Effect of dietary protein on cholesterol homeostasis in diabetic rats

Bhalchandra J. Kudchodkar,* Mi-Ja Choi Lee,† Sun-Min Lee,* Nancy M. DiMarco,† and Andras G. Lacko*

Department of Biochemistry,* Texas College of Osteopathic Medicine, University of North Texas, Fort Worth, TX 76107, and Department of Nutrition and Food Sciences,† Texas Woman's University, Denton, TX 76204

Abstract Normal and streptozotocin (STZ)-diabetic rats were studied in order to examine the effects of altering the type of dietary protein on cholesterol homeostasis. Rats were fed a nonpurified or a purified diet containing either casein or soybean protein. The results obtained on the specific aspects of lipid metabolism were remarkably similar in control rats fed the nonpurified (Purina Lab Chow) diet or the purified diet with the soybean protein. However, most of the findings obtained with the above two groups were different from those obtained with rats fed the purified diet containing casein. In the latter group, plasma cholesterol was elevated following a 15-day feeding period as compared to the other two dietary groups. The excess plasma cholesterol in the casein-fed group was found in two lipoprotein fractions with densities of 1.023–1.045 g/ml and 1.045–1.086 g/ml, respectively. The latter lipoprotein fraction was also enriched with apolipoprotein E. The casein-fed animals also showed a lower fractional rate of plasma cholesterol esterification and an abnormal accumulation of cholesterol in the body despite inhibition of cholesterol synthesis in the liver and in the intestines. Twelve to 15 days after the induction of diabetes, plasma cholesterol increased to a similar extent in the rats on all three diets. However, the distribution of cholesterol among the lipoprotein fractions was markedly different. The percentage of cholesterol in fractions of d < 1.086 g/ml was increased while that carried in the fraction of d 1.086–1.161 g/ml decreased in the rats fed the nonpurified diet and the casein diet. In contrast, there was no change in the distribution of lipoprotein cholesterol between the diabetic and the control rats fed the soybean protein diet. The hepatic synthesis of cholesterol was unaltered in diabetic rats fed the nonpurified diet and the purified diet with soybean protein, but was increased 2.4-fold in diabetic rats fed casein. Intestinal cholesterol synthesis was increased in all three dietary groups. The increase was highest in the rats fed casein and lowest in rats fed soybean protein. The rate of sterol synthesis in the kidneys was not significantly affected by the diet or diabetes. In all three dietary groups diabetes led to an abnormal accumulation of cholesterol in the body. This accumulation was highest in the casein-fed rats and lowest in those fed the soybean protein diet. The cholesterol content of the kidneys was markedly increased by dietary casein. Dietary casein increased the hepatic as well as intestinal synthesis of fatty acids and their incorporation into triglycerides in the control and in diabetic rats as compared to the rats fed the other two diets. These data suggest that diabetic hypercholesterolemia is due to the increased transport of cholesterol, synthesized de novo by the intestine along with that absorbed (endogenous and/or exogenous) by the intestine. Dietary soybean protein may be beneficial in controlling hyperlipidemia but not the hyperglycemia associated with STZ-induced diabetes. —**Kudchodkar, B. J., M-J. C. Lee, S-M. Lee, N. M. DiMarco, and A. G. Lacko.** Effect of dietary protein on cholesterol homeostasis in diabetic rats. *J. Lipid Res.* 1988. **29:** 1272–1287.

Supplementary key words streptozotocin • diabetes • plasma lipoproteins • plasma LCAT • cholesterol absorption • cholesterol synthesis • tissue cholesterol • kidney cholesterol • lipid synthesis

Both hypertriglyceridemia and hypercholesterolemia are often associated with diabetes (1). For these and possibly other reasons, diabetes has been recognized as one of the major risk indicators of premature atherosclerosis (1). Although rats, the most commonly used animal model in diabetes research, are normally resistant to dietary cholesterol-induced hypercholesterolemia and atherosclerosis (2), they become susceptible to this dietary stress following streptozotocin- (STZ) or alloxan-induced diabetes (3, 4). Recent findings suggest that intestinal events may trigger both hypertriglyceridemia and the hypercholesterolemia seen in diabetic rats (5–8). Accordingly, increased intestinal synthesis and lymphatic transport of cholesterol and triglycerides have been observed in diabetic rats (5–8). In addition, increased absorption and transport of both biliary and dietary cholesterol have been found in these animals (5, 9). The metabolism of bile acids is also abnormal in diabetic rats as evidenced by a marked increase in the synthesis and pool size of cholic acid (5, 10)

Recent studies have shown that high protein diets may be effective in the control of hyperglycemia in humans (11, 12) as well as in rats (13). These high protein diets gener-

Abbreviations: STZ, streptozotocin; VLDL, very low density lipoprotein(s); LDL, low density lipoprotein(s); HDL, high density lipoprotein(s); LCAT, lecithin:cholesterol acyltransferase; FR, fractional rate; apo, apolipoprotein.

ally contain protein from animal sources (e.g., casein) which has been shown to be hypercholesterolemic in nondiabetic animals (14, 15). On the other hand, dietary plant proteins (e.g., soybean protein) have been found to be hypocholesterolemic in humans as well as in animals with hypercholesterolemia (14, 15). Furthermore, plant proteins have been shown to prevent the development of hypercholesterolemia generally seen in rats upon aging (16) or upon consuming a high fat/high cholesterol atherogenic diet (14, 15). Although the mechanism of the hypocholesterolemic effect of dietary soybean protein has not been fully elucidated, a likely mechanism involves the facilitation of the fecal excretion of neutral sterols and bile acids due to their reduced absorption (14–16). Because diabetes increases both cholesterol and bile acid reabsorption in rats (5, 9, 10), it was of interest to examine the effects of the dietary sources of protein on cholesterol homeostasis in STZ-induced diabetic rats.

EXPERIMENTAL

Animals

Male weanling, Sprague-Dawley rats weighing about 50 g were obtained from SASCO, Inc. (Omaha, NE). Animals were housed individually in stainless-steel cages with wire-mesh bottoms in a temperature-controlled room (20–23°C) with a 12-hr light/dark cycle. They were fed a pelletized commercial nonpurified diet (Purina Lab Chow, Ralston Purina Co., St. Louis, MO) and water ad libitum.

Induction of diabetes

Experiments were performed at three different times. At all three times animals were handled similarly, except that in one of the experiments the animals weighed between 280 and 350 g compared to the animals in the other two experiments, where they weighed between 180 and 200 g. Each time the animals with slightly higher (about 10–20 g) body weights were made chemically diabetic by an intraperitoneal injection of STZ at a dose of 50 mg/kg body weight (7–9). Higher weight animals were chosen for induction of diabetes because these animals were more likely to withstand the weight loss induced by drug treatment (13). The nondiabetic control animals received the injection of buffer alone. After the injection, the animals had free access to water and the nonpurified diet. Four days later, glycosuria in the STZ-treated group was confirmed qualitatively using Ketodiastix reagent strips and quantitatively by analyzing the glucose content of blood samples collected from the tail vein into heparinized micro hematocrit capillary tubes. Animals were considered to be diabetic when their nonfasting plasma glucose levels exceeded 300 mg/dl. Other criteria of diabetes were increased food and water intake and urine output.

Diet

Five days following STZ treatment, rats were randomly assigned to one of the three groups of diabetic rats or one of the three groups of nondiabetic rats. One group of nondiabetic and diabetic rats was continued on the nonpurified diet, while the other two nondiabetic and diabetic groups were switched to experimental purified diets. The two purified diets were similar in composition with the exception of the protein source, which was either casein or soybean protein isolate (22.5%). Other components of the purified diets were dextrose (53.2%), corn oil (4.5%), minerals and vitamins (6%), and celufil (3.8%). The protein (22.5%), carbohydrate (53%), and the fat (4.5%) content of the nonpurified diet originated from mixed sources (animals, fish, and vegetables) and its cholesterol content has been reported to be negligible (between 0.25 and 0.32 mg/g food) (17). The nutrient sources of the purified diets are precisely formulated and these diets are virtually cholesterol-free. The purified diets were obtained from U.S. Biochemical Corporation (Cleveland, OH) in a pelletized form. Diets were fed ad libitum for a period of 11–15 days before use. Food consumption and body weights were recorded every 3 days during the study.

Collection of blood and other body tissues

At the end of the experiment, animals were anesthetized with ether. Blood was taken from the inferior vena cava and transferred to chilled tubes containing EDTA (ethylene diamine tetraacetic acid) at a final concentration of 1 mg/ml. Plasma was obtained by low speed centrifugation at 4°C. Aliquots of plasma were taken for the determination of glucose, cholesterol, and triglyceride levels as well as for the determination of radioactivity, lecithin:cholesterol acyltransferase (LCAT) activity, and for the isolation of lipoproteins. After the removal of blood, the body was perfused with cold saline. The liver, kidneys, spleen, heart, lungs, testes, and epididymal fat pads were removed, rinsed with cold saline, blotted, and weighed. The gastrointestinal tract was removed. The stomach, small intestines, and colon were cut and cleaned thoroughly with water and saline, blotted, and weighed. After removing the organs, the animals were skinned, blotted, and skin weights were recorded. The tail was cut and discarded. The carcass (bone, muscle, central nervous system, etc.) was blotted free of moisture, cut into small pieces, and weighed. All tissues were stored at −20°C before analysis. All tissues were used for the determination of total and esterified cholesterol (liver and intestines) and radioactivity (synthesis and absorption studies).

MATERIALS

Sources of chemicals and reagent supplies were as follows: [1,2-^{3}H]cholesterol (40 Ci/mmol), [4-^{14}C]cholesterol (50 mCi/mmol), and [1-^{14}C]octanoate (58 mCi/mmol), New England Nuclear, Boston, MA; Ecolite, scintillation fluid, West Chem, San Diego, CA; chemicals for gel electrophoresis, Bio-Rad Laboratories, Richmond, CA. Enzymatic kits for cholesterol and glucose were from Boehringer Mannheim, Indianapolis, IN, for triglycerides from Amresco, South, Euclid, OH; cholesterol, triglycerides, standards, streptozotocin, silica gel plates for thin-layer chromatography, and crystalline bovine serum albumin (essentially fatty acid-free) were from Sigma Chemicals Co., St. Louis, MO; Ketodiastix, Miles Laboratories, Elkhart, IN; heparin-Sepharose CL-6B, Pharmacia Inc., Piscataway, NJ; Aquacide, I.A., Calbiochem, La Jolla, CA. All other chemicals were obtained from Fischer Scientific Company, Dallas, TX and were reagent grade or better.

METHODS

Relative rates of synthesis of tissue sterols and other lipids

In vivo incorporation of [1-^{14}C]octanoate into tissue sterols and other lipids was used as an index of the relative rate of their synthesis (18). The synthetic rates of tissue sterols and other lipids were determined only for liver, small intestines, and kidneys. After being fed the experimental diets for a period of 14–15 days, fasting animals were given an intraperitoneal injection of 10 μCi of [^{14}C]octanoate dissolved in physiological saline. Injection was given between 8:00 and 9:00 AM and the animals were killed 1 hr later. Tissues were weighed and stored at −20°C until analysis.

