PLASMA TOTAL CHOLESTEROL AND TRIGLYCERIDE RESPONSES OF HAMSTERS FED OAT BRAN AND PINTO BEAN DIETS

by

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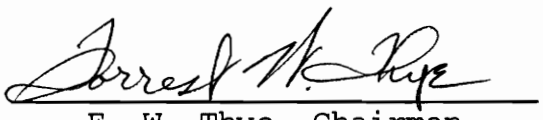

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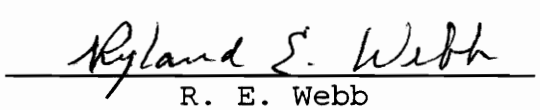

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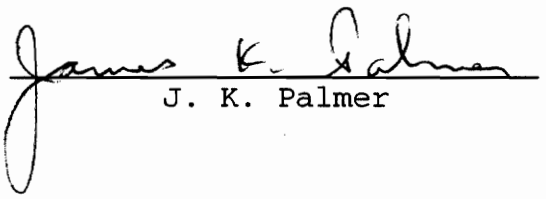

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Human Nutrition and Foods

(ABSTRACT)

The effect of a 53% oat bran diet and a 30% pinto bean diet on hamster plasma total cholesterol (TC) and triglycerides (TG) was investigated. Hamsters were made hypercholesterolemic (average value = 206 mg/dL) and then fed one of four experimental diets, the hypercholesterolemic control (HC), an insoluble fiber (alphacel) control (FC), the oat bran (OB), or the pinto bean (PB) diet for three weeks, with the latter three containing 8.5%, 10.0%, and 7.6%, total dietary fiber, respectively. Plasma TC and TG were measured for each animal before and after the experimental diets. At the end of the experimental period, plasma TC levels of hamsters fed the OB diet (179 mg/dL) were significantly lower than those fed either the HC (203 mg/dL, $p<0.05$) or FC (221 mg/dL, $p<0.001$) diets. Plasma TG levels of hamsters fed the OB diet (200 mg/dL) were significantly lower than those fed the FC diet (358 mg/dL, $p<0.01$). Thus, it was concluded that oat bran significantly lowered plasma TC and TG in hypercholesterolemic hamsters, while pinto beans did not.

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INTRODUCTION

Coronary Artery Disease (CAD) is the leading cause of death in the United States and Western Europe. There are three universally accepted primary modifiable risk factors for this disease: cigarette smoking, hypertension, and hyperlipidemia (Criqui, 1986). Hypercholesterolemia is the most widely acknowledged and dominant risk factor, linked to excessive intake of saturated fats on the one hand and CAD on the other (Stehbens, 1990). Evidence for triglycerides (TG) as an independent risk factor for CAD is inconclusive and somewhat controversial, however at this time, several reports provide persuasive data to support this hypothesis (Castelli, 1988; Gotto, 1990; Wilson, 1990).

Dietary fiber from plant material has been found to be an important food component that is resistant to hydrolysis by endogenous enzymes in the digestive tract of mammals (Schneeman, 1989). Plant fiber is classified as water soluble or water insoluble. Insoluble and soluble fiber fractions are thought to act differently in the digestive tracts of animals, and are therefore thought to have different physiological effects. Both soluble and insoluble fibers cause an increased bulk of softer stool due to their increased water-retaining capabilities (Lipsky et al, 1990). When comparing the effect of these two types of dietary fiber on blood lipids, it appears that the insoluble dietary fiber has little effect, if any, on serum cholesterol levels. The majority of

researchers acknowledge the hypocholesterolemic effect of soluble fiber from various plant sources. The mechanism by which soluble fibers lower serum cholesterol is currently unknown (Lipsky et al, 1990).

The specific role of dietary fiber on hypertriglyceridemia has not been determined, although fiber intake does appear beneficial. Studies by Anderson and Tietyen-Clark (1986) indicated that high-fiber diets had both short and long-term effects on decreasing serum TG levels.

Oat bran and bean products have been shown to have a hypocholesterolemic effect in humans. Studies by Anderson et al (1984a) and Kirby et al (1981), indicated that oat bran lowers serum low density lipoprotein cholesterol (LDL-C) without affecting serum high density lipoprotein cholesterol (HDL-C) in humans. More recent studies have shown similar results. Using hypercholesterolemic men, Anderson et al (1991) found that oat bran lowered serum total cholesterol (TC) and LDL-C with no change in HDL-C. Anderson and Chen (1979) found that bean products and water-soluble fiber extracts of beans had significant hypocholesterolemic effects. Another study by Anderson et al (1984b) showed that a bean supplemented diet selectively lowered serum LDL-C in hypercholesterolemic men.

In using animal models for cholesterol research, one must be aware that many animals react differently between and within species to administration of cholesterol in the diet. The effect of dietary

cholesterol and fat on plasma lipids is varied among different animal models. In their review article, Beynan et al (1987) state that humans are less sensitive to dietary cholesterol than random-bred rabbits or monkeys. Humans may be more sensitive to dietary cholesterol than rats since it is necessary to include extreme amounts of cholesterol (1-2%) with cholate (0.5%) in rat diets to enhance their cholesterolemic response (Beynan et al, 1987). It appears that the rat has a rate of cholesterol synthesis in both hepatic and extrahepatic tissues which is exceptionally high (Turley and Dietschy, 1982). The rat also appears to have a highly efficient mechanism for converting excess cholesterol into bile acids (Wilson, 1964). Additionally, the rat biliary cholesterol secretion and bile acid output are tightly coupled, so that many dietary and pharmacological treatments used in humans do not have an effect on this process in the rat (Turley and Dietschy, 1980; Turley and Dietschy 1982). That is, the rat has a very efficient mechanism for controlling cholesterol in it's body, so that dietary manipulations using cholesterol may not be as applicable to humans as with other animal models.

Hamsters, on the other hand, have been found to respond to dietary cholesterol of a much lower content than rats. Sicart et al (1986) found a significant increase in plasma TC with addition of only 0.1% cholesterol in the diet of hamsters. Additionally, the rate of hepatic cholesterol synthesis in the hamster is low compared to the rat, and is in fact more similar to the human (Spady and

Dietschy, 1985). The fact that the hamster has a relatively high level of plasma low density lipoprotein (LDL) with a 1:1 ratio of plasma LDL:HDL (high density lipoprotein) as compared to other rodents (Tsuda et al, 1983), coupled with the evidence that administration of dietary cholesterol increases LDL plasma lipoproteins (Tsuda et al, 1983; Spady and Dietschy 1983; Spady and Dietschy, 1985) makes the hamster a good model for cholesterol research (Spady and Dietschy, 1988).

The present study was undertaken to determine the effect of purified diets containing either oat bran or pinto beans to provide 8.6% total dietary fiber on plasma TC and TG in the hamster. Hamster feed intake, weight gain, and plasma lipoprotein results from pooled samples were also examined. If the results from this study correlate well with similar human studies, further support would be given to the hypothesis that the hamster is a good animal model for hyperlipidemic research.

REVIEW OF LITERATURE

Coronary Artery Disease

Cardiovascular diseases are responsible for nearly one out of every two deaths in the United States (Chobanian, 1991). Coronary Artery Disease (CAD) is the leading cause of death in the United States and Western Europe. While CAD has a multifactorial etiology, there are three universally accepted primary modifiable risk factors for this disease: cigarette smoking, hypertension, and hyperlipidemia (Criqui, 1986). That is, the greater the level of any one of these, the greater the likelihood of a coronary event. In addition, if more than one of these risk factors is present, the risk for CAD is markedly increased (Rifkind, 1990). Conversely, reducing the number and level of these risk factors can prevent death and disability from cardiovascular diseases (Chobanian, 1991). Hypercholesterolemia is the most widely acknowledged and dominant risk factor, linked to excessive intake of saturated fats on the one hand and CAD on the other (Stehbens, 1990).

Blood Lipids and Coronary Artery Disease Risk

Total Cholesterol

Serum total cholesterol (TC), which is comprised of low density lipoprotein (LDL), high density lipoprotein (HDL) and very low density lipoprotein (VLDL) cholesterol, is an independent risk factor for CAD (Hulley et al, 1980). The higher the serum

cholesterol level, the greater the risk for CAD (Chobanian, 1991). The relationship between serum TC and CAD appears to be weak, however (Oliver, 1981), and there is no relationship between serum TC and sudden cardiac death or unrecognized infarcts (Medalie, 1976). In adults 20 years of age and older, serum TC levels are classified as "desirable blood cholesterol" (<200 mg/dL), "borderline-high blood cholesterol" (200-239 mg/dL), or "high blood cholesterol" (≥240 mg/dL) (National Cholesterol Education Program Expert Panel, 1988).

LDL-Cholesterol

Plasma LDL-cholesterol (LDL-C) comprises approximately 70% of plasma TC and has a strong positive relationship to CAD (Gordon et al, 1977), which may help to explain the weak association of plasma TC with CAD. Elevations in serum LDL-C levels accelerate atherogenesis (Chobanian,1991). Therefore, increased levels of LDL-C in the blood are causally related to an increased risk of CAD. LDL-C has been identified as the major target for cholesterol-lowering therapy. That is, there is substantial evidence that lowering LDL-C levels will reduce the incidence of CAD. Levels of LDL-C of 160 mg/dL or greater are classified as "high-risk LDL-C" and those of 130 to 159 mg/dL as "borderline-high-risk LDL-C" (National Cholesterol Education Program Expert Panel, 1988).

HDL-Cholesterol

Various human studies over the years have pointed to a strong inverse relationship between plasma HDL cholesterol (HDL-C) and CAD (Rifkind, 1990). That is, the higher the plasma HDL level, the lower the risk of CAD. Little animal research has been done on this relationship, however, and a consensus has yet to be reached regarding the prevention of CAD by increasing plasma HDL-C levels. It was only recently that a report by the National Cholesterol Education Program Expert Panel (NHLBI of the NIH) assigned a risk-factor status to a low plasma HDL-C level (Rifkind, 1990). HDL's usually contain 20-30% of the TC in the blood (National Cholesterol Education Program Expert Panel, 1988).

VLDL-Cholesterol and Triglycerides

VLDL contain 10-15% of the total serum cholesterol (National Cholesterol Education Program Expert Panel, 1988). Hypertriglyceridemia is defined as triglyceride (TG) levels greater than 250 mg/dL (Dalen, 1988). Because plasma TG are transported by, and make up 70% (by weight) of the VLDL cholesterol (VLDL-C) (Gotto, 1990), the two are closely related.

Criqui (1986) stated that plasma VLDL-C and TG levels were strongly correlated, but that neither had an independent cardiovascular disease association. Recently, opinions have changed among researchers. Reevaluation of the results from the Framingham Study in the early 1970's showed that plasma TG did have a positive

correlation, at least in women, where women with high levels of plasma TG (123-611 mg/dL) were at greater risk for CAD (Gotto, 1990, Wilson, 1990). Wilson (1990) concluded that the measurement of plasma TG levels may prove useful over and above it's value in calculating plasma LDL-C, at least in women. Gotto (1990) reported that, based on his research, he thinks that hypertriglyceridemia and CAD are definitely related. Although the evidence for TG as an independent risk factor for CAD is inconclusive and somewhat controversial at this time, several reports provide persuasive data to support this hypothesis. Castelli (1988) also suggested that by looking at data collected from the Framingham Study, there was evidence of the relation between plasma LDL-C and plasma VLDL-C levels and CAD.

Cholesterol Transport

The transport of plasma cholesterol and other fatty substances throughout the body is best described by the model put forth by Brown and Goldstein (1984). The fat-transport system can be divided into two pathways, exogenous and endogenous. Most of the cholesterol utilized by the body is produced by the liver and transported via the endogenous pathway (80%). The remaining 20% comes from the diet, and is transported via the exogenous pathway.

The exogenous pathway begins in the intestine where triglycerides and cholesterol are absorbed from the intestine. Dietary fats are packaged into lipoprotein particles called

chylomicrons which transport triglycerides to adipose and muscle tissue. What is left after removal of the triglycerides in extrahepatic tissues by lipoprotein lipase is a chylomicron remnant, which also contains cholesteryl esters, vitamins and apoproteins, and is removed from circulation by specific receptors found only on liver cells (Havel, 1986).

The endogenous fat-transport system begins at the liver upon secretion of the large VLDL particle. The VLDL contains mostly triglycerides synthesized in the liver, with a small amount of cholesteryl esters, which travel to capillaries of adipose and muscle tissue where most of the triglycerides are removed by lipoprotein lipase. The VLDL remnant (also called intermediate density lipoprotein, or IDL) that is left can either be removed by the liver or converted in circulation to the LDL (Brown and Goldstein, 1984).

LDL's are the major cholesterol-carrying lipoproteins in plasma. These LDL particles carry the cholesterol which contribute to atherosclerotic plaque. Theoretically and epidemiologically, the more LDL circulating in the bloodstream, the more rapid the development of coronary artery disease and atherosclerosis (Brown and Goldstein, 1984).

The removal of LDL's from plasma occurs primarily at the liver, but also at the extrahepatic tissues. HDL's pick up cholesterol at the extrahepatic tissues (and other sources) and transfer it to IDL's and LDL's. Cholesterol carried by these

lipoproteins can then be removed by the liver (Grundy, 1986).

Most of the cholesterol taken up by the liver is converted to bile acids which are secreted into the upper small intestine where they emulsify dietary fats. Once this has been done, bile acids are largely reabsorbed from the intestine, returned to the bloodstream, taken up by the liver, and once again secreted into the upper intestine. The recycling of bile acids in this way limits the liver's need for cholesterol (Brown and Goldstein, 1984).

Some cholesterol is also secreted in the bile to join with dietary cholesterol in the intestine. Once in the intestine, this cholesterol may be eliminated by fecal excretion or partially reabsorbed (Brown and Goldstein, 1984; Mitchell et al, 1991).

The small intestine is a major site of cholesterol biosynthesis and lipoprotein degradation, and therefore plays an important role in maintaining cholesterol balance within the body. Cholesterol synthesis by the intestinal mucosa contributes directly to the plasma pool of cholesterol. The small intestine is the only organ that regulates the amounts of exogenous dietary and endogenous biliary cholesterol that enters the body daily (Field et al, 1990).

LDL Receptor Regulation of Cholesterol

LDL receptors on hepatic and extrahepatic cells play an important role in the regulation of the endogenous production and utilization of cholesterol in the body (Brown and Goldstein, 1984). The availability of unesterified cholesterol within the cell

regulates the number of LDL receptors on the cell surface. When delivery of cholesterol by lipoproteins (or by other means) to regulatory sites is high, the receptors on the cell surface are down-regulated. That is, fewer receptor sites are available. Hence, plasma LDL concentration increases. Conversely, when cellular cholesterol is depleted, receptors on the cell are up-regulated, so more receptors are available. Therefore, plasma LDL concentrations will eventually decrease as LDL is accepted by the cell. In general, the greater the number of LDL receptors on hepatocytes, the more efficient the uptake of VLDL remnants, so fewer remnants are available in the blood to form LDL particles. Additionally, the greater the number of hepatocytic LDL receptors, the more efficient the removal of LDL from the blood (Havel, 1988).

Diet and LDL Receptor Regulation of Cholesterol

Dietary cholesterol taken into the liver via chylomicron remnants tends to down-regulate hepatocytic LDL receptors. The extent of down-regulation, however, depends on the liver's ability to excrete this cholesterol, primarily through formation of bile acids, into the bile. Humans seem to vary in their capacity to excrete cholesterol in this way, and therefore dietary cholesterol has a variable and usually limited effect on their plasma cholesterol levels (Havel, 1988).

In humans, saturated fats increase plasma LDL levels and tend to increase HDL levels as well. Havel (1988) feels that dietary

saturated fat may have effects on cholesterol or lipoprotein metabolism that extend beyond the down-regulation of hepatocytic LDL receptors. More research is needed in this area to determine the mechanism behind the effect of dietary saturated fatty acids on circulating blood lipids and lipoproteins in the human.

Hypo- and Hyperresponsiveness to Dietary Cholesterol

In using animal models for cholesterol research, one must be aware that many animals react differently between and within species to administration of cholesterol in the diet. Beynan and coworkers (1987) determined that within each species of random-bred animals there are hypo- and hyperresponders to dietary cholesterol. That is, upon administration of a diet containing cholesterol (and sometimes cholate) there are individuals who show a consistently high or low cholesterolemic response. Hypo- and hyperresponsiveness has been confirmed in random-bred rabbits, rats, and squirrel monkeys, and is thought to occur in guinea pigs (Beynen et al, 1987) and hamsters (Phillips, 1990).

In addition to variability among random-bred animals, certain strains within species are bred to respond in a certain way to dietary cholesterol. That is, they have a genetic predisposition to react to dietary cholesterol in a designated manner. Inbreeding has produced hyperresponsive strains of rabbits, rats, mice, and pidgeons (Beynen et al, 1987) as well as in hamsters (Sicart et al, 1986).

These phenomena may be present in humans as well, where hypo/hyperresponsiveness to dietary cholesterol is apparent within as well as between racial/ethnic groups. The existence of these phenomena in humans, however, has proven very difficult to substantiate experimentally (Beynen et al, 1987).

