COMPARISON OF FECAL LIPID PATTERNS IN HUMANS FED BEEF TALLOW AND CORN OIL AS SOLE SOURCES OF DIETARY FAT

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ABSTRACT

The present study was undertaken to determine whether the hypocholesterolemic effect reported for beef tallow is due to increased fecal steroid excretion as has been reported for corn oil. Since liquid formula diets alter lipid excretion and have a hypocholesterolemic effect regardless of the fat in the forumla, a solid test diet was designed resembling the normal diet but with 40% of the calories from either beef tallow or corn oil. Fecal lipid excretion patterns of six healthy young men fed the tallow and corn oil diets were determined for a 5-day period during each of the two 22-day metabolic studies. The apparent digestibility of corn oil was significantly higher ($P < 0.005$) than that of beef tallow. Lower apparent digestibility of palmitic and stearic acids accounted for the lower digestibility of beef tallow. Non-saponifiable lipid excretion was significantly greater ($P < 0.025$) for corn oil than for tallow, due to a significant increase ($P < 0.005$) in the excretion of plant sterols and neutral steroids derived from endogenous cholesterol. The excretion of major bile acids was appreciably lower than that of neutral steroids, and did not differ significantly between diets. Since cholesterol-derived neutral steroid excretion was significantly lower on a solid diet containing beef tallow than when the same diet contained corn oil, the hypocholesterolemic mechanism of the two fats may differ.

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INTRODUCTION

The effect of dietary fat on cholesterol metabolism has assumed increasing importance because of the correlation between elevated serum cholesterol and atherosclerosis. There have been numerous reports confirming the observations that saturated fats in the diet elevate, whereas unsaturated fats lower, the serum cholesterol concentration in man. However, there has not been a definitive explanation of the mechanism of the hypocholesterolemic effect of polyunsaturated fat in the diet. Theoretically, this action could result from any one or a combination of the following alterations in cholesterol metabolism: (a) a decrease in endogenous cholesterol synthesis; (b) a reduction in absorption of cholesterol; (c) an increase in fecal excretion of cholesterol and/or bile acids; and (d) a redistribution of blood cholesterol between plasma and other tissues. Although the mechanism responsible for the hypocholesterolemic action of dietary polyunsaturated fat remains unresolved, there seems to be considerable evidence that these fats promote increased excretion of fecal steroids.

Beef tallow, one of the major sources of saturated fat in the North American diet, has been found to lack the cholesterol-elevating effect expected of a saturated fat. It has been suggested that the failure to observe an increase in serum cholesterol in response to beef tallow is associated with the relatively high proportion of stearic acid in this

fat. Since increased fecal steroid excretion has been shown to accompany the cholesterol-lowering effect of corn oil, it was considered of interest to compare the fecal lipid excretion patterns of young men fed tallow or corn oil as the sole source of dietary fat. In addition since liquid formulae test diets have been associated with alterations in steroid excretion patterns irrespective of the fat contained in the formula, it was considered necessary to formulate a solid test diet of customary foods which would permit the testing of a single saturated or unsaturated fat source.

REVIEW OF LITERATURE

A. INTRODUCTION

In spite of the high correlation among dietary fat, elevated serum cholesterol levels and the incidence of atherosclerosis (Stamler et al. 1966), there is still a definite lack of understanding regarding the detailed mechanisms whereby cholesterol metabolism is regulated. Polyunsaturated fatty acids have been shown to lower the plasma cholesterol when substituted for saturated fatty acids in the diet (Ahrens et al. 1957; Hegsted et al. 1965), whereas long chain, saturated fatty acids tend to elevate blood cholesterol levels. However, several reports (Ahrens et al. 1957; Connor et al. 1964; Erickson et al. 1964 and Keys et al. 1965c) have indicated that, in man, stearic acid tends to lack the cholesterol-elevating effect of saturated fatty acids. Cocoa butter, a fat rich in stearic acid, did not produce the cholesterol-raising effect expected on the basis of its total content of long-chain saturated fatty acids (Grande et al. 1970). The mechanism whereby unsaturated fats, or saturated fats high in stearic acid, may alter plasma cholesterol is still unknown.