Relative rates of cholesterol absorption

Two separate studies were performed. Body weights of the rats were lower in the first study (nondiabetic, 230–260 g; diabetic, 170–220 g) than those used in the second study (nondiabetic, 280–360 g; diabetic, 225–290 g). In addition, rats in the first study were fed experimental diets for a period of 11 days, while in the second study they were fed the diets for a period of 14 days before measuring cholesterol absorption. In both studies, absorption of [^{3}H]cholesterol was estimated by measuring the difference between [^{3}H]cholesterol intake and [^{3}H]cholesterol (and metabolites) recovered in the whole body 38–40 hr after administration of the label (19). [1,2-^{3}H]Cholesterol, purified by thin-layer chromatography, was dissolved in corn oil and a known amount (2.1 μCi) was given by gastric intubation just before the onset of the dark period. Twenty-four hours after the animals received the label, they were fasted for 14–16 hr and then killed. Blood and tissues were obtained and radioactivity was determined as described below. Tissues were weighed and stored at −20°C until analysis.

Isolation of plasma lipoproteins

Plasma, treated with sodium azide (0.01% w/v), was kept at 4°C and used for the isolation of lipoproteins. Lipoproteins were isolated within 24 hr of obtaining the plasma by using a density gradient ultracentrifugation method essentially as described by Terpstra, Woodward, and Sanchez-Muniz (20). The procedure was calibrated in our laboratory by repeatedly determining the density of successive 1-ml fractions that were removed from the top of the tubes by a narrow-bore Pasteur pipette. In the final procedure the following six fractions of different volumes and densities were collected for the determination of the distribution of cholesterol and triglycerides: fraction 1, d < 1.01 g/ml (1 ml); fraction 2, d 1.01–1.023 g/ml (2 ml); fraction 3, d 1.023–1.045 g/ml (2 ml); fraction 4, d 1.045–1.086 g/ml (2 ml); fraction 5, d 1.086–1.161 g/ml (2 ml); and fraction 6, d 1.161–1.181 g/ml (1 ml). The mean percentage recovery of cholesterol and triglycerides (sum of fractions compared to plasma values) was 94 ± 4 (range 90–105) and 89 ± 3 (range 85–95), respectively.

Heparin-Sepharose chromatography

Fraction 4 (d 1.045–1.086 g/ml) from each dietary group with and without diabetes, was pooled, dialyzed extensively (at 4°C 0.01 M Tris-HCL, 0.15 M NaCl buffer, pH 7.4, containing 0.01% EDTA) and fractionated by heparin-Sepharose chromatography according to the method of Shelburne and Quarfordt (21). A buffer containing 2 mM phosphate (pH 7.4) with an initial NaCl concentration of 0.05 M and a final salt concentration of 1.5 M was used in developing the gradient separations.

Lecithin:cholesterol acyltransferase (LCAT) activity

The initial rate of endogenous cholesterol esterification was measured by the method of Stokke and Norum (22). To estimate the amount of LCAT present, the plasma samples were also assayed by the method of Glomset and Wright (23) modified to employ high density lipoproteins (d 1.125–1.21 g/ml) isolated from human plasma as substrate. The details of this method have been described previously (24).

Analysis of tissues

Total lipids from liver and intestines were extracted by the method of Folch, Lees, and Sloane Stanley (25). The washed chloroform layer was evaporated to dryness and the lipids were redissolved in a known volume of chloroform. An aliquot was taken for the separation of lipids by

thin-layer chromatography. Plates were developed using hexane–diethyl ether–glacial acetic acid 80:20:1.5 (v/v/v). The lipid bands were visualized by iodine vapor and identified by comparison to standards run alongside the samples. The bands corresponding to phospholipids, triglycerides, and fatty acids were scraped into counting vials and their radioactivity was determined.

Esterified and unesterified cholesterol (along with mono- and diglycerides) were eluted with ether. Ether was evaporated and the residue was dissolved in isopropanol. Aliquots were then taken for the measurement of cholesterol and radioactivity. Before counting, the unesterified cholesterol was precipitated using 1% digitonin in 50% ethanol (26). Total cholesterol content and radioactivity of all other tissues were determined after their saponification with 30% KOH in methanol (17). For smaller tissues the entire volume was used for the extraction (3× with hexane) of nonsaponifiables. The carcass and the skin extracts were diluted to a known volume with methanol and one-fourth of the volume was extracted three times with hexane. All the hexane extracts were washed with water, dried, and dissolved in a known amount of isopropanol. An aliquot was used for counting radioactivity and another for determining mass. Internal standards of [^{3}H]cholesterol (for cholesterol synthesis study) and [^{14}C]cholesterol (for cholesterol absorption study) were added before extraction to correct for methodological losses. The radioactivity of all samples was determined by counting in a Packard Scintillation counter using Ecolite scintillation fluid. Quenching was corrected for by using an external standard.

Analytical procedures

Plasma glucose and plasma and lipoprotein triglyceride were measured by using respective enzymatic reagent kits. Plasma and lipoprotein fractions were extracted with isopropanol before measuring free cholesterol enzymatically and total cholesterol chemically (27). Total, free, and esterified cholesterol (separated by TLC) of other body tissues were quantitated chemically (27). Protein concentration was determined as described by Markwell et al. (28) using bovine serum albumin as standard. Gradient polyacrylamide gel electrophoresis (PAGE) was performed in the presence of 0.1% (w/v) SDS, according to the method of Laemmli (29).

Calculations

Esterified cholesterol levels in plasma and lipoprotein fractions were calculated by subtracting free cholesterol values from total cholesterol values. Fractional rate of [^{3}H]cholesterol absorption was calculated as follows: percent [^{3}H]cholesterol absorbed = (total [^{3}H]cholesterol found in the body ÷ total [^{3}H]cholesterol fed) × 100. Whole body cholesterol mass equalled the sum of the total cholesterol mass in each tissue. In this study, it was deemed desirable to separate retention of cholesterol due to growth from the abnormal accumulation of cholesterol in the tissues studied. Data available in the literature suggest that in growing rats fed chow (nonpurified) diet the cholesterol content of a tissue is generally proportional to the weight of that tissue. Therefore, an increase of cholesterol per unit weight of tissue is most likely to be due to an abnormal retention of cholesterol in that tissue. This assumption may not be valid in diabetic rats, where some tissues lost weight while others gained weight. In addition, data available in the literature on tissue cholesterol levels of starving animals (30) suggest that loss of tissue weight may not result in a proportionate loss of cholesterol from that tissue. Consequently, the cholesterol content of the tissues under these conditions shows an increase when it is expressed per unit weight of the tissue. We have therefore calculated the prediabetic weight for each tissue by multiplying the prediabetic body weight by the mean tissue weight (g/g body weight) for the rats fed nonpurified control diet. The cholesterol content of each tissue was then calculated by multiplying tissue weight (g) by the cholesterol content (mg/g) of that tissue in control rats. The difference between the calculated value (expected cholesterol content of the prediabetic tissue) and the actual observed value was assumed to represent the change in the tissue cholesterol content due to diabetes in rats fed the nonpurified diet and due to diabetes and diet in animals fed the purified diet (specific diets were fed only after the induction of diabetes). If the tissue was losing weight, then an increase in the cholesterol content of that tissue was considered abnormal. If a tissue was gaining weight, then the expected change in cholesterol mass due to growth was calculated in order to determine whether there was any abnormal accumulation of cholesterol in the growing tissue. If there was no difference between the calculated and observed value for cholesterol mass, then the increase in the cholesterol content of the growing tissue was considered to be due to growth. If there was an increase over and above the value expected due to the growth of the tissue, then it was considered to be due to an abnormal accumulation of cholesterol in that tissue.

Statistical methods

One-way analysis of variance was performed to see whether any significant differences occurred among dietary groups within the nondiabetic and diabetic groups. Newman-Keul's test was performed to determine the significant difference between pairs of means. The Student's unpaired *t*-test was performed to examine the differences between the nondiabetic and diabetic rats within each dietary group. Correlational analyses were performed using linear regression procedure (31). A prob-

ability of 0.05 or less was accepted as statistically significant. Values reported in the text and in the tables represent mean ± standard error of the mean (SEM).

RESULTS

Animals with higher body weights were used in one experiment to study the effects of diabetes on the distribution of cholesterol and triglycerides in plasma lipoprotein fractions and on tissue cholesterol levels. Cholesterol absorption was also determined in these rats. Whereas the levels of plasma glucose and the rate of cholesterol absorption of the heavier rats were similar to those of lighter rats used in the other two studies, the plasma cholesterol and triglyceride levels were higher in the heavier rats by about 5–15 mg/dl. Upon induction of diabetes, the degree of change in plasma lipids, glucose, and in cholesterol absorption was similar to that seen in smaller animals. Since the heavier animals were evenly distributed among the six experimental groups, the data on plasma lipids, plasma glucose, and the fractional rate of absorption from these animals were combined with that from lighter rats.

Food intake and body weight

The food intake and the weight gain of nondiabetic rats fed the nonpurified or the purified diets were similar (**Table 1**). Following STZ treatment, the food consumption increased to a similar extent in all three dietary groups (Table 1). However, regardless of diet, all the diabetic rats had lower body weights compared to the animals of the respective control groups (Table 1).

Plasma glucose

Fasting plasma glucose was not significantly influenced by the different diets in control rats (Table 1). Although the levels increased in all three dietary groups upon STZ administration (Table 1), the hyperglycemia was much less pronounced in the group fed the purified diet containing casein (194.5 ± 11.8 mg/dl) as compared to the groups fed the purified diet containing soybean protein (270 ± 12.3 mg/dl) or the nonpurified diet (276.2 ± 22.3 mg/dl).

Plasma triglycerides and their distribution among lipoproteins

Plasma triglyceride levels (Table 1) were similar in the two groups fed the purified diets (casein, 114 ± 3.8 mg/dl; soybean protein 120.6 ± 7.2 mg/dl) and were significantly higher than those of the group fed nonpurified diet (99 ± 3.3 mg/dl). The onset of diabetes resulted in significant increases in the plasma triglyceride levels of all three dietary groups (Table 1).