In their review article, Beynan et al (1987) state that humans are less sensitive to dietary cholesterol than random-bred rabbits or monkeys. Humans may be more sensitive to dietary cholesterol than rats since it is necessary to include extreme amounts of cholesterol (1-2%) with cholate (0.5%) in rat diets to enhance their cholesterolemic response. Contrary to this response, hamsters have been found to respond to dietary cholesterol of a much lower content than rats. Sicart et al (1986) found a significant increase in plasma TC with addition of only 0.1% cholesterol in the diet of hamsters. A discussion of the effect of diet on blood lipids and lipoproteins in various animal models will follow.

The Effect of Dietary Cholesterol and Fat on Blood Lipids and Lipoproteins in Animal Models

Garg et al (1985) investigated the idea that redistribution of cholesterol between plasma and tissue pools accounts for the hypocholesterolemic effect of polyunsaturated fatty acids (PUFA's) in man and animals. Control rats were fed a commercially available reference diet. Experimental animals were fed the reference diet supplemented with one of the following: 2% cholesterol, 15% coconut

oil (saturated fat enriched diet), coconut oil plus cholesterol, 15% sunflower seed oil (unsaturated fat enriched diet), or sunflower seed oil plus cholesterol. Diets were fed for four weeks. Results showed that feeding cholesterol with sunflower seed oil resulted in the greatest accumulation of cholesterol in the liver and was significantly more than with the saturated fat enriched diet plus cholesterol (12.0 mg/g versus 4.5 mg/g). Plasma TC was increased to the same extent in both of the high fat, high cholesterol groups when compared to the cholesterol control group (sunflower plus cholesterol = 94.1 mg/dL, coconut plus cholesterol = 83.5 mg/dL, cholesterol control = 60.2 mg/dL) (Garg et al, 1985).

The authors concluded that the liver cholesterol, not plasma cholesterol appears to be directly correlated with dietary cholesterol depending on the quality and quantity of fat used in the diet. They stated that these results suggested that whatever cholesterol is absorbed into the blood from the gut is immediately removed from circulation by the liver and possibly by other organs, and that these results leave no doubt that the nature of fat in the diet has a pronounced effect on cholesterol metabolism in the liver (Garg et al, 1985).

The effect of cholesterol feeding on plasma lipoprotein profiles was investigated by Tsuda et al (1983). Comparisons were made between two types of rats, 8 week old Sprague-Dawley and 7 week old ExHC (a strain established to be highly responsive to dietary cholesterol), 6 week old mice, and 6 week old hamsters. Cholesterol

was fed at 1% in the diet, which also included 5% olive oil and 0.2% sodium cholate. Diets were fed for one week.

Results showed that plasma HDL's were major lipoproteins in all animals (exact values were not reported). Hamsters had the most abundant plasma LDL's with the ratio of cholesterol content between plasma HDL and LDL being about 1:1. After cholesterol feeding, the cholesterol content of plasma LDL's increased in all animals. The greatest increase was seen in ExHC rats, while the least increase in plasma LDL was seen in mice. Plasma levels of cholesterol prior to administration of the cholesterol diet were similar for both strains of rats (approx. 65 mg/dL). Mean plasma TC levels after one week were 162 mg/dL in Sprague-Dawley rats, 421 mg/dL in the ExHC rats. Plasma TC for the cholesterol fed mice was 197 mg/dL as compared to 115 mg/dL in the control group. Hamster control plasma TC was 164 mg/dL and increased to 389 mg/dL after one week on the cholesterol diet (Tsuda et al, 1983).

Sicart et al (1986) set out to determine to what extent hamsters could maintain an unchanged distribution of plasma cholesterol among lipoproteins upon becoming more hypercholesterolemic. Hamsters used were of a variety (FEC) which develop high levels of plasma cholesterol with aging. Cholesterol content in peripheral tissues of these hamsters does not differ from that in normocholesterolemic hamsters and they do not exhibit atherosclerotic lesions in the arterial tissue.

Six month old male FEC hamsters were fed either a standard

diet or one enriched with either 0.1% or 0.5% cholesterol for four weeks. Results showed control plasma TC levels of 153 mg/dL. Animals fed both 0.1% and 0.5% cholesterol diets had increased ($p<0.05$) plasma TC levels when compared to the control (196 mg/dL and 327 mg/dL respectively). The increase in plasma TC was accompanied by a redistribution of the sterol among the various lipoproteins. The control diet showed approximately 50% of the cholesterol carried in HDL (78.0 mg/dL) and 50% in VLDL and LDL (25.9 mg/dL and 47.5 mg/dL respectively). Administration of cholesterol significantly increased plasma HDL-C for both doses as compared to control, but were not significantly different between cholesterol doses (0.1% = 93.0 mg/dL, 0.5% = 98.7 mg/dL, control = 78.0 mg/dL). Plasma LDL-C appeared to increase but was not significant for either experimental diet (0.1% = 55.0 mg/dL, 0.5% = 54.0 mg/dL, control = 47.5 mg/dL). The proportion of cholesterol carried by VLDL increased significantly only with the 0.5% cholesterol diet (0.1% = 27.3 mg/dL, 0.5% = 144 mg/dL, control = 25.9 mg/dL) when compared to control levels (Sicart et al, 1986).

The authors stated that the observed increase in percentage of cholesterol was weaker than in species such as the rabbit, where greater changes are observed with similar levels of dietary cholesterol (Sicart et al, 1986). It is important to note that in this study the increase in cholesterol from 0.1% to 0.5% affected only the plasma VLDL-C.

Hayes and coworkers (1980) investigated the effect of dietary

factors on gallstone induction in hamsters. In the process, they explored the formulation of purified diets that would meet the nutritional requirements of hamsters. They found it necessary to include fiber in the diets of hamsters to sustain health. Additionally, high levels of simple sugars induced 'wet tail' disease - chronic diarrhea which usually results in anorexia and death. Addition of wheat bran and cellulose to the diet and a decrease in the simple sugar content prevented the appearance of 'wet tail' disease (Hayes et al, 1980). It has also been determined that a fat mixture of 10:6:3 butter:canola oil:corn oil (2:1:3.3, P:M:S ratio) making up 5-10% of the diet would provide essential fatty acids and allow for elevation of plasma TC in hamsters (Phillips, 1990).

The effect of dietary cholesterol and fat on plasma lipids is varied among different animal models. It appears that the rat has a rate of cholesterol synthesis in both hepatic and extrahepatic tissues which is exceptionally high (Turley and Dietschy, 1982). The rat also appears to have a highly efficient mechanism for converting excess cholesterol into bile acids (Wilson, 1964). Additionally, the rat biliary cholesterol secretion and bile acid output are tightly coupled, so that many dietary and pharmacological treatments used in humans do not have an effect on this process in the rat (Turley and Dietschy, 1980; Turley and Dietschy 1982). In other words, the rat has a very efficient mechanism for controlling cholesterol in it's body, so that dietary manipulations using

cholesterol may not be as applicable to humans as with other animal models.

Alternatively, the rate of hepatic cholesterol synthesis in the hamster is low compared to the rat, and is in fact more similar to the human (Spady and Dietschy, 1985). The fact that the hamster has a relatively high level of plasma LDL with a 1:1 ratio of plasma LDL:HDL as compared to other rodents (Tsuda et al, 1983), coupled with the evidence that administration of dietary cholesterol increases LDL plasma lipoproteins (Tsuda et al, 1983; Spady and Dietschy 1983; Spady and Dietschy, 1985) makes the hamster a good model for cholesterol research (Spady and Dietschy, 1988).

Dietary Fiber

Dietary fiber from plant material has been found to be an important food component that is resistant to hydrolysis by endogenous enzymes in the digestive tract of mammals (Schneeman, 1989). The majority of dietary fiber components are found in or are associated with the cell walls of plants. Included in these are the structural compounds such as cellulose, hemicelluloses, pectins, and lignin. The nonstructural polysaccharides include various gums and mucilages that are associated with the endosperm and intracellular space (Southgate, 1976; Southgate, 1982). Chemical compositions of the various dietary fiber components differ and are listed in Table 1. Dietary fiber is composed of cellulose, noncellulose polysaccharides (NCP's), and lignin (Schneeman, 1989).

Table 1. Chemical Classification of Dietary Fiber

Fiber Component	Chemical Components	
	Main Chain	Side Chain
Polysaccharides		
Cellulose (1,4 beta-linked)	Glucose	----[a]
Noncellulose		
Hemicellulose		
Arabinoxylan	Xylose	Arabinose
Galactomannan	Mannose	Galactose
Glucomannon	Galactose	Glucuronic acid
Pectic substances	Galacturonic acid	Galactose, glucose Rhamnose Arabinose Xylose Fucose
Beta-glucans (1,3 beta-and 1,4 beta-linked)	Glucose	----[a]
Mucilages	Galactose, mannose Glucose, mannose Arabinose, xylose Galacturonic acid, rhamnose	Galactose
Gums	Galactose Glucuronic acid, mannose Galacturonic acid, rhamnose	Xylose Fucose Galactose
Algal polysaccharides	Mannose Xylose Guluronic acid, mannuronic acid Glucose	Galactose
Nonpolysaccharides		
Lignin	Sinapyl alcohol Coniferyl alcohol p-Coumaryl alcohol	

[a] No side chain

Adapted from Schneeman BO. Dietary Fiber. Food Technol 1989;43:133-9.

A description of various dietary fiber components, taken from Schneeman (1989) follows.

Cellulose is a linear polymer of glucose with beta 1-4 linkages. It is the major structural component in plant cell walls and is essentially insoluble in water.

Beta-glucans are glucose polymers with both beta 1-3 and beta 1-4 linkages in various proportions depending on the source. As a result, this molecule is more soluble in water than cellulose. Barley and oats are two cereals rich in beta-glucans.

Pectins are substances composed primarily of rhamnogalacturonans (D-galacturonic acid and rhamnose), containing side chains of other carbohydrates. Pectic substances can be either soluble (pectins) or insoluble (protopectins) in water, and are part of the cell walls and middle lamella of plants. The solubility and/or gelling properties of pectic substances can be altered and may affect the physiological response to pectin as a source of fiber. Many fruits are good sources of pectic substances.

Hemicelluloses are a group of heterogeneous polysaccharides containing various sugars in the backbone and side chains. They are soluble in dilute alkali.

Lignin is a complex nonpolysaccharide polymer containing phenylpropane units. They are insoluble and resistant to human digestion.

Other components are present in plants but may not fit the classical definition of dietary fiber. They are, however, important

in understanding the physiological responses to diets containing fiber-rich foods. These components include phenolic compounds, phytic acid, digestive enzyme inhibitors, Maillard compounds, and starch that is resistant to digestion.

Upon analysis of dietary fiber, researchers can determine insoluble and soluble fiber fractions. This has been useful in determining physiological effects of each. That is, insoluble and soluble fiber fractions are thought to act differently in the digestive tracts of animals, and are therefore thought to have different physiological effects. Plant materials differ markedly in their amounts of insoluble and soluble fiber fractions.

Mechanism of Action of Dietary Fiber on Blood Lipids and Lipoproteins

Both soluble and insoluble fibers cause an increased bulk of softer stool due to their increased water-retaining capabilities. Soluble fibers retard gastric emptying and decrease food digestion and nutrient absorption. The mechanism by which soluble fibers lower serum cholesterol is currently unknown (Lipsky et al, 1990). Several mechanisms have been proposed:

Research has shown that various fibers bind bile acids and cholesterol in vitro (Eastwood and Hamilton, 1968; Kritchevsky and Story, 1974; Story and Kritchevsky, 1976). Gelation of some soluble fibers could occur in the small intestine, preventing absorption of bile acids and cholesterol (Topping, 1991; Anderson

and Tietyen-Clark, 1986; Kritchevsky, 1977). This would result in an increase in neutral steroid excretion, thereby reducing dietary input to cholesterol pools (Story, 1985).

Soluble fiber slows upper intestinal transit time and interacts with biliary and dietary lipids (Ebihara and Schneeman, 1989) by interfering with micelle formation in the proximal small intestine, resulting in decreased absorption of cholesterol and fatty acids (Vahouny, 1982; Vahouny, 1988; Ikeda et al, 1989).

Most fiber polysaccharides are fermented by bacteria in the colon (Cummings, 1984a; Cummings, 1984b) into short-chain fatty acids. These fatty acids are rapidly absorbed into the blood stream and may inhibit hepatic cholesterol synthesis. Short-chain fatty acids may also decrease hepatic cholesterol concentrations and secretions by interfering with compensatory mechanisms (Chen et al, 1984).

The hypocholesterolemic effect of soluble fiber may be due to a combination of all of the above proposed mechanisms. It is evident that more research is necessary in this area to determine an acceptable mechanism for the effect of dietary fiber on levels of circulating lipids and lipoproteins in the human body.

The Effect of Dietary Fiber on Blood Lipids and Lipoproteins in the Human

Plant fiber is classified as water soluble or water insoluble. When comparing the effect of these two types of dietary fiber, it

appears that the insoluble dietary fiber has little effect, if any, on serum cholesterol levels. For example, the addition of 36 grams of wheat fiber (primarily insoluble fiber) for a three week metabolically controlled study increased fecal bulk significantly, but did not change serum cholesterol or TG levels (Jenkins et al, 1975). Subjects in this study were normocholesterolemic young healthy males. Conversely, some sources of soluble fiber seem to have a hypocholesterolemic effect in humans.

Approximately 50% of the fiber in the oat groat is soluble (Anderson, 1985). Studies by Kirby et al (1981) and Anderson et al (1984a), indicated that oat bran significantly lowered serum LDL-C without affecting serum HDL-C in humans. Kirby et al (1981) randomly assigned eight hyperlipidemic men to either a control or oat bran diet for ten days. The two diets were identical in energy, carbohydrate, protein, fat, and cholesterol content. The oat bran diet contained 100 grams of oat bran per day, providing two times as much dietary fiber as the control diet. Serum TC and LDL-C levels were significantly lower (13% and 14%, respectively) with the oat bran diet as compared to the control diet. HDL-C levels were similar between the two diets. Anderson et al (1984a) studied the effect of oat bran diets on hypercholesterolemic men for 21 days in a metabolic ward. Diets were similar to those used by Kirby et al (1981), with the test diet containing 100 grams of oat bran per day. The oat bran diet significantly lowered serum TC and LDL-C by 19% and 23%, respectively, with no significant changes in HDL-C.

More recent studies have shown similar results. Using hypercholesterolemic men, Anderson et al (1991) found that the addition of 100 grams of oat bran per day significantly lowered serum TC and LDL-C by 12.8% and 12.1%, respectively, over three weeks. No significant change was observed in HDL-C levels. A diet containing 40 grams of wheat bran per day showed no change in these parameters. Control and test diets were identical in nutrient and energy needs, differing only in the amounts of total dietary fiber (TDF) and soluble fiber. The oat bran, wheat bran, and control diets provided 34g TDF (13.4g soluble fiber), 34g TDF (7.8g soluble fiber), and 14g TDF (3g soluble fiber) per day, respectively (Anderson et al, 1991).

Similarly, Kestin et al (1990) showed significant decreases in serum TC (233 mg/dL versus 245 mg/dL) and LDL-C (164 mg/dL versus 176 mg/dL) with diets containing 11.8 grams of dietary fiber from oat bran when compared to a low-fiber control. Mildly hypercholesterolemic men were fed the control diet (<12g nonstarch polysaccharide), or diets containing 11.8 grams of dietary fiber from either oat bran, wheat bran, or rice bran for four weeks. No change in serum TC or lipoprotein cholesterol levels was found using wheat bran. Oat bran and rice bran showed an increase in the ratio of HDL-C to TC when compared to wheat bran (Kestin et al, 1990).

The majority of researchers acknowledge the hypocholesterolemic effect of soluble fiber from various plant sources, however some scientists have questioned that oat bran has a direct

hypocholesterolemic effect. Swain et al (1990) questioned whether oat bran diets lowered serum cholesterol by replacing fatty foods in the diet or by a direct hypocholesterolemic effect of the fiber in oat bran. They used isocaloric supplements (100g/day) of either a high-fiber oat bran or a low-fiber refined wheat product added to self-selected diets of the subjects. The subjects were given each type of supplement for six-week periods in a double-blind, crossover trial. Results showed no significant differences in serum TC (mean = 172 mg/dL), LDL-C (mean = 106 mg/dL), or HDL-C (mean = 52 mg/dL) between the fiber groups. Both regimens were associated with 7% reductions in serum TC (mean baseline 186 mg/dL), however. The researchers concluded that oat bran has little cholesterol-lowering effect, and that high- and low-fiber dietary grain supplements reduce serum cholesterol about equally, probably because they replace dietary fats (Swain et al, 1990).

Questions result from this research including the use of subjects who were mostly young, lean, female dietitians or dietitians-in-training. Also, except for the test supplements, the subjects were free to select their own diets. The validity of this research is in question, particularly whether these findings can be generalized to older, heavier males and females who are hypercholesterolemic.

Anderson and Chen (1979) found that bean products and water-soluble fiber extracts of beans had significant hypocholesterolemic effects. A later study by Anderson and Chen (1983) showed serum TC

concentrations of bean-supplemented hypercholesterolemic men to be significantly lower (19%) than those on the control diet (242 mg/dL versus 299 mg/dL, respectively). Additionally, serum LDL-C (169 mg/dL versus 219 mg/dL, respectively) was significantly lowered more than serum HDL-C (28 mg/dL versus 33 mg/dL, respectively) with bean supplementation as compared to the control diet. Subjects were fed a control diet or one week, followed by the bean-supplemented diet for three weeks. Control and bean-supplemented diets had similar energy, carbohydrate, protein, fat, and cholesterol content. The bean supplemented diet provided 115 grams of dried beans per day, and contained more soluble (20g/day) and total (50g/day) plant fiber than the control diet (soluble fiber 5g/day, total fiber 20g/day), however (Anderson and Chen, 1983).

