

Comparative Effects of Purified and Human-Type Diets on Cholesterol Metabolism in the Rat^{1,2}

BARBARA COONEY O'BRIEN AND RAYMOND REISER
*Consumer Research Center and Department of
Biochemistry and Biophysics, Texas Agricultural
Experiment Station, Texas A&M University System,
College Station, Texas 77843*

ABSTRACT Purified diets and others composed of foods commonly eaten by humans either low or 0.5% in cholesterol and low in fiber or containing plant or animal acid polysaccharides and 0.5% cholesterol were fed to adult female Sprague-Dawley rats for 10 weeks. All eight diets contained 28% beef fat and were comparable with respect to total calories as well as the relative amounts of fat, carbohydrate, and protein. The average serum cholesterol level of the rats which consumed the high cholesterol diet without added acid polysaccharides was 187 mg/dl. Without added cholesterol it was significantly lower (95 mg/dl). Average serum cholesterol levels of rats fed high cholesterol purified diets with pectin (a plant acid polysaccharide) or with chondroitin sulfate isomers (an animal acid polysaccharide) were similar and intermediate (118 mg/dl and 126 mg/dl, respectively), but were not significantly different ($P > 0.05$) from serum cholesterol levels of rats fed the low cholesterol purified diet or the human-type diets. There was no difference in average serum cholesterol levels among the four groups of rats ingesting the human-type diets, they being equal to that of rats fed the low cholesterol, low fiber purified diet. The homeostatic effect of the human-type diets could not be explained on the basis of enhanced fecal excretion of neutral sterols or bile acids nor increased activity of cholesterol 7 α -hydroxylase. The specific activity of hepatic 3-hydroxy-3-methylglutaryl coenzyme-A reductase tended to be lower in rats fed the human-type diets. *J. Nutr.* 109: 98-104, 1979.

INDEXING KEY WORDS diet · cholesterol metabolism · pectin · chondroitin sulfate

The response of serum lipids, especially cholesterol, to dietary fat and cholesterol has been the subject of intense investigation, and controversy, for more than two decades. Earlier studies showed that human serum cholesterol was virtually independent of diet fat and cholesterol (1, 2). However, coincidental with the use of purified diets in metabolic studies (3), significant responses of human serum cholesterol to saturated fat with and without added cholesterol in the diet were reported (4-8).

Although the purified diets used to study human serum cholesterol responses to diet

were adequate with respect to all known nutrients, we now recognize the nutritional importance of the nondigestible components of the diet, which are defined here as substances resistant to enzymes secreted by mammals. Some of these nondigestible diet constituents are hypocholesterolemic, such as pectin (9, 10) and plant sterols (11). The objective of the present study was to

Received for publication June 21, 1978.

¹Supported by the National Live Stock and Meat Board and by the Texas Cattle Feeders Association.

²A preliminary report of this study was presented at a meeting of the Federation of American Societies of Experimental Biology, Federation Proceedings 47, 2803 (1978).

compare the effects on serum cholesterol of purified and human-type diets high in animal fat and cholesterol. These two types of diets were essentially isoenergetic and energetically equivalent in fat, carbohydrate and protein. In particular, this investigation was concerned with the effect on serum cholesterol of typical dietary nondigestible acid polysaccharides from plant and animal tissue. These will be referred to subsequently as "fiber" for convenience with the realization that this may not be the most definitive term. Pectin was selected as an appropriate plant fiber since purified pectin has been shown to be hypocholesterolemic when consumed as part of a cholesterol-supplemented purified diet (9). Green beans provided the source of pectin for the corresponding human-type diet. Purified chondroitin sulfate isomers were used as the animal-derived nondigestible component in the purified diet and beef shank in the human-type diet.

MATERIALS AND METHODS

Animals. Female Sprague-Dawley rats³ which were 9 months old at the beginning of the feeding periods were used in these experiments. The rats were housed in wire bottom, stainless steel cages, three rats per cage, and fresh food and water were offered daily ad libitum. The rats were kept in an environmentally controlled room on a 12-hour light-dark cycle which was dark from 0300 to 1500 hours.

Diets. The compositions of the basal purified and human-type diets are presented in table 1. These diets were designed to be isoenergetic with equivalent calories of fat, carbohydrate, and protein. The experimental diets are shown in table 2 as modifications of the basal purified and human-type diets. The experimental diets were the basal diets (LoChol-LoFiber), the basal diets with 0.5% cholesterol (HiChol-LoFiber), the basal diets with 0.5% cholesterol and pectin (HiChol-Plant Fiber) and, finally, the basal diets with 0.5% cholesterol and glycosaminoglycans (HiChol-Animal Fiber).