Less than 5% of the plasma triglycerides was found in the lipoprotein fractions of d > 1.045 g/ml. This distribution was not affected by diet or by the onset of diabetes. Diet but not diabetes caused a change in the distribution of triglycerides among the lipoprotein fractions of d < 1.045 g/ml. In the animals fed soybean protein, about 6% of the triglycerides was located in the lipoprotein fraction with d 1.023–1.045 g/ml; about 4% in the fraction with d 1.01–1.023 g/ml, and about 88% in the fraction with d < 1.01 g/ml. In contrast, in the casein-fed animals, 11% was found in the fraction with d 1.023–1.045 g/ml, 7% in the fraction with d 1.01–1.023 g/ml, and only 78% in the fraction with d < 1.01 g/ml. The corresponding values

TABLE 1. Body weights, food intake, plasma cholesterol, triglycerides, and glucose levels in nondiabetic and diabetic rats fed a nonpurified diet or a purified diet containing casein or soybean protein

	Experimental Group					
			Purified Diet			
	Nonpurified Diet		Casein		Soybean Protein	
Parameter	Nondiabetic	Diabetic	Nondiabetic	Diabetic	Nondiabetic	Diabetic
Body weight (g)	250 ± 7 (13)	235 ± 9 (14)	254 ± 9 (13)	207 ± 9* (13)	261 ± 11 (13)	213 ± 8* (13)
Food intake (g/day)	23.3 ± 1.0 (10)	35.7 ± 1.4* (10)	22.6 ± 0.4 (10)	33.2 ± 1.2* (10)	23.4 ± 0.9 (10)	33.3 ± 1.3* (10)
Concentration in plasma						
Cholesterol (mg/dl)	86.4 ± 3.1[a] (9)	115.6 ± 3.1[d]* (10)	108.1 ± 3.5[b] (10)	138.7 ± 3.9[e]* (10)	80.0 ± 3.5[a] (9)	108.1 ± 4.8[d]* (9)
Triglycerides (mg/dl)	99.0 ± 3.3[a] (11)	175.8 ± 14.0* (12)	114.0 ± 3.8[b] (11)	200.9 ± 17.6* (11)	120.6 ± 7.2[b] (10)	165.2 ± 84* (10)
Glucose (mg/dl)	144.0 ± 8.9 (9)	276.2 ± 22.3[d]* (10)	141.3 ± 7.7 (9)	194.5 ± 11.8[e]* (10)	155.2 ± 8.6 (9)	270.1 ± 12.3[d]* (10)

Data represent the mean ± SEM of values obtained in the number of animals shown in parentheses. Values without common superscript letters denote significant differences ($P < 0.05$) among diets.

*Denotes significant difference ($P < 0.05$) between nondiabetic and diabetic groups on the same diet.

for the animals fed the nonpurified diet were 7%, 6%, and 84% of the total plasma triglycerides, respectively. The differences among the three dietary groups in the triglyceride content of the lipoprotein fraction of d < 1.01 g/ml were statistically significant (data not shown).

Plasma cholesterol and its distribution among lipoproteins

Total cholesterol levels of the plasma were significantly higher in the casein-fed group (108.1 ± 3.5 mg/dl) as compared to those fed soybean protein (80 ± 3.5 mg/dl) or the nonpurified diet (86.4 ± 3.1 mg/dl). The difference in plasma cholesterol levels between the latter two groups was not significant (Table 1). In each dietary group, the onset of diabetes was associated with increased plasma cholesterol levels (Table 1). Although the diabetic group fed the casein diet had the highest levels of plasma cholesterol, the increase from the control values (30 mg/dl) was similar to that seen in the groups fed soybean protein (28 mg/dl) or the nonpurified diet (29 mg/dl).

The distribution of total cholesterol and the percentage of esterified cholesterol in the isolated plasma lipoprotein fractions are given in **Table 2.** Only a minor portion (3–5% of the total) of the cholesterol was found in the lipoprotein fraction of d 1.161–1.181 g/ml. The cholesterol content of this fraction was not affected by either diet or the onset of diabetes (Table 2). In the control rats fed the nonpurified diet, the levels of plasma cholesterol as well as its distribution among the lipoprotein fractions were remarkably similar to the group fed the purified diet containing soybean protein. The only difference was a small but significant increase in the amount of cholesteryl esters in the fraction of d <1.01 g/ml in the former group (Table 2). In the group fed the casein diet, the cholesterol levels increased in all lipoprotein fractions except in the one with d 1.086–1.161 g/ml. The greatest and most significant increases were seen in the fractions with d 1.023–1.045 g/ml and d 1.045–1.086 g/ml. These fractions were also significantly enriched with esterified cholesterol (Table 2). The proportion of cholesterol among the fractions of density 1.023–1.045, 1.045–1.086, and 1.086–1.161 g/ml was 18%, 34%, and 32% in the rats fed casein diet, and 12%, 32%, and 40% in the corresponding density fractions for the rats fed soybean protein or the nonpurified diet (Table 2).

In the rats fed the nonpurified diet or the casein diet, STZ treatment resulted in increases in the amount of

TABLE 2. Cholesterol levels in different density fractions of plasma lipoproteins in nondiabetic and diabetic rats fed a nonpurified diet or a purified diet containing casein or soybean protein

Density Fraction	Parameter	Experimental Group: Nonpurified Diet — Nondiabetic	Nonpurified Diet — Diabetic	Purified Diet: Casein — Nondiabetic	Casein — Diabetic	Soybean Protein — Nondiabetic	Soybean Protein — Diabetic
g/ml							
<1.01	Total cholesterol (mg/dl)	7.3	17.0^{d*}	9.5	18.3^{d*}	7.6	9.4^{e}
		(8.0)	(14.3)^{d*}	(8.4)	(13.1)^{d*}	(9.0)	(8.5)^{e}
	Esterified cholesterol (%)	46.3^{a}	48.8^{a*}	46.4^{a}	53.0^{d*}	42.6^{b}	42.1^{e}
1.01–1.023	Total cholesterol (mg/dl)	2.4^{a}	5.1^{de*}	4.2^{b}	6.0^{d}	2.5^{a}	2.6^{e}
		(2.6)	(4.3)^{d*}	(3.7)	(4.3)^{d}	(2.9)	(2.3)^{e}
	Esterified cholesterol (%)	60.9	66.6*	62.7	67.0	59.8	64.1*
1.023–1.045	Total cholesterol (mg/dl)	12.3^{a}	20.4^{de*}	20.2^{b}	25.6^{d}	9.5^{a}	14.6^{e}
		(13.6)^{a}	(17.3)^{d*}	(17.9)^{b}	(18.1)^{d}	(11.3)^{c}	(13.1)^{e}
	Esterified cholesterol (%)	69.7^{a}	75.6^{d*}	75.5^{b}	76.9^{d}	68.3^{a}	68.8^{e}
1.045–1.086	Total cholesterol (mg/dl)	27.7^{a}	40.2^{de*}	38.8^{b}	47.1^{d*}	26.8^{a}	35.9^{e*}
		(30.5)^{a}	(34.2)*	(34.4)^{b}	(33.6)	(31.8)^{c}	(32.6)
	Esterified cholesterol (%)	76.4^{a}	82.2^{d*}	81.2^{b}	82.5^{d*}	74.5^{a}	77.0^{e*}
1.086–1.161	Total cholesterol (mg/dl)	36.5	31.6^{d*}	35.5	39.0^{de}	34.4	44.4^{e*}
		(40.6)^{a}	(26.9)^{d*}	(31.6)^{b}	(27.8)^{d*}	(40.7)^{a}	(40.5)^{e}
	Esterified cholesterol (%)	81.8	81.8	82.0	82.1	81.2	83.2
1.161–1.181[†]	Total cholesterol (mg/dl)	4.3	3.5	4.6	4.6	3.6	3.2
		(4.8)	(2.9)	(4.0)	(3.3)	(4.3)	(3.0)

Data represent the mean value for three animals lin each group except the nonpurified diet-fed diabetic group where it is for four animals. For visual clarity SEM values have not been given. Values without common superscript letter denote significant difference ($P < 0.05$) among diets. Numbers in parentheses represent the mean value for the percentage distribution of lipid among the different density fractions.

[†] Esterified cholesterol concentration was not measured.

*Denotes significant difference ($P < 0.05$) between nondiabetic and diabetic groups on the same diet.

cholesterol in all lipoprotein fractions except in the one with the density 1.086–1.161 g/ml. As a result, the proportion of cholesterol carried in the fraction with d 1.086–1.161 g/ml significantly decreased while that carried in others increased. In contrast, diabetes had no effect on the distribution of cholesterol among plasma lipoprotein fractions in rats fed the soybean protein diet. The excess cholesterol was distributed among all the fractions (Table 2).

Distribution of apolipoprotein E among the isolated lipoprotein fractions

Analysis of the lipoprotein fractions of densities 1.045–1.086 and 1.086–1.161 g/ml, by SDS-PAGE revealed that, in control rats, both of these fractions contained apoCs, apoA-I, apoE, and apoA-IV, and some minor amounts of apoB. However, the same lipoprotein fractions obtained from the diabetic rats had markedly decreased contents of apoE. These changes were not due to the redistribution of apoE to the fractions of d $<$ 1.045 g/ml as these fractions also contained markedly low amounts of apoE (data not shown). Because the lipoprotein fraction with d 1.086–1.161 g/ml contained some serum albumin, only the fraction with d 1.045–1.086 g/ml from control and diabetic rats was subfractionated by heparin-Sepharose chromatography for the estimation of the apoE-enriched component. Equal amounts of protein (300 μg) were loaded on the column and the subfractions were eluted with a stepwise gradient as described in Methods. Elution profiles are shown in **Figs. 1 A–C.** Among the control rats, the heparin-Sepharose-bound, apoE-enriched fraction was highest in the group fed the casein diet and lowest in rats fed the soybean protein diet. In diabetic animals, this fraction was markedly reduced from the respective control levels in all three dietary groups (Figs. 1 A–C). In diabetic animals, whether or not the bound fraction contained apoE or apoB was not determined.

Plasma cholesterol esterification

The fractional rate of plasma endogenous cholesterol esterification (FR) was 15.3 ± 0.7% · hr^{-1} for the group fed the nonpurified diet. This value was not significantly different from the FR of the group fed the soybean protein diet (15.6 ±0.93% · hr^{-1}). The FRs in both the above groups were significantly higher than that observed for the group fed the casein diet (10.3 ± 0.92% · hr^{-1}). Upon induction of diabetes, FR increased to 12.6 ± 1.2% · hr^{-1} in the group fed the casein diet. In the groups fed nonpurified diet and the soybean protein diet, diabetes had no significant effect on the esterification of endogenous plasma cholesterol (FR = 14.0 ± 1.2% · hr^{-1} and 14.2 ± 1.11% · hr^{-1}, respectively). The FR (% · hr^{-1} measured using an exogenous substrate was affected neither by the diet nor by diabetes. In the nondiabetic versus diabetic