Similar to studies by Anderson and Chen (1979, 1983), significant responses of serum TC, LDL-C, and HDL-C were found by Anderson et al (1984b) using oat bran and bean supplemented diets in hypercholesterolemic men. Twenty hypercholesterolemic men were randomly assigned to one of three diets, a control, or diets supplemented with oat bran or pinto beans for 21 days. Diets were equivalent in energy, fat, and cholesterol, however oat bran and pinto bean diets had two times more total, and three times more soluble fiber than the control. Results showed that oat bran and pinto bean diets significantly lowered serum TC by 19% for both diet groups. Serum LDL-C levels were significantly decreased in both oat bran and pinto bean diet groups (23% and 24%, respectively).

Researchers concluded that oat bran or bean supplements may have an important role in nutritional management of selected hypercholesterolemic patients (Anderson et al, 1984b).

The specific role of dietary fiber on hypertriglyceridemia has not been determined, although fiber intake does appear beneficial. Research on the effect of specific types of fiber (insoluble or soluble) on serum or plasma TG remains inconclusive.

Anderson and Chen (1983) fed hypercholesterolemic men a control diet for seven days, followed by a bean-supplemented diet for 21 days. The two diets were similar in energy, carbohydrate, protein, fat, and cholesterol contents, differing only in the amounts of total, soluble, and insoluble fiber. The bean and control diets contained total dietary fiber of 50 grams and 20 grams per day, respectively, and soluble fiber of 20 grams and 5 grams per day, respectively. No significant differences in serum TG levels were found between the two diets (average value = 230 mg/dL) (Anderson and Chen, 1983).

Anderson, et al (1984b) found similar results with bean supplementation over 21 days. Bean supplementation of 115 grams per day in hypercholesterolemic men resulted in no significant change in serum TG levels (average level = 230 mg/dL). Supplementation with 100 grams of oat bran per day, however, significantly decreased serum TG levels when compared to controls (235 mg/dL versus 289 mg/dL). Control and test diets provided equivalent energy, fat, and cholesterol, but test diets contained twice more total and three

times more soluble fiber than the control diet. Likewise, Kirby, et al (1981) investigated the effect of oat bran on eight hypercholesterolemic men. Control and oat bran diets were similar in carbohydrate, protein, fat, and cholesterol, with the test diet containing 100 g/day of oat bran. Diets were fed for 10 days. The diets had no significant effects on either fasting or postprandial serum TG concentrations. Postprandial serum TG concentrations averaged 218 mg/dL on control diets and 209 mg/dL on oat-bran diets.

Conversely, Anderson et al (1991) showed a 10% decrease in serum TG levels of hypercholesterolemic men with oat bran (110g/day) and wheat bran (40g/day) supplementation, but was only significant in wheat bran subjects when compared to controls (exact values were not reported). Diets were fed for 21 days and were identical in energy content and nutrients. Kestin et al (1990) found no significant differences in plasma TG levels in mildly hypercholesterolemic men consuming wheat bran, oat bran, or rice bran supplemented diets as compared to control levels (baseline value = 143 mg/dL). The control diet contained less than 12 grams of nonstarch polysaccharide per day (low-fiber), while experimental diets contained 11.8 grams of dietary fiber per day from each of the three cereal brans. All diets were fed for four weeks in a double-blind, crossover design.

Anderson and Tietyen-Clark (1986) suggested that their studies indicated that high-fiber diets had both short and long-term effects on decreasing serum TG levels. Ten hypertriglyceridemic men

(multiple fasting serum TG levels >1000 mg/dL) were placed on a high-fiber/carbohydrate (HCF) diet and then a high-fiber maintenance (HFM) diet and were monitored up to 6 months. The HCF diet provided 70% of energy as carbohydrate, 18% protein, 12% fat, 50 mg/day cholesterol, and 70 grams/day dietary fiber (source not specified). Serum TG levels decreased significantly from 2508 mg/dL to 451 mg/dL over a 10 to 14 day period on the HCF diet. The HFM diet was adapted for home use and provided 55-60% of energy as carbohydrate, 20% protein, 20-25% fat, <200 mg/day cholesterol, and approximately 50 grams/day dietary fiber. After following the HFM diet for an average of 6 months, fasting serum TG levels for these 10 men averaged 226 mg/dL (Anderson and Tietyen-Clark, 1986).

The Effect of Different Sources of Dietary Fiber on Blood Lipids and Lipoproteins in the Rat

Wheat Bran

The effect of wheat bran on plasma lipids in the rat has been studied by several researchers. Vigne et al (1987) fed diets containing 25% fat, 1.2% cholesterol, and either no fiber (control) or an additional 10% wheat bran to male Sprague-Dawley rats for six weeks. Serum TC, TG, LDL-C, HDL-C, and VLDL-C were determined for each group. Results showed a significant decrease in serum TG (wheat bran = 43 mg/dL, control = 87 mg/dL) and VLDL-C (wheat bran = 36 mg/dL, control = 56 mg/dL) with the addition of 10% wheat bran. No significant differences were found in either serum TC

(wheat bran = 109 mg/dL, control = 130 mg/dL), LDL-C (wheat bran = 12 mg/dL, control = 17 mg/dL) or HDL-C (wheat bran = 54 mg/dL, control = 52 mg/dL).

Unwin (1986) found that addition of 12% wheat bran to a rat diet containing 5% corn oil and 0.25% cholesterol increased plasma TC levels when compared to controls (111 mg/dL versus 94 mg/dL). Control and experimental diets were fed for five weeks prior to blood analysis. These results are somewhat questionable, however, because of the low level of dietary cholesterol and the lack of cholate in the diet which have been shown to be necessary in adequate amounts to enhance the cholesterolemic response in rats (Beynen, 1987). Additionally, rats used in this study were female, complicating the picture with a different hormonal pattern than males.

Results of studies on the effect of wheat bran on plasma lipids in the rat appear to be varied (Kritchevsky et al, 1984; Vigne et al, 1987; Unwin, 1986). This may be due in part to inconsistent levels of fat and cholesterol in the diets among research studies. Other inconsistencies could be due to differences in the gender of animals as well as an individual variability within genders. While wheat bran fiber seems to be very effective in increasing fecal bulk, most researchers agree that it is less effective on lowering plasma cholesterol in the rat (Schneeman, 1989; Nishina and Freedland, 1990).

Gram

The use of gram as a hypocholesterolemic agent in rats has been studied by several researchers. Mathur et al (1964) showed that addition of either whole gram flour, defatted gram flour, or fat extract of gram to a diet containing 20% nut oil, 1% cholesterol and 0.2% cholate decreased serum TC levels in male albino rats (whole gram flour = 106.0 mg/dL, defatted gram flour = 111.3 mg/dL, fat extract of gram = 89.2 mg/dL versus control = 174.7 mg/dL). Rats were fed a hypercholesterolemia inducing diet alone for the first 6 weeks, after which the whole gram, defatted gram, and lipid extract replacements were made for the following 6 weeks.

Similarly, Jayakumari and Kurup (1979) studied the effect of blackgram fiber on serum TC levels in the rat. Diets contained 15% ground nut oil, 2% cholesterol, 0.5% sodium cholate, and either no fiber (control) or 30% isolated blackgram fiber. Diets were fed for three months to male Sprague-Dawley rats. Serum TC was significantly lower in the blackgram fiber group compared to the control group (99 mg/dL versus 164 mg/dL).

More recently, Birender et al (1987) showed hypocholesterolemic effects of several varieties of gram in weanling albino rats over a four week period. Diets containing 20% hydrogenated groundnut oil, 1% cholesterol, and enough raw gram flour or casein (control) to provide 15% protein (73% green-seeded gram, 61% Kabli gram, 58% Bengal gram). Five percent cellulose was added to the control diet. Plasma TC was measured and all gram varieties showed

considerable hypocholesterolemic effects. Green-seeded gram (118 mg/dL) and Kabli gram (137 mg/dL) had higher hypocholesterolemic effects as compared to Bengal gram (187 mg/dL), and all results were significant when compared to the control (243 mg/dL). Unfortunately, fiber content in this study was not determined for the experimental diets. Therefore, conclusions cannot be reached regarding the effect of dietary fiber from each type of gram on plasma TC in the rat.

Oat Products

Interest in oat gum was spawned when it was discovered that one third of the plant fiber content of oat bran is oat gum. Chen et al (1981) studied the effects of oat bran and oat gum on plasma TC and lipoproteins in the male Sprague-Dawley rat. Diets were fed for a three week period and consisted of 6% fat, 1% cholesterol, 0.2% cholate, and 10% fiber from oat bran (36.5% oat bran in the diet) or oat gum. The control diet contained 10% cellulose. They determined that oat gum significantly decreased plasma TC (83 mg/dL) as did oat bran (107 mg/dL) when compared to a cellulose control (140 mg/dL). Plasma HDL-C increased the same with oat gum (37 mg/dL) as with oat bran (34 mg/dL) when compared to the control (21 mg/dL). Interestingly, plasma TG decreased with oat gum (56 mg/dL), but did not with oat bran (102 mg/dL) as compared to controls (110 mg/dL). The researchers concluded that the cholesterol-lowering effect of oat bran appeared to be related to it's water-soluble gum

content.

In a different study, male Sprague-Dawley rats were fed a diet containing 10% corn oil, 1% cholesterol, 0.2% cholate, and 6% dietary fiber from either cellulose (control) or oat bran for three weeks. The distribution of cholesterol among the lipoproteins was measured. Results showed that the oat bran group had significantly lower plasma TC (84.8 mg/dL versus 115.2 mg/dL), lower plasma LDL-C (18.0 mg/dL versus 29.5 mg/dL), and lower plasma VLDL-C (39.6 mg/dL versus 65.2 mg/dL) than the cellulose control. Additionally, the oat bran group had higher plasma HDL-C than controls (27.2 mg/dL versus 21.5 mg/dL) (Ney et al, 1988). Although the dietary fiber content in these diets was less, the results were in agreement with those of Chen et al (1981), discussed earlier.

Shinnick et al (1988) found similar results, using diets containing similar amounts of cholesterol and cholate, where a decrease in plasma TC was found using a variety of oat products including oat bran over three weeks. They found that as little as 4% dietary fiber from a processed oat flour significantly lowered plasma TC concentrations in the rat as compared to a 5% cellulose control (101.3 mg/dL versus 150.4 mg/dL). No differences were noted in plasma TG.

Beans

Research on the effect of dietary fiber from beans on hypercholesterolemia in the rat has been scant. One study using

relatively common beans was not controlled for dietary fiber content, but rather for protein content. In this study by Chang et al (1986), the effect of diets containing navy, pinto, great northern, kidney, baby lima, and mung beans, on rat plasma TC was investigated. Diets were fed for four weeks and were isoproteinic at 10% (from the various sources) with the control diet containing casein at the same level. Diets also contained 15% fat, 2% cholesterol, and 1% cellulose. No analysis of dietary fiber content in diets was made. Results showed that the replacement of casein by the bean flours did not cause any reduction in plasma TC in rats (control = 83 mg/dL, navy = 86 mg/dL, pinto = 86 mg/dL, great northern = 82 mg/dL, kidney = 86 mg/dL, baby lima = 75 mg/dL, mung = 85 mg/dL).

Agarwal and Chauhan (1988) studied the effect of Khejri beans on hyperlipidemia in the rat. Diets were equivalent in fiber content at 10% from either cellulose (control) or Khejri beans, and 1% cholesterol. Diets were fed for a duration of six weeks. Results showed no significant differences between control and Khejri bean diet group serum TC (233.3 mg/dL versus 226.7 mg/dL respectively) or TG (264.2 mg/dL versus 253.3 mg/dL respectively).

Conversely, Rigotti et al (1989) found that the addition of fiber from an unknown bean source (obtained from Campex in Gorbea, Chile) decreased serum TC (72 mg/dL versus 99 mg/dL), TG (70 mg/dL versus 129 mg/dL) significantly when compared to a cellulose control diet. Lipoprotein cholesterol appeared to decrease in all

fractions, but reached a significant difference only in VLDL-C concentration (values not reported). Diets were formulated to contain 5% fat and 4% fiber. The diets did not, however, contain cholesterol.

Cellulose and Hemicellulose

The effect of isolated fiber fractions on hypercholesterolemia in rats has also been investigated. Cellulose consistently displays no effect on plasma TC, TG, or lipoproteins in rats (Vigne et al, 1987; Nishina and Freedland, 1990; Borel et al, 1989). Because cellulose is an insoluble fiber fraction, it provides a good control for investigations of various other fiber fractions and their effect on plasma lipids and lipoproteins. Similarly, hemicellulose has been shown to have no effect on the amounts of lipids and cholesterol found in the plasma (Borel et al, 1989).

Pectin

Pectin given to rats at 8% in the diet for 8-10 weeks with dietary fat at 5% and no added cholesterol resulted in no significant decrease in plasma TC (64.6 mg/dL versus 52.8 mg/dL respectively) or TG (13.5 mg/dL versus 11.0 mg/dL respectively) when compared to cellulose controls (Nishina and Freedland, 1990). When cholesterol is added to the diets, the results change, however. Chen et al (1981) found that pectin added to diets at 10% which also contained 6% fat, 1% cholesterol, and 0.2% cholate significantly

decreased plasma TC (78 mg/dL versus 140 mg/dL) and TG (51 mg/dL versus 110 mg/dL) and increased plasma HDL-C (34 mg/dL versus 21 mg/dL) as compared to a cellulose control over 8-10 weeks.

Similar results were found by Judd and Truswell (1985) who used diets containing 1% cholesterol and 10% pectin or maize starch (control = 85% cellulose + 15% hemicellulose) to demonstrate the hypo-cholesterolemic effect of pectin. Experimental diets contained 10% pectin with varying amounts of fats (5%, 10%, 15%, and 20%) from groundnut oil. The control diet contained 10% fat. All experimental diets decreased plasma TC significantly when compared to the control diet group (control = 122 mg/dL, 5% = 65 mg/dL, 10% = 67 mg/dL, 15% = 78 mg/dL, 20% = 86 mg/dL).

Vigne et al (1987), however, found no significant hypocholesterolemic effect of pectin at 10% in the diet for six weeks when compared to a cellulose control for serum TC (108 mg/dL versus 131 mg/dL), TG (76 mg/dL versus 74 mg/dL), LDL-C (12 mg/dL versus 15 mg/dL), and HDL-C (51 mg/dL versus 51 mg/dL). The only significant difference was with VLDL-C which decreased with pectin (38 mg/dL versus 58 mg/dL) (Vigne et al, 1987). The differing effect of pectin between research studies could be due to the variable levels of fat used in the diets. It appears that pectin has a hypocholesterolemic effect at low levels of fat and little, if no effect at higher levels of fat in the diet.

The Effect of Dietary Fiber on Blood Lipids and Lipoproteins in Other Animals

Guinea Pigs

Pectin isolated from prickly pear was given to male guinea pigs to determine the effect on plasma cholesterol and TG levels. Two diets were formulated from a single batch of nonpurified diet by adding 0.25% (wt/wt) cholesterol or 0.25% cholesterol + 1% prickly pear pectin (wt/wt). After 25 days of dietary treatment, plasma was collected and analyzed (Fernandez et al, 1990).

Guinea pigs fed the pectin diet exhibited a significant 26% reduction in plasma TC levels and a 33% decrease in plasma LDL and HDL-C levels as compared to levels in control animals. Plasma VLDL-C concentrations appeared elevated in pectin-fed animals but the difference was not significant. Plasma TG levels did not differ between animals fed the two diets (Fernandez et al, 1990).

Minipigs

Ahrens et al (1986) studied the effect of oral pectin administration on blood lipids in minipigs. Male adult hypercholesterolemic minipigs were fed a diet which contained approximately 14% fat, 1% cholesterol, 0.5% cholic acid, 5.7% cellulose,and either no pectin or citrus pectin to equal 75 grams per 24 hour period. Diets were fed for four weeks. Serum TC and lipoprotein cholesterol were determined. Serum TC was significantly lower in pigs consuming pectin than in the control (165 mg/dL versus

326 mg/dL), while LDL-C (70 mg/dL versus 142 mg/dL), HDL-C (3 mg/dL versus 86 mg/dL), and VLDL-C (17 mg/dL versus 69 mg/dL) were all significantly lower in the group fed pectin as well. Important to note is the fact that sample size was very small (n=2) in each group, and that no control fiber (eg: cellulose) was added to the control diet in the same amount as pectin.

Chicks

Hypocholesterolemic effects of oat bran fractions in chicks has been investigated by Welch et al (1988). Oat bran was separated into five fractions: oil; insoluble (rich in starch and insoluble fiber); protein-rich; gum; and soluble residue. Diets contained 5.7% oil and 0.22% cholesterol. Two separate experiments were performed, feeding oat bran at 40% (expt.#1) and 30% (expt.#2) and oat bran fractions for 19 and 14 days respectively. Fractions were remixed into the diets in both experiments to equal concentrations proportional to their yields from the original bran. Fiber sources were substituted in place of cornstarch in the control diets (40% and 30% controls). Plasma TC and HDL-C were determined separately for each experiment.