The meat in the human-type diets was cooked at 95° for 3 hours in steam-jacketed 151.4-liter containers⁴ with sufficient distilled water to slurry. The remaining in-

TABLE 1
Composition of basal purified and human-type diets

| Purified | | Human-type | |
|--------------------------|-------------|------------------------------|-------------|
| Ingredient | Composition | Ingredient | Composition |
| | % | | % |
| Casein ¹ | 20.5 | Beef tenderloin ⁷ | 24.8 |
| Cornstarch ² | 46.3 | White bread ⁸ | 21.0 |
| Beef tallow ³ | 28.0 | Refined rice ³ | 27.2 |
| Mineral mix ⁴ | 4.0 | Milk ⁵ | 6.0 |
| Vitamin mix ⁵ | 1.0 | Beef tallow ³ | 16.0 |
| Methionine ⁶ | 0.2 | Mineral mix ⁴ | 4.0 |
| | | Vitamin mix ⁵ | 1.0 |

¹ National Casein Company, Tyler, Texas. ² United States Biochemical Corporation, Cleveland, Ohio. ³ Courtesy of Mr. Harold Peeler, Jacob Stern & Sons, Inc., Houston, Texas. ⁴ Amounts in mg/100 g diet: calcium carbonate, 1,526; cobalt chloride, 0.920; copper sulfate, 1.90; ferrous sulfate, 108; manganese sulfate, 17.6; magnesium sulfate, 229; potassium iodide, 3.16; potassium phosphate, 1,556; sodium chloride, 557; zinc chloride, 1.04. Fluoride in water supply, 1 ppm. ⁵ Vitamin fortification mix purchased from Tekland Mills, Madison, Wisconsin, which provided the following amounts per 100 g diet: choline dihydrogen citrate, 370 mg; inositol, 11 mg; *p*-aminobenzoic acid, 11 mg; α -tocopheryl acetate, 48 mg; niacin, 9.9 mg; calcium pantothenate, 616 mg; thiamin hydrochloride, 2.2 mg; folic acid, 0.20 mg; biotin, 0.044 mg; pyridoxine hydrochloride, 2.2 mg; menadione, 5.0 mg; riboflavin, 2.2 mg; B-12, 3 μ g; retinyl palmitate, 19,841 IU; ergocalciferol, 22,205 IU. ⁶ Sigma Chemical Company, St. Louis, Missouri. ⁷ Courtesy of Department of Animal Science, Texas A&M University. ⁸ Courtesy of Food Services, Texas A&M University.

gredients were added after the meat had cooked for 2 hours. The human-type diets were then freeze-dried and sealed in cans in 1-kg portions until fed to the rats.

Experimental design. The experimental diets were fed to groups of 12 rats for 10 weeks. The rats were weighed biweekly. At termination there were 6 to 10 healthy rats in each diet group. Some of the rats showed symptoms of chronic respiratory infection and others developed tumors. Tissues from rats maintaining stable body weights and free from tumors were analyzed. Three-day fecal samples were collected for neutral sterol and bile acid analysis during the final week of the feed-

³ ARS/Sprague-Dawley, Madison, Wisconsin.

⁴ Courtesy of Oilseed Products Division, Texas A&M University, College Station, Texas.

TABLE 2

Experimental diets as modifications of basal diets

| LoChol-Fiber | HiChol-LoFiber | HiChol-Plant Fiber | HiChol-Animal Fiber |
|--------------|---------------------------------|---------------------------------|-------------------------------------|
| | | Purified ¹ | |
| None | 0.5% Cholesterol ² | 0.5% Cholesterol ³ | 0.5% Cholesterol ³ |
| | | 5% Pectin ⁴ | 5% Chondroitin Sulfate ⁵ |
| | | Human-type ² | |
| None | 4% Brains (bovine) ⁶ | 4% Brains (bovine) ⁶ | 4% Brains (bovine) ⁶ |
| | | 30.5% Green Beans ⁷ | 24% Beef Shanks ⁸ |

¹ Additions were made at the expense of cornstarch. ² Modifications were made by appropriate changes in the basal ingredients to maintain an isocaloric distribution of protein, carbohydrate, and fat. ³ Amerchol, Amerchol Park, Edison, New Jersey. ⁴ United States Biochemical Corporation, Cleveland, Ohio. ⁵ Sigma Chemical Company, St. Louis, Missouri. ⁶ Readfields Meat Company, Bryan, Texas. ⁷ Canned green beans courtesy of Food Services, Texas A&M University. ⁸ Courtesy of Animal Science Department, Texas A&M University.

ing period. Serum and liver cholesterol and liver neutral glycerides, hepatic 3-hydroxy-3-methylglutaryl coenzyme-A (HMG CoA) reductase [mevalonate: NADP oxidoreductase (acylating CoA) EC 1.1.1.34], and cholesterol 7 α -hydroxylase [cholesterol, reduced NADP: oxygen oxidoreductase (7 α -hydroxylating) EC 1.1.4] were assayed at termination.