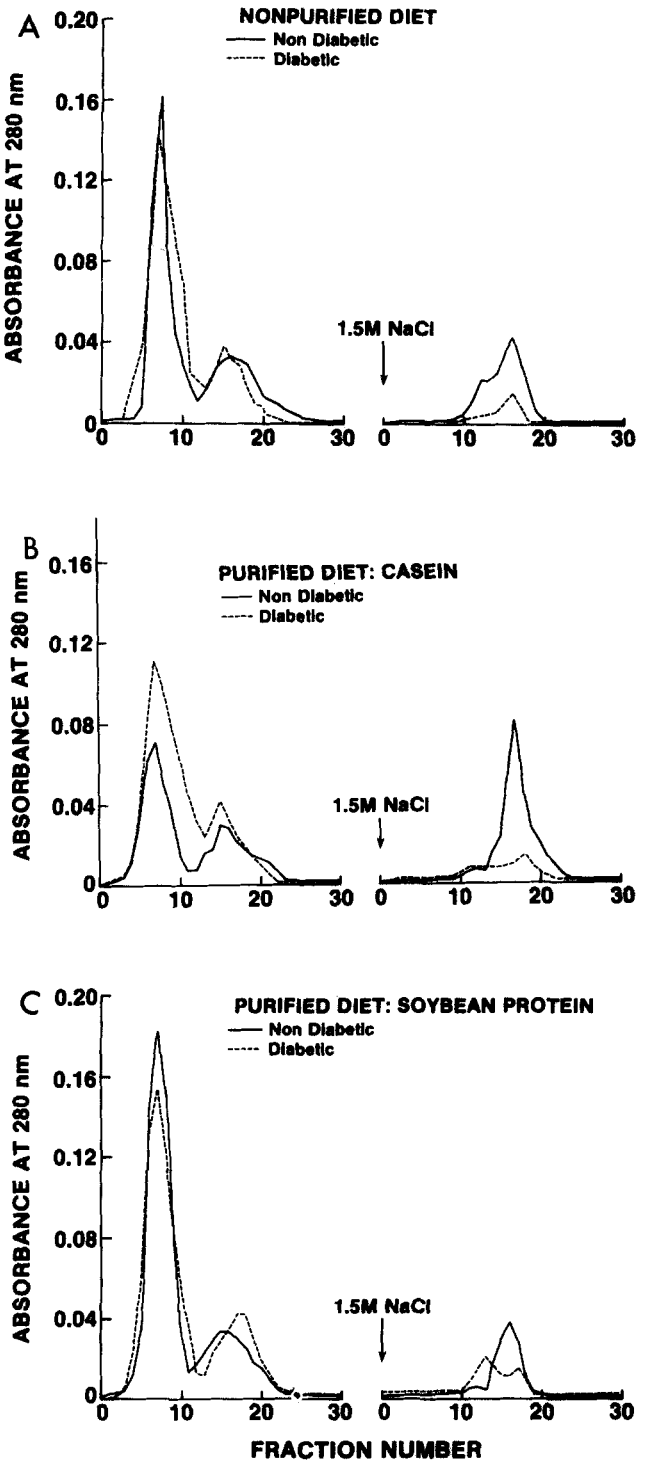

Fig. 1. Distribution of apolipoprotein E-enriched fraction in the lipoprotein fraction with density 1.045–1.086 g/ml. Three hundred microgram samples of protein from nondiabetic (——) and diabetic (----) rat plasma lipoprotein fraction of d 1.045–1.086 g/ml were applied to a heparin-Sepharose column (2.5 × 12 cm) and eluted with stepwise salt gradient (0.05 M and 1.5 M NaCl). The flow rate was 30 ml/hr and 2-ml fractions were collected. Panel A: rats fed the nonpurified diet; panel B: rats fed the purified diet containing casein; panel C: rats fed the purified diet containing soybean protein.

animals these values were, respectively: 4.7 ± 0.7 and 4.6 ± 0.2 in the nonpurified diet group, 4.8 ± 0.4 and 5.1 ± 0.8 in the casein-fed group, and 5.3 ± 0.8 and 5.8 ± 0.3 in the soybean protein-fed group.

Cholesterol absorption

In the control rats, the fractional rate (FR) of cholesterol absorption was significantly higher in the group fed the casein diet (71.7 ± 1.3%) than in the group fed soybean protein diet (64.2 ± 0.8%) or in the group fed the nonpurified diet (65.1 ± 1.3%). The differences between the latter two groups were not significant. In each of the three dietary groups, diabetes resulted in an increase in the fractional rate of cholesterol absorption. This increase was significant in the groups fed the nonpurified diet and the group fed the purified diet with soybean protein, but not in the group fed the casein diet. The FRs of absorption following STZ treatment were: 73.6 ± 1.8%, 75.9 ± 2.1%, and 71.4 ± 0.9%, respectively, for the groups fed the nonpurified diet or the purified diet containing casein or soybean protein.

Hepatic cholesterol content and the rates of lipid synthesis

The weights of the livers of the control rats were not significantly different among the three dietary groups. In diabetic rats, the liver weights were significantly increased in groups fed the nonpurified diet and the soybean protein diet (**Table 3**). The changes in the hepatic unesterified cholesterol (UC) associated with diabetes were due to the increase in the weight of the tissue. In control rats, the mass of hepatic esterified cholesterol (expressed as mg or mg/g of tissue) was significantly higher in rats fed casein diet as compared to those fed soybean protein or the nonpurified diet. The onset of diabetes was associated with a significant increase in the cholesteryl ester content of the liver in each dietary group (whether expressed as mg or mg/g of liver tissue). The increase was smallest in the group fed the soybean protein diet.

The rate of cholesterol synthesis (as measured by the in vivo incorporation of [1-^{14}C]octanoate) was significantly lower in rats fed the casein-containing diet as compared to the rats fed the soybean protein or the nonpurified diet (Table 3). The rate of cholesterol synthesis per gram of liver tissue was not affected by STZ treatment in the groups fed the soybean protein diet or the nonpurified diet, but was increased 2.4-fold in the group fed the casein diet. The total radioactivity found in the esterified cholesterol fraction was similar in all three dietary groups and was not significantly affected by the onset of diabetes (Table 3).

In the control animals, the synthesis of fatty acids was higher in the group fed the casein diet than in the groups fed the soybean protein or the nonpurified diet. In each group, the onset of diabetes was associated with a significant decrease in the rate of fatty acid synthesis. However, this decrease was only 14% in the animals fed casein compared to 30% in the animals fed the soybean protein diet and 42% in the animals fed the nonpurified diet. Incorporation of the newly synthesized fatty acids into hepatic triglycerides was significantly higher in the animals fed casein as compared to those fed soybean protein or the nonpurified diet. The radioactivity incorporated into triglycerides decreased as a result of STZ treatment in all three dietary groups (Table 3). In the control animals, the rate of newly synthesized fatty acid incorporation into phospholipids was similar in all three dietary groups. The onset of diabetes was associated with in-

TABLE 3. Hepatic cholesterol levels and the in vivo rate of incorporation of [1-^{14}C]octanoate in lipids in nondiabetic rats fed a nonpurified diet or a purified diet containing casein or soybean protein

			Purified Diet			
	Nonpurified Diet		Casein		Soybean Protein	
Parameter	Nondiabetic	Diabetic	Nondiabetic	Diabetic	Nondiabetic	Diabetic
Weight (g) (n = 13)	8.1 ± 0.2	10.0 ± 0.3^{d*}	7.6 ± 0.2	7.70 ± 0.4^{e}	7.5 ± 0.30	8.4 ± 0.3^{e*}
Cholesterol (n = 13)						
Unesterified (mg/g)	2.26 ± 0.05	2.17 ± 0.07	2.22 ± 0.05	2.23 ± 0.03	2.23 ± 0.03	2.27 ± 0.06
Esterified (mg/g)	0.43 ± 0.10^{a}	0.61 ± 0.03^{d*}	0.69 ± 0.03^{b}	0.79 ± 0.05^{e*}	0.40 ± 0.02^{a}	0.46 ± 0.02^{f*}
Incorporation of [^{14}C]octanoate in lipids (dpm × 10^2) (n = 5)						
Unesterified cholesterol (dpm/g)	13.6 ± 1.4^{a}	13.7 ± 0.6	5.7 ± 0.8^{b}	13.7 ± 1.1*	15.8 ± 2^{a}	14.2 ± 1.4
Esterified cholesterol (dpm/g)	1.6 ± 0.1	1.1 ± 0.2*	2.3 ± 0.5	1.5 ± 0.2	1.5 ± 0.1	1.2 ± 0.2
Fatty acids (dpm/g)	14.2 ± 2.0^{a}	6.2 ± 0.7^{d*}	29.1 ± 1.0^{bc}	24 ± 1.5^{e*}	21.5 ± 2.9^{ac}	13.3 ± 0.8^{f*}
Triglycerides (dpm/g)	1.9 ± 0.20^{a}	1.0 ± 0.1^{d*}	6.6 ± 1.1^{b}	4.8 ± 0.4^{e}	2.8 ± 0.4^{a}	1.8 ± 0.3^{d*}
Phospholipids (dpm/g)	45.9 ± 4.9	49.9 ± 4.1^{de}	47.1 ± 4.2	57.0 ± 4.2^{d}	45.4 ± 4.0	42.6 ± 1.2^{e}

Data represent mean ± SEM values obtained in the number (n) of animals shown in parentheses. Values without common superscript letters denote significant differences ($P < 0.05$) among diets.

*Denotes significant difference ($P < 0.05$) between nondiabetic and diabetic groups on the same diet.

creased rates of incorporation of fatty acids into phospholipids in the animals fed the casein diet and the nonpurified diet but not in those fed the soybean protein diet.

Cholesterol content and the rate of lipid synthesis of the small intestine

The weight of the intestines did not differ significantly among the three control groups. However, the onset of diabetes resulted in a significant increase in the weight of the intestines in each group. The (UC) content of the intestines was not significantly different among the control groups (**Table 4**). Significant increases in tissue UC seen within each group upon STZ treatment became nonsignificant when the data were expressed on the basis of the unit weight of the tissue (mg of UC/g of intestinal tissue). The esterified cholesterol (CE) content of the intestinal tissue was significantly higher in the casein-fed rats as compared to rats fed the soybean protein diet but not the rats fed the nonpurified diet. The differences between the latter two groups were not significant. Within each group, the onset of diabetes resulted in significant increases of tissue CE content based on the total amount of CE present but not when expressed as the mg of CE per gram of tissue (Table 4).

Among the three nondiabetic groups, the animals fed the casein diet had lower rates of intestinal cholesterol synthesis compared to the other two groups. However, these differences were not statistically significant. The rate of intestinal cholesterol synthesis was significantly increased in each group following STZ injection. The increase was 2.4-fold in the group fed casein and about 1.5-fold in the other two groups. The incorporation of label ([1-^{14}C]octanoate) into CE was not significantly different among the three control groups. Within each group, the incorporation of the label into CE was increased with the onset of diabetes (Table 4).

In the nondiabetic animals, the rate of intestinal fatty acid synthesis was significantly higher in the animals fed casein as compared to the other two groups. These synthetic rates increased in each group following STZ treatment but the increases were not significant in the animals fed the soybean protein diet and the nonpurified diet once the data were normalized with respect to tissue weight (Table 4). The incorporation of the newly synthesized fatty acids into triglycerides was higher in the animals fed casein compared to the soybean protein-fed group, which in turn was higher than in the group fed the nonpurified diet. The onset of diabetes was associated with decreased incorporation of radioactivity into the triglyceride fraction in each dietary group compared to their respective controls. The decrease was 73% in animals fed the soybean protein diet, 43% in the casein-fed, and 46% in the nonpurified diet-fed groups (Table 4). The rate of phospholipid synthesis in was higher in the group fed the casein diet compared to the other two groups. The increase in the rate of phospholipid synthesis observed upon STZ treatment was abolished when the data were normalized on the basis of tissue weights (Table 4).