Total body cholesterol is normally regulated by the interaction of three processes: absorption, synthesis and excretion. Hence the following modes of action have been proposed (Spritz et al. 1965) to explain the cholesterol-lowering effects of polyunsaturated fatty acids. (1) An increased excretion of cholesterol as neutral steroid could occur. This could be due to decreased absorption of cholesterol from the diet and

intestinal lumen; (2) An increase in bile acid formation and excretion is a possible mechanism. This could be achieved by decreased bile reabsorption or increased conversion of cholesterol to bile acids; (3) Cholesterol synthesis could be decreased. In addition, a redistribution of cholesterol from plasma into tissues has been suggested as a fourth possible mechanism. A redistribution of cholesterol into tissues, however, has not been documented in man. A brief review of cholesterol metabolism is necessary in order to understand the possible effect of these mechanisms in lowering plasma cholesterol.

B. CHOLESTEROL ABSORPTION

Absorbed cholesterol is derived from two sources, exogenous and endogenous. Exogenous cholesterol is derived from the diet, which supplies from 0.5 to 2 gm. of cholesterol in Western countries (Keys et al. 1965b). Endogenous cholesterol is mainly derived from bile, which supplies about 1 gm. cholesterol per day (Phillips 1960), and cholesterol synthesized by the cells of the ileum. A substantial fraction of the cholesterol from the latter source is discharged into the lumen (Wilson and Reinke 1968) but since this occurs mainly in the terminal ileum, which is beyond the site of maximal cholesterol absorption (Borgstrom 1960; Simmonds et al. 1967), it may not be a major source of reabsorbed cholesterol.

The rate and extent of cholesterol absorption is postulated to be determined by the level of fat and the physical form of cholesterol in the diet, the size of the bile acid pool, the relative activity of cholesterol

esterase and the rate of chylomicron formation (Dietschy and Wilson 1970).

While a high fat diet has been shown to enhance cholesterol absorption (Kim and Ivy 1952; Wilson 1962), the exact mechanism of this enhancement has not been elucidated. The process may involve the promotion of increased bile flow, the provision of additional amphipathic substances (such as monoglycerides), or the increased rate of cholesterol absorption may reflect an increased rate of chylomicron formation after fat feeding.

Diversion of bile acids from the intestinal lumen essentially stops cholesterol absorption (Siperstein et al. 1952), while expansion of the bile acid pool by feeding exogenous bile acids results in a greater than normal rate of cholesterol absorption (Duel 1955). This effect of bile acids on cholesterol absorption has been attributed to their role in promoting micellar solubilization of sterols in the intestinal lumen, and stimulation of pancreatic cholesterol esterase (Vahouny et al. 1959). In addition, it has been suggested (Dietschy and Wilson 1970; Sylven and Borgstrom 1968) that bile acids may facilitate movement of cholesterol from the mucosal cell into the lymph.

Level of cholesterol in the diet has been shown to alter the percent of cholesterol absorbed. The human intestine has a limited ability to absorb cholesterol. Grundy and Ahrens (1969) found that about half of the dietary cholesterol was absorbed when the total intake was between 290 and 452 mg./day. However, Grundy et al. (1969) demonstrated that repeated ingestion of high levels of cholesterol resulted in a marked decline in the proportion of cholesterol absorbed. In fact, Grundy et al. (1969) estimated,

on the basis of studies with two subjects, that maximal dietary cholesterol absorption was about 320 and 340 mg./day. The limited ability of man to absorb more than about 500 mg. of dietary cholesterol daily had previously been suggested by Taylor and Ho (1967).

Dietary cholesterol does not appear to contribute much more than one third of the circulating cholesterol on an average diet. Wilson and Lindsey (1965) demonstrated that no more than 38% of the circulating cholesterol was derived from dietary cholesterol in studies on two subjects. Grundy and Ahrens (1969) confirmed these observations. The contribution from dietary cholesterol ranged from 10 - 36% when 290 - 1600 mg. was eaten daily by 5 subjects.

Because of the limited ability of the human to absorb cholesterol and the minor contribution of absorbed dietary cholesterol to plasma cholesterol levels, alterations in cholesterol absorption are not considered the primary control on serum cholesterol levels. The percentage absorption of cholesterol remains fairly constant under normal conditions. An alteration in bile acid metabolism would, however, affect cholesterol absorption.