Methods. In order to eliminate the effects of diurnal variations on enzyme activities, the rats were killed by decapitation within 15 minutes of 0900 hours. The rats were killed during the dark period because the activities of HMG CoA reductase and cholesterol 7 α -hydroxylase are higher than during the light period (12, 13). The livers were quickly excised, weighed and placed in ice cold beakers. Isolation of liver microsomes has been previously described (11). Portions of the liver homogenates were retained for the analyses of cholesterol and neutral glycerides. Hepatic HMG CoA reductase was assayed according to the method of Dugan et al. (14) as modified by Reiser et al. (15). Cholesterol 7 α -hydroxylase was assayed as described by Mitropoulos and Balasubramaniam (16). Microsomal protein was determined by the method by Lowry et al. (17). Microsomal cholesterol was determined spectrophotometrically according to Searcy and Bergquist (18) following alkaline hydrolysis of the tissue sample (19). Cholesterol concentrations in liver homogenates and in

serum were analyzed by the same methods used for microsomal cholesterol analysis. Liver neutral glycerides were determined using the method of Foster and Dunn (20) and calculated as triglycerides using a standard triolein solution.⁵

Fecal samples from each treatment group were pooled. Duplicate 1-g samples of dried and ground feces were saponified and extracted as described by Miettinen et al. (21). Trimethylsilyl derivatives⁶ of the fecal neutral sterols, cholesterol and coprostanol, were separated by gas-liquid chromatography (GLC) using a 91.4 cm glass column (2 mm id) packed with 100 to 120 mesh Gas Chrom Q coated with 3% OV-101⁷ at 250°, a flow rate 70 ml He/minute and were quantitated with 5 α -cholestane as internal standard. The bile acid content of the residue, after extraction of the neutral sterols, was further saponified under pressure as described by Grundy et al. (22). The bile acids were purified by thin-layer chromatography (TLC) and quantitated by GLC according to the method of Bartov et al. (23).

The pectin contents of the human-type diets as well as of fresh and canned green beans were determined according to the procedure of Lin et al. (24). The ground tenderloin and ground beef shank used in

⁵ Sigma Chemical Company, St. Louis, Missouri.

⁶ Tri Sil. Pierce Chemical Company, Rockford, Illinois.

⁷ Applied Science Laboratories, Inc., State College, Pennsylvania.

the human-type diets were analyzed for glycosaminoglycans by hydrolyzing the dried, defatted meat in 4 N HCl for 17 hours at 100° with galactosamine as internal standard (25) and quantitated spectrophotometrically as described by Ludwig and Benmaman (26).

Statistical analysis. The significance of differences between means was evaluated by Scheffé's multiple comparison method following analysis of variance (27).

RESULTS AND DISCUSSION

The contrasting effects on serum cholesterol of diet cholesterol ingested as part of a purified diet or as part of a diet of mixed ingredients is clearly demonstrated in table 3. Although pectin and chondroitin sulfate isomers in the cholesterol-supplemented purified diet do attenuate the cholesterol-emic effect of diet cholesterol, neither fiber is as effective as the diets of common food-stuffs. The lack of any difference in response of serum cholesterol to diet cholesterol between any of the human-type diet groups was striking. One cannot reasonably attribute the observed serum cholesterol homeostasis in rats fed the human-type diet to the fiber contents of the diets. There is little natural fiber in the basal low fiber diet. The diet designated as "high cholesterol-plant fiber" was found to contain less than 1% pectin, and the diet designated "high cholesterol-animal fiber" contained approximately 0.04% glycosaminoglycans as calculated from analysis of the beef shank.

Although the purified and human-type diets were energetically equivalent in fat, carbohydrate, and protein, the sources of these nutrients were quite different in the two types of diets. Hamilton and Carroll (28) demonstrated the influence of different sources of dietary protein, carbohydrate, and fiber on serum cholesterol of rabbits consuming low fat, low cholesterol diets during a 4-week feeding period. In general, animal protein in the diet (extracted whole egg, nonfat milk, lactalbumin, beef protein concentrate, pork protein concentrate, or raw egg white) increased serum cholesterol significantly more than plant protein (wheat gluten, peanut protein concentrate, peanut meal, soybean protein concentrate, or soybean

TABLE 3
Some metabolic responses in rats fed purified and human-type diets for 10 weeks