Cholesterol content and lipid synthetic rates in kidney tissue

The weights of the kidneys were similar in the three nondiabetic groups. However, following the onset of diabetes, renal tissue weights were significantly increased in

TABLE 4. Intestinal cholesterol levels and the in vivo rate of incorporation of [1-^{14}C]octanoate in lipids in nondiabetic rats fed a nonpurified diet or a purified diet containing casein or soybean protein

	Experimental Group					
			Purified Diet			
	Nonpurified Diet		Casein		Soybean Protein	
Parameter	Nondiabetic	Diabetic	Nondiabetic	Diabetic	Nondiabetic	Diabetic
Weight (g) (n = 8)	6.53 ± 0.26	10.34 ± 0.78*	5.83 ± 0.20	8.73 ± 0.47*	6.27 ± 0.2	8.63 ± 0.71*
Cholesterol (n = 8)						
Unesterified (mg/g)	2.23 ± 0.07	2.19 ± 0.5	2.23 ± 0.03	2.26 ± 0.07	2.25 ± 0.08	2.34 ± 0.07
Esterified (mg/g)	0.13 ± 0.01^{ac}	0.15 ± 0.02	0.16 ± 0.01^{a}	0.15 ± 0.01	0.11 ± 0.01^{bc}	0.12 ± 0.07
Incorporation of [^{14}C]octanoate in lipids (dpm × 10^2) (n = 5)						
Unesterified cholesterol (dpm/g)	15.8 ± 1.5	24.8 ± 5.0*	12.7 ± 0.8	30.9 ± 5.2*	15.3 ± 1.8	23.3 ± 3.1*
Esterified cholesterol (dpm/g)	1.7 ± 0.22	3.0 ± 0.9	1.7 ± 0.2	3.3 ± 0.5*	1.5 ± 0.3	2.4 ± 0.4
Fatty acids (dpm/g)	14.9 ± 0.35^{a}	13.8 ± 0.26^{d}	29.2 ± 1.95^{bc}	46.1 ± 6.4^{e}*	18.0 ± 1.9^{c}	22.0 ± 3.2^{d}
Triglycerides (dpm/g)	9.2 ± 2.0	5.0 ± 0.05^{d}*	18.6 ± 3.5	10.7 ± 1.8^{e}*	12.9 ± 1.3	3.5 ± 0.28^{d}*
Phospholipids (dpm/g)	75.0 ± 5.6^{a}	64.4 ± 2.7^{d}	108.0 ± 5.0^{b}	122.6 ± 9.3^{e}	77.9 ± 4.7^{a}	83.5 ± 10.4^{d}

Data represent mean ± SEM values obtained in the number (n) of animals shown in parentheses. Values without common superscript letters denote significant differences ($P < 0.05$) among diets.

*Denotes significant difference ($P < 0.05$) between nondiabetic and diabetic groups on the same diet.

each group (**Table 5**). Cholesterol content of the kidneys was higher in the casein-fed animals compared to the other two groups. Total cholesterol content of kidneys was increased significantly in all three dietary groups following the onset of diabetes. When normalized to tissue weight, the total cholesterol content of the kidneys was similar to the respective control values in the groups fed the nonpurified and the soybean protein diet. However, the renal cholesterol content of the casein-fed animals increased further upon STZ treatment. Although renal sterol synthesis per unit weight tended to be lower in casein-fed control animals, the differences seen among the three groups were not significant. The onset of diabetes was associated with a small decrease in the sterol synthesis of kidneys in the animals fed the nonpurified diet and a small increase in the animals fed casein diet. There was no change with the onset of diabetes in the renal sterol synthesis of the animals on the soybean protein diet (Table 5).

Total fatty acid synthesis was increased significantly in the casein-fed control animals compared to the other two dietary groups. Total fatty acid synthesis increased following STZ treatment in all three dietary groups. On the basis of unit weight, the fatty acid synthetic rates following the onset of diabetes were similar in each group compared to their respective control values (Table 5).

Relationship between tissue weight and cholesterol content

In control rats, the diet had no significant effect on total body weight (Table 1) or on the wet weight of most of the tissues examined. The wet weights of most tissues were remarkably similar when normalized to total body weight (g/100 g body weight). The weights of colon, lungs, and kidneys tended to be lower and that of the skin higher in the groups fed the purified diet (**Table 6**).

The average (mean ± SEM) cholesterol concentrations (mg/g wet tissue weight) obtained for a number of tissues of control rats are presented in Table 6. The cholesterol content of the whole body as well as that of the individual tissues were remarkably similar between animals fed the nonpurified diet or the purified diet containing the soybean protein. Two exceptions were the kidneys and lungs in which the cholesterol contents were higher in the animals fed the nonpurified diet compared to the group fed the soybean protein diet. When the data were computed on the basis of equal body weight (315 g), the casein-fed animals showed an "abnormal" accumulation of 52 mg of cholesterol in the whole body as compared to the animals fed soybean protein during the 15-day study period. The excess cholesterol was located mostly in the carcass (30 mg), the remainder being distributed as follows: skin (7 mg), blood (6 mg), liver (3 mg), kidneys (1 mg), lungs (1 mg), and epididymal fat (0.4 mg).

The animals lost weight following the onset of diabetes (25% in animals fed casein, 21% in animals fed soybean protein, and 17% in animals fed the nonpurified diet). The changes in individual tissue weights (calculated as outlined in the Methods section) were also affected by the type of diet, i.e., purified versus nonpurified diet. Within the purified diet groups, the source of dietary protein, i.e., casein versus soybean, had no major effect. Whereas the liver, stomach, intestines, colon, and kidneys gained weight in animals fed the nonpurified diet. On both types of diets, all other tissues studied lost weight to a similar extent with the exception of the epididymal fat pads, where the loss was severe (about 70% on the purified diet and about 40% on the nonpurified diet). On both types of diets the loss of weight for heart tissue was about 10% and for the other tissues, between 20 and 30% (**Table 7**).

Cholesterol contents expressed on the basis of tissue weight (mg/g) increased markedly with the onset of dia-

TABLE 5. Kidney cholesterol levels and in vivo rate of incorporation of [1-^{14}C]octanoate in lipids in nondiabetic rats fed a nonpurified diet or a purified diet containing casein or soybean protein

	Experimental Group					
			Purified Diet			
	Nonpurified Diet		Casein		Soybean Protein	
Parameter	Nondiabetic	Diabetic	Nondiabetic	Diabetic	Nondiabetic	Diabetic
Weight (g) (n = 8)	1.88 ± 0.10	2.48 ± 0.08*	1.91 ± 0.09	2.30 ± 0.16*	1.96 ± 0.16*	2.28 ± 0.17
Cholesterol (mg/g) (n = 8)	3.26 ± 0.13	3.22 ± 0.08^{a}	3.53 ± 0.15	3.77 ± 0.07^{c}	3.11 ± 0.12	3.12 ± 0.05^{a}
Incorporation of [^{14}C]octanoate (n = 5)						
Cholesterol (dpm/g)	248 ± 24	168 ± 16^{a}*	201 ± 19	294 ± 27^{c}*	269 ± 47	261 ± 43^{ac}
Total fatty acids (dpm/g)	2520 ± 216^{a}	2663 ± 167^{ac}	3537 ± 315^{b}	3564 ± 326^{d}	2275 ± 192^{a}	2214 ± 301^{c}

Data represent mean ± SEM values obtained in the number (n) of animals shown in parentheses. Values without common superscript letters denote significant differences ($P < 0.05$) among diets.

*Denotes significant difference ($P < 0.05$) between nondiabetic and diabetic groups on the same diet.

TABLE 6. Tissue weights and cholesterol content in nondiabetic rats fed a nonpurified diet or a purified diet containing casein or soybean protein

	Nonpurified Diet (n = 3)		Purified Diet			
			Casein (n = 3)		Soybean Protein (n = 3)	
	Weight	Cholesterol	Weight	Cholesterol	Weight	Cholesterol
	% BW	*mg/g tissue*	*% BW*	*mg/g tissue*	*% BW*	*mg/g tissue*
Whole body*	100 ± 0.00	1.28 ± 0.04^{ab}	100 ± 0.00	1.43 ± 0.01^{a}	100 ± 0.00	1.26 ± 0.05^{b}
Carcass	63.2 ± 0.81	1.07 ± 0.06	63.5 ± 1.20	1.20 ± 0.02	63.1 ± 1.1	1.05 ± 0.07
Skin	19.5 ± 0.36	1.59 ± 0.05	20.8 ± 0.45	1.71 ± 0.03	20.8 ± 0.93	1.60 ± 0.03
Liver	3.0 ± 0.15	2.55 ± 0.06^{a}	2.63 ± 0.12	2.86 ± 0.08^{b}	2.63 ± 0.12	2.54 ± 0.05^{a}
Stomach	0.43 ± 0.02	2.40 ± 0.08	0.46 ± 0.03	2.50 ± 0.05	0.44 ± 0.02	2.41 ± 0.12
Intestines	2.2 ± 0.32	2.34 ± 0.06	2.0 ± 0.17	2.39 ± 0.03	1.97 ± 0.15	2.35 ± 0.08
Colon	0.8 ± 0.09	1.77 ± 0.05	0.59 ± 0.08	1.74 ± 0.16	0.60 ± 0.07	1.65 ± 0.15
Kidney	0.76 ± 0.04	3.07 ± 0.13^{ab}	0.68 ± 0.03	3.34 ± 0.08^{a}	0.66 ± 0.01	2.82 ± 0.05^{b}
Spleen	0.21 ± 0.02	3.01 ± 0.05	0.19 ± 0.02	3.13 ± 0.04	0.19 ± 0.01	3.04 ± 0.06
Lungs	0.57 ± 0.06	3.51 ± 0.03^{a}	0.53 ± 0.03	3.90 ± 0.08^{b}	0.47 ± 0.03	3.31 ± 0.08^{a}
Heart	0.36 ± 0.02	1.26 ± 0.13	0.34 ± 0.02	1.28 ± 0.10	0.34 ± 0.01	1.27 ± 0.08
Testes	1.2 ± 0.08	1.58 ± 0.05	1.31 ± 0.17	1.51 ± 0.04	1.21 ± 0.02	1.48 ± 0.06
Epididymal fat	1.06 ± 0.09	0.48 ± 0.01	1.13 ± 0.13	0.57 ± 0.06	1.16 ± 0.23	0.44 ± 0.02

Data represent mean ± SEM values obtained in the number (n) of animals shown in parentheses. Values without common superscript letters denote significant differences ($P < 0.05$) among diets.

*Whole body weights at the time of killing were: nonpurified diet, 297 ± 9 g; casein, 315 ± 2 g; and soybean protein diet, 334 ± 11 g.