Results showed that the native oat bran diets had no significant effect on plasma HDL-cholesterol (control = 110 mg/dL), but lowered plasma TC when compared to the controls (40%: 161 mg/dL versus 297 mg/dL, 30%: 167 mg/dL versus 341 mg/dL). Upon comparison of individual fractions with the control and native oat bran diets

(30% and 40%), both the gum and protein fractions lowered the plasma TC to levels not significantly different from the oat bran (40%: control = 297 mg/dL, oat bran = 161 mg/dL, protein = 207 mg/dL, gum = 163 mg/dL; 30%: control = 341 mg/dL, oat bran = 104 mg/dL, protein = 110 mg/dL, gum = 109 mg/dL). The effect of gum was greater than that of the protein in the first experiment. Diet had no significant effect on plasma HDL-C levels. The researchers concluded, therefore, that oat gum as well as oat protein appeared to have a hypocholesterolemic effect in the chick (Welch et al, 1988).

Monkeys and Baboons

The effects of dietary fiber in vervet monkeys fed "western" diets was investigated by Kritchevsky et al (1988). "Western" diets contained 46.2% calories from fat, 39.8% as carbohydrate, and 14% as protein. Also added to the diets was either 10% cellulose or 10% apple pectin, and 0.15% cholesterol. The control diet contained 5% fat, 65% carbohydrate, and 20% protein. Diets were fed for 34 weeks. Final serum TC levels in monkeys fed "western" diets with 10% fiber were elevated significantly when compared to the control values (10% cellulose = 163 mg/dL, 10% pectin = 185 mg/dL, control = 119 mg/dL) but were not significantly different between the two test groups. Only average serum LDL-C and HDL-C levels over the course of the study were reported. Final values for serum LDL-C and HDL-C were not measured. Serum LDL-C levels in monkeys fed pectin were

significantly lower than those fed cellulose (10% cellulose = 239 mg/dL, 10% pectin = 155 mg/dL, control = 63 mg/dL). Only serum HDL-C levels were significantly higher in the monkeys fed pectin than control levels (58 mg/dL versus 39 mg/dL). Serum TG levels were unaffected by diet (Kritchevsky et al, 1988).

The effect of apple pomace as a hypocholesterolemic agent in baboons given a high-fat diet was investigated by Sly et al (1989). Two groups of 18 post-adolescent baboons were placed on a high-fat diet (20% beef tallow) with 0.1% cholesterol and either 20% apple pomace (processing residue from production of apple juice) or paper pulp (to provide equal bulking). The apple pomace diet was thought to contain 5.1% insoluble and 2.3% soluble fiber by weight, while the paper pulp diet provided 10.3% insoluble fiber with essentially no soluble dietary fiber. The apple pomace diet contained 1.5% pectin. Diets were fed for six months.

Results showed that serum TC levels rose significantly above starting values in both groups as soon as they were placed on the high-fat cholesterol-containing diets (apple pomace: 150 mg/dL versus 119 mg/dL initial value; paper pulp: 179 mg/dL versus 121 mg/dL initial value). In the group receiving apple pomace, serum TC levels rose to a significantly lower level than the paper pulp group. This difference between the two groups occurred early in the study and persisted for the duration of the study. Serum HDL-C did not change for the apple pomace group. The paper pulp group, however, saw a significant increase in levels of HDL-C

(73 mg/dL versus 55 mg/dL initial value). No significant differences were seen in serum TG (Sly et al, 1989).

The Effect of Dietary Fats and Cholesterol on Blood Lipids and Lipoproteins and Lipoprotein Transport in the Hamster

It is generally accepted among researchers that the addition of dietary saturated fat and/or cholesterol to the diet of male hamsters will increase plasma or serum TC and LDL-C. Spady and Dietschy (1989) fed a diet enriched in cholesterol and saturated TG to male hamsters over 24 months. Results showed that rates of LDL production increased, total body LDL receptor activity was suppressed, and plasma LDL-C levels rose.

Using male Golden Syrian hamsters, Ohtani et al (1990) demonstrated that levels of plasma TC and LDL-C increased significantly in animals fed a diet containing 0.1% cholesterol when compared to a control diet containing 0.05% cholesterol (178 mg/dL versus 144 mg/dL and 50 mg/dL versus 41 mg/dL, respectively). Levels of plasma VLDL-C and HDL-C in experimental animals tended to increase but were not significantly different from control animals. An increase in VLDL-C secretion from hepatocytes was also observed in animals fed 0.1% cholesterol.

All diets contained 0.57% linoleic acid, 0.16% palmitic acid, 0.30% oleic acid, and 0.12% other fatty acids, and were fed ad libitum for 14 days. The experimental diets were prepared by adding various amounts of cholesterol and/or fatty acid to the control

diet. The addition of 5% palmitic acid to the 0.1% cholesterol diet enhanced the effect of dietary cholesterol on plasma VLDL-C and LDL-C and secretion of VLDL-C from hepatocytes. Dietary linoleic acid of 5% in the 0.1% cholesterol diet enhanced the effect of dietary cholesterol on VLDL-C secretion from hepatocytes, but diminished the effect on the plasma LDL-C level. Hepatic LDL receptor activity was greatly suppressed by the control diet, with a further supression by the 0.1% cholesterol diet with or without palmitic acid. Dietary linoleic acid prevented the suppression of hepatic LDL receptor activity induced by cholesterol (Ohtani et al, 1990).

Similarly, Tsuda et al (1983) fed male hamsters a high cholesterol diet containing 1% cholesterol, 0.2% sodium cholate, and 5% olive oil. Results showed HDL to be a major lipoprotein of normal hamster plasma. The amount of plasma VLDL was also high when expressed as cholesterol. After feeding the experimental diet for one week, plasma TC increased to 389 mg/dL from 164 mg/dL of animals on a powdered basal diet. The increase in plasma TC was reflected in increases of both HDL-C and LDL-C (exact values were not reported).

Hayes et al (1992) studied the effect of dietary fat and cholesterol on plasma lipoprotein distribution in the male Syrian hamster. Diets were fed for 13 weeks, and were either fat-free or contained 5% butterfat + 0.4% cholesterol. Results showed a significant increase in both plasma TC (271 mg/dL versus 128 mg/dL)

and plasma TG (310 mg/dL versus 186 mg/dL) with the diet containing fat + cholesterol. Plasma lipoproteins were not measured.

The results of some studies showed an increase in plasma and serum TC and some lipoproteins with addition of dietary fat and cholesterol over time. Jackson et al (1990) maintained hamsters on diets containing either 2% (w/w) cholesterol or 20% (w/w) hydrogenated coconut oil for up to 12 weeks. Control diets contained 0.1% cholesterol. Results showed that both experimental diets induced hypercholesterolemia.

Measurements of plasma cholesterol were taken at 1 week, 2 weeks, 4 weeks and 12 weeks. Results of plasma TC, LDL-C, HDL-C, and VLDL-C were displayed in graph format, and exact levels were not reported. The control level of plasma TC remained stable over the entire 12 weeks. In the cholesterol-fed animals, the level of plasma TC rose slowly and remained stable and significantly higher than the control or fat-fed group. The level of plasma TC in the fat-fed group rose rapidly to a level significantly higher than the control group, but then gradually declined to a level just above that of the control group after 12 weeks (Jackson et al, 1990).

The plasma lipoprotein distribution was determined in all animals. The animals that showed an increase in plasma TC showed a corresponding increase in all plasma lipoprotein fractions. The 2% cholesterol diet resulted in a significant increase in amounts of plasma LDL-C, HDL-C, and VLDL-C. The 20% coconut oil fed animals showed an increase in plasma LDL-C that was significant after one

week but disappeared at the end of 12 weeks. Plasma HDL-C levels were increased in all fat-fed animals throughout the experimental period. There was no increase in plasma VLDL-C in fat-fed animals. The researchers postulated that the hamsters were able to compensate for the effects of the saturated fat on plasma cholesterol by a metabolic mechanism (Jackson et al, 1990).

Similarly, the effect of dietary fat and cholesterol on serum TC, LDL-C, and HDL-C over 12 months was studied by Nistor et al (1987). Male Golden Syrian hamsters were fed a hyperlipidemic diet consisting of standard chow supplemented with 3% cholesterol and 15% butter.

Results showed that in control animals, mean serum levels were as follows: TC = 64 mg/dL, LDL-C = 28 mg/dL, HDL-C = 36 mg/dL. During the first two weeks on the experimental diet, serum TC did not change, but doubled in the third week and increased almost four-fold after the fourth week. At 10 months, serum TC reached a 17-fold increase. Experimental levels of serum LDL-C increased four-fold after four weeks and 13-fold after 10 months. Serum HDL-C in experimental animals only doubled after four and 12 weeks. Exact values for experimental serum lipids and lipoproteins were not reported (Nistor et al, 1987).

Several researchers have studied the effect of different doses of dietary cholesterol on serum or plasma and liver lipids and lipoproteins in male Golden Syrian hamsters. Singhal et al (1983) found a dose-related increase in serum and liver TC with addition of

cholesterol at 0.15% or 1% to a chow diet containing 0.01% cholesterol. Experimental diets were fed for 1 month. Serum TC increased from 114 mg/dL to 215 mg/dL to 342 mg/dL in the control, 0.15% cholesterol, 1% cholesterol diet groups, respectively. Liver TC increased from 1.82 mg/g to 13.7 mg/g to 22.2 mg/g for the control, 0.15% cholesterol, 1% cholesterol diets, respectively.

Male Golden Syrian hamsters were fed diets enriched with 0.1%, 0.25%, or 1% cholesterol for 1 month by Spady and Dietschy (1988). Results showed a suppression of receptor-dependent LDL transport in the liver (43%, 63%, 77% respectively). Receptor-independent LDL transport was not significantly altered by dietary cholesterol or TG. Plasma LDL-C increased from 22 mg/dL (control) to 43 mg/dL, 72 mg/dL, and 126 mg/dL on diets of 0.10% cholesterol, 0.25% cholesterol, and 1.0% cholesterol respectively.

The Effect of Dietary Fiber on Blood Lipids, Lipoproteins, and Liver Cholesterol Metabolism in the Hamster

Studies on the effect of dietary fiber on blood lipid and lipoprotein levels and liver cholesterol metabolism in the hamster are not as numerous as those using the rat as an animal model. In one study, male Golden hamsters were fed one of four diets for four weeks. A standard diet (control - a balanced pelleted hamster diet containing 0.001% cholesterol and 6% fibers provided by various cereals), the standard diet enriched with 5% pectin or in combination with 0.1% cholesterol, or the standard diet enriched

with 0.1% cholesterol only. The addition of cholesterol to the diet caused a significant rise in plasma TC when compared to the control (142 mg/dL versus 93 mg/dL). Pectins given in combination with cholesterol did not inhibit this rise (137 mg/dL versus 93 mg/dL). Pectin alone had no effect on plasma TC as compared to the control (94 mg/dL versus 93 mg/dL) (Sable-Amplis and Sicart, 1986a).

Several studies have demonstrated the hypocholesterolemic effect of apple and apple products in hamsters. The effect of apple supplementation on plasma and liver cholesterol content on two strains of hamsters was investigated by Sable-Amplis et al (1979). The two strains of hamsters were normal animals and fatty-liver animals (spontaneously hypercholesterolemic animals that accumulate cholesteryl esters in the liver when they are fed a standard hamster diet). Normal animals and fatty-liver animals were fed either a commercial standard hamster diet or the same with free access to apples for 30 days. Hamsters consumed approximately 32 grams of apple per day per 100 grams body weight. The fiber level ingested was not reported.

The apple-supplemented diet significantly reduced plasma TC (102 mg/dL versus 130 mg/dL) and increased plasma TG (281 mg/dL versus 219 mg/dL) in normal animals. Apple supplementation in normal animals also significantly reduced liver cholesterol content (235 mg/100g versus 368 mg/100g liver). In fatty-liver animals with apple supplementation, a significant decrease in both plasma TC and TG was observed (125 mg/dL versus 182 mg/dL and 256 mg/dL versus

417 mg/dL, respectively). The fatty liver animals also showed a dramatic decrease in liver cholesterol content from 2081 mg/100g to 311 mg/100g liver. The authors noted that the levels of plasma and liver lipids of fatty-liver hamsters became comparable to those of normal hamsters with apple supplementation (Sable-Amplis et al, 1979).

A similar study by Sicart et al (1983) observed the effect of apple supplementation on plasma lipoprotein profiles and liver cholesterol in normal and hypercholesterolemic hamsters with high levels of liver cholesterol (FEC hamsters). Animals were fed either a commercial standard hamster diet or the same diet supplemented with apple ad libitum. The basal diet and the edible fresh apple contained 4% and 0.9% total fiber, respectively. Apple-supplemented animals ingested an average of 33 grams of apple per day. Duration of the experiment was not reported. Results of plasma and liver cholesterol levels in normal and FEC hamsters were similar to the previously mentioned study by Sable-Amplis et al (1979) where apple supplementation decreased plasma TC and liver cholesterol in both strains of animals.

Cholesterol transport by each lipoprotein fraction in normal and FEC hamsters responded similarly. Addition of apple to the diet resulted in significant decreases in LDL-C (normal: 13.4 mg/dL versus 25.8 mg/dL; FEC: 23.6 mg/dL versus 47.5 mg/dL) and VLDL-C (normal: 9.6 mg/dL versus 18.5 mg/dL; FEC: 7.1 mg/dL versus 25.9 mg/dL). No changes in HDL-C were noted with apple supplementation

in either strain of hamster (Sicart et al, 1983). Similar results were found with apple supplementation in FEC hamsters in studies by Sable-Amplis and Sicart (1986b) over a six month period, and by Sicart and Sable-Amplis (1987) over five months.

Kahlon et al (1990) investigated the influence of rice bran, oat bran, and wheat bran on plasma TC and TG in hamsters. Eight diets were fed to male Golden Syrian hamsters for 21 days. The control diet contained 10% cellulose and 0.5% cholesterol. Experimental diets were modifications of the control diet that provided 10% total dietary fiber (in place of cellulose) from either stabilized rice bran (RB), defatted RB, parboiled rice bran (PB), defatted PB, fiber from RB and wheat bran (RWB, 2:1 ratio respectively), fiber from defatted RB and wheat bran (defatted RWB, 2:1 ratio respectively), or oat bran (OB).

After 21 days, RB and OB diets resulted in significantly lower plasma TC and liver cholesterol values as compared to the control diet [plasma TC (mg/dL): RB = 274, OB = 294, control = 402; liver cholesterol (mg/g liver): RB = 31, OB = 40, control = 57]. The PB diet and rice bran diets with added wheat bran fiber significantly lowered plasma TC (PB = 302 mg/dL, RWB = 334 mg/dL, defatted RWB = 331 mg/dL). Defatting rice bran resulted in a loss of it's plasma cholesterol-lowering effect. Defatted RB and rice bran with added wheat bran fiber (RWB) significantly decreased levels of liver cholesterol (defatted RB = 45 g/g liver, RWB = 49 g/g liver). Plasma TG were not affected by any of the diets. The researchers

postulated that the cholesterol-lowering effect of rice bran appears to be associated with the oil fracton rather than total or soluble dietary fiber (Kahlon et al, 1990).

Kahlon et al (1992) found similar results using rice bran at various levels, defatted rice bran, and rice bran oil for 21 days in male Golden Syrian hamsters. The cholesterol level was 0.3% in all diets. Similar to the study by Kahlon et al (1990), full-fat rice bran was the only treatment that significantly lowered both plasma and liver cholesterol from levels found in cellulose. Plasma TC lowering by rice bran was significantly correlated to the level of rice bran in the diet. Researchers stated that the data suggest that recombined defatted rice bran and rice bran oil are not as effective as full-fat rice bran in producing significant cholesterol reductions in hamsters (Kahlon et al, 1992).

Jonnalagadda et al (1993) used male Golden Syrian hamsters to study plasma total cholesterol, triglycerides, and lipoprotein cholesterol in animals fed various fiber diets. A purified hypercholesterolemic diet (0.1% cholesterol, 10% fat, 4% cellulose), was fed for four weeks, with a subsequent one week recovery period. At week five, animals were randomly assigned to diet groups including the control, or one of four experimental diets containing approximately 10% total dietary fiber from either cellulose, guar gum, oat bran, or xylan. All diets contained 0.1% cholesterol, 10% fat, and were fed for an additional four weeks.

After four weeks on the fiber diets, results showed

significant decreases in plasma TC in the oat bran, guar gum, and xylan groups (16%, 12%, and 15%, respectively). Plasma TG and HDL-C decreased significantly only in the guar gum animals (46% and 12%, respectively). The combined plasma VLDL-C + LDL-C concentrations were significantly lowered by the oat bran, xylan, and cellulose diets (38%, 34%, and 40%, respectively). The authors concluded that oat bran, guar gum, and xylan appeared to be effective hypocholesterolemic agents in hamsters (Jonnalagadda et al, 1993).

The effect of dietary fat, cholesterol, and fiber on the production of pigment or cholesterol gallstones in hamsters was investigated by Hayes et al (1992). Male Golden Syrian hamsters were fed either 0.4% cholesterol + 5% butterfat-supplemented purified diets, or cholesterol and fat-free diets with or without fiber (25% total fiber = 10% cellulose + 15% wheat bran) for 13 weeks. Over the course of the study, plasma TC and TG were measured at week 5, and plasma TC, TG, LDL-C, HDL-C, VLDL-C, and IDL-C were measured at 13 weeks.