C. CHOLESTEROL SYNTHESIS

Cholesterol is synthesized de novo from acetate. Every mammalian tissue is capable of some degree of de novo cholesterol synthesis, but the primary sites of synthesis are the liver and the ileum of the gastro-intestinal tract. Together they account for about 95% of total endogenous cholesterol synthesis (Dietschy and Wilson 1968; Lindsey and Wilson 1965),

with the rate of sterol synthesis in the human intestine being similar to that of the liver (Dietschy and Wilson 1970).

Rate of cholesterol biosynthesis is regulated by three principal physiologic variables: the amount of cholesterol in the diet; the caloric intake of the animal; and the functional integrity of the enterohepatic circulation of bile acids. Cholesterol feeding has been shown to produce both a prompt and a marked suppression of cholesterol synthesis by liver (Dietschy and Wilson 1968). No tissue other than liver, however, shows such marked suppression of sterol synthetic activity (Dietschy and Siperstein 1967). Thus it follows that the gastrointestinal tract, in particular the ileum, becomes the major site for endogenous sterol synthesis when high levels of cholesterol are ingested.

Bile acids have been observed to affect cholesterogenesis. Dietschy and Wilson (1968, 1970) have demonstrated that biliary diversion, in the rat and monkey, enhanced cholesterol synthesis in the liver by 2½ - 3 fold and in the small intestine by as much as 10 fold. Similar measurements on the rate of cholesterogenesis following biliary diversion have not been reported for man. However, using balance techniques in man, Grundy et al. (1966) have presented evidence which suggests that expansion of the bile acid pool decreases the rate of endogenous cholesterol synthesis, whereas interruption of the bile acid enterohepatic circulation enhances cholesterol synthesis.

The primary effect of polyunsaturated fats does not appear to be a direct effect on cholesterol synthesis. The bulk of the evidence suggests

that the primary effect of polyunsaturated fats is on excretion of cholesterol as neutral sterols and/or bile acids, although an alteration in excretion would affect total cholesterol.

D. CHOLESTEROL DEGRADATION AND EXCRETION

Cholesterol excretion and degradation take place via the gastrointestinal tract predominantly by two mechanisms - excretion of cholesterol itself and catabolism to bile acids. These moieties and their bacterial transformation products are thought to account for the vast majority of cholesterol turnover in the normal state.

D.1. Bile Acids. The converson of cholesterol to bile acids serves as a major pathway for the degradation of cholesterol in mammals. This process occurs in the liver exclusively, the primary bile acids formed being cholic acid, chenodeoxycholic acid, and lithocholic acid. Bile acids are conjugated to glycine or taurine and secreted into the bile as sodium or potassium salts. Most of the bile salts that enter the intestinal lumen are subsequently reabsorbed via the enterohepatic circulation although 150-350 mg./day is lost in the feces (Rosenfeld and Hellman 1962). Deoxycholic acid, a product of bacterial action on cholate, also is present in appreciable quantities in intestinal bile. In fact, the bile acids excreted in the feces constitute a complex mixture of compounds formed by the action of intestinal microorganisms.

D.2. Neutral Sterols. In general, there are two sources of fecal neutral sterols: diet and endogenous production. Endogenous sterols (primarily cholesterol) enter the intestine in bile secretion, as intestinal secretion and from sloughed intestinal cells. Some fecal sterols are identical to the sterols present in animal tissues, for example, cholesterol; cholestanol; lathosterol; 7- dehydrocholestanol and methosternol. Other fecal neutral sterols are the products of the action of bacteria in the colon on cholesterol and other sterols of tissue origin. The most abundant bacterial conversion product in feces is coprostanol but others such as epicoprostanol, cholestanone and coprostanone also are present (Masoro 1968).

Dietary sterols are composed primarily of cholesterol and various plant sterols. Plant sterols are poorly absorbed in man and almost all enter the feces as neutral sterols. As previously mentioned, only about 50% of dietary cholesterol is absorbed under normal dietary conditions. Therefore about half the dietary cholesterol is excreted as neutral sterol although most of this is probably modified by the colonic bacteria to compounds such as coprostanol.

The biliary and intestinal secretions plus sloughed mucosal cells deliver about 2 gm. of cholesterol per day to the intestinal lumen (Masoro 1968). However only about half the cholesterol that enters the intestine from endogenous sources is lost in the feces; the remainder, like the bile acids, is reabsorbed.

D.3. Dietary Factors Affecting Steroid Excretion

D.3.a. Carbohydrates. Dietary carbohydrate has been shown to affect neutral sterol excretion. Recovery of dietary plant sterol was found to be complete when a diet of mixed general foods was consumed, whereas only about 25-58% of the ingested plant sterols were recovered when liquid formula diets were fed (Denbesten et al. 1970). The addition of fresh celery or pulverized cellulose to the formula diet partially corrected the plant sterol loss in the feces (80% being recovered). Addition of both cellulose and lactose to the formulae led to complete recovery of ingested plant sterols in the feces. Denbesten et al. (1970) hypothesized that the metabolism of intestinal tract bacteria is altered when certain constituents were not present in the diet, and that these bacteria may degrade the sterol nucleus. Different dietary carbohydrates have been found to affect the half-life and pool size of cholic acid in rats (Portman and Murphy 1958). The half-life of cholic acid was longer on diets containing sucrose than those containing starch or sucrose plus Celluflour. Wells et al. (1960) have shown that feeding of lactose-containing diets induces an increased absorption of cholesterol and an increased excretion of bile acids in rats. The changes in half-life and pool of cholic acid in rats induced by different carbohydrates may be secondary to effects of carbohydrates on intestinal flora (Portman 1960). No data are available on the type of bile acids present in human feces on different carbohydrate diets.

Addition of nondigestible cellulosic residue to the diet increases cholic acid production (Portman and Murphy 1958; Tennent et al. 1960).

In a variety of animals, supplementing the diet with alfalfa, pectin, artichoke roots and mucillagenous polysaccharides reduces serum cholesterol (Keys et al. 1961; Eastwood 1969), presumably by increasing bile acid excretion. Lignin, a major component of vegetable fiber, adsorbs bile acids (Eastwood and Hamilton 1968).

D.3.b. Plant Sterols. In addition to contributing to the total fecal neutral sterol, plant sterols may cause an increase in cholesterol excretion. The sitosterols present in vegetable oils such as corn oil have been shown to have an appreciable cholesterol-lowering effect in man (Beveridge et al. 1956). Grundy et al. (1969) have shown that consumption of large amounts of plant sterols stimulates the excretion of endogenous neutral sterol.

D.3.c. Fat. Substitution of polyunsaturated fats for saturated fats in the diets of man and animals has been shown by numerous investigators to increase fecal steroid excretion (Spritz et al. 1965; Nestel 1970).

There is considerable controversy in the literature as to whether the increase in steroid excretion is due to an increase in bile acid excretion, and an increase in neutral steroid excretion, or an increase in the excretion of both of these fecal steroid components.

Wilson (1961) demonstrated that in rats, addition of 20% linoleic acid to a fat-free diet results in increased excretion of neutral sterols, while addition of 20% palmitic acid does not influence fecal sterol excretion. Gerson et al. (1961) found an increase in coprostanol excretion

when rats were transferred from a fat-free diet to one containing crude or refined corn oil, while excretion of cholesterol remained unchanged.

Cholesterol excretion studies in man indicate that bile acid excretion increased when polyunsaturated fatty acids were substituted for saturated fatty acids in the diet (Lewis 1958; Roels and Hashim 1962; Haust and Beveridge 1958; Haust and Beveridge 1963). However, other investigators have reported an increase in neutral sterol excretion with polyunsaturated fats such as corn oil (Rosenfeld and Hellman 1959; Antonis and Bersohn 1962). And still other studies have demonstrated an increased excretion of both neutral and acidic (bile acid) sterols on substitution of polyunsaturated fats for saturated fats in the human diet (Gordon et al. 1957; Kaleta et al. 1959; Goldsmith et al. 1960; Wood et al. 1966; Moore et al. 1968; Connor et al. 1969).

Numerous investigators have suggested that increased fecal cholesterol losses account for the fate of cholesterol observed leaving the plasma when polyunsaturated fats are substituted for saturated fats (Hellman et al. 1957; Grundy and Ahrens 1970). However several groups of investigators have found no significant differences in the fecal excretion of bile acids by exchanging dietary fats (Lindstedt et al. 1965; Hellstrom and Lindstedt 1966; Ali et al. 1966). Eneroth et al. (1964) noted no significant change in the excretion of neutral steroids with polyunsaturated fats. In some studies, excretion of both bile acids and neutral steroids was not significantly altered by polyunsaturated fats (Avigan and Steinberg 1965;

Spritz et al. 1965).