TABLE 7. Changes in tissue wet weight and cholesterol content in diabetic rats compared to nondiabetic rats fed a nonpurified diet

	Experimental Group								
				Purified Diet					
	Nonpurified Diet			Casein			Soybean Protein		
Tissue	Weight	Cholesterol		Weight	Cholesterol		Weight	Cholesterol	
	g	*mg*	*mg/day*	*g*	*mg*	*mg/day*	*g*	*mg*	*mg/day*
Whole body	− 56*	40.6*	2.70	− 83.3	38.4	2.56	− 67.6	18.5	1.23
Carcass	− 47	11.3	0.75	− 54.2	5.4	0.36	− 47.6	− 2.1	− 0.14
Skin	− 17	10.0	0.67	− 21.1	23.3	1.55	− 17.6	14.5	0.97
Liver	+ 1.5	4.0	0.27	− 1.1	1.3	0.09	+ 0.3	0.9	0.06
Stomach	+ 0.2	0.5	0.03	+ 0.4	1.0	0.07	+ 0.3	1.0	0.07
Intestines	+ 5.5	12.1	0.82	+ 2.8	6.4	0.43	+ 3.2	8.2	0.55
Colon	+ 1.2	2.7	0.18	− 0.5	− 0.8	− 0.05	− 0.3	− 0.4	− 0.03
Kidney	+ 0.4	1.3	0.09	+ 0.3	2.7	0.18	+ 0.3	0.8	0.05
Spleen	− 0.2	− 0.2	− 0.01	− 0.3	− 0.7	− 0.05	− 0.3	− 0.5	− 0.03
Lungs	− 0.4	− 0.5	− 0.03	− 0.4	− 0.2	− 0.01	− 0.4	− 1.3	− 0.09
Heart	− 0.1	0.0	0.00	− 0.2	− 0.2	− 0.01	− 0.1	− 0.1	− 0.01
Testes	− 0.6	− 0.9	− 0.06	− 1.4	− 1.2	− 0.08	− 0.4	− 1.1	− 0.07
Epididymal fat	− 1.4	0.0	0.0	− 2.5	− 0.1	− 0.01	− 2.2	− 0.3	0.02

*Prediabetic weight and cholesterol content of each tissue of the diabetic rats was calculated as described in Methods. The difference between the calculated and observed values represents the change due to diabetes. When a tissue lost weight, the increase in cholesterol content of that tissue was considered to be due to an abnormal accumulation, e.g., in diabetic rats fed casein, the observed value for skin cholesterol was 126.4 mg while the calculated value for the prediabetic skin cholesterol was 1031.1 mg (prediabetic body wt (332.7 g) × skin wt. (0.195 g/g BW) × skin cholesterol content (1.59 mg/g) = 103.1 mg). Since skin lost weight (− 21.1 g) the observed increase of 23.3 mg in cholesterol was considered to be due to abnormal accumulation. When a tissue gained weight, expected changes in cholesterol mass due to growth were calculated. If the calculated value did not differ from the observed, then the increase was considered to be normal and due to the growth of the tissue, e.g., in the same diabetic rats fed casein, the intestinal cholesterol was increased by 6.4 mg. Since tissue weight was increased by 2.8 g, the expected increase in cholesterol due to growth was 6.6 mg. (increase in tissue wt. (2.8 g) × cholesterol content of the intestines (2.34 mg/g) = 6.6 g mg). Thus all the increase in the cholesterol of the intestinal tissue of the diabetic rat was considered to be due to growth. If the increase in the cholesterol content of the growing tissue was above that expected, that increase was considered to be due to an abnormal accumulation of cholesterol in that growing tissue, e.g., in the above rats the weight of kidneys was increased by 0.3 g; therefore the expected increase in cholesterol due to growth was 0.92 mg (increase in kidney wt. (0.3 g) × cholesterol content of kidney (3.07 mg/g) = 0.92 mg). The observed increase was 2.7 mg. Therefore, the abnormal increase in cholesterol in kidneys of diabetic rat was 1.78 mg. (2.7 mg − 0.92 mg expected due to growth).

betes, especially in those tissues where most of the weight losses occurred. On the other hand, the cholesterol content of those tissues that gained weight remained fairly similar to their respective control values. For example, in diabetic rats fed the nonpurified diet, the cholesterol content of skin (which lost weight) was 2.47 mg/g compared to 1.59 mg/g in control rats, while that of intestines (which gained weight) was 2.30 mg/g compared to 2.34 mg/g in control rats. Thus, it appeared that the changes in relative tissue cholesterol content (mg cholesterol/g of tissue) associated with the onset of diabetes were mostly due to tissue weight losses rather than to an increase in actual cholesterol content of the respective tissue. For example, the weight of the epididymal fat pads in control rats was 3.3 g which decreased to 1.9 g (−42%) in the diabetic animals. However, the cholesterol content of this tissue, which was 1.6 mg, did not change with the onset of diabetes. These observations indicate that although the tissues gained cholesterol in proportion to the weight gain due to growth (even in the diabetic animals), they did not lose cholesterol in proportion to the loss of their weights following STZ administration. Prediabetic cholesterol content of the whole body and the individual tissues was therefore calculated as outlined in Methods. On this basis (observed−expected), it was found that diabetes led to an increase in the cholesterol content of the body, which was 41 mg in the animals fed the nonpurified diet, 38 mg in animals fed casein, and only 19 mg in animals fed the soybean protein diet (Table 7). Of this increase, 20 mg, 8 mg, and 11 mg of cholesterol could be accounted for by the growth of the tissues in the respective dietary groups. Therefore, the abnormal accumulation of cholesterol due to metabolic changes induced by diabetes, was 21 mg in the group fed nonpurified diet, 30 mg in the casein-fed group, and only 8 mg in the group fed the soybean protein diet. In addition to liver tissue, the abnormal accumulation of cholesterol occurred mainly in the carcass and in the skin in the group fed the nonpurified diet, in the carcass, skin, and kidneys in the group fed casein diet, and only in the skin for the group fed the soybean protein diet.

Correlations observed among the parameters of cholesterol metabolism

Complete sets of data on all parameters studied were not available in all animals. When the data from three dietary groups were analyzed together as one group, significant ($P < 0.05$) correlations were seen among some of the parameters of body cholesterol metabolism. Plasma cholesterol levels (mg/dl) were directly correlated with the fractional rate of cholesterol absorption ($r = 0.73$, n = 52), to the absorbed [^{3}H]cholesterol found in plasma 38–40 hr after the administration of the label ($r = 0.84$, n = 43), and to the cholesteryl ester (CE) content of the liver, especially when CE content was expressed on the basis of percentage of total liver cholesterol ($r = 0.67$, n = 52). Liver CE content was also directly related to the fractional rate of cholesterol absorption ($r = 0.79$, n = 52). Intestinal weight did not correlate with the fractional rate of cholesterol absorption and showed a significant but weak correlation with plasma cholesterol ($r = 0.52$, n = 52).

DISCUSSION

The purpose of the present investigation was to compare the effects of animal (casein) and plant (soybean isolate) proteins on the metabolism of cholesterol in STZ-induced diabetic rats and to gain additional insights into the mechanism of diabetic hyperlipidemia. The results obtained on the specific aspects of tissue lipid metabolism were remarkably similar in the control rats fed the nonpurified diet (Purina Lab Chow) or the purified diet with soybean protein. The only discernible difference between these two groups was that the VLDL fraction (d < 1.01 g/ml) in the former had higher cholesteryl ester and lower triglyceride content.

Following the onset of diabetes, most of the parameters of lipid synthesis studied were similar in the rats fed the nonpurified diet and the soybean protein-containing purified diet. However, major differences were seen in the distribution of cholesterol among the isolated lipoprotein fractions and in tissue weights, which in turn affected the relative cholesterol content of the tissues. In the rats fed the nonpurified diet, the weights of the intestine, colon, and liver were markedly increased with the onset of diabetes. Earlier studies have shown that in rats fed the nonpurified diet, cholesterol levels in the whole body as well as skin increase upon the onset of diabetes (4, 17). During this investigation changes in whole body cholesterol content were also observed. For instance, of the 40 mg increase in whole body cholesterol seen in rats fed the nonpurified diet, 20 mg was due to tissue growth and the remaining 20 mg was attributable to the abnormal deposition in the carcass (muscle, bones, nervous system) and skin, presumably induced by the metabolic changes associated with diabetes. In the rats fed the soybean protein diet, whole body cholesterol increased by 19 mg, of which 11 mg was attributable to tissue growth and the remaining 8 mg (mainly in the skin) to abnormal deposition following STZ treatment.

The differences in the distribution of cholesterol among the lipoprotein fractions and in the increased accumulation of cholesterol (Tables 2 and 7), between the diabetic rats fed the nonpurified diet and the soybean protein-containing diet, may be related to the difference in the dietary fat and cholesterol intake. Whereas the soybean protein diet contained polyunsaturated fat and was cholesterol-free, the nonpurified diet contained some saturated fat and cholesterol (0.2–0.3 mg/g (17)). The changes

in the distribution of cholesterol among lipoproteins observed in diabetic rats fed the nonpurified diet (Table 2) were very similar to the changes found by others in rats that were made hypercholesterolemic by an atherogenic diet (2, 32). These data suggest that the cholesterol-free (purified) diet containing soybean protein may be more suitable as a control diet for the assessment of changes in cholesterol metabolism. In the ensuing discussion the effects of the casein diet are compared mostly with that of the soybean protein diet. Because the only difference between these two diets was the source of protein, the differences observed in cholesterol metabolism could be ascribed to the direct or indirect influence of the dietary protein source on this process.

In agreement with earlier reports (14–16), among the control groups, the intake of dietary casein resulted in higher levels of plasma cholesterol. The increase occurred mainly in the plasma lipoprotein fractions with densities 1.023–1.045 and 1.045–1.086 g/ml. These fractions were also enriched in cholesteryl esters. Although the total amount of cholesterol in the fraction with d < 1.01 g/ml was similar in the two dietary groups, the amount of cholesteryl esters was significantly increased in the casein-fed group. Furthermore, the plasma lipoprotein fraction isolated between the densities of 1.045–1.086 g/ml was enriched with apoE-containing lipoproteins (Figs. 1A–C). An apoE-rich HDL population designated as HDL_1 is a normal constituent present in rat plasma. As this fraction is mainly isolated in the density range of 1.04–1.085 g/ml (33), our data suggest that casein feeding increases the HDL_1 levels in rat plasma. Increased levels of apoE in plasma HDL have been found in rats fed a casein-containing diet (34). The mechanism whereby plasma LDL and the apoE-rich HDL are elevated upon casein feeding remains to be elucidated. Decreased tissue apoB/E receptor activity may be a factor, as the function of this receptor has been shown to decline in rabbits consuming casein (35).

Although the plasma cholesterol levels were increased to a similar extent in both dietary groups upon the induction of diabetes, there were marked differences in the distribution of cholesterol among lipoprotein fractions. In the casein-fed rats, the excess plasma cholesterol was found mainly in the lipoprotein fractions isolated at d < 1.086 g/ml; these fractions were also relatively rich in cholesteryl esters. The cholesterol content of the lipoprotein fraction d 1.086–1.161 g/ml was significantly increased in soybean protein-fed rats but not in the casein-fed rats. There were also significant differences in the levels and the distribution of triglycerides in all lipoprotein fractions isolated at d < 1.045 g/ml. These data suggest that the source of dietary protein has a marked effect on the distribution of cholesterol among lipoprotein fractions in both control and diabetic rats. In the case of all three diets, the onset of diabetes was associated with marked increases in the cholesterol content of the lipoprotein fraction of d 1.045–1.086 g/ml. However, the apoE rich-HDL content of this fraction was greatly reduced, which is in accord with previous studies (36, 37). Whether the decrease is due to the increased removal or decreased synthesis of apoE (or apoE-containing lipoproteins) remains to be determined. Diabetes per se may not be a factor affecting the synthesis of apoE in the liver, as the apoE concentration in plasma is markedly increased in cholesterol-fed diabetic rats (37).

In accord with previous studies (15, 16), dietary casein decreased the fractional rate (FR) of plasma cholesterol esterification. The decreased FR may be indicative of changes in the nature of substrate lipoproteins, as the plasma LCAT activity measured using an exogenous substrate (indicative of plasma LCAT mass (38)) was not affected. Plasma HDL is the preferred substrate for LCAT reaction, especially in rats. Plasma HDL, however, is composed of different subpopulations (33). Whether all these subpopulations of rat plasma HDL are equally efficient as substrates for LCAT is presently unknown. Data obtained from studies of human plasma suggest that the substrate potential of apoE-rich HDL is lower than that of apoA-rich HDL (39). If this is also true in rats, then the increased levels of the apoE-rich HDL subclass in casein-fed rats may be responsible for decreased FR of plasma LCAT. This postulate is supported by the findings that the FR of plasma LCAT in rats is decreased in aging, copper deficiency, hypothyroidism, and cholesterol feeding; the conditions in which the levels of apoE-rich HDL or the apoE content of the HDL have been found to increase (24, 32, 40–42). Furthermore, when rats were fed a fat-free purified diet containing casein, the FR of plasma cholesterol esterification was $18\% \cdot hr^{-1}$ compared to $12.6\% \cdot hr^{-1}$ seen in the rats fed the same diet but containing fat. The plasma cholesterol levels were lower in rats fed the fat-free diet and there was also a marked reduction in the apoE-containing fraction of HDL isolated between densities 1.045 and 1.086 g/ml (Kudchodkar, B. J., unpublished observations). Additional factors are likely to be involved in regulating the plasma LCAT activity because marked reductions in apoE-rich HDL and increased levels of apoA-rich HDL were found in diabetic animals without a concomitant change in the FR of plasma cholesterol esterification.

In control rats, one of the major effects of the dietary casein was the accumulation of cholesterol in tissues unrelated to growth, found mainly in the blood, liver, carcass, skin, kidney, and lungs (Table 6). Following the onset of diabetes, the accumulation of cholesterol in casein-fed rats was especially marked in the kidneys, suggesting that the dietary casein may have a specific effect on the cholesterol metabolism in this tissue. Because the synthesis of cholesterol in the kidneys was not affected by the diet or diabetes, the increased levels of cholesterol in

this tissue may be due to the deposition of plasma cholesterol, which in turn may be a consequence of the altered composition of plasma lipoproteins. The same mechanism may be responsible for the accumulation of cholesterol seen in other tissues following the onset of diabetes, as the rate of cholesterol synthesis in most tissues other than the liver and intestines was not significantly affected by the onset of diabetes, at least in rats fed the nonpurified diet (43).

Although a decrease in hepatic and other tissue lipoprotein receptor activity can explain the increase in the levels of plasma cholesterol (44, 45), it cannot account for the increase in total body cholesterol levels in the absence of any change in the tissue cholesterol synthesis or the growth of the tissue. In control rats on the casein diet, the hepatic synthesis of cholesterol was markedly decreased. The intestinal synthesis of cholesterol was also decreased in these animals but to a smaller extent. In light of this evidence, it was surprising to find increased cholesterol in the body of these rats. This seemingly paradoxical situation could be explained by feedback inhibition of tissue cholesterol synthesis. Under these conditions, the amount of cholesterol synthesized would decrease but the pool of tissue cholesterol would remain unchanged (or may even increase), if the increased cholesterol input (from the lipoprotein pool) was not compensated for either by a decrease in cholesterol synthesis and/or by an increase in removal and catabolism (46). In accord with earlier studies, the fractional rate of endogenous cholesterol absorption increased in casein-fed rats (15, 16). Earlier studies have also shown that the fecal excretion (15, 16) but not the biliary secretion of cholesterol was reduced (15) in these animals. These observations indicate a net increase in the reabsorption (retention) of cholesterol by the casein-fed animals. Because more cholesterol is retained in the body, tissue cholesterol pools would expand in the casein-fed rats compared to their counterparts fed the soybean protein diet.

Increased transport of absorbed cholesterol to the liver via chylomicron remnants has been shown to increase the hepatic pool of cholesteryl esters, which in turn is believed to inhibit cholesterol synthesis and apoB/E receptor activity in the liver and consequently lead to higher plasma cholesterol levels (44, 45). Indeed, the cholesteryl ester content of the liver increased in the animals fed the casein diet. There was also a negative correlation between the hepatic synthesis of cholesterol and its CE content ($r = -0.64$, $n = 15$) and a strong positive correlation ($r = 0.79$, $n = 27$) between plasma cholesterol levels and the hepatic CE content. Thus, among the control rats, most of the changes in cholesterol metabolism seen in casein-fed rats could be explained on the basis of increased net absorption of cholesterol from the intestinal lumen.

Hepatic as well as the intestinal synthesis of fatty acids and its incorporation into triglycerides and phospholipids were significantly increased in the casein-fed control rats. Increased hepatic synthesis of fatty acids and VLDL secretion have been noted earlier (15, 47). Our data suggest that there may also be an increase in the intestinal secretion of triglyceride-rich lipoproteins in the casein-fed animals. Thus changes in the metabolism of triglyceride-rich lipoproteins may be responsible for the altered distribution of cholesterol among the lipoprotein fractions, as VLDL is known to be a precursor of IDL, LDL, and subpopulation(s) of HDL (33).

A similar mechanism may also be operating in diabetic rats since the rate of cholesterol absorption increases following the onset of diabetes. Because fed labeled cholesterol may exchange with the unlabeled intestinal cholesterol, the higher values for the fractional rates of absorption in diabetic rats may be due to increased intestinal weight and increased cholesterol mass. However, the observations made during this study do not support such an explanation. For example, no correlation was observed between the fractional rate of cholesterol absorption and the weight of the intestines or its cholesterol content. Furthermore, in animals fed casein, the weight of the intestines and its cholesterol content (both before and after induction of diabetes) were similar to those from rats fed soybean protein (Table 4). However, the percent cholesterol absorption was significantly higher in the casein-fed animals. These observations and the data presented earlier (46, 48) indicate that the phenomenon of exchange is not likely to be a major interfering factor in the determination of the rate of cholesterol absorption. Although the excretion of cholesterol and bile acids was not determined during these studies, the data available on the diabetic rats fed the nonpurified diet suggest that the onset of diabetes leads to an increase in the secretion of biliary cholesterol but not in its excretion in the feces (5, 10, 17). Such a condition could lead to a net increase in the absorption of endogenous cholesterol along with the dietary cholesterol, if available from the diet (nonpurified), since the fractional rate of cholesterol absorption increases following the onset of diabetes. In addition, diabetes is known to be associated with an increase in the synthesis of cholesterol in the intestines, a considerable part of which may be transported to the liver via intestinal lipoproteins (6, 7). For example, in a 200 g control rat fed the nonpurified diet, cholesterol synthesized by the intestines is about 6 mg per day (18). Upon induction of diabetes, the intestinal synthetic rate in our study was increased by 57%, i.e., an additional 3.4 mg was synthesized daily in the diabetic state. The daily retention of cholesterol by the intestines due to growth was, however, only 0.8 mg per day (Table 7). Because diabetes does not result in an increase in the excretion of neutral sterols (10, 17), up to 2.6 mg of the additionally synthesized cholesterol may have been transported to the liver via intestinal lipoproteins. In

the casein-fed diabetic animals, hepatic and intestinal cholesterol synthesis were markedly increased. Intestinal synthesis of fatty acids and their incorporation into triglycerides and phospholipids were also higher compared to diabetic rats fed the other two diets, suggesting increased synthetic rates and secretion of intestinal lipoproteins in the casein-fed rats.

The reason(s) for marked increases in hepatic and in intestinal cholesterol synthesis in casein-fed diabetic rats is not clear. These synthetic rates were not related to growth inasmuch as the increases in tissue weights were essentially the same as those for the rats fed soybean protein and were lower than those for the rats fed the nonpurified diet. Recently it has been shown that both hepatic and intestinal cholesterol synthesis are increased in glucose-fed diabetic rats compared to glucose-fed normal controls (49). The purified diet in our studies contained glucose as the sole source of carbohydrate. Marked increases in hepatic and intestinal synthesis of cholesterol seen in casein-fed diabetic animals may therefore be due to increased efficiency in the utilization of glucose by these diabetic rats. Possibly, as a result, the plasma glucose levels in casein-fed diabetic rats were significantly lower than those of the diabetic rats fed the other two diets (Table 1).

A striking finding in diabetes is the marked increase in the cholic acid pool, due to increased synthesis as well as increased reabsorption (5, 10, 17). Cholic acid is known to affect the cholesterol, triglyceride, and phospholipid metabolism in the rat (50–52). Therefore, it is possible that the changes seen in plasma lipoprotein cholesterol and triglycerides and in tissue cholesterol levels upon the induction of diabetes may be secondary to the changes in the synthesis and reabsorption of cholic acid.

Supported in part by grants from TCOM, organized Research funds, The Robert A. Welch Foundation (B-935), the National Institute of Health (HL-27620), Texas Woman's University Organized Research and Grants Administration funds, and from Natural Fibers and Food Protein Commission of Texas. Rosina Paige and Diane Bradish provided excellent secretarial assistance.

Manuscript received 13 November 1987, in revised form 14 March 1988, and in re-revised form 10 May 1988.