| | Purified | | | | | Human-type | | | | |
|--|--------------------------|--------------------------|----------------------------|----------------------------|----------------------------|----------------------------|----------------------------|----------------------------|----------------------------|----------------------------|
| | LoCh-LoF ^b | HiCh-LoF ^b | LoCh-PF ^b | HiCh-PF ^b | HiCh-AnF ^b | LoCh-LoF ^b | HiCh-LoF ^b | HiCh-PF ^b | HiCh-AnF ^b | HiCh-AnF ^b |
| Serum cholesterol (mg/dl) | 95 ± 10 ^{a,c} | 187 ± 58 ^b | 118 ± 12 ^a | 126 ± 37 ^a | 126 ± 37 ^a | 93 ± 17 ^a | 92 ± 18 ^a | 101 ± 18 ^a | 101 ± 18 ^a | 99 ± 17 ^a |
| Liver cholesterol (mg/g) | 3.1 ± 0.4 ^a | 14.6 ± 5.8 ^b | 8.2 ± 3.0 ^{a,c} | 12.4 ± 7.9 ^{b,c} | 12.4 ± 7.9 ^{b,c} | 3.6 ± 0.4 ^a | 4.3 ± 0.5 ^a | 4.2 ± 0.4 ^a | 4.2 ± 0.4 ^a | 4.0 ± 0.6 ^a |
| Liver neutral glycerides (mg/g) | 9.4 ± 2.5 ^a | 10.7 ± 3.2 ^a | 13.1 ± 2.1 ^a | 15.3 ± 5.3 ^a | 15.3 ± 5.3 ^a | 11.7 ± 3.0 ^a | 15.0 ± 3.8 ^a | 8.5 ± 2.0 ^a | 8.5 ± 2.0 ^a | 9.8 ± 3.7 ^a |
| HMG CoA reductase (nanomoles mevalonate acid produced/mg protein/min) | 0.66 ± 0.25 ^a | 0.61 ± 0.16 ^a | 0.56 ± 0.17 ^a | 0.72 ± 0.18 ^a | 0.72 ± 0.18 ^a | 0.28 ± 0.17 ^a | 0.23 ± 0.21 ^a | 0.35 ± 0.13 ^b | 0.35 ± 0.13 ^b | 0.24 ± 0.13 ^b |
| Cholesterol 7 α -hydroxylase (nmoles 7 α -hydroxycholesterol produced/mg protein/20 min) | 0.35 ± 0.18 ^a | 1.28 ± 0.46 ^b | 0.87 ± 0.23 ^{a,c} | 0.76 ± 0.17 ^{a,c} | 0.76 ± 0.17 ^{a,c} | 0.48 ± 0.19 ^{a,c} | 0.53 ± 0.14 ^{a,c} | 0.73 ± 0.21 ^{a,c} | 0.73 ± 0.21 ^{a,c} | 0.70 ± 0.19 ^{a,c} |
| Fecal neutral sterols ³ (mg rat/day) | 8.7 | 29.5 | 34.7 | 36.1 | 36.1 | 6.0 | 28.1 | 31.3 | 31.3 | 25.9 |
| Fecal bile acids ³ (mg rat/day) | 5.8 | 12.1 | 14.6 | 10.7 | 10.7 | 7.8 | 16.3 | 19.4 | 19.4 | 9.4 |

^a LoCh-LoF, low cholesterol-low fiber; HiCh-LoF, high cholesterol-low fiber; LoCh-PF, high cholesterol-plant fiber; HiCh-PF, high cholesterol-plant fiber; HiCh-AnF, high cholesterol-animal fiber. ^b Mean \pm SD. Horizontal values not sharing a common superscript letter are significantly different at $P < 0.05$. Values are average of 6 to 10 rats. ^c Feces from each diet group were pooled and duplicate samples were analyzed. Individual measurements were within 5% of the mean.

protein isolate), animal protein 105 to 235 mg/dl and plant proteins 15 to 80 mg/dl. Among the animal protein sources investigated, nonfat milk had a significantly greater effect than beef protein on average serum cholesterol, 230 and 160 mg/dl respectively. Among the sources of carbohydrate that were fed to the rabbits (sucrose, glucose, lactose, wheat starch, corn starch, rice starch, and potato starch) in casein-containing diets, corn starch was less cholesterolemic than wheat starch, 185 versus 260 mg/dl, and more cholesterolemic than rice starch, 185 versus 140 mg/dl. Potato starch-fed rabbits had the lowest average serum cholesterol among the test groups, 95 mg/dl. However, if the differences in serum cholesterol that were observed in the present study were due simply to differences in protein and carbohydrate sources, one would have expected a difference in the average serum cholesterol level between rats that were fed the low cholesterol purified diet and the low cholesterol human-type diet. There was no difference. If plant protein were more hypocholesterolemic than animal protein, one might expect the human-type diet containing green beans to elicit the lowest average serum cholesterol, but that was not observed. However, Hamilton and Carroll (28) found that a 3:1 mixture of zein (corn protein) and lactalbumin fed to rabbits resulted in an average serum cholesterol level which was in the range of rabbits fed animal protein, 170 mg/dl.

No doubt the mineral and vitamin contents of the purified and human-type diets used in this study were different. Both the purified and human-type diets were supplemented with minerals and vitamins (see footnotes, table 1). However, one would expect that the human-type diets would contain more minerals and vitamins and possibly some minerals which were not present in the purified diets, i.e., chromium, selenium, molybdenum. Selenium and chromium are now recommended in rat diets (29).

There was no effect of diet cholesterol on liver cholesterol of rats which ate the cholesterol containing human-type diets although there was a pronounced effect when cholesterol was consumed as part of a puri-

fied diet (table 3). Although pectin appeared to somewhat mitigate the accumulation of cholesterol in the liver, chondroitin sulfate isomers did not. There was no difference in the liver neutral glycerides (table 3) among any groups of rats.

The regulatory hepatic enzymes of cholesterol synthesis, HMG CoA reductase, and of cholesterol degradation, cholesterol 7 α -hydroxylase, were assayed in an attempt to elucidate the homeostatic effect of the human-type diets. These data are shown in table 3. Rats fed the same type of diet, purified or human-type, had the same average activities of HMG CoA reductase. However, there is a suggestion that rats fed the purified diets have higher activities of this enzyme than those fed the human-type diets. It is possible that a higher level of hepatic HMG CoA reductase activity in rats ingesting the purified diets compared to rats fed the human-type diets accounts for the higher serum cholesterol level in rats fed cholesterol-supplemented purified diets. However, if endogenous cholesterol were the source of serum cholesterol, one would expect that rats fed the low cholesterol, purified diet to have equally high serum cholesterol since the enzyme activity was equally high. This was not observed.