No adequate reason has yet been advanced that reconciles the inconsistencies in the above studies. Part of the problem may be differences in methodology. Early techniques for measuring sterol balance and identification of fecal lipids were inadequate. These methods and their inadequacies have been well reviewed by Danielsson (1963) and Nestel (1970) and will not be discussed here. Grundy and Ahrens (1969) and Quintao et al. (1971) have recently reviewed the major techniques used to measure cholesterol turnover and have compared their relative merits. Probably the two most reliable methods for fecal steroid analysis are the isotope balance technique and chemical balance techniques using chromatographic procedures including gas-liquid chromatography to purify, partition and quantitate dietary and fecal steroids (Miettinen et al. 1965; Grundy et al. 1965). These methods quantify accurately individual neutral steroids and bile acids and they distinguish between plant sterol and cholesterol excretion products. Nevertheless, differences in technique do not completely account for the variation in results. Isotope balance techniques have both shown (Moore et al. 1968) and failed to show conclusively (Grundy and Ahrens 1970) changes with polyunsaturated fats. Connor et al. (1969) using the chromatographic analysis procedures of Miettinen et al. (1965) and Grundy et al. (1965) obtained results which were at variance with those obtained by Spritz et al. (1965) using the same techniques.

Choice of subjects may be another factor in the different results. Conner et al. (1969) used normal subjects while Spritz et al. (1965) used hypercholesteremic subjects. However, both increased steroid excretion (Goldsmith et al. 1960; Wood et al. 1966; Grundy and Ahrens 1966; Moore et al. 1968), and unaltered steroid excretion (Lindstedt et al. 1965; Avigan and Steinberg 1965; Ali et al. 1966; Grundy and Ahrens 1970), have been observed with both normal (Lindstedt et al. 1965; Wood et al. 1966; Ali et al. 1966; Moore et al. 1968) and hyperlipemic subjects (Goldsmith et al. 1960; Avigan and Steinberg 1965; Grundy and Ahrens 1966; Grundy and Ahrens 1970) when polyunsaturated fats were substituted for saturated fats in the diet.

It may be that some hypercholesterolemic subjects do not respond to alterations in dietary fat source in the same manner as normolipemic individuals. It has been noted, for example, that subjects suffering from Type II hypercholesterolemia have diminished bile acid turnover and low cholic acid excretion on normal diets when compared to individuals with normal blood cholesterol levels (Hellstrom and Lindstedt 1966; Nestel 1970). Kottke (1969) reported a difference in cholic acid turnover between Type II and Type IV hypercholsterolemic patients. Lofland et al. (1968) found that the fecal excretion of steroids and the turnover of cholesterol were consistently greater among monkeys who maintained normal serum cholesterol levels with saturated fat diets as compared to those who developed hypercholesterolemia. It is hypothesized that faulty cholesterol excretion may characterize some hypercholesterolemic states.

The kind of test diet may be an important factor in steroid excretion patterns. As previously mentioned, liquid formula diets devoid of vegetable fiber result in increased bile acid excretion and incomplete plant sterol recovery when compared to a solid diet of mixed foods. In addition, there have been reports in the literature suggestive of a hypocholesterolemic effect associated with the transition from mixed foods to liquid formula diets (Hashim et al. 1959; Ahrens et al. 1957; McOsker et al. 1962). The effects due to formula diets cannot be accounted for by changes in body weight or by fatty acid and cholesterol composition of the formula diets; in some of these studies, the initial effect overshadowed subsequent responses to manipulation of fatty acid composition. Whether these changes are the result of more frequent, spaced feedings, of alterations in intestinal flora, or of other confounding variables are unanswered questions. Lindstedt et al. (1965) noted alterations in bile acid turnover following transition from solid to liquid formula diets. The relative contribution of chenodeoxycholic acid found in bile samples was lower on liquid formula diets than on solid diets. In two additional subjects, transition from a solid diet to a formula diet was associated with large changes in serum cholesterol level with parallel rather than reciprocal changes in cholic acid turnover. The effect of formula diets on cholesterol metaboliam remains to be deliniated.

Digestibility of the fat in the test diet may have an effect on the steroid excretion patterns. A level of 40% of total calories from fat is in agreement with the usual North American intake (Scheig 1969). However,