REFERENCES

1. Garcia, M. J., P. M. McNamara, T. Gordon, and W. B. Kannel. 1974. Morbidity and mortality in diabetics in the Framingham population. Sixteen year follow-up study. *Diabetes.* **23:** 105–111.
2. Mahley, R. W., and K. S. Holcombe. 1977. Alterations of plasma lipoproteins and apoproteins following cholesterol feeding in the rat. *J. Lipid Res.* **18:** 314–324.
3. Kalant, N., J. I. Teitelbaum, A. A. Cooperberg, and A. A. Harland. 1964. Dietary atherogenesis in alloxan diabetes. *J. Lab. Clin. Med.* **63:** 147–157.
4. Russell, P. T., J. C. Scott, and J. T. VanBruggen. 1962. Effects of dietary fat on cholesterol metabolism in the diabetic rat. *J. Nutr.* **76:** 460–466.
5. Nervi, F. O., A. Gonzalez, and V. D. Valdivieso. 1974. Studies on cholesterol metabolism in the diabetic rat. *Metabolism.* **23:** 495–503.
6. Young, N. L., C. D. Saudek, and S. A. Crawford. 1982. Total hydroxymethylglutaryl CoA reductase activity in the small intestine and liver of insulin-deficient rats. *J. Lipid Res.* **23:** 266–275.
7. Feingold, K. R., G. Z. Sigmond, M. Hughes-Fulford, S. R. Lear, and A. H. Moser. 1985. The effect of diabetes mellitus on the lymphatic transport of intestinal sterols. *Metabolism.* **34:** 1105–1109.
8. Popper, D. A., Y. F. Shiau, and M. Reed. 1985. Role of small intestine in pathogenesis of hyperlipidemia in diabetic rats. *Am. J. Physiol.* **249:** G161–167.
9. Young, N. L., D. R. Lopez, D. J. McNamara, and G. Benavides. 1985. Evaluation of the contribution of dietary cholesterol to hypercholesterolemia in diabetic rats and of sitosterol as a recovery standard for cholesterol absorption. *J. Lipid Res.* **26:** 62–69.
10. Uchida, K., H. Takase, M. Kadowaki, Y. Nomura, T. Matsubara, and N. Takeuchi. 1979. Altered bile acid metabolism in alloxan diabetic rats. *Jpn. J. Pharmacol.* **29:** 553–562.
11. O'dea, K. 1984. Marked improvement in carbohydrate and lipid metabolism in diabetic Australian Aborigines after temporary reversion to traditional lifestyle. *Diabetes.* **33:** 596–603.
12. Seino, Y., S. Seino, M. Ikeda, S. Matsukara, and H. Iniura. 1983. Beneficial effects of high protein diet in treatment of mild diabetes. *Hum. Nutr. Appl. Nutr.* **37A:** 226–230.
13. Seigel, E. G., V. E. Trapp, C. B. Wollheim, A. E. Renold, and F. H. Schmidt. 1980. Beneficial effects of low-carbohydrate, high-protein diets in long-term diabetic rats. *Metabolism.* **29:** 421–428.
14. Carroll, K. K. 1978. Dietary protein in relation to plasma cholesterol levels and atherosclerosis. *Nutr. Rev.* **36:** 1–5.
15. Sugano, M. 1983. Hypocholesterolemic effect of plant protein in relation to animal protein: mechanism of action. *In* Animal and Vegetable Proteins in Lipid Metabolism and Atherosclerosis. *Curr. Top. Nutr. Dis.* **8:** 19–49.
16. Park, M. C., B. J. Kudchodkar, and G. U. Liepa. 1987. Effects of dietary animal and plant proteins on the cholesterol metabolism in immature and mature rats. *J. Nutr.* **117:** 30–35.
17. Young, N. L., D. J. McNamara, C. D. Saudek, J. Krasovsky, D. R. Lopez, and G. Levy. 1982. Hyperphagia alters cholesterol dynamics in diabetic rats. *Diabetes.* **32:** 811–819.
18. Turley, S. D., J. M. Andersen, and J. M. Dietschy. 1981. Rates of sterol synthesis and uptake in the major organs of the rat in vivo. *J. Lipid Res.* **22:** 551–569.
19. Sodhi, H. S., B. J. Kudchodkar, P. Varughese, and D. Duncan. 1974. Validation of the ratio method for calculating absorption of dietary cholesterol in man. *Proc. Soc. Exp. Biol. Med.* **145:** 107–111.
20. Terpstra, A. H. M., C. J. H. Woodward, and F. J. Sanchez-Muniz. 1981. Improved techniques for the separation of serum lipoproteins by density gradient ultracentrifugation: visualization by prestaining and rapid separation of serum lipoprotein from small volumes of serum. *Anal. Biochem.* **111:** 149–157.
21. Quarfordt, S. H., R. S. Hain, L. Jakoi, S. Robinson, and F. Shelburne. 1978. The heterogeneity of rat high density lipoproteins. *Biochem. Biophys. Res. Commun.* **83:** 786–793.

22. Stokke, K. T., and K. R. Norum. 1971. Determination of lecithin:cholesterol acyltransferase in human blood plasma. *Scand. J. Clin. Lab. Invest.* **27:** 21–27.
23. Glomset, J. A., and J. A. Wright. 1964. Some properties of a cholesterol esterifying enzyme in human plasma. *Biochim. Biophys. Acta.* **89:** 266–276.
24. Carlile, S. I., and A. G. Lacko. 1981. Strain differences in the age-related changes of rat lipoprotein metabolism. *Comp. Biochem. Physiol.* **70 B:** 754–758.
25. Folch, J., M. Lees, and G. H. Sloane Stanley. 1957. A simple method for the isolation and purification of total lipids from animal tissues. *J. Biol. Chem.* **226:** 497–509.
26. Kudchodkar, B. J., J. J. Albers, and E. L. Bierman. 1983. Effect of positively charged sphingomyelin liposomes on cholesterol metabolism of cells in culture. *Atherosclerosis.* **46:** 353–367.
27. Parekh, A. C., and D. H. Jung. 1970. Cholesterol determination with ferric acetate–uranium acetate and sulfuric acid–ferrous sulfate reagents. *Anal. Chem.* **42:** 1423–1427.
28. Markwell, M. A. K., S. M. Hass, N. E. Tolbert, and L. L. Bieber. 1981. Protein determination in membrane and lipoprotein samples: manual and automated procedures. *Methods Enzymol.* **72:** 296–303.
29. Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature.* **227:** 680–685.
30. Hutchens, T. T., J. T. VanBruggen, R. M. Cockburn, and E. S. West. 1954. The effect of fasting upon tissue lipogenesis in the intact rat. *J. Biol. Chem.* **208:** 115–122.
31. Nie, N. H., C. H. Hull, J. G. Jenkins, K. Steinbrenner, and D. H. Beut. 1975. Statistical Package for Social Science. 2nd ed. McGraw-Hill, New York.
32. DeLamatre, J. G., and P. S. Roheim. 1981. Effect of cholesterol feeding on apoB and apoE concentration and distributions in euthyroid and hypothroid rats. *J. Lipid Res.* **22:** 297–306.
33. Eisenberg, S. 1984. High density lipoprotein metabolism. *J. Lipid Res.* **25:** 1017–1058.
34. Lefevre, M., and B. O. Schneeman. 1984. High density lipoprotein composition in rats fed casein or soyprotein isolate. *J. Nutr.* **114:** 768–777.
35. Chao, Y. S., T. T. Yamin, and A. W. Alberts. 1982. Effects of cholestyramine on low density lipoprotein binding sites on liver membranes from rabbits with endogenous hypercholesterolemia induced by a wheat starch-casin diet. *J. Biol. Chem.* **257:** 3623–3627.
36. Bar-On, H., P. S. Roheim, and H. A. Eder. 1976. Serum lipoproteins and apolipoproteins in rats with streptozotocin-induced diabetes. *J. Clin. Invest.* **57:** 714–721.
37. Arbeeny, C. M., D. Edelstein, S. R. Freedman, and H. A. Eder. 1980. Serum lipoproteins of diabetic rats fed a high cholesterol diet. *Diabetes.* **29:** 774–777.
38. Albers, J. J., C. H. Chen, and J. L. Adolphson. 1981. Lecithin:cholesterol acyltransferase (LCAT) mass: its relationship to LCAT activity and cholesterol esterification rate. *J. Lipid Res.* **22:** 1206–1213.
39. Marcel, Y. L., C. Vezina, D. Emond, and G. Suzue. 1980. Heterogeneity of human high density lipoproteins: presence of lipoproteins with and without apoE and their roles as substrate for lecithin:cholesterol acyltransferase reaction. *Proc. Natl. Acad. Sci. USA.* **77:** 2969–2973.
40. Lau, B. W. C., and L. M. Klevay. 1981. Plasma lecithin:cholesterol acyltransferase in copper-deficient rats. *J. Nutr.* **111:** 1698–1703.
41. Lee, S. M., B. J. Kudchodkar, and A. G. Lacko. 1987. Study of tissue lipoprotein receptor activity in aging rats. *Fed. Proc.* **46:** 2158 (abstract).
42. Lefevre, M., C. L. Keen, B. Lonnerdal, L. Hurley, and B. O. Schneeman. 1986. Copper deficiency-induced hypercholesterolemia: effects on HDL-subfractions and hepatic lipoprotein receptor activity in the rat. *J. Nutr.* **116:** 1735–1746.
43. Feingold, K. R., M. H. Wiley, G. MacRae, A. H. Moser, S. R. Lear, and M. D. Siperstein. 1982. The effect of diabetes mellitus on sterol synthesis in the intact rat. *Diabetes.* **31:** 388–395.
44. Brown, M. S., and J. L. Goldstein. 1986. A receptor-mediated pathway for cholesterol homeostasis. *Science.* **232:** 34–37.
45. Mahley, R. W., and T. L. Innerarity. 1983. Lipoprotein receptors and cholesterol homeostasis. *Biochim. Biophys. Acta.* **737:** 197–222.
46. Kudchodkar, B. J. 1986. The role of endogenously reabsorbed cholesterol in the regulation of plasma and other body tissue cholesterol pools. *In* Nutritional Effects on Cholesterol Metabolism. A. C. Beynen, editor. Transmondial, Voortheizen. 171–201.
47. Iritani, N., K. Nagashima, H. Fukuda, A. Katsurada, and T. Tanaka. 1986. Effects of dietary proteins on lipogenic enzymes in rat liver. *J. Nutr.* **116:** 190–197.
48. Kudchodkar, B. J. 1983. Relationship of cholesterol metabolism to the metabolism of plasma lipoproteins: perspectives from methodology. *Adv. Lipid Res.* **20:** 45–105.
49. Feingold, K. R., and A. H. Moser. 1985. Effect of glucose or fructose feeding on cholesterol synthesis in diabetic animals. *Am. J. Physiol.* **249:** G634–641.
50. Uchida, K., Y. Nomura, and N. Takeuchi. 1980. Effects of cholic acid, chenodeoxycholic acid and their related bile acids on cholesterol, phospholipids and bile acid levels in serum liver, bile and feces of rats. *J. Biochem.* **87:** 187–194.
51. Cohen, B. I., R. F. Raicht, and E. H. Mosbach. 1977. Sterol metabolism studies in rat. Effects of primary bile acids (sodium taurochenodeoxycholate and sodium taurocholate) on sterol metabolism. *J. Lipid Res.* **18:** 223–231.
52. Nilsson, S., and T. Scherstén. 1977. Influence of cholic and chenodeoxycholic acid on biliary cholesterol secretion in man. *Eur. J. Clin. Invest.* **7:** 383–388.