In comparing the response of cholesterol 7 α -hydroxylase to the high cholesterol, low fiber purified and human-type diets, rats fed the purified diet had significantly higher activity of this liver enzyme than rats fed the corresponding human-type diet. One might infer from this comparison that more cholesterol is absorbed for enzyme substrate when cholesterol is consumed in the purified diet. However, the excretion of total neutral sterols (table 3) does not support that inference. Likewise, differences in total bile acid excretions (table 3) could not account for the serum cholesterol homeostasis observed when rats were fed cholesterol supplemented human-type diets. The results of this study do not eliminate the possibility that cholesterol in the human-type diet was more poorly absorbed since no attempt was made to isolate cholesterol degradation products other than coprostanol. Cholesterol absorption in rats fed the two types of diets, purified and

human-type, should be compared using the dual isotope method of Zilversmit (30).

In vitro determinations of enzyme activities are not reliable measures of in vivo syntheses or degradations since in vitro activities are maximized by optimum cofactor and substrate availabilities. In order to evaluate the effects on cholesterol synthesis and degradation of the two types of diets used in these experiments, in vivo incorporation of a labeled cholesterol precursor, such as [^{14}C]glucose, should be determined in rats fed comparable purified and human-type diets (31).

The results of this study demonstrate the importance of the nonlipid constituents of the diet in the control of cholesterol metabolism, not just fat and cholesterol. The original hypothesis that the nondigestible components of a high cholesterol, high fat diet of common foodstuffs were responsible for the stabilization of serum cholesterol was not supported. None of the human-type diets used in this study contained more than 1% pectin or glycosaminoglycans. Experiments are presently underway to test the effects on serum and liver lipids of the individual components of the high cholesterol, low fiber human-type diet used in this study by sequentially substituting the ingredients of the high cholesterol, low fiber purified diet with the practical sources of carbohydrate and protein used in the human-type diet.

ACKNOWLEDGMENT

The authors are grateful to Mr. Jerry Graham of the Right Away Foods Company, Edinburg, Texas, for freeze-drying and canning the cooked diets of natural foodstuffs used in this study.

LITERATURE CITED

- Weinhouse, S. (1944) General Reviews: The Blood Cholesterol. *Arch. Path.* 35, 438-500.
- Messinger, W. J., Porosowska, Y. & Steele, J. M. (1950) Effect of feeding egg yolk and cholesterol on serum cholesterol levels. *Arch. Int. Med.* 86, 189-195.
- Ahrens, E. H., Jr., Dole, V. P. & Blankenhorn, D. H. (1954) The use of orally-fed liquid formulas in metabolic studies. *Am. J. Clin. Nutr.* 2, 336-342.
- Beveridge, J. M. R., Connell, W. F. & Mayer, G. A. (1956) Dietary factors affecting the level of plasma cholesterol in humans: The role of fat. *Can. J. Biochem. Phys.* 34, 441-455.
- Brönte-Stewart, B., Antonis, A., Eales, L. & Brock, J. F. (1956) Effects of feeding different fats on serum-cholesterol levels. *Lancet* *i*, 521-526.
- Ahrens, E. H., Jr., Hirsch, J., Insull, W., Jr., Tsaltas, T. T., Blomstrand, R. & Peterson, M. L. (1957) The influence of dietary fats on serum-lipid levels in man. *Lancet* *ii*, 943-953.
- Beveridge, J. M. R., Connell, W. F., Mayer, G. A. & Haust, H. L. (1960) The response of man to dietary cholesterol. *J. Nutr.* 71, 61-65.
- Connor, W. E., Hodges, R. E. & Bleiler, R. A. (1961) The serum lipids in men receiving high cholesterol and cholesterol-free diets. *J. Clin. Invest.* 40, 894-901.
- Tsai, Alan C., Elias, J., Kelley, J. J., Lin, R. S. C. & Robson, J. R. K. (1976) Influence of certain dietary fibers on serum and tissue cholesterol levels in rats. *J. Nutr.* 106, 118-123.
- Kay, R. M. & Truswell, A. S. (1977) Effect of citrus pectin on blood lipids and fecal steroid excretion in man. *Am. J. Clin. Nutr.* 30, 171-175.
- O'Brien, B. C., Skutches, C. L., Henderson, G. R. & Reiser, R. (1977) Interrelated effects of food lipids on steroid metabolism in rats. *J. Nutr.* 107, 1444-1454.
- Edwards, P. A. & Gould, R. G. (1972) Turnover rate of hepatic 3-hydroxy-3-methylglutaryl coenzyme-A reductase as determined by use of cycloheximide. *J. Biol. Chem.* 247, 1520-1524.
- Danielsson, H. (1972) Relationship between diurnal variations in biosynthesis of cholesterol and bile acids. *Steroids* 20, 63-72.
- Dugan, R. E., Slakey, L. L., Brieds, L. L. & Porter, J. W. (1972) Factors affecting the diurnal variation in the level of β -hydroxy- β -methylglutaryl coenzyme-A reductase and cholesterol-synthesizing activity in rat liver. *Arch. Biochem. Biophys.* 152, 21-27.
- Reiser, R., Henderson, G. L. & O'Brien, B. C. (1977) Persistence of dietary suppression of 3-hydroxy-3-methylglutaryl coenzyme-A reductase during development in rats. *J. Nutr.* 107, 1131-1138.
- Mitropoulos, K. A. & Balasubramaniam, S. (1972) Cholesterol 7 α -hydroxylase in rat liver microsomal preparations. *Biochem. J.* 128, 1-9.
- Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) Protein measurement with Folin phenol reagent. *J. Biol. Chem.* 193, 165-175.
- Searcy, R. L. & Bergquist, L. M. (1960) A new color reaction for the quantitation of serum cholesterol. *Clin. Chem. Acta* 51, 192-199.
- Abell, L. L., Levy, B. B., Brodie, B. B. & Kendall, F. E. (1952) A simplified method for the estimation of total cholesterol in serum and demonstration of its specificity. *J. Biol. Chem.* 195, 337-366.
- Foster, L. B. & Dunn, R. J. (1973) Stable