After 5 weeks, plasma TC increased significantly with the addition of dietary fiber to the diet (162 mg/dL versus 93 mg/dL). Plasma TG were not significantly affected by dietary fiber. After 13 weeks, plasma TC was significantly greater in the fiber-fed animals (151 mg/dL versus 128 mg/dL). Plasma LDL-C increased significantly with the addition of fiber (19 mg/dL versus 12 mg/dL). Plasma HDL-C, VLDL-C, IDL-C, and TG levels showed no differences. Ninety-three percent of the hamsters fed the fat-supplemented diet

had gallstones (mostly cholesterol), whereas 62% of the hamsters fed the fat-free diet had gallstones (almost all pigmented). Adding fiber to the fat-free diet eliminated cholesterol stones and enhanced pigment stone formation (Hayes et al, 1992).

No conclusions were discussed regarding the effect of fiber on plasma lipids and lipoprotein fractions. Researchers did conclude, however, that their fat and cholesterol diets essentially assured induction of cholesterol gallstones, whereas the fat and cholesterol-free diets (especially with added wheat bran) favored production of pigment stones, although an overall lower incidence rate. The authors stated that a major difference between cholesterol and pigment gallstone induction was the concentration of liver cholesterol. When the liver was cholesterol laden, bile was supersaturated and cholesterol gallstones predominated (Hayes et al, 1992).

OBJECTIVES

1. To evaluate the effect of purified control, oat bran and pinto bean diets on hamster feed intake and weight gain.

2. To determine the effect of purified control, oat bran and pinto bean diets on plasma total cholesterol and triglyceride levels in the hamster.

3. To evaluate the effect of purified control, oat bran and pinto bean diets on plasma lipid and lipoprotein levels in pooled samples in the hamster.

4. To relate the amounts of soluble, insoluble, and total dietary fiber in the experimental diets to plasma lipid and lipoprotein levels of hamsters in the different diet treatments.

JOURNAL ARTICLE

PLASMA TOTAL CHOLESTEROL AND TRIGLYCERIDE RESPONSES OF HAMSTERS FED OAT BRAN AND PINTO BEAN DIETS

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ABSTRACT The effect of a 53% oat bran diet and a 30% pinto bean diet on hamster plasma total cholesterol (TC) and triglycerides (TG) was investigated. Hamsters were made hypercholesterolemic and then fed one of four experimental diets, the hypercholesterolemic control (HC), an insoluble fiber (alphacel) control (FC), the oat bran (OB), or the pinto bean (PB) diet for three weeks, with the latter three containing 8.5%, 10.0%, and 7.6% total dietary fiber, respectively. Plasma TC and TG were measured for each animal before and after the experimental diets. Plasma TC levels were significantly different between hamsters fed the HC (203 mg/dL) and OB (179 mg/dL) diets ($p<0.05$), and between those fed the FC (221 mg/dL) and OB diets ($p<0.001$). Plasma TC levels of animals fed the PB diet averaged 199 mg/dL. Plasma TG levels were significantly different only between hamsters fed the FC (358 mg/dL) and OB (200 mg/dL) diets ($p<0.01$). Thus, it was concluded that oat bran significantly lowered plasma TC and TG in hamsters, while pinto beans did not.

KEY WORDS Hamster, oat bran, beans, dietary fiber, plasma cholesterol, plasma triglycerides

INTRODUCTION

The hypocholesterolemic effect of dietary fiber has been studied extensively. Various types of dietary fiber have differing effects on blood lipid and lipoprotein levels. Diets including fiber from sources rich in water-soluble fiber, as oat bran and pinto beans, have been shown to significantly lower serum total cholesterol (TC) and low density lipoprotein cholesterol (LDL-C) (1,2) with little or no effect on serum triglycerides (TG) in hypercholesterolemic men (3). The mechanism behind the hypocholesterolemic effect of soluble fiber remains unclear (4). Conversely, diets including fiber from sources high in water-insoluble fiber, as wheat bran and cellulose, most often have a minimal though variable effect on serum TC and LDL-C levels and have been shown to significantly lower serum TG in hypercholesterolemic men (2,3).

The hamster has been shown to respond similarly to the human in hypercholesterolemia research (5), yet few studies have used the hamster as a small animal model to study the effect of diet and in particular various sources of dietary fiber on blood lipids and lipoproteins. The purpose of this study was to determine the effect of purified diets containing either oat bran or pinto beans on plasma TC and TG in the hamster. Hamster feed intake, weight gain, and plasma lipoprotein results from pooled samples were also examined.

MATERIALS AND METHODS

Animal Model

Seventy-four 9 to 11 week old Golden Syrian male hamsters (102 to 121 grams) were purchased from Harlan Sprague Dawley, Inc., Indianapolis, Indiana. Upon arrival at the university vivarium, hamsters were quarantined for one week. The hamsters were housed individually in plastic cages lined with corn cob bedding.and covered with wire lids. Throughout the study, the hamsters were fed and watered ad libitum, and remained in a temperature (21-21°C) and humidity (40-50%) controlled room, with a 12 hour light-dark cycle. Hamster food consumption and body weight were recorded every other day. Care and handling of animals was according to policies and procedures established by the Virginia Tech Animal Care Committee (Virginia Polytechnic Institute and State University, Blacksburg, VA).

Experimental Design

At the beginning of the experiment, all hamsters were weighed (base weight), fasted overnight, and a blood sample taken to establish normal plasma TC levels. Immediately after bleeding, and for the duration of three weeks, all animals were fed a hypercholesterolemic (HC) diet. At the end of the third week, all hamsters were fasted overnight and a blood sample was taken to determine the plasma TC response. Twenty percent of the hamsters

did not respond to the HC diet with elevated plasma TC levels. Fifty-six hamsters who showed an elevated cholesterol response of at least 20% above baseline levels were ranked by plasma TC levels and randomly assigned to one of four experimental diet groups (randomized, block design), each containing fourteen hamsters. The first group remained on the HC diet, the second group was fed an insoluble fiber control (FC) diet, the third group was fed a 53% oat bran (OB) diet, and the fourth group was fed a diet containing 30% pinto beans (PB), the latter three containing 8.5%, 10.0%, and 7.6% total dietary fiber, respectively (6). The hamsters remained on their experimental diets for three weeks, then fasted overnight, and a blood sample collected before they were euthanized under anesthesia (sodium pentobarbital, 40-50 mg/kg). (Detailed descriptions of all methodologies can be found in Appendix A).

Diets

All diets were calculated to contain 16% crude protein, 10% fat (2:1:3.3, P:M:S ratio) (6), 0.1% cholesterol, and recommended amounts of vitamins, minerals, and other biochemicals necessary for proper hamster nutrition (7). The HC diet contained 4.0% insoluble fiber, while the FC, OB, and PB diets were calculated to contain approximately 8.6% total dietary fiber. The OB diet contained 53% Quaker Oat Bran (The Quaker Oats Co., Chicago, IL). Pinto beans that were thoroughly cooked and freeze-dried made up 30% of the PB diet. Calculated diet components are listed in Table 2.

Table 2. Calculated Composition of Experimental Diets.

Component	HC	FC	OB	PB
	% (dry wt basis)			
Casein[1]	16.0	16.0	6.3	9.9
Fat[2]	10.0	10.0	6.9	9.7
Cornstarch[3]	36.5	32.0	4.8	20.0
Dextrose[4]	28.0	28.0	28.0	28.0
Alphacel[5]	4.0	8.5	0.0	0.0
Cholesterol[6]	0.1	0.1	0.1	0.1
DL-Methionine[7]	0.1	0.1	0.1	0.1
Choline Chloride[8]	0.3	0.3	0.3	0.3
Vitamin Mix[9]	1.0	1.0	1.0	1.0
Mineral Mix[10]	4.0	4.0	4.0	4.0
Oat Bran[11]	0.0	0.0	53.0	0.0
Pinto Beans[12]	0.0	0.0	0.0	30.0

[1,4-8] Casein, Vitamin Free; Dextrose; Alphacel non-nutritive bulk; cholesterol U.S.P.; DL-Methionine U.S.P.; Choline Chloride; ICN Biomedicals Inc., Costa Mesa, CA.

[2] 10:6:3 by weight, butter:Puritan Oil:Mazola Corn Oil, (unsalted butter, Land O'Lakes Inc., Arden Hills, MN; Puritan Oil,Proctor and Gamble, Cincinnati, OH; Mazola Corn Oil, Best Foods, CPC International Inc., Englewood Cliffs, NJ).

[3] Argo Cornstarch, Best Foods CPC International, Inc., Englewood Cliffs, NJ.

[9] Vitamin Mix composed of (per kg mix) the following: Thiamin Hydrochloride 600mg; Riboflavin 600mg; Pyridoxine Hydrochloride 700mg; Nicotinic Acid 3g; D-Calcium Pantothenate 1.6g; Folic Acid 200mg; D-Biotin 20mg; Cyanocobalamin 1mg; Retinyl Palmitate 1.6g; DL-alpha-Tocopherol Acetate 20g; Cholecalciferol 250mg;

(continued)

(continued - Table 2)

Menaquinone 5mg; Sucrose 972.9g, ICN Biomedicals, Inc., Costa Mesa, CA.

[10] Mineral Mix composed of (per kg mix) the following: Calcium Phosphate Dibasic 500g; Sodium Chloride 74g; Potassium Citrate Monohydrate 220g; Potassium Sulfate 52g; Magnesium Oxide 24g; Manganous Carbonate 3.5g; Ferric Citrate 6g; Zinc Carbonate 1.6g; Cupric Carbonate 0.3g; Potassium Iodate 0.01g; Chromium Potassium Sulfate 0.55g; Sucrose 118g, ICN Biomedicals, Inc., Costa Mesa, CA.

[11] Quaker Oat Bran, The Quaker Oats Co., Chicago, IL.

[12] Thoroughly cooked and freeze-dried.

Diets were prepared individually in batches, sampled for analysis, and stored in a freezer until feeding time. Diets were fed to the hamsters in ceramic cups which remained in the cages.

Diet Analysis

Proximate analysis (crude protein, fat, ash, and moisture) was determined on each of the four diets, as well as on the cooked and freeze-dried pinto beans (CFPB) and commercial oat bran (COB). Protein was determined by the Kjeldahl method (8). Fat was measured by ether extract analysis at the Virginia Tech Forage Testing Laboratory (Blacksburg, VA) (9). Ash was determined by incineration of samples overnight at 550-600°C in a muffle furnace (8). Moisture in the samples was measured by drying at 100°C overnight (8). Carbohydrate was calculated by difference.

Further analysis was done on the CFPB and COB to determine the composition of the carbohydrate component. *Starch* in the CFPB and COB was analyzed by an enzymatic procedure using glucoamylase (10), with determination of resulting reducing sugars by the use of bicinchoninate reagent (11). The amount of *cellulose* in the samples was determined by the Updegraff procedure (12). *Pectin* (hexuronic acid) was quantified by using a colorimetric reagent, 3-5-dimethylphenol, which is selective for a chromagen that is formed from uronic acids in concentrated sulfuric acid at 70°C. Addition of this reagent at 20°C produces a chromophore absorbing at 450nm (13). After hydrolysis with 1N sulfuric acid, the amount of

cell wall sugars (arabinose, xylose, glucose, galactose, mannose) in the samples was determined by gas chromatography (GC). The amount of sugar was calculated based on the peak height and internal standard (14). *Soluble sugars* (fructose, glucose, sucrose, maltose) were extracted from the CFPB and COB using 80% ethanol. Determination by high-pressure liquid chromatography (HPLC) followed, using a Waters microBondapak Carbohydrate column (Millipore Corporation, Milford, MA). The HPLC eluting solvent was acetonitrile:water (75:25) (15).

The amount of *soluble and insoluble fiber* was determined by the use of a FiberZym kit (Novo BioLabs, Denmark) which is a slightly modified and improved version of the method described by Prosky et al (8,16). Further modifications were made to separate soluble and insoluble fiber (17). Quantification of the soluble and insoluble fiber fractions included corrections for ash and protein. Ash was measured using a muffle furnace as in proximate analysis. Protein determinations were carried out using a Labconco Rapid Kjeldahl System (Labconco, Kansas City, MO) with cupric sulfate as a catalyst (18).

Blood Collection and Analysis

Three blood samples were taken from each hamster throughout the experiment. Blood samples were drawn just prior to administration of the HC diet (0 time), just prior to administration of the four experimental diets (3 weeks), and at the end of the experiment

(6 weeks). At each drawing, approximately 1.5 ml of blood was taken from the orbital sinus of the hamsters (19), which were anaesthetized with either halothane or ketamine. Blood samples were collected in test tubes containing disodium EDTA, held on ice and then centrifuged at 3000 rpm in a refrigerated centrifuge at 4°C for 30 minutes. The separated plasma (approximately 0.5-0.7ml) was pipeted into a PVC test tube and stored at 4°C until further analysis.

Plasma TC and TG were determined for each blood sample. Plasma TC was determined by using the Liebermann-Burchard colorimetric reaction (20). A Stanbio kit (#2000) supplied by Stanbio Co., San Antonio, TX, was used to determine plasma TG. This assay involved the use of glycerophosphate oxidase in a quantitative, fully enzymatic, colorimetric technique (21).

To determine LDL-C, high density lipoprotein cholesterol (HDL-C), and very low density lipoprotein cholesterol (VLDL-C), the remaining plasma was pooled. A volume of 0.5ml was pooled from each of seven plasma samples within a feeding group, to equal a total volume of 3.5ml. The result created two observations for each experimental diet group. Plasma TC was determined for each observation using the Liebermann-Burchard reaction (20). Lipoprotein fractions within each pooled observation were separated using ultracentrifugation at hydrated density of 1.006 for 18 hours (22). After ultracentrifugation, the fraction containing both HDL-C and LDL-C was analyzed to determine the combination (HDL-C + LDL-C)

using the Liebermann-Burchard reaction (20). The plasma low density lipoprotein (LDL) was precipitated from the above fraction using a heparin-manganese chloride solution (23). The plasma HDL-C was analyzed and determined, as above, in the remaining supernatant. Concentrations of plasma VLDL-C and LDL-C were calculated by difference as follows:

$$\text{VLDL-C} = \text{TC} - (\text{HDL-C} + \text{LDL-C})$$

$$\text{LDL-C} = (\text{HDL-C} + \text{LDL-C}) - \text{HDL-C}$$

Statistical Analysis

The General Linear Models (GLM) procedure (24) was used for all analyses of variance (ANOVA). If the ANOVA was found to be significant, the Least Squares Means (LSM) procedure (24) was used to determine where these differences occurred. All differences were considered to be significant if the p-value was < 0.05.

ANOVA was used to analyze plasma TC and TG over weeks 0, 3, 6, and between all diets. ANOVA was also applied to daily weight gain and food intake data between weeks 0-3 and 4-6, and between all diets.

RESULTS

During the course of this study, three hamsters died or were sacrificed due to illness. These deaths resulted in a reduced number of animals within some treatment groups (Table 3). The statistical model used to analyze the data allowed for this difference in sample size.

Feed Intake

The average daily feed intake of hamsters within each diet group at weeks 0-3 and weeks 4-6 are presented in Table 3. The analysis of variance (ANOVA) for feed intake showed no significant differences over time or between treatments. (See Appendix B for individual hamster average feed intake).

Average Weight and Weight Gain

Figure 1 shows average hamster weight at weeks 0, 3, and 6 for all diet groups. ANOVA was done to compare hamster weights between diet groups at week 0, week 3, and week 6, and between weeks for all treatments. At week 3, hamster weight for all diet groups was significantly greater than at week 0 ($p<0.001$). From week 3 to week 6, average weight of the PB and OB animals continued to increase ($p<0.05$), while weights of the HC and FC animals did not. At week 6, the only significant difference between treatments was found between the PB and FC animals ($p<0.05$), where PB animals

Table 3. Average Daily Feed Intake (grams/day) of Hamsters for Weeks 0-3 and Weeks 4-6 on a Control and Three Fiber Diets

Group*	Week 0-3	Week 4-6
	Mean ± SEM	
HC (n=13)	8.2 ± 0.4	7.5 ± 0.3
FC (n=13)	8.7 ± 0.4	7.4 ± 0.3
OB (n=14)	7.7 ± 0.4	6.9 ± 0.3
PB (n=13)	8.4 ± 0.4	7.6 ± 0.3

* HC = Hypercholesterolemic control
FC = Fiber Control
OB = Oat bran
PB = Pinto bean

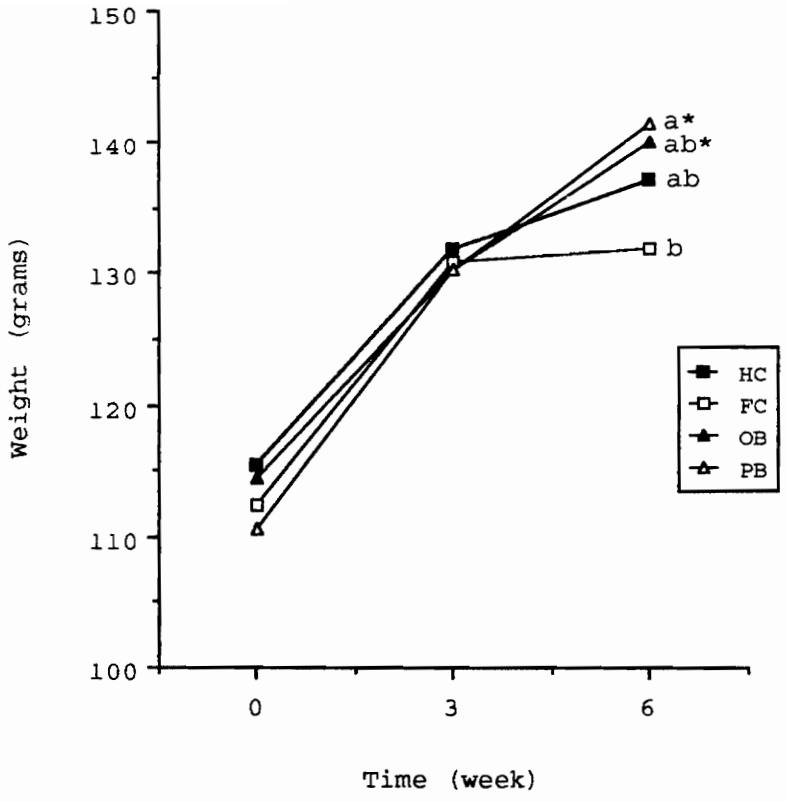

Figure 1. Average Hamster Weight (grams) at Weeks 0, 3, 6 on a Control and Three Fiber Diets

HC = Hypercholesterolemic control
FC = Fiber control
OB = Oat bran
PB = Pinto bean
[ab] values with different superscripts are significantly different at $p<0.05$
* values are significantly different from week 3 values ($p<0.05$)

weighed more than the FC animals (142grams versus 132grams, respectively). (See Appendix C for average hamster body weight values for each experimental diet group, and Appendix D for individual hamster body weight values).

Hamster weight gain was determined for weeks 0-3 and weeks 4-6 and did not differ between diets for weeks 0-3. For weeks 4-6, no significant difference was found for hamster weight gain between the OB diet and PB animals. Both the OB and PB animals gained significantly ($p<0.001$) more weight than the HC diet animals (OB = 9.8g, PB = 11.4g, HC = 4.4g). The OB, PB, and HC animals all gained significantly ($p<0.05$) more weight than the FC animals (FC = 0.9g). (See Appendix E for average hamster weight gain values for each experimental diet.)

Proximate Analysis of Experimental Diets, Commercial Oat Bran, and Cooked and Freeze-Dried Pinto Beans

Fat content of all diets was close to the goal and calculated value of 10% (8.9% - 9.7%). The actual protein content of the diets was considerably lower than the calculated value (9.6% - 10.9% versus 16%), however all diets were shown to have similar amounts of protein. Table 4 shows the composition of experimental diets, COB, and CFPB as determined by proximate analysis.

Table 4. Composition of Experimental Diets, Commercial Oat Bran (COB), and Cooked and Freeze-Dried Pinto Beans (CFPB) - Results of Proximate Analysis

	Protein	Fat	Ash	Moisture	Carbohydrate[a]
			(%)		
COB[b]	21.6	6.7	3.1	8.0	61.6
CFPB	22.6	1.2	4.4	9.1	62.7
HC	9.6	9.7	3.2	5.4	72.1
FC	10.7	9.4	3.0	5.5	71.4
OB	10.4	8.9	4.3	5.5	70.9
PB	10.9	9.2	4.0	4.2	71.7

[a] carbohydrate calculated by difference
[b] COB = Commercial Oat Bran
CFPB = Cooked and Freeze-Dried Pinto Beans
HC = Hypercholesterolemic control
FC = Fiber control
OB = Oat bran
PB = Pinto bean

Composition of the Carbohydrate Fraction of Commercial Oat Bran and Cooked and Freeze-Dried Pinto Beans

The COB and CFPB contained similar amounts of total carbohydrate (COB = 53.0% versus CFPB = 51.4%) (Table 5). The carbohydrate fraction in COB contained 24% more starch than the CFPB (45.8% versus 34.8%). The carbohydrate fraction of the CFPB, however, contained greater amounts of pectin, cellulose and hemicellulose than the COB. Oat bran is known to contain a beta-glucan fraction, whereas pinto beans do not. The beta-glucan fraction of our COB was determined to be 3.1% by calculating the level of glucose minus starch. Levels of sugars varied among the COB and CFPB.

Fiber Content of Experimental Diets, Commercial Oat Bran, and Cooked and Freeze-Dried Pinto Beans

Table 6 displays the summary of fiber content of the experimental diets, COB, and CFPB. Because of the small sample size (n=2), no statistical analyses were performed on the fiber data. Analyzed total dietary fiber content of the OB and PB diets was not equivalent to calculated values (see Table 2). The HC and FC diets contained calculated amounts of total dietary fiber made up of Alphacel non-nutritive bulk (cellulose) at 4.0% and 8.5% respectively. The OB diet appeared to contain the greatest amount of dietary fiber at 10%, distributed equally between insoluble and soluble fiber fractions. The PB diet appeared to contain slightly

Table 5. Composition of the Carbohydrate Fraction of Commercial Oat Bran and Cooked and Freeze-Dried Pinto Beans

Component	Oat Bran	Pinto Beans
	(%)	
Starch	45.8	34.8[a]
Pectin	trace	2.5
Cellulose	trace	4.0
Hemicellulose[b]	5.9[c]	7.8
Sucrose[d]	1.3	2.3
Total Carbohydrate[e]	53.0	51.4
Sugars Derived by Hydrolysis of Carbohydrate Fraction:		
Arabinose	1.3	4.3
Xylose	1.4	0.7
Glucose	48.9	38.8
Galactose	trace	2.5
Mannose	0.1	0.3

[a] starch determined by glucose minus cellulose (38.8 - 4.0 = 34.8)
[b] hemicellulose determined by summation of arabinose, xylose, galactose, and mannose
[c] partly determined by glucose minus starch: 48.9 - 45.8 = 3.1 (which represents the beta-glucan fraction)
[d] sucrose occurred as a free sugar and was determined by HPLC
[e] carbohydrate determined by summation of above results

Table 6. Summary of Fiber Content in Experimental Diets, Commercial Oat Bran (COB), and Cooked and Freeze-Dried Pinto Beans (CFPB)

Compound	Insoluble Fiber	Soluble Fiber	Total Fiber
		(%)	
COB[a]	9.4	9.5	18.9
CFPB	19.1	6.3	25.4
HC[b]	4.0	0	4.0
FC[b]	8.5	0	8.5
OB[c]	5.0	5.1	10.0
PB[c]	5.7	1.9	7.6

[a] COB = Commercial Oat Bran
CFPB = Cooked and Freeze-Dried Pinto Beans
HC = Hypercholesterolemic control
FC = Fiber control
OB = Oat bran
PB = Pinto bean

[b] contribution from Alphacel non-nutritive bulk added to the diet in this amount

[c] values calculated using results from fiber analysis on COB and CFPB and their respective amounts in diets (OB diet = 53% COB, PB diet = 30% CFPB)

less total dietary fiber (7.6%) than the FC diet, with 75% of this appearing as insoluble fiber and the remaining 25% as soluble fiber.

Insoluble fiber content between the OB and PB diets was similar, less than the FC diet, and greater than the HC diet. Soluble fiber content in the OB diet, however, was two and a half times that in the PB diet. The analysis of COB and CFPB reflected the distribution of dietary fiber between soluble and insoluble fiber fractions in the OB and PB diets.

Plasma Total Cholesterol

No significant differences in plasma TC concentrations were found between treatments at week 0 or week 3 (Table 7). At week 3, plasma TC levels were significantly higher than at week 0 ($p<0.001$). From week 3 to week 6, plasma TC levels decreased significantly ($p<0.01$) only for the OB animals.

At week 6 the OB animals had significantly ($p<0.05$) lower plasma TC levels than either the HC or the FC animals. Plasma TC levels for the PB animals were not significantly different from any other treatment at week 6, however these differences approached significance with FC ($p=0.0775$) and OB ($p=0.0976$) animals. (See Appendix F for individual hamster plasma TC levels).

Plasma Triglycerides

Average hamster plasma TG for week 0, week 3, and week 6 are shown in Table 8 (see Appendix G for individual hamster plasma TG

Table 7. Average Plasma Total Cholesterol (mg/dL) of Hamsters for Weeks 0, 3, 6 on a Control and Three Fiber Diets

Group*	Week 0	Week 3	Week 6
		Mean (mg/dL) ± SEM	
HC (n=13)	$85^{1} \pm 4$	$208^{2} \pm 8$	$203^{2a} \pm 8$
FC (n=13)	$87^{1} \pm 4$	$205^{2} \pm 8$	$221^{2a} \pm 8$
OB (n=14)	$81^{1} \pm 4$	$205^{2} \pm 7$	$179^{3b} \pm 8$
PB (n=13)	$76^{1} \pm 4$	$207^{2} \pm 8$	$199^{2ab} \pm 8$

* HC = Hypercholesterolemic control
FC = Fiber control
OB = Oat bran
PB = Pinto bean

[123] means within rows with different superscripts are significantly different at $p<0.05$

[ab] means within columns with different superscripts are significantly different at $p<0.05$

Table 8. Average Plasma Triglycerides (mg/dL) of Hamsters for Weeks 0, 3, 6 on a Control and Three Fiber Diets

Group*	Week 0	Week 3	Week 6
	Mean (mg/dL) ± SEM		
HC (n=13)	73^{1a} ± 7	221^{2} ± 36	286^{3ab} ± 41
FC (n=13)	104^{1b} ± 7	269^{2} ± 36	358^{2a} ± 41
OB (n=14)	72^{1a} ± 7	298^{2} ± 34	200^{3b} ± 40
PB (n=13)	72^{1a} ± 7	216^{2} ± 36	267^{2ab} ± 41

* HC = Hypercholesterolemic control
FC = Fiber control
OB = Oat bran
PB = Pinto bean

[123] means within rows with different superscripts are significantly different at $p<0.05$

[ab] means within columns with different superscripts are significantly different at $p<0.05$

levels). At the beginning of the study, the FC animals started out with significantly higher ($p<0.01$) plasma TG levels (104mg/dL) than the other three treatments (HC = 73mg/dL, OB and PB = 72mg/dL). Plasma TG for all diet groups then increased significantly between week 0 and week 3 ($p<0.01$), with no significant differences between treatments at week 3. At week 6, plasma TG levels increased again for the HC animals ($p<0.05$), but did not significantly change for the FC and PB animals. Plasma TG levels for the OB animals decreased significantly between week 3 and week 6. At week 6, the only significant difference between treatments for plasma TG was found between the FC and OB groups ($p<0.01$).

Plasma Lipoprotein Cholesterol - Pooled Samples

In order to obtain enough plasma to perform the plasma lipoprotein cholesterol analysis, plasma samples from individual hamsters had to be pooled. This allowed 10 observations for week 0, and 2 observations for each treatment for week 3 and week 6. Because of this small sample size for weeks 3 and 6, statistical analyses were not feasible. Table 9 shows the levels of cholesterol found in each lipoprotein fraction for the various pooled samples (see Appendix H for tables of pooled hamster plasma lipid and lipoprotein values).

At week 3, all of the various plasma lipoprotein fractions (TC, HDL-C, LDL-C, VLDL-C) demonstrated a similar trend across the four treatments. Plasma TC, HDL-C, and VLDL-C more than doubled,

Table 9. Average Cholesterol Levels in Lipoproteins (mg/dL) of Pooled Plasma Samples of Hamsters for Weeks 0, 3, 6 on a Control and Three Fiber Diets

Diet:[a]		HC	FC	OB	PB
			(mg/dL)		
Week 0[b] (n=10)	TC[c]	88			
	HDL-C	25			
	LDL-C*	63			
	VLDL-C*	0			
Week 3 (n=2)	TC	194	196	195	193
	HDL-C	70	67	70	72
	LDL-C*	90	101	83	89
	VLDL-C*	34	28	42	32
Week 6 (n=2)	TC	218	233	193	212
	HDL-C	78	68	53	67
	LDL-C*	106	86	123	104
	VLDL-C*	34	79	17	41

[a] HC = Hypercholesterolemic control
FC = Fiber control
OB = Oat bran
PB = Pinto bean
[b] baseline values prior to any diet
[c] TC = total cholesterol
HDL-C = high density lipoprotein cholesterol
LDL-C = low density lipoprotein cholesterol
VLDL-C = very low density lipoprotein cholesterol
* LDL-C and VLDL-C calculated by difference

and LDL-C increased by one half, from week 0. At week 6, plasma TC increased slightly from week 3 for all treatments, and was similar between treatments, except OB which remained the same.

From week 3 to week 6, plasma HDL-C levels generally stayed the same, and plasma LDL-C levels increased across the treatments except for the FC group. Plasma VLDL-C levels were highly variable in their response from week 3 to week 6.

DISCUSSION

Research studies involving dietary manipulations with possible unknown side effects, as those involving hypercholesterolemia and new sources of dietary fiber, necessitate the use of a feasible small animal model. Various rodents have been used in this capacity, particularly the rat. Humans may be more sensitive to dietary cholesterol than rats since it is necessary to include extreme amounts of cholesterol (1-2%) with cholate (0.5%) in rat diets to enhance their cholesterolemic response (25). It appears that the rat has a rate of cholesterol synthesis in both hepatic and extrahepatic tissues which is exceptionally high (26). The rat also appears to have a highly efficient mechanism for converting excess cholesterol into bile acids (27). Additionally, the rat biliary cholesterol secretion and bile acid output are tightly coupled, so that many dietary and pharmacological treatments used in humans do not have an effect on this process in the rat (26, 27). That is, the rat has a very efficient mechanism for controlling cholesterol in it's body, so that dietary manipulations using cholesterol may not be as applicable to humans as with other animal models.

The hamster, however, has been shown to respond similarly to the human in hypercholesterolemia research (5). Hamsters respond to dietary cholesterol of a much lower content than rats. Sicart et al (28) found a significant increase in plasma TC with addition of only 0.1% cholesterol in the diet of hamsters. Additionally, the rate of

hepatic cholesterol synthesis in the hamster is low compared to the rat, and is in fact more similar to the human (29). The fact that the hamster has a relatively high level of plasma low density lipoprotein (LDL) with approximately a 1:1 ratio of plasma LDL:HDL (high density lipoprotein) as compared to other rodents (30), coupled with the evidence that administration of dietary cholesterol increases LDL plasma lipoproteins (29, 30, 31) makes the hamster a good model for cholesterol research (5). Therefore, the hamster was used in the present study to investigate the hypocholesterolemic effect of oat bran and pinto beans on plasma lipids and lipoproteins. It is necessary to point out the presence of the fore-stomach in the hamster which might have an effect on manipulations using dietary fiber and should be considered when comparing results to other species.

Average daily feed intake was consistent between treatments and during the weeks of the entire experiment, and was comparable to other studies using hamsters of a similar age (32, 33). Average weight increased throughout the experiment for the PB and OB animals. Average weight of the HC and FC animals increased from week 0 to week 3, and remained constant from week 3 to week 6. Hamster weight gain did not differ between diets for weeks 0-3. The OB and PB animals experienced a good weight gain for weeks 4-6. The HC animals did not gain weight as well as the OB and PB animals, while the FC animals had the lowest weight gain during this time. It is possible that the higher amount of cellulose (Alphacel non-

nutritive bulk) in the FC diet may have had an adverse effect on weight gain, where the greater the content of cellulose in the diet, the less the weight gain. This hypothesis, however, contradicts findings by Kahlon et al (34). Four week old male Golden Syrian hamsters were fed either a control diet containing 10% cellulose or an oat bran diet containing 10% total dietary fiber (4.3% soluble fiber from oat bran) for 21 days. Both diets contained 0.5% cholesterol. No differences were found between feed intake, weight gain, or final weight for the two diets. Similar results were observed in hamsters fed diets containing 0.3% cholesterol and 10% fiber from either cellulose or rice bran (35). Studies using rats reveal similar results as well. Separate experiments using oat bran and cellulose at 6% (36) or 10% (37) in diets containing 1% cholesterol and 0.2% cholate have shown no differences in animal feed intake or weight gain between diets.

Additional unknown nutritional components in the COB and CFPB added to the OB and PB diets, respectively, may have enhanced the weight gain and overall health of these animals. This hypothesis is supported by Sable-Amplis et al (32) in an experiment where apples were added to hamster diets. Apple supplementation of a commercial diet accelerated the growth of young hamsters.

Proximate analysis showed fat content of all diets to be close to the experimental goal and calculated value of 10%. Protein content of the diets appeared to be lower than the goal and calculated value of 16%, however all diets had similar amounts of

protein ranging from 9.6% - 10.9%. Allen et al (33) fed diets containing 12, 18, or 36% casein to 3 month old Golden Syrian hamsters for 19 weeks. No significant differences were found for weight gain or food intake between diets. It has also been observed that different sources of protein have varying effects on plasma lipid and lipoprotein levels in the hamster. Terpstra et al (38) showed a hypocholesterolemic effect of soybean protein versus casein in hamsters fed cholesterol-free or cholesterol enriched semipurified diets. Hamsters fed diets containing 25% casein had higher plasma TC, TG, LDL-C, and VLDL-C levels than animals fed a 25% soybean protein diet.

It is unlikely that the protein level had an effect on hamster weight gain or food intake in the present experiment. The diets contained protein from different sources, however. The HC and FC diets contained protein solely from casein, while the OB and PB diets included protein from COB and CFPB respectively, as well as from casein. It is possible that protein from COB and CFPB may have affected the plasma lipid and lipoprotein profiles differently, but this is unlikely due to the low level of protein in the diets. Additionally, this effect would be difficult to determine since casein was included in the OB and PB diets. No studies have demonstrated the effect of protein from COB and CFPB on plasma lipid and lipoprotein levels in the hamster.

The carbohydrate fraction of COB and CFPB as determined by analysis and summation of individual components (Table 5) was

similar (53.0% and 51.4% respectively). The possibility of error from each analysis is additive in the final calculation of total carbohydrate for each conventional solid food. It is very likely that fractions of each component were lost, due to the fact that recovery of samples in each analysis is rarely 100%. The carbohydrate fraction (which includes dietary fiber) of COB and CFPB as determined by proximate analysis (Table 4) was similar as well, but was 10% higher than the above calculation (61.6% and 62.7% respectively). Total carbohydrate was determined here by difference, that is, total sample weight less the protein, fat, ash, and moisture. This measurement presumes a greater amount of carbohydrate. Again, recovery of samples in proximate analysis is less than 100%. From these results, we can assume that COB and CFPB have similar amounts of carbohydrate, with a general quantification estimate of 51% - 63%. Because there is error built into each determination of carbohydrate (by analysis and addition of individual components or by difference from proximate analysis), more study is needed in this area to resolve this discrepancy.

Fiber content from Alphacel non-nutritive bulk (cellulose) in the HC and FC diets was assumed to be the same as calculated values. Cellulose was used in the fiber control diet because it is an insoluble fiber fraction and consistently displays no effect on plasma TC, TG, or lipoproteins in rats (39, 40, 41). Cellulose therefore provides a good control for investigations of various other fiber fractions and their effect on plasma lipids and

lipoproteins.

Analyzed total dietary fiber content of the OB and PB diets (10% and 7.6% respectively) was not equivalent to calculated values (8.6%). This could be a result of several factors. Error could have resulted in the measuring and/or mixing of diet ingredients, inaccurate determinations of components in the commercial oat bran and freeze-dried pinto beans prior to mixing resulting in inaccurate calculations in diet recipes, or low recovery and loss of sample during final fiber analysis.

An accurate and fast method for dietary fiber analysis has yet to be developed. Several analytical methods for dietary fiber determinations are available. Some laboratories using the method performed in this study have encountered difficulty with samples that are high in starch and protein. In fact, most of the popular and commonly used analytical procedures for determining the fiber composition of foods underestimate total fiber content (42).

It should be pointed out that research studies dealing with purified diets containing sources of dietary fiber rarely perform analyses on the diets to determine the accuracy of the composition of their diet. This can be a great source of error. Additionally, commercial or freeze-dried food products added to purified diets should be analyzed before incorporation into the diets. This would assure closer calculations of the amounts of these products and their constituents in the experimental diets. The development of an accurate method for dietary fiber determinations including

separation of soluble and insoluble fiber fractions is imperative for the accuracy of future studies involving the mechanisms and effects of dietary fiber.

The OB diet appeared to contain the greatest amount of dietary fiber at 10%, distributed equally between insoluble and soluble fiber fractions. The PB diet appeared to contain slightly less total dietary fiber than the FC diet at 7.6%, with 75% of this appearing as insoluble fiber and the remaining 25% as soluble fiber. Even though total dietary fiber appeared to be less in the PB diet, insoluble fiber content between the OB and PB diets was similar. Soluble fiber content in the OB diet, however, was two and a half times that in the PB diet. The possibility of an effect of cooking and freeze-drying the pinto beans on the distribution of total dietary fiber between insoluble and soluble fiber fractions is noted. The analysis of COB and CFPB reflected the results of the distribution of dietary fiber between soluble and insoluble fiber fractions in the OB and PB diets.

Insoluble and soluble fiber fractions are thought to act differently in the digestive tracts of animals, and are therefore thought to have different physiological effects. Both insoluble and soluble fibers cause an increased bulk of softer stool due to their increased water-retaining capabilities. Soluble fibers retard gastric emptying and decrease the rate of food absorption and digestion (4).

When comparing the effect of insoluble and soluble dietary

fiber, it appears that the insoluble dietary fiber has little effect, if any, on serum cholesterol levels. The majority of researchers have demonstrated and acknowledge the hypocholesterolemic effect of soluble fiber from various plant sources. The mechanism by which soluble fiber lowers serum cholesterol is currently still unknown (4).

In the present study, hamsters were made hypercholesterolemic when fed a diet containing 0.1% cholesterol and 9 to 10% fat for three weeks. No significant differences were found in plasma TC levels between treatments at week 0 or week 3. Similar results were reported in studies using hamsters fed varying amounts of cholesterol (0.1%, 0.15%, 0.5%, and 1%) (43, 44).

The effects of the fiber diets on plasma TC were observed at week 6. The OB diet showed the only significant effect, a decrease in plasma TC levels from week 3 to week 6 and as compared to the HC and FC treatments at week 6.

Kahlon et al (34) used Golden Syrian hamsters to demonstrate the hypocholesterolemic effects of oat bran. Animals were younger and lighter than those used in the present study, and animals were not made hypercholesterolemic prior to administration of experimental diets. Diets were fed for three weeks and contained 0.5% cholesterol (higher than the present study), 10.7% fat, and 10% total dietary fiber from oat bran or cellulose (control), similar to that in the present study. Soluble fiber content in their oat bran diet was approximately 4.3%, similar to the 5.1% soluble fiber in

the present study. Results were in the same direction as the present study in that they showed a decrease in plasma TC levels with oat bran as compared to the cellulose control (294.0 mg/dL versus 401.8 mg/dL respectively), but the magnitude of the decrease was considerably greater. Although the results were similar, actual levels of plasma TC are difficult to compare to those of the present study since animals were not made hypercholesterolemic prior to administration of the OB diet, and the level of dietary cholesterol was five times more in the Kahlon et al (34) study.

Jonnalagadda et al (1993) studied the effect of various fiber diets including oat bran on plasma TC in Golden Syrian hamsters. The study design was similar to the present study, with the diets containing 0.1% cholesterol and approximately 10% fat. Hamsters were made hypercholesterolemic by a diet containing 4% cellulose, and then fed diets containing 10% total dietary fiber from cellulose or oat bran. Animals responded similarly to the present study, however plasma TC levels were slightly higher, in general. The oat bran diet showed a significant decrease in plasma TC levels when compared to the cellulose control diet (212.7 mg/dL versus 228.2 mg/dL, respectively). The oat bran diet contained 4.2% soluble, and 5.7% insoluble dietary fiber, similar to our values (45).

Results similar to the present study have been observed in rats fed diets containing 1% cholesterol, 0.2% cholate and dietary fiber from oat bran ranging from 4% to 10% (36, 37, 46). Hypercholesterolemic men have shown similar reductions in plasma TC

levels as well (2, 3).

Humans and rats have been used to study the effects of beans and bean products on blood cholesterol. Anderson and Chen (47) found that bean products and water-soluble fiber extracts of beans had significant hypocholesterolemic effects. Subsequent studies by Anderson and Chen (48) and Anderson et al (1) showed a 19% reduction in serum TC with bean supplemented diets in hypercholesterolemic men when compared to those on control diets. Experimental and control diets within these studies did not have similar amounts of total dietary fiber, however.

Results from rat studies using dietary fiber from beans are varied and inconsistent. Studies are few and have different experimental designs and diets than the present study, lack consistency in dietary fiber content, do not contain dietary cholate (necessary to create hypercholesterolemia in the rat), or contain no dietary cholesterol.

Although plasma TC levels for the PB animals were not significantly different from the other treatments at week 6, they did approach significance with the FC and OB treatments. In the present study, total dietary fiber contributed by cooked and freeze-dried pinto beans comprised only 7.6% of the PB diet of hypercholesterolemic hamsters in attempt to simulate the hypocholesterolemic effect of pinto beans as seen by Anderson and Chen (48) in hypercholesterolemic men. The amount of total dietary fiber provided by 30% cooked and freeze-dried pinto beans in the diet, as

incorporated into the Anderson and Chen (48) diet, was supposed to be 8.6% of the diet. Unfortunately, the fiber analysis of the PB diet showed a lesser amount of total dietary fiber in our PB diet (7.6%). As a result, the corresponding amount of soluble fiber from pinto beans was only 1.9%.

If we assume, as did Anderson and Chen (48) and Anderson and Gustafson (49), that the hypocholesterolemic effect of pinto beans is due to the soluble fiber content, the lack of an effect on the plasma TC levels in the present study may be partially explained by the lesser amount of total dietary fiber, and particularly the soluble fiber, in our PB diet as compared to that of Anderson and Chen (48). Had the FC, OB and PB diets had similar amounts of total dietary fiber (8.6%), the amount of soluble fiber in the PB diet would have been greater, and may have resulted in a significant decrease in plasma TC levels in PB animals.

The OB diet had 2.5 times the amount of soluble fiber than the PB diet and may partially account for the plasma TC differences seen in the present study. Additionally, COB is rich in oat gum, a beta-glucan (50), a glucose polymer that is thought to play a role in the hypocholesterolemic effects of oat bran (35). Our OB diet contained 3.1% beta-glucan which may have contributed to the hypocholesterolemic effect of this diet. Conversely, pinto beans contain no beta-glucans. More research on the beta-glucan fraction of oat bran would prove useful in the future. Results from the present study regarding the effect of the PB diet on plasma TC levels are

inconclusive and difficult to compare to human or rat studies.

The specific role of dietary fiber on hypertriglyceridemia has not been determined, although fiber intake does appear beneficial. Studies by Anderson and Tietyen-Clark (50) indicated that high-fiber diets had both short and long-term effects on decreasing serum TG levels.

With administration of the HC diet, all treatments showed significant increases in plasma TG levels from week 0 to week 3, with no differences between diet groups at week 3. Plasma TG levels were high (>200 mg/dL) at week 3, and remained so for the duration of the experiment. Responses of plasma TG varied among the diet treatments from week 3 to week 6.

At week 6 of the present study, the only significant difference in plasma TG levels between treatments was found between the FC and OB diet groups. It is possible that the mere fact that the OB diet contained more total dietary fiber (10%) than any of the other diets could have resulted in this difference. The FC diet contained 8.5% total dietary fiber from cellulose, but TG levels in this diet group were higher at week 0, and may have been higher for the duration of the experiment as a result, therefore showing a significant difference from levels in the OB treatment at week 6.

The effect of dietary fiber on plasma or serum TG values is highly variable between studies and between species. Hypercholesterolemic diets containing 0.5% cholesterol, 10.7% fat, and 10% fiber from wheat bran or oat bran, were fed to Golden Syrian

hamsters for 21 days. Results showed no effect on plasma TG levels when compared to a 10% cellulose control (34). Similarly, Golden Syrian hamsters made hypercholesterolemic and then fed diets containing 0.1% cholesterol, 10% fat, and 10% total dietary fiber from either cellulose or oat bran for four weeks showed no change in plasma TG values (45).

Results from studies using the rat are similar to hamster studies. Studies using rats fed a hypercholesterolemic diet containing either no fiber (control) or 10% fiber from wheat bran showed significant decreases in serum TG levels in the wheat bran animals (39). Additionally, no significant differences were observed in TG levels using hypercholesterolemic diets in rats containing 4% (36) or 10% (37) fiber from oat bran, or 10% fiber from khejri beans (51) when compared to control diets containing comparable amounts of fiber from cellulose.

In humans, it appeared that fiber from oat bran lowered serum TG levels in hypercholesterolemic men (1, 3). Bean supplemented diets appeared to have no effect on serum TG levels of hypercholesterolemic men (1, 48). Wheat bran (primarily insoluble fiber) decreases serum TG levels in hypercholesterolemic men (3).

If wheat bran has this effect, and is primarily insoluble fiber, it is possible that cellulose, an insoluble fiber, could have a similar effect. Cellulose is often used as the fiber source in control diets as a negative or neutral control. When investigating the effect of various dietary fibers and fiber fractions on plasma

or serum TG levels, cellulose may not be a good fiber to use as a neutral control, because cellulose itself may affect TG levels. However in the present study with hamsters, plasma TG increased markedly, though not significantly by week 6 in animals fed the cellulose control diet.

We had hoped to determine HDL-C, LDL-C, and VLDL-C for each individual plasma sample. This was not possible, however, because high TG levels (>200 mg/dL) in the plasma samples created problems with direct precipitation of the LDL-C + VLDL-C fraction, and resulting analysis of the HDL-C fraction by the Liebermann-Burchard reaction (20). A fatty layer developed on top of the plasma during centrifugation, and no precipitation of the LDL-C + VLDL-C fraction occurred. Therefore, only plasma TC and TG were determined for individual samples, while plasma lipoprotein fractions were determined from pooled plasma samples. High plasma TG levels may have been a source of error in pooled plasma samples as well, although precipitation of the LDL-C + VLDL-C fraction was achieved after pooled plasma was ultracentrifuged.

The procedure used in the present study to determine plasma lipoprotein fractions (22) required at least 3.5 ml of plasma to perform the assay. It was therefore necessary to pool plasma samples from hamsters within treatment groups to achieve this volume. This allowed ten observations for week 0 and only two observations per treatment for weeks 3 and 6. Because of this small sample size, statistical analysis on these results was not feasible.

The confidence that we can put in the resulting values is therefore small, however, we can discuss tendencies.

Plasma TC levels for pooled samples (Table 9) at week 3 were more than two times higher than those at week 0, similar to individual plasma TC levels, and approximately equivalent between treatments. The pooled plasma TC levels at week 3 were approximately 10% lower than in individual hamster plasma samples (Table 7). Plasma TC levels at week 6 appeared to increase from week 3 in all treatments except the OB diet group, which remained the same. Pooled plasma TC levels at week 6 were approximately 5-10% higher than individual hamster levels.

Normocholesterolemic hamsters have been found to have a ratio of cholesterol content between HDL and LDL as being about 1:1 (30). As our animals became hypercholesterolemic, the ratio of HDL-C to LDL-C was approximately 1:1.3 in pooled plasma samples at week 3 and 1:1.6 at week 6. Nevertheless, all plasma lipoprotein fractions (HDL-C, LDL-C, and VLDL-C) appeared to increase in the present study with administration of the HC diet from week 0 to week 3. Similar increases in plasma or serum HDL-C, LDL-C, and VLDL-C have been shown using a range of dietary cholesterol content from 0.1% to 3% in hamster diets (5, 30, 52-55).

From week 3 to week 6 of the present study, the effect of the experimental diets on plasma lipoproteins can be observed. Plasma HDL-C levels generally appeared to remain the same, while plasma LDL-C levels appeared to increase across the treatments. Plasma

VLDL-C levels were highly variable in their response from week 3 to week 6.

Few studies have investigated the effect of dietary fiber on plasma lipoproteins in the hamster. The addition of 10% total dietary fiber from oat bran to the diet of hypercholesterolemic hamsters (0.1% cholesterol in diet) showed no change in plasma HDL-C, but significantly decreased the combined plasma VLDL-C + LDL-C concentration by 38% (45). Similarly, a study using apple supplementation of a standard pelleted diet and either normal or spontaneously hypercholesterolemic (FEC) hamsters showed that the addition of apples to the diet caused significant decreases in plasma LDL-C and VLDL-C. No differences were observed in plasma HDL-C with apple supplementation. Results were similar for each strain of hamster. The diet itself, however, did not contain cholesterol (56).

High plasma TG values may have been a source of error in pooled plasma samples as observed in individual plasma samples. High plasma TG values may have played a role in the variability of plasma VLDL-C responses observed from week 3 to week 6, since VLDL is made up largely of TG. Pooled plasma TG values (Appendix H) were highly variable in their response from week 3 to week 6, as with pooled plasma VLDL-C levels. The use of a table top ultracentrifuge, spinning much smaller volumes (0.2 to 0.5 ml), would have been the preferred method for lipoprotein separation since this would have allowed analysis of individual plasma samples. Additionally,

an enzymatic method for plasma lipoprotein cholesterol analysis may have given us more accurate results with the high TG samples.

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SUMMARY AND CONCLUSION

Coronary Artery Disease is the leading cause of death in the United States and Western Europe. There are three universally accepted primary modifiable risk factors for this disease: cigarette smoking, hypertension, and hyperlipidemia (Criqui, 1986). Hypercholesterolemia is the most widely acknowledged and dominant risk factor (Stehbens, 1990).

Serum total cholesterol, which is comprised of low density lipoprotein, high density lipoprotein, and very low density lipoprotein cholesterol, is an independent risk factor for CAD. As serum cholesterol levels increase, the greater the risk of CAD for the individual.

Plasma LDL-cholesterol comprises approximately 60 to 70% of plasma TC and is strongly and positively related to CAD. Elevations in serum LDL-C levels accelerate atherogenesis. LDL-C has been identified as the major target for plasma cholesterol-lowering therapy because there is substantial evidence that lowering LDL-C levels will reduce the incidence of CAD.

Various human studies over the years have pointed to a surprisingly strong, inverse relationship between plasma HDL cholesterol and CAD. Although plasma HDL-C comprises only 20 to 25% of the total cholesterol, it has been shown that the higher the plasma HDL level, the lower the risk of CAD.

VLDL contain 10-15% of the total serum cholesterol. Because

plasma TG are transported by, and make up 70% (by weight) of the VLDL cholesterol, the two are closely related. Evidence to date to support triglycerides as an independent risk factor for CAD is inconclusive and somewhat controversial.

The rat has been used extensively as an animal model in hyperlipidemia research. Humans, however, may be more sensitive to dietary cholesterol than rats since it is necessary to include extreme amounts of cholesterol (1-2%) with cholate (0.5%) in rat diets to enhance their cholesterolemic response. The rat has a very efficient mechanism for controlling cholesterol in it's body, so that dietary manipulations to affect plasma cholesterol may not be as applicable to humans as with other animal models.

Hamsters, on the other hand, have been found to respond to dietary cholesterol at a much lower concentration than rats. The hamster was used in this study because it has a relatively high level of plasma LDL as compared to other rodents, and has been shown to have a ratio of cholesterol content between HDL and LDL of approximately 1:1 on a normal diet. Additionally, research has shown that administration of dietary cholesterol increases plasma TC and LDL-C in hamsters similar to humans. This would make the hamster a good model for cholesterol research.

Oat bran and bean products have been shown to have a hypocholesterolemic effect in humans. Because of the similarity in responses to dietary cholesterol and amounts produced by the liver in man and hamster, it was hoped that the hamster would have similar

plasma lipid and lipoprotein responses to dietary fiber as well.

Dietary fiber has been found to be an important food component consisting of plant material that is resistant to hydrolysis by endogenous enzymes in the digestive tract of mammals. Plant fiber is classified as water soluble or water insoluble. The majority of researchers acknowledge the hypocholesterolemic effect of soluble fiber from various plant sources. While the specific role of dietary fiber in lowering plasma TG has not been determined, fiber intake does appear beneficial.

The present study was undertaken to determine the effect of purified diets containing either oat bran or pinto beans on plasma TC and TG in the hamster. Hamster feed intake, weight gain, and plasma lipoprotein results from pooled samples were also examined. Prior to the beginning of the study, the hamsters were fed a chow diet for one week. Animals were fed and watered ad libitum for the duration of the study.

At the beginning of the experiment, all hamsters were weighed (base weight), fasted overnight, and a blood sample taken to establish normal plasma total cholesterol levels. All blood samples (1.5 ml) were drawn from the orbital sinus of the hamster. Immediately after bleeding, and for the duration of three weeks, all animals were fed a hypercholesterolemic diet containing 0.1% cholesterol and 10% fat. At the end of the third week, all hamsters were fasted overnight and a blood sample was taken to determine the plasma TC response. An estimated 20% nonresponder rate was

observed. Fifty-six hamsters who showed an elevated cholesterol response were ranked by plasma TC level and randomly assigned to one of four experimental diets (randomized, block design), each containing fourteen hamsters.

The first group remained on the HC diet, the second group was fed an insoluble fiber control diet, the third group was fed a 53% oat bran diet, and the fourth group was fed a diet containing 30% cooked and freeze-dried pinto beans. The latter three diets contained 8.5%, 10.0%, and 7.6% total dietary fiber, respectively. All diets contained 10% fat, and 0.1% cholesterol. The hamsters remained on their experimental diets for three weeks, at which time they were fasted overnight, and a blood sample was taken to determine any changes in plasma cholesterol levels. The plasma samples were analyzed for TC and TG. Pooled plasma samples were analyzed for TC, HDL-C, LDL-C, and VLDL-C.

Average daily feed intake was constant between treatments and throughout the entire experiment. Average weight increased throughout the experiment for the PB and OB animals. Average weight of the HC and FC animals increased from week 0 to week 3, and remained constant from week 3 to week 6. Hamster weight gain did not differ between diets for weeks 0-3. The oat bran (OB, 9.8 grams) and pinto bean (PB, 11.4 grams) animals experienced a good weight gain for weeks 4 to 6, significantly more than the hypercholesterolemic control (HC) animals (4.4 grams). The insoluble fiber control (FC) animals (0.9 grams) gained the least

($p<0.05$) amount of weight during this time.

As expected, all treatment group plasma TC levels were significantly increased about 2.5 times from week 0 to week 3. No significant differences were found in plasma TC levels between treatments at week 0 or week 3. The effects of the fiber diets on plasma TC were observed at week 6. The OB diet showed the only significant decrease in plasma TC levels from week 3 (205 mg/dL) to week 6 (179 mg/dL) and was significantly lower than the HC (203 mg/dL) and FC (221 mg/dL) treatments at week 6. Although plasma TC levels for the PB animals (199 mg/dL) were not significantly different from the other treatments at week 6, they did approach significance with the FC and OB treatments.

With administration of the HC diet, all treatments showed significant increases in plasma TG levels from week 0 to week 3, with no differences between diet groups at week 3. Plasma TG levels were high (>200 mg/dL) at week 3, and remained so for the duration of the experiment. Plasma TG levels of OB animals decreased significantly from week 3 (298 mg/dL) to week 6 (200 mg/dL). At week 6, the only significant difference in plasma TG levels between treatments was found between the FC and OB diet groups, where OB plasma TG levels were lower than FC levels (358 mg/dL).

Plasma TC levels for pooled samples at week 3 appeared to be more than two times higher than those at week 0, and approximately equivalent between treatments. This general response was similar to individual plasma TC levels. The pooled plasma TC levels at week 3

were approximately 10% lower than the average of individual hamster plasma samples.

Plasma TC levels at week 6 appeared to increase from week 3 in all treatments except the OB diet group, which remained the same. The response of the pooled plasma TC levels to the diets was roughly the same as that seen in the individual plasma TC levels of hamsters in that the plasma TC levels of the OB animals were the lowest of the four treatments. Pooled plasma TC levels at week 6 were approximately 5-10% higher than individual hamster levels.

From week 0 to week 3, all of the various plasma lipoprotein fractions (TC, HDL-C, LDL-C, VLDL-C) increased, and all but the plasma LDL-C more than doubled. From week 3 to week 6, the effect of the experimental diets on plasma lipoproteins were observed. Plasma HDL-C levels generally remained the same, while plasma LDL-C levels increased across the treatments. Plasma VLDL-C levels were highly variable in their response from week 3 to week 6.

In conclusion, the results of this study indicated that the OB diet had beneficial results in reducing a modifiable CAD risk factor by significantly lowering plasma TC levels. Similar results using oat bran have been observed in hypercholesterolemic men. Studies using the rat have consistently shown significant decreases in plasma TC levels using oat bran with hypercholesterolemic-inducing diets. Although less numerous, research studies using the hamster have demonstrated the hypocholesterolemic action of oat bran supplementation on plasma TC levels as well.

Plasma TC levels for animals on the PB diet approached significance, and this absence of effect may have been associated with a lesser amount of total dietary fiber, and therefore soluble fiber, in the PB diet as compared to the OB diet. Pinto bean supplementation in humans has been shown to lower plasma TC levels in hypercholesterolemic subjects. Animal studies using beans or dietary fiber from beans to decrease plasma TC levels have been few with inconsistent results.

The OB diet was the only effective treatment to decrease plasma TG values. This response is similar to studies in humans, where fiber from oat bran has been found to lower serum TG levels in hypercholesterolemic men. Animal studies have shown no significant effect on plasma TG using oat bran. Oat gum (the water-soluble portion of oat bran), however, has been shown to significantly lower plasma TG in rats.

No effect on plasma TG levels was observed in the present study in hamsters consuming the PB diet. Similarly, bean supplemented diets have been shown to have no effect on serum TG levels in hypercholesterolemic men. As with plasma TC levels, animal studies using beans or dietary fiber from beans to decrease plasma TG levels are few with inconsistent results.

The confidence that we can put in the pooled plasma lipoprotein values is small, because statistical analysis was not feasible due to the small number of observations (n=2) per treatment. Pooled plasma lipoprotein values, however, were similar

to other studies using hamsters in response to the HC diet from week 0 to week 3 and to the fiber diets from week 3 to week 6.

This study demonstrated the need for further research to investigate the effect of dietary fiber from different sources on hypercholesterolemia in the hamster. The results from hamsters consuming a 53% oat bran diet in the present study correlate well with similar human studies. Results using a 30% pinto bean diet have a questionable correlation with simialr human studies, possibly because of the lesser amount of soluble dietary fiber as compared to the oat bran diet. Therefore, it appears that the hamster is a good small animal model for hyperlipidemia research, as well as for dietary fiber research, when using oat bran as the hypocholesterolemic agent. The use of pinto beans in this capacity needs to be investigated further.

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APPENDICES

APPENDIX A

LABORATORY METHODOLOGY - DIET ANALYSIS

DETERMINATION OF MOISTURE:

Procedure:

1. Weigh a small metal pan that has been dried in 100°C oven for 30 min and cooled in dessicator.
2. Weigh 1-2 grams sample into pan in duplicate.
3. Place pan and sample into 100°C oven for 8 hours or overnight.
4. Remove from oven and place in dessicator for at least 30 minutes to cool.
5. Weigh dried pan and dried sample together.

Calculations:

$$\text{weight dry sample} = \text{weight (dry pan + dry sample)} - \text{weight dry pan}$$

$$\% \text{ moisture} = \frac{\text{weight wet sample} - \text{weight dry sample}}{\text{weight wet sample}} \times 100$$

DETERMINATION OF ASH:

Determine percent ash on duplicate samples using the following method. Moisture has previously been expelled from sample at 100°C as above.

Procedure:

1. Weigh dry crucible.
2. Weigh 2-3 grams dry sample into ashing crucible. Sample should be ground or in powder form.
3. Place crucible in muffle furnace at 550 - 600 °C for at least 6-8 hours or overnight.
4. Cool in dessicator for 1 hour and weigh.

Calculations:

$$\% \text{ ash} = \frac{\text{weight (crucible + ash)} - \text{weight crucible}}{\text{weight sample}} \times 100$$

DETERMINATION OF PROTEIN - KJELDAHL:

Measure 0.5-2.0 grams of sample (equivalent to 15-40 mg of nitrogen) in duplicate. Use either a weighing pan or filter paper. Prepare two blanks (include filter paper if used).

Procedure:

Digestion:

1. Place sample in the Kjeldahl flask (if filter paper was used, fold sample and paper and place in flask).
2. Add 10 grams Na_2SO_4 - $CuSO_4$ mixture (1 measure) and 4 glass beads to each flask. Add this and filter paper to 2 clean, dry flasks to be used as blanks.
3. Pour 25 ml H_2SO_4 down the side of the flask (slowly turning flask as you pour) to wash down any sample adhering to the neck of the flask. If sample is larger than 2 grams, add 10 ml H_2SO_4 for each additional gram of sample.
4. Place flask on burners - turn heat to "2". Heat at this setting until samples stop frothing and begin to clear (less black in color). Throughout this period turn flasks frequently to rinse down carbonaceous material.
5. Turn heat to "4" and heat until sample turns clear green in color.
6. When all samples have turned clear green in color, turn heat up to "7" and heat for 30 mintues.
7. After 30 minutes, turn heat off and allow samples to cool on the heating rack until vapors are no longer apparent.
8. When flasks can be handled with your hand, move the flasks to the rack and stopper them tightly. Allow samples to cool completely.
9. When samples are completely cool, add 250 ml of distilled water. Add slowly at first, mixing during the addition. Re-stopper the flask.

Samples at this point may sit overnight. If you choose to continue with the distillation procedure on the same day, you must cool samples thoroughly before continuing on with the distillation.

Distillation:

1. Pour about 25 ml of 4% boric acid and 25 ml H_2O into 500 ml Erlenmeyer flask (use graduated cylinder).
2. Add 4 drops of mixed Indicator (methyl red-methylene blue).
3. Place flask under the distillation rack and insert the delivery tube (plastic or glass) under the surface of the liquid.
4. Turn on water to the condenser (manual). Check to be sure cold water is flowing.
5. Turn on the burners to "2" to let them warm up.

6. Swirl each flask to mix contents. To each Kjeldahl flask add approximately 1/16 teaspoon of granular zinc, then immediately add 70-80 ml of 50% NaOH - add slowly down the side of the flask to layer the NaOH below the diluted sulfuric acid digest.
7. Connect the flask with the distillation rack.
8. Swirl slowly and then vigorously to mix the contents of the flask.
9. Turn the heat up to "5" immediately and place the label on the receiving flask. If the mixture does not turn blue, then the acid was not neutralized, and more NaOH should be added to the samples.
10. Distill until about 200 ml are in the collection flask.
11. Lower the collection flask so the tube is out of the liquid. Distill to 225 ml total.
12. Turn off the heat.

Titration:

1. Titrate in order of coming off the distillation. Titrate each sample with 0.1N HCl. Titrate blanks first.
2. Turn off water (about 15 minutes after heat is turned off).
3. Titrate to a slight purple (lavender) color. Titrate all samples until the color matches the end-point of the blank.

Calculations:

One equivalent of HCl reacts quantitatively with one equivalent of N as ammonium borate. Therefore:

Normality of acid x 14.000 = mg of N equivalent to 1 ml of acid

Total N = (ml HCl - ml blank) x equivalent of N (above)

$$\% \text{ Protein} = \frac{\text{Total N (mg)} \times 6.25^{*}}{\text{sample weight}} \times 100$$

* Nitrogen is the element quantitatively determined in the Kjeldahl protein analysis. Protein, on the average, contains 16% nitrogen. To determine how much protein is in a sample we must multiply by a "protein factor" 6.25. This figure is derived by dividing 100 by 16.

DETERMINATION OF FAT - SOXHLET: (Forage Lab, VA Tech)

Procedure:

1. Put 2-3 grams of sample in 20g dry matter bottles.
2. Dry sample overnight (with top off) in 90°C oven.
3. Store in dessicator (with top on). Turn on heat and water.
4. Dry cups and thimbles in 90°C oven 15 minutes or more, tare. Weigh sample into thimbles (0.5-1.0 g) immediately after taking out of dessicator. Place in holder and then on instrument.
5. Measure out petroleum ether (50 ml) into cups. Place in holder and then on instrument.
6. Slowly lower thimble into petroleum ether. Open stopcock.
7. Let boil for 30 minutes.
8. Raise thimbles, let rinse in pet. ether 60 min.. NOTE: If boiling time has accidentally been varied, rinse time must always be double boiling time.
9. After rinsing, close condenser valves, collect solvent for 15 minutes.
10. Press air button and open evaporation valve for 15 minutes.
11. Turn off air button and close evaporation valve.
12. Remove cups and thimbles.
13. Place in 90°C oven for only 10 min.
14. Place in dessicator until cool and weigh.
15. If continuing to do another set, check level of pet ether in top and add more up to 50 ml mark if needed. Repeat above procedure.
16. Turn off heat. After 30 min. remove ether. Turn off water.

Note:
1. If sample is light (fluffy), use only 40 ml pet ether.
2. If sample is high in fat, use 0.25-0.5 grams.of sample. If sample is low in fat, use 1.0-2.0 grams of sample for determination.

Cleaning of equipment:
After weighing and calculating, clean cups with 80% ethanol. Knock out sample from thimbles and blow out residue with air hose.

Calculations:

$$\% \text{ Fat} = \frac{\text{weight sample - weight residue}}{\text{weight sample}} \times 100$$

DETERMINATION OF STARCH:

Reagents:

1. Buffer: 0.05M Citrate buffer
2. BCA Reagent:

   Solution A: 1.0g $CuSO_4 \cdot 5H_2O$ (cupric sulfate)
   3.7g aspartic acid
   in 1 liter water

   Solution B: 38.0g $Na_2CO_3 \cdot 10H_2O$ (sodium carbonate)
   2.0g BCA (2,2'-Bicinchoninate, sodium salt)
   in 1 liter water

   Mix solutions A and B 1:1 before using, filter, keep in dark
3. GAM
4. Glucose standard 0.01mg/ml

Procedure:

1. Weigh 10.0 mg sample into 25.0 ml volumetric in duplicate (one for control, one for sample). Also, weigh 10.0 mg cornstarch into 25.0 ml volumetric flask.
2. Add 2 ml water to each volumetric.
3. Add buffer to each sample: 2.25 ml to controls, 2.0 ml to samples and cornstarch.
4. Boil 10 minutes, cool.
5. Add 0.25 ml GAM (diluted 0.5 ml + 9.5 ml buffer) to samples and cornstarch, but not to controls.
6. Incubate at room temperature for 4 hours.
7. Bring controls and samples up to 25.0 ml with water. Bring cornstarch solution up to 250 ml with water.
8. Run blank, standards, and samples in duplicate. In test tubes:

   Blank: 1.0 ml water
   Standards: Glucose - 2.5, 5.0, 7.5 micrograms/ml in a volume of 1 ml.
   Sample: 0.05 ml sample
   0.95 ml water
   Sample Control: 0.05 ml sample (no GAM added)
   0.95 ml water

9. Add 2.0 ml BCA Reagent to each test tube, vortex.
10. Boil 5 minutes, cool in tap water.
11. Read Absorbance at 550 nm.

Calculations:

1. Make a standard curve from glucose standards.
2. Determine concentration of starch in samples from standard curve, correcting for controls.
3. Calculate percent starch in sample by correcting concentration in sample for dilution factors, cornstarch recovery, and moisture content.

DETERMINATION OF CELL WALL SUGARS BY GAS CHROMATOGRAPHY AFTER HYDROLYSIS WITH 1N H_2SO_4:

Reagents:

1. 1N H_2SO_4

2. GC Reagent - Internