^{a}	0.388

[1] Polyunsaturated:saturated fat ratio. [2] Mean weight in grams for 28-day feeding period. [3] Mean square error. Mean with different superscript alphabet are significantly different from each other as determined by Duncan's multiple range test ($P < 0.05$).

in fat deposition due to saturation of dietary fat was not observed in the present study.

Liver weights

Altering the level and type of dietary fat and carbohydrate had no significant effect on liver weights in weanling rats (tables 7 and 8). Analysis of mean liver weights for the four diet groups did not indicate any significant difference among treatment means, linear contrasts did not indicate any differences due to dietary fat or carbohydrate, and there was no difference between treatment means using DMRT.

The inability to find differences in mean liver weights in the present study may be partially explained by findings of Naismith and Rana (72). They indicated that sucrose and fructose feeding resulted in heavier ($P < 0.01$) livers in comparison to starch feeding because of increased total enzyme activity and higher total triglycerides in the liver with these sugars. They reported liver weights of rats fed various carbohydrates: for fructose, 14.16 g; sucrose, 12.26 g; and starch, 10.49 g. These values are higher than those observed in the present study (table 8) since their animals (72) were about a week older. Laube et al. (12) also observed no difference in mean liver weights of rats, which were 11.0 g, 11.0 g, and 10.2 g, respectively, on the 68% sucrose, equal mixture of fructose-glucose, and starch diets. However Laube et al. (12) did find a higher percentage of liver fat with sucrose and fructose-glucose diets, 9.8% and 9.7% compared to 5.9% with the starch diet. The inability of the degree of saturation of fat to alter liver weights in rats in the present study is supported in findings by Bruckdorfer et al. (54) who reported differences ($P < 0.01$) in liver weights of rats fed sucrose versus starch diets. Use of arachis oil or hydrogenated coconut oil with the two

carbohydrates had no effect on liver weights, but the interaction of dietary fat and carbohydrate was significantly different ($P < 0.01$).

Serum cholesterol

Decreasing the level and saturation of fat had no effect on serum cholesterol levels as indicated by a linear comparison of the two levels of fat in table 9. Dietary carbohydrate had some effect, but the difference did not reach statistical significance. The interaction of fat and sugar resulted in no significant differences in mean serum cholesterol levels among diets (table 9) or between treatment means using DMRT (table 10).

A probable reason for the inability to find any differences in serum cholesterol levels of rats fed diets in the present study was that the hypolipidemic effect of increasing PUFA in diets 3 and 4 from 5% to 10% of the calories was negated by the effect of increasing the levels of sucrose/fructose from 20% to 25%. These sugars are considered to be hyperlipidemic.

The inherent ability of rats to resist changes in blood cholesterol levels with diets high in fats and sugars is supported by findings by several researchers (12, 54, 72, 74) and by results of the present study. Naismith and Rana (72) reported no significant difference in plasma cholesterol levels in rats fed 68.2% by weight fructose or sucrose diets, although these plasma cholesterol levels were higher ($P < 0.01$) than those of rats fed a starch diet. Hallfrisch et al. (74) however reported no difference in serum cholesterol levels of Wistar rats fed 30% sucrose or starch diets with fat at 40% (P:S, 0.3); nor did Laube et al. (12) find any difference in serum cholesterol levels with starch versus sugars diets. The study by Bruckdorfer et al. (54) is further proof of the lack of response in rats to changes in dietary carbohydrate and fat and the

TABLE 9

Analysis of variance on serum cholesterol, HDL cholesterol, HDL cholesterol/cholesterol ratio and triglycerides

Source	d.f.[2]	Mean square and significance[1]			
		Cholesterol	HDL-ch[3]	HDL-ch/ch × 100	Triglycerides
Replication	2	563.43	83.98	222.66	319.0
Diet	3	50.05$^{n.s.}$	188.34$^{n.s.}$	153.38*	111.5$^{n.s.}$
Replication × diet	6	104.9$^{n.s.}$	84.44$^{n.s.}$	16.33$^{n.s.}$	108.08$^{n.s.}$
Contrasts; diets					
1,2 vs 3,4	1	0.257$^{n.s.}$	119.1$^{n.s.}$	164.08*	190.95$^{n.s.}$
1,3 vs 2,4	1	146.98$^{n.s.}$	390.06^{+}	182.25*	82.23$^{n.s.}$

[1]$^{*}P < 0.05$, $^{+}P < 0.10$, $^{n.s.}P > 0.10$. [2]Degrees of freedom. [3]Cholesterol.