- reagents for determination of serum triglycerides by a colorimetric Hantsch condensation method. *Clin. Chem.* *19*, 338-340.
21. Miettinen, J. A., Ahrens, E. H., Jr., & Grundy, S. M. (1965) Quantitative isolation and gas-liquid chromatographic analysis of total dietary and fecal neutral steroids. *J. Lipid Res.* *6*, 411-424.
 22. Grundy, S. M., Ahrens, E. H., Jr., & Miettinen, J. A. (1965) Quantitative isolation and gas-liquid chromatographic analysis of total fecal bile acids. *J. Lipid Res.* *6*, 397-410.
 23. Bartov, I., Reiser, R. & Henderson, G. R. (1973) Hypercholesterolemic effect in the female rat of egg yolk versus crystalline cholesterol dissolved in lard. *J. Nutr.* *103*, 1400-1405.
 24. Lin, M. J. Y., Sosulski, F. W., Humbert, E. S. & Downey, R. K. (1975) Distribution and compositions of pectins in sunflower plants. *Can. J. Plant Sci.* *55*, 507-513.
 25. Ogston, A. G. (1964) On the estimation of glucosamine in hyaluronic acid. *Anal. Biochem.* *8*, 337-343.
 26. Ludowieg, J. & Benmaman, J. D. (1967) Colorimetric differentiation of hexosamines. *Anal. Biochem.* *19*, 80-88.
 27. Bancroft, T. A. (1968) "Topics in Intermediate Statistical Methods," Vol. I, pp. 101-103, Iowa State University Press, Ames, Iowa.
 28. Hamilton, R. M. G. & Carroll, K. K. (1976) Plasma cholesterol levels in rabbits fed low fat, low cholesterol diets. Effects of dietary proteins, carbohydrates and fibre from different sources. *Atherosclerosis* *24*, 47-62.
 29. Report of the American Institute of Nutrition Ad Hoc Committee on Standards for Nutritional Studies (1977) *J. Nutr.* *107*, 1340-1348.
 30. Zilversmit, D. B. (1972) A single blood sample dual isotope method for the measurement of cholesterol absorption in rats. *Proc. Soc. Exp. Biol. Med.* *140*, 862-865.
 31. Kelley, J. J. & Tsai, A. C. (1978) Effect of pectin, gum arabic and agar on cholesterol absorption, synthesis, and turnover in rats. *J. Nutr.* *108*, 630-639.

Progress Report, 1977-78

Project supported by

National Live Stock and Meat Board

and

Texas Cattle Feeders Association

Stabilization of Blood Lipids and Steroid Metabolism
by Balanced Diets of Natural Foodstuffs

Raymond Reiser
Principal Investigator

The present investigation was designed to elucidate the results of a previous study supported by the National Live Stock and Meat Board and by the Texas Cattle Feeders Association. This earlier experiment demonstrated in rats the contrasting effects on cholesterolemia of isoenergetic purified and human-type diets which contained 28% beef fat and 0.5% added cholesterol and were low in nondigestible constituents. The average serum cholesterol concentration of rats consuming the purified diet was 187 mg/dl while rats ingesting the human-type diet had an average serum cholesterol concentration of 92 mg/dl, equivalent to the mean serum cholesterol level of rats fed isoenergetic purified and human-type diets containing no added cholesterol, 95 mg/dl and 93 mg/dl respectively. These results were reported at the meeting of the Federation of American Societies of Experimental Biology in April, 1978 and have been published (1). The objective of the present study was to determine the ingredient(s) in the human-type diet which was (were) responsible for its hypocholesterolemic effect.

In addition to beef fat and cholesterol, the purified diet contained casein, corn starch, vitamins, and minerals while the energetically equivalent human-type diet contained beef tenderloin, white bread, polished rice, whole milk, vitamins, and minerals. In the earlier experiment only the human-type diet was cooked and freeze-dried. Although the objective of the earlier study was to assess the cholesterolemic effect of nondigestible constituents in purified and human-type diets, the results of the investigation clearly showed that the hypocholesterolemic effect of the human-type diet used in that study was not due to nondigestible components.

The hypercholesterolemic purified diet was used as the basis for comparison. The carbohydrate source, corn starch, was substituted with rice, bread, or rice and bread in the proportions used in the human-type diet while the protein source, casein, was altered only to the extent necessary

to maintain equivalent protein content in the diets. The casein was then substituted with beef tenderloin or tenderloin and whole milk while the major carbohydrate source was corn starch. Then, diets containing tenderloin were varied with respect to carbohydrate: rice, bread, or rice and bread. In another diet nondigestible constituents, plant sterols, pectin, and chondroitin sulfate, were added to the human-type diet. All of these diets were virtually energetically equivalent and contained 28% beef fat and 0.5% added cholesterol (dissolved in the fat). The control diets used in this experiment were a stock rat diet, the purified diet without added cholesterol, the human-type diet without added cholesterol, and the tenderloin and corn starch diet without added cholesterol. The compositions of the 13 prepared diets used in this study are given in table 1. Excepting for the stock rat diet, all the diets were supplemented with vitamins and minerals, cooked at 95° for 1 hour with sufficient water to slurry, freeze-dried and sealed in cans in 1-kg portions. We are grateful to the Right Away Foods Company, Edinburg, Texas for freeze-drying and canning the food.

The diets were fed to female Sprague-Dawley rats (11 month old retired breeders) for 10 weeks. Body weights were recorded biweekly. During the 8th week of the feeding period, food consumption was recorded although it was measured approximately throughout. During the 9th week of the feeding period, three-day fecal samples were collected. The 11 or 12 healthy rats in each diet group were killed by decapitation. Blood samples and livers were retained for cholesterol analysis. Fecal neutral sterols and bile acids were determined. These data are shown in table 2.

The ingredient of the human-type diet which appears to be primarily responsible for its hypocholesterolemic effect is bread. When casein is

the major protein source in the high cholesterol diets, the average serum cholesterol concentration of rats ingesting bread-containing diets is significantly lower than when corn starch or rice alone are consumed; bread alone, 109 mg/d, and bread and rice, 121 mg/dl compared to corn starch, 170 mg/dl, and rice, 144 mg/dl. All high cholesterol, casein-containing diets elicited a higher serum cholesterol level than that of rats fed the stock rat diet, 86 mg/dl. However, when rats were fed the high cholesterol diet in which tenderloin was the major protein source and bread was the source of carbohydrate, their average serum cholesterol level, 92 mg/dl, was the same as that of the stock-fed rats and the same as those of rats fed the diets without added cholesterol. The bread used in these diets was low in fiber content. Furthermore wheat starch has been reported by Dr. K.K. Carroll and his coworkers (2) to be more hypercholesterolemic than either corn starch or rice. The plant protein content of wheat and rice is approximately equivalent at 7.5%. Since the wheat flour is probably not the hypocholesterolemic agent in bread, it appears likely that an ingredient used in the preparation of the bread is responsible for its hypocholesterolemic effect.

In comparing the cholesterolemic response of rats consuming these various diets it is important to notice that there is no hypercholesterolemic response associated with the ingestion of beef tenderloin. However dietary casein in high cholesterol diets clearly elicits hypercholesterolemia in the absence of some attenuating dietary carbohydrate. For example, rats which consumed the high cholesterol diet containing tenderloin, milk, and corn starch had the same high serum cholesterol level, 167 mg/dl, as that of rats consuming the high cholesterol casein-corn starch diet, 170 mg/dl.

The equivalent diet without milk produced a serum cholesterol level of 126 mg/dl.

The accumulation of cholesterol in the livers of the high cholesterol purified diet group was significantly higher, 28.4 mg/g, than in any other group. Rats consuming the high cholesterol human-type diet supplemented with plant sterols, pectin, and chondroitin sulfate had the same average liver cholesterol concentration, 4.9 mg/g, as rats consuming the low cholesterol prepared diets which were slightly, but significantly, higher than that of stock-fed rats, 3.8 mg/g. The high cholesterol tenderloin-bread diet significantly attenuates the accumulation of cholesterol in the liver.

The fecal data do not provide any insight into the hypocholesterolemic effect of bread.

References

1. O'Brien, Barbara Cooney and Reiser, Raymond (1979). Comparative effects of purified and human-type diets on cholesterol metabolism in the rat. *J. Nutr.* 109, 98-104. (reprint enclosed)
2. Hamilton, R.M.G. and Carroll, K.K. (1976) Plasma cholesterol levels in rabbits fed low fat, low cholesterol diets. Effects of dietary proteins, carbohydrates and fibre from different sources. *Atherosclerosis* 24, 47-62.

TABLE 1
Percent Composition of Prepared Diets

| Ingredients | Diets ¹ | | | | | | | | | | | | |
|-----------------------|-------------------------------|------|--------------|------|-------------|--------------------|-----------------|-------|--------------|----------------|--------------------|------------------------------|------------------------|
| | High Cholesterol ² | | | | | | Beef Tenderloin | | | | | Low Cholesterol ³ | |
| | Casein | | Rice & Bread | | Corn Starch | Milk & Corn Starch | Rice | Bread | Bread & Rice | Nondigestibles | Casein Corn Starch | Human-Type | Tenderloin Corn Starch |
| Casein | 18.4 | 14.5 | 10.2 | 12.8 | -- | -- | -- | -- | -- | 18.4 | -- | -- | |
| Tenderloin | -- | -- | -- | -- | 26.1 | 20.3 | 20.8 | 14.6 | 18.4 | 18.4 | -- | 21.1 | 26.4 |
| Whole Milk | -- | -- | -- | -- | -- | 15.7 | -- | -- | -- | -- | -- | 5.0 | -- |
| Corn Starch | 47.4 | -- | -- | -- | 47.4 | 40.0 | -- | -- | -- | -- | -- | 47.9 | 47.3 |
| Bread | -- | -- | 58.6 | 23.2 | -- | -- | -- | 58.5 | 23.0 | 20.3 | -- | 17.8 | -- |
| Rice | -- | 52.4 | -- | 31.7 | -- | -- | 51.9 | -- | 31.4 | 27.6 | -- | 30.6 | -- |
| Beef Tallow | 28.5 | 27.4 | 25.5 | 26.6 | 21.0 | 18.5 | 21.7 | 21.4 | 21.7 | 21.7 | 28.5 | 20.5 | 21.3 |
| Cholesterol | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | -- | -- | -- |
| Vitamins ⁴ | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 |
| Minerals ⁵ | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 |
| Methionine | 0.2 | 0.2 | 0.2 | 0.2 | -- | -- | -- | -- | -- | -- | 0.2 | -- | -- |
| Soy Sterols | -- | -- | -- | -- | -- | -- | -- | -- | -- | 0.5 | -- | -- | -- |
| Chondroitin Sulfate | -- | -- | -- | -- | -- | -- | -- | -- | -- | 3.0 | -- | -- | -- |
| Pectin | -- | -- | -- | -- | -- | -- | -- | -- | -- | 3.0 | -- | -- | -- |
| Cholesterol Content | 0.7 | 0.6 | 0.8 | 0.7 | 0.7 | 0.6 | 0.8 | 0.7 | 0.7 | 0.8 | 0.1 | 0.1 | 0.2 |

1 Diet designations consistent with those used in Table 2
 2 0.5g crystalline cholesterol dissolved in beef tallow/100g feed
 3 No cholesterol added
 4 Vitamin Fortification Mix, Teklad Mills, Madison, Wisconsin
 5 Jones-Foster Mineral Mix, United States Biochemical Corp., Cleveland, Ohio

TABLE 2

Some Responses in Rats Fed Various Human-Type Diet In
Diets

| Parameter | High Cholesterol | | | | Tenderloin | | | |
|---|-----------------------|-----------------------|-----------------------|-----------------------|-----------------------|-----------------------|-----------------------|---------------------|
| | Casein Corn Starch | Rice | Bread | Rice & Bread | Corn Starch | Milk & Corn Starch | Rice | Bread |
| Food Intake (g/rat/day) | 8.8±1.3a ¹ | 8.5±0.8a | 10.4±1.1 ^b | 9.1±1.1 ^{ab} | 10.0±1.2 ^b | 12.1±0.9 ^c | 10.6±2.2 ^b | 11.0±2. |
| Initial Body Weight (g) | 294±30a | 295±28a | 296±28a | 297±28a | 297±29a | 297±29a | 298±28a | 299±28a |
| Final Body Weight (g) | 318±44 ^{ab} | 327±34 ^{ab} | 308±37 ^a | 308±28a | 322±33 ^{ab} | 333±30 ^b | 336±57 ^{ab} | 318±23 ^a |
| Liver Weight (g) | 9.0±1.2 ^a | 8.6±0.6a | 8.6±0.6a | 8.4±1.1a | 8.5±1.1a | 8.8±1.3 ^a | 8.8±1.1 ^a | 8.5±1.0 |
| Serum Choles- terol (mg/dl) | 170±32 ^a | 144±38 ^{ab} | 109±19 ^{cf} | 121±30 ^{cd} | 126±25 ^{cd} | 165±46 ^a | 140±38 ^{ad} | 92±17 ^e |
| Liver Choles- terol (ng/g) | 28.4±9.8 ^a | 14.4±5.7 ^b | 10.9±2.8 ^b | 12.5±4.5 ^b | 14.6±4.4 ^b | 15.8±6.4 ^b | 12.7±5.5 ^b | 7.7±2. |
| Fecal Weight ² (g/rat/day) | 0.54 | 0.64 | 0.87 | 0.78 | 0.44 | 0.88 | 0.79 | 0.77 |
| Fecal Neutral Sterols (mg/rat/d) ³ | 20.6 | 7.5 | 19.3 | 24.2 | 16.5 | 10.8 | 4.5 | 15.8 |
| Fecal Bile Acids (mg/rat/d) ³ | 4.5 | 5.8 | 10.9 | 4.5 | 3.6 | 13.0 | 10.0 | 12.7 |

¹ Mean ±SD. Horizontal values not sharing a common superscript letter are significantly dif

² Dry Weight

³ Feces from each diet group were pooled