TABLE 10

Effect of diets on serum cholesterol, HDL cholesterol, HDL cholesterol/cholesterol ratio and triglycerides

Mean[2]	40% Fat (P:S, 0.4)[1]		30% Fat (P:S, 1.0)[1]		M.S.E.[3]
	20% Sucrose	20% Fructose	25% Sucrose	25% Fructose	
Cholesterol	83.01^{a}	79.54^{a}	83.41^{a}	78.8^{a}	104.9
HDL cholesterol	62.18^{a}	53.1^{a}	63.33^{a}	59.23^{a}	84.44
HDL ch/ch[4] × 100	75.04^{a}	67.35^{b}	76.11^{a}	75.17^{a}	16.33
Triglycerides	63.0^{a}	56.75^{a}	55.16^{a}	55.37^{a}	108.08

[1]Polyunsaturated:saturated fat ratio. [2]mg/dl. [3]Mean square error. [4]Cholesterol. Mean with different superscript alphabet are significantly different from each other as determined by Duncan's multiple range test ($P < 0.05$).

interaction of these factors on serum cholesterol levels. Bruckdorfer et al. observed no difference in plasma cholesterol levels as a result of feeding Sprague Dawley rats starch or sucrose diets with saturated fat or PUFA.

Research evidence indicates that unlike rats, humans are more likely to respond to changes in dietary fat and carbohydrate with changes in blood cholesterol levels. The interaction of dietary fat and carbohydrate, particularly the hypocholesterolemic effect of high levels of PUFA in a sucrose rich diet and the hypercholesterolemic effect of saturated fat in a sucrose rich diet has been demonstrated by several authors (46, 53, 73), but this interaction was not observed in the present study. Birchwood et al. (46) showed that dietary sucrose fed at approximately 40% of the total calories did not increase serum cholesterol levels when PUFA was high in the diet (P:S, 1.4). The substitution of saturated fat (P:S, 0.1) in the diet used by Birchwood et al. (46) by Antar et al. (73) resulted in a significant increase in serum cholesterol levels ($P < 0.001$) with sucrose versus starch at 40% of the calories in the diets. Mann et al. (53) observed that doubling the normal level of sucrose to 34% of the calories resulted in an increase ($P < 0.05$) in serum cholesterol levels compared with the control patterned after the American diet, while increasing the level of PUFA to 75% of the total fat with sucrose at 34% did not result in a significant difference in serum cholesterol levels as compared with the control.

Serum high density lipoprotein (HDL) cholesterol

Serum HDL cholesterol levels were not significantly different among diets (table 9) and mean separation using DMRT did not indicate any difference among diets (table 10). However linear contrasts of the two

dietary variables revealed that with sucrose in the diet, HDL cholesterol levels were higher ($P < 0.10$) than when fructose was substituted for sucrose (table 9). Altering the level and saturation of fat had no impact on HDL cholesterol in the serum of rats used in the present study. Because of the fairly recent discovery of a negative relationship between HDL cholesterol levels and heart disease, researchers have started to investigate the impact of dietary factors on HDL cholesterol levels. However, such data was not reported in any of the rat studies reviewed.

In human studies a reduction in HDL cholesterol levels has been observed in studies which also report a reduction in cholesterol levels. Vessby et al. (56) reported a 15% decrease ($P < 0.05$) in HDL cholesterol with a 23% decrease in cholesterol levels with a high PUFA diet and fat at 35% of the energy (P:S, 2.0). Shepherd et al. (45) observed a drop in HDL cholesterol levels with a high PUFA diet but they claim that this drop was not a result of a decline in the percentage of cholesterol in the lipoprotein but in an overall reduction in plasma HDL concentrations.

In contrast to these reported findings of a reduction in HDL cholesterol levels with high PUFA diets and similar to the findings of the present study, Harris et al. (50) reported no change in HDL cholesterol levels in humans fed vegetable oil (P:S, 3.4), salmon oil (P:S, 1.3) or a control fat (P:S, 0.4) with fat at 40% of the calories. Chait et al. (55) also observed no change in HDL cholesterol levels in hyperlipidemic patients fed saturated fat (P:S, 0.2) or PUFA (P:S, 2.4) at 40% of the calories.

In the present study altering the level of sucrose had little effect on HDL cholesterol level of rats which was 62.18 mg/dl with sucrose at 20% and 63.33 mg/dl with sucrose at 25% (table 10). In contrast

Coulston et al. (9) observed a decline ($P < 0.05$) in HDL cholesterol level of rats fed a diet with sucrose at 25% of the calories (carbohydrate 60%) compared with a diet with sucrose at 22% (carbohydrate 40%).

Serum HDL cholesterol/total cholesterol ratio

This ratio was calculated by dividing the serum HDL cholesterol level by the total serum cholesterol level and expressing this fraction as a percentage.

$$\frac{\text{HDL cholesterol}}{\text{Total cholesterol}} \times 100$$

When this ratio was analyzed there was a difference ($P < 0.05$) among diets (table 9). Using DMRT to separate means, the ratio for diet 2 was lower ($P < 0.05$) than the ratios for the other 3 diets (table 10). The reason for this result is not known as data available in published literature is not adequate to support this finding of a lower ratio with a 40% fat (P:S, 0.4), 20% fructose diet. Linear contrasts indicated a difference ($P < 0.05$) between the two levels of fat and two types of sugar. The reason for these differences is not known; it could be due to a considerably lower ratio with diet 2.

Reiser et al. (67) reported this ratio as: HDL cholesterol/(total cholesterol-HDL cholesterol). With a 33% sucrose diet this ratio was lower ($P < 0.05$) than those computed for 18% and 5% sucrose diets. This was only true for males; female subjects showed no such difference with the three diets. Coulston et al. (9) reported a decrease ($P < 0.05$) in HDL cholesterol to cholesterol ratio with a 25% sucrose diet in comparison with a 22% sucrose diet. In the present study HDL cholesterol to cholesterol was slightly higher with the 25% sucrose diet as compared with the 20% sucrose diet, which is contrary to findings by Coulston

et al. (9). It was 76.11% for rats fed diet 3 and 75.04% for rats fed diet 1 (table 10). Harris et al. (50) and Chait et al. (55) did not compute this ratio but when comparing their reported values for HDL cholesterol and total cholesterol there was an increase in the proportion of HDL cholesterol to cholesterol with use of high PUFA diets. In the present study the HDL cholesterol to cholesterol ratio was higher ($P < 0.05$) with the 30% fat (P:S, 1.0) in comparison with the 40% fat (P:S, 0.4) diets (table 9). It was 76.11% for diet 3 and 75.17% for diet 4 (table 10) in comparison with 75.04% for diet 1 and 67.35% for diet 2 (table 9).

Serum triglycerides

Results obtained for serum triglycerides were similar to those observed for serum cholesterol. A comparison of the two levels of fat and two types of sugar (table 9) revealed no significant differences, neither did the interaction of these two factors among diets. Mean triglyceride levels for the four diets also were not significant when DMRT was applied as shown in table 10.

The inability to demonstrate differences in triglyceride levels in rats on the four diets in the present study may be attributed to several factors: all diets contained sucrose or fructose and both of these sugars are hypertriglyceridemic (12, 72). Naismith and Rana (72) reported 50% higher plasma triglyceride levels with sugars versus starch in the diet. With a sucrose diet triglyceride concentration was 88 mg/dl, with a fructose diet 78 mg/dl and with a starch diet (51 mg/dl). These levels were higher than those observed in the present study (table 10). Naismith and Rana (72) fed their animals for 50 days in comparison with 28 days in the present study and the level of carbohydrate they used was

68.2% by weight in comparison with 47.2% in diets 1 and 2 and 53.6% with diets 3 and 4 in the present study (table 1). Using 68% carbohydrate and Wistar rats, Laube et al. (12) reported a 505 mg/dl fasting triglyceride concentration with a starch diet, which was lower ($P < 0.05$) than those observed with a sucrose diet (650 mg/dl) and fructose-glucose diet (641 mg/dl). These levels are considerably higher than those observed for Sprague Dawley rats which indicates breed differences.

The inability to observe significant decreases in triglyceride levels with increasing the percentage of PUFA in diets 3 and 4 from 5% to 10% of the calories may be explained by the fact that the level used in the present study was considerably lower than levels used in human studies carried out by several workers (45, 46, 55, 56). Although some workers (46, 53) have demonstrated the hypotriglyceridemic effect of the interaction of PUFA and carbohydrate, in the present study the effect of PUFA may have been negated by increasing the level of sucrose/fructose in the diets from 20% to 25% of the calories.

The hypotriglyceridemic effect of a high PUFA diet was demonstrated by Birchwood et al. (46) and Antar et al. (73). They showed that with a high PUFA diet (P:S, 1.4), sucrose was not hypertriglyceridemic but with saturated fat (P:S, 0.1) there was an increase ($P < 0.01$) in triglyceride levels in hyperlipidemic patients. Mann et al. (53) also reported increases ($P < 0.05$) in serum triglycerides when sucrose in the diet was double the normal level (34% of calories) in the American diet, but no change from normal levels of serum triglyceride when PUFA in a high sucrose diet was increased to 75% of the total fat. This finding is in agreement with those of Chait et al. (55) who observed a 35% decrease ($P < 0.001$) with a high PUFA (P:S, 2.4) diet in comparison with a low

PUFA (P:S, 0.2) diet; Vessby et al. (56) observed a 25% reduction in serum triglyceride levels with a high PUFA (P:S, 2.0) diet; and Shepherd et al. (45) reported a 13% reduction ($P < 0.01$) with a high PUFA diet (P:S, 4.0).

In rats, Bruckdorfer et al. (54) reported lower triglyceride values with a starch-high PUFA diet. The total plasma triglyceride concentration was about 30 mg/dl with the starch-high PUFA diet in comparison to about 48 mg/dl with a sucrose-saturated fat diet, significant at the 1% level. In the present study serum triglyceride level for diet 1 was 63 mg/dl, which was higher than the value obtained for diet 3 (55.16 mg/dl) (table 10) although this difference did not reach statistical significance, it was in agreement with the findings of Bruckdorfer et al. (54) of a hypertriglyceridemic effect of a sucrose-saturated fat diet.

CONCLUSIONS

From observations in the present study the following conclusions were made:

1. The type of sugar had a significant effect on body weight gain and epididymal fat pad deposition. When sucrose rather than fructose was present in the diet, rats converted calories more efficiently into body weight. Dietary sucrose also promoted fat deposition.
2. The level and degree of saturation of fat had no effect on body weight gain, fat deposition or conversion of calories to body weight gain.
3. The level and degree of saturation of fat and the type of sugar had no effect on caloric intake. When diets differed in caloric yields, rats tended to consume enough diet to meet caloric requirements.
4. Liver weights in rats were not affected by the level of degree of saturation of fat or the type of sugar in the diet.
5. The level and degree of saturation of fat and the type of sugar had no effect on serum cholesterol and triglyceride levels.
6. Dietary sucrose compared with dietary fructose increased the level of high density cholesterol (HDL) cholesterol in the serum. The level and degree of saturation of fat had no effect on HDL cholesterol levels.
7. The level and degree of saturation of fat and the type of sugar both affected the ratio of HDL cholesterol to total cholesterol in the serum. Sucrose and polyunsaturated fats (PUFA) significantly increased the ratio of HDL cholesterol to cholesterol.

A follow-up to the present study could be to investigate the impact of a reduction in the percentage of dietary sugar to 10% of the calories

as proposed in the "Dietary Goals for the United States" on serum lipids. In the present study, the impact of decreasing fat from 40% to 30% and increasing PUFA from 5% to 10% of the calories was probably negated by the hyperlipidemic effect of increasing sugar from 20% to 25% of the calories.

SUMMARY

A randomized complete block (RCB) design was used to assign 36 male weanling rats to diets with two levels of fat and distribution of fatty acids patterned after the current American diet and the proposed U.S. Dietary Goals and with two sources of sugar. There were 3 replications to each of the four treatments, and 3 animals to each replication. The effects of the four dietary treatments on serum lipid levels, epididymal fat pad weights and liver weights were investigated after a 28-day feeding period. The four diets were: diet 1, fat 40% of the calories with a P:S of 0.4 and sucrose at 20%; diet 2, fat 40% (P:S, 0.4) and fructose 20%; diet 3, fat 30% (P:S, 1.0) and sucrose 25%; diet 4, fat 30% (P:S, 1.0) and fructose 25%. At the termination of the study the rats were fasted overnight and sacrificed. Blood was drawn from the abdominal artery and the livers and epididymal fat pads were removed and weighed. The parameters measured were: weight gain, feed intake, epididymal fat pad weights, liver weights, serum cholesterol, serum high density lipoprotein (HDL) cholesterol and serum triglycerides.

Weights were different ($P < 0.05$) among diets. Rats on the sucrose diets (diets 1,3) gained more ($P < 0.05$) weight than rats on the fructose diets (diets 2,4) and deposited more fat ($P < 0.05$) as measured by epididymal fat pad weights.

An important factor which contributed to a higher weight gain in sucrose-fed versus fructose-fed rats, was the ability of rats on the sucrose diets to convert feed intake more efficiently ($P < 0.01$) into body weight gain. When feed efficiency among diets was calculated as weight gain as a) a function of grams of feed intake or as b) a function of caloric intake, the difference was significant ($P < 0.01$). The level

and degree of saturation of fat did not affect weight gained, fat deposition or feed efficiency measured as function of caloric intake.

The four dietary treatments had no significant effect on liver weights, total serum cholesterol, serum HDL cholesterol or serum triglyceride levels. Serum HDL cholesterol levels were influenced by the type of sugar. Rats on the sucrose diets had higher ($P < 0.10$) HDL cholesterol levels than rats on the fructose diets. The level and degree of saturation of fat had no effect on HDL cholesterol levels.

The ratio of HDL cholesterol to total serum cholesterol was different ($P < 0.05$) among diets. Rats fed the 40% fat, 20% fructose diet had a lower ratio ($P < 0.05$) than rats fed the other three diets. The level and degree of saturation of fat and the type of sugar affected ($P < 0.05$) the percentage of HDL cholesterol to cholesterol.

The results of this study indicated that levels and degree of saturation of fat and type of sugar used in the diets had a significant effect on weight gain, feed efficiency and fat deposition in rats, but had no significant effect on serum lipid levels and liver weights.

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APPENDIX

ANALYTICAL PROCEDURES

Total serum cholesterol

Ferric chloride-sulfuric acid reaction (Leffler, modified) (17).

REAGENTS:

1. Iron reagent. Dissolve 2.5 g ferric chloride ($FeCl_3 \cdot 6H_2O$) in 100 ml of phosphoric acid (assay 85%). Store in a glass stoppered bottle. This solution is stable indefinitely.
2. Glacial acetic acid, acetone, isopropyl alcohol and concentrated sulfuric acid, all AR grade. Keep the sulfuric acid bottle tightly stoppered to prevent absorption of moisture from the air.
3. Cholesterol standards. Dissolve 100, 200, 300 and 400 mg of cholesterol (NBS certified standard or preparation of similar quality) in isopropyl alcohol and dilute each solution to 100 ml. The 200 ml/dl standard is used routinely. All four standards should be used to prepare a standard curve or to check linearity.

PROCEDURE:

1. Pipet exactly 200 µl of serum into the bottom of a test tube.
2. Pipet exactly 200 µl of standard (200 mg/dl) into a second tube.
3. Add 5.0 ml of isopropyl alcohol rapidly from a pipet or automatic dispensing device.
4. Mix thoroughly and centrifuge for 10 minutes at 2,000 rpm. The standard does not require centrifugation.
5. Pipet 1.0 ml of each supernatant into the bottom of a dry glass stoppered 12 or 15 ml centrifuge tube or teflon caped tube.
6. Pipet 1.0 ml of isopropyl alcohol into a clean dry tube which will hold the reagent blank.
7. Add 3.0 ml glacial acetic acid to all tubes and mix.

8. Add 0.3 ml of iron reagent and mix.
9. At 15 second intervals, add 3.0 ml of concentrated sulfuric acid to one tube at a time; let the acid flow freely down the side of the tube.
10. Let the tubes stand 10 minutes to cool. Do not hasten cooling by immersing in cold water.
11. Transfer solutions to respective cuvettes and let stand for 2 to 3 minutes for any bubbles to rise.
12. Without delay, measure absorbances of standard (A_s) and unknown (A_u) against the reagent blank at 560 nm.

CALCULATIONS:

$$\text{mg cholesterol/dl} = \frac{A_u}{A_s} \times 200$$

Serum high density lipoprotein (HDL) cholesterol

Fractionation of lipoprotein by selective precipitation (17).

REAGENTS:

1. Heparin, 5 g/dl aqueous solution.
2. Manganous chloride, 1 mol/liter. Dissolve 19.8 g of $MnCl_2 \cdot 4H_2O$ in water and dilute to 1 dl.

PROCEDURE:

To 1.0 ml of plasma or serum add 40 µl of heparin solution mix, and then add 50 µl of manganous chloride solution. Mix, let stand for 15 minutes and then centrifuge for 10 minutes at 2,000 rpm. Analyze the clear supernatant fluid for total cholesterol, and multiply the result by 1.09 to correct for dilution of the sample. This represents HDL cholesterol.

Serum triglycerides

Sigma Chemical Company, Saint Louis, Missouri. Kit No. 405.

REAGENTS:

1. Triglyceride purifier. Activated alumina.
2. Isopropyl, anhydrous.
3. Triolein standard. Contains 300 mg triolein (glycerol trioleate) dissolved in 1 dl anhydrous isopropanol.
4. Potassium hydroxide solution, 1 N.
5. Sodium m-periodate solution. Prepared by reconstituting a vial (125 g) of sodium m-periodate with 50 ml 2 N acetic acid solution.
6. Ammonium acetate solution 2 M.
7. H' color reagent. Prepare by mixing 20 ml ammonium acetate solution, 40 ml isopropanol (anhydrous) and 0.15 ml acetylacetone. Age overnight.

PROCEDURE: Extraction

1. Label 3 or more tubes, add 0.8 g (± 0.2) triglyceride purifier to each tube. To blank add 5.0 ml isopropanol and 0.2 ml water; to standard 4.8 ml isopropanol, 0.2 ml water and 0.2 ml triolein standard; to test 5.0 ml isopropanol and 0.2 ml serum or plasma.
2. a) Shake with a mechanical mixer, or manually, for at least 5 minutes. b) Allow vials to stand for a few seconds until absorbent starts to settle. To facilitate separation of fluid from solids in the following step, give each vial a single sharp snap with the wrist while grasping the capped vial from the top. This will tend to wash most of the solids down to the bottom of the vial.
3. Centrifuge at about 3,000 rpm for 5 minutes to obtain clear supernatant.

Saponification

1. Label 3 or more clean tubes: blank, standard, test 1, test 2, etc.
2. Carefully transfer 2.0 ml of clear supernatant to bottom of the correspondingly labeled tube.
3. Into each tube pipet just above the liquid level 0.5 ml potassium hydroxide solution. Mix by swirling, do not invert.
4. Incubate all tubes at 60° (± 4°) for 5 minutes.
5. Remove tubes from water bath and cool to room temperature with tap water.

Oxidation

1. To each tube add 0.5 ml periodate solution. Mix immediately after each addition. Start timer after addition to the first tube and note time interval between additions.

Color development

1. Ten minutes after addition of periodate solution to first tube, add to each tube 3 ml reagent H'.
2. Cover tubes and place in a 60° (± 4°) water bath for 30 minutes.
3. Remove tubes from water bath and cool to room temperature with tap water.
4. Transfer contents of tubes to correspondingly labelled cuvettes. Read absorbance (A) of the standard and test versus blank as reference at the same wavelength used to prepare calibration curve. Reading should be completed within 20 minutes.

CALCULATIONS:

Determine the value for both the standard and test from the calibration curve. The absorbance (A) of the standard should correspond to a

value between 280 and 320 mg/dl as read on your calibration curve. If it does the following equation may be used:

$$\text{Triglycerides mg/dl} = \frac{\text{A (test)}}{\text{A (standard)}} \times 300$$

TABLE 11A

Initial body weight, final body weight, weight gain, feed intake, feed efficiency, liver weight and epididymal fat pad weights

Replication	Animal	Body weight			Feed intake		Feed efficiency		Liver wt. (g)	Epididymal fat pad wt. (g)
		Initial (g)	Final[1] (g)	Weight gain[2] (g)	(g)	kcal	% Feed in[3]	% kcal[4]		
				Diet 1: fat 40%, sucrose 20%						
1	1	51	230	179	329	1531	0.54	11.70	8.7	2.2
1	2	48	223	175	317	1475	0.55	11.87	6.9	1.7
1	3	44	225	181	329	1531	0.55	11.83	6.9	1.7
2	1	50	238	188	343	1596	0.55	11.78	9.0	2.4
2	2	66	225	189	368	1712	0.51	11.04	8.1	2.2
2	3	46	227	181	330	1535	0.55	11.79	7.9	2.6
3	1	49	243	194	349	1624	0.56	11.95	8.7	2.8
3	2	56	264	208	390	1814	0.53	11.46	8.3	2.4
3	3	42	230	188	353	1642	0.53	11.45	7.4	1.7
Mean		50	237	187	345	1606	0.54	11.65	8.0	2.2
				Diet 2: fat 40%, fructose 20%						
1	1	47	205	158	305	1419	0.52	11.14	7.2	1.5
1	2	44	224	180	333	1549	0.54	11.62	7.6	1.7
1	3	53	225	172	338	1572	0.51	10.94	7.4	2.3
2	1	57	216	159	331	1540	0.48	10.33	7.9	1.8
2	2	66	240	174	364	1693	0.48	10.28	7.3	1.8
2	3	47	222	175	324	1507	0.54	11.61	7.5	1.9
3	1	40	203	163	311	1447	0.52	11.27	6.8	1.9
3	2	53	228	175	336	1563	0.52	11.20	7.1	2.0
3	3	56	240	184	359	1670	0.51	11.02	7.6	2.5
Mean		51	223	171	333	1551	0.51	11.04	7.4	1.9

(continued)

TABLE 11A (continued)

Replication	Animal	Body weight			Feed intake		Feed efficiency		Liver wt. (g)	Epididymal fat pad wt. (g)
		Initial (g)	Final[1] (g)	Weight gain[2] (g)	(g)	kcal	% Feed in[3]	% kcal[4]		
				Diet 3: fat 30%, sucrose 25%						
1	1	46	225	179	357	1546	0.50	11.57	6.8	2.2
1	2	52	235	183	368	1594	0.50	11.48	6.7	2.2
1	3	43	215	172	351	1521	0.49	11.31	6.3	1.4
2	1	55	234	179	357	1547	0.50	11.57	8.7	2.2
2	2	55	235	180	397	1720	0.45	10.47	7.8	2.3
2	3	51	225	174	357	1547	0.49	11.25	8.6	2.4
3	1	55	233	178	364	1577	0.49	11.29	7.6	2.1
3	2	49	229	180	381	1650	0.47	10.91	7.4	3.0
3	3	42	216	174	343	1486	0.51	11.71	6.7	1.5
Mean		50	227	178	364	1576	0.49	11.29	7.4	2.1
				Diet 4: fat 30%, fructose 25%						
1	1	52	222	170	345	1495	0.49	11.37	6.7	1.9
1	2	49	218	169	334	1447	0.51	11.68	6.5	1.7
1	3	51	235	184	372	1612	0.49	11.42	7.4	1.8
2	1	54	227	173	368	1594	0.47	10.85	7.9	2.1
2	2	47	240	193	399	1728	0.48	11.17	8.2	1.9
2	3	55	224	169	349	1512	0.48	11.18	7.3	1.8
3	1	57	231	174	360	1560	0.48	11.16	7.6	2.1
3	2	47	230	183	380	1646	0.48	11.12	7.8	1.8
3	3	45	200	155	334	1447	0.46	10.71	6.8	1.4
Mean		51	225	174	333	1560	0.48	11.18	7.4	1.8

[1]Day 28 of experiment. [2]Final weight - initial weight. [3]Weight gain (g)/feed intake (g).
[4]Weight gain (g)/kcal × 100.

TABLE 12A

Total serum cholesterol, serum HDL cholesterol, HDL cholesterol/cholesterol and serum triglycerides

Replication	Animal	Serum cholesterol			Serum triglycerides (mg/dl)
		Total (mg/dl)	HDL (mg/dl)	HDL/total × 100	
		Diet 1: fat 40%, sucrose 20%			
1	1	75.9	61.2	80.7	73.8
1	2	77.2	53.5	69.3	59.0
1	3	77.2	57.4	74.3	54.1
2	1	89.7	72.4	80.8	70.2
2	2	91.0	65.0	71.4	46.0
2	3	62.0	55.4	89.3	60.0
3	1	95.3	71.4	74.9	82.5
3	2	88.0	61.6	70.0	79.0
3	3	90.7	61.6	68.0	42.5
Mean		83.0	62.2	75.0	63.0
		Diet 2: fat 40%, fructose 20%			
1	1	86.2	67.3	78.1	53.1
1	2	90.0	55.1	61.4	55.0
1	3	79.3	52.3	66.0	77.5
2	1	62.1	51.7	83.3	56.1
2	2	69.0	50.3	72.9	53.2
2	3	89.7	55.4	61.8	67.7
3	1	82.9	46.6	56.2	56.0
3	2	77.1	50.4	65.3	41.0
3	3	80.0	48.9	61.1	51.0
Mean		79.5	53.1	67.3	56.8
		Diet 3: fat 30%, sucrose 25%			
1	1	72.4	52.0	71.8	63.9
1	2	58.6	45.0	76.9	66.4
1	3	93.1	76.3	81.9	63.0
2	1	89.0	72.4	81.4	43.6
2	2	74.5	62.8	84.3	52.5
2	3	75.9	57.6	76.0	70.2
3	1	100.0	71.4	71.4	45.0
3	2	98.0	69.2	70.6	36.0
3	3	89.3	62.1	70.7	55.0
Mean		83.4	63.3	76.1	55.2

(continued)

TABLE 12A (continued)

Replication	Animal	Serum cholesterol			triglycerides (mg/dl)
		Total (mg/dl)	HDL (mg/dl)	HDL/total × 100	
		Diet 4: fat 30%, fructose 25%			
1	1	75.9	50.9	67.1	39.3
1	2	65.5	45.9	70.0	78.7
1	3	79.3	64.0	80.6	71.3
2	1	67.6	53.2	78.7	50.3
2	2	77.2	60.1	77.8	53.2
2	3	75.9	59.1	77.9	60.5
3	1	85.0	67.7	79.6	44.0
3	2	90.0	66.2	73.5	46.0
3	3	92.9	66.2	71.2	55.0
Mean		78.8	59.2	75.2	55.4

EFFECTS OF DIETARY FAT AND CARBOHYDRATE ON WEIGHT GAIN AND SERUM LIPIDS IN RATS

by

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AN ABSTRACT OF A MASTER'S THESIS

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ABSTRACT

A randomized complete block (RCB) design was used to assign 36 male weanling rats to diets with two levels of fat and distribution of fatty acids patterned after the current American diet and the proposed U.S. Dietary Goals and with two sources of sugar. There were 3 replications to each of the four treatments, and 3 animals to each replication. The effects of the four dietary treatments on serum lipid levels, epididymal fat pad weights and liver weights were investigated after a 28-day feeding period. The four diets were: diet 1, fat 40% of the calories with a P:S of 0.4 and sucrose at 20%; diet 2, fat 40% (P:S, 0.4) and fructose 20%; diet 3, fat 30% (P:S, 1.0) and sucrose 25%; diet 4, fat 30% (P.S, 1.0) and fructose 25%. At the termination of the study the rats were fasted overnight and sacrificed. Blood was drawn from the abdominal artery and the livers and epididymal fat pads were removed and weighed. The parameters measured were: weight gain, feed intake, epididymal fat pad weights, liver weights, serum cholesterol, serum high density lipoprotein (HDL) cholesterol and serum triglycerides.

Weights were different ($P < 0.05$) among diets. Rats on the sucrose diets (diets 1,3) gained more ($P < 0.05$) weight than rats on the fructose diets (diets 2,4) and deposited more fat ($P < 0.05$) as measured by epididymal fat pad weights.

An important factor which contributed to a higher weight gain in sucrose-fed versus fructose-fed rats, was the ability of rats on the sucrose diets to convert feed intake more efficiently ($P < 0.01$) into body weight gain. When feed efficiency among diets was calculated as weight gain as a) a function of grams of feed intake or as b) a function of caloric intake, the difference was significant ($P < 0.01$). The level

and degree of saturation of fat did not affect weight gained, fat deposition or feed efficiency measured as function of caloric intake.

The four dietary treatments had no significant effect on liver weights, total serum cholesterol, serum HDL cholesterol or serum triglyceride levels. Serum HDL cholesterol levels were influenced by the type of sugar. Rats on the sucrose diets had higher ($P < 0.10$) HDL cholesterol levels than rats on the fructose diets. The level and degree of saturation of fat had no effect on HDL cholesterol levels.

The ratio of HDL cholesterol to total serum cholesterol was different ($P < 0.05$) among diets. Rats fed the 40% fat, 20% fructose diet had a lower ratio ($P < 0.05$) than rats fed the other three diets. The level and degree of saturation of fat and the type of sugar affected ($P < 0.05$) the percentage of HDL cholesterol to cholesterol.

The results of this study indicated that levels and degree of saturation of fat and type of sugar used in the diets had a significant effect on weight gain, feed efficiency and fat deposition in rats, but had no significant effect on serum lipid levels and liver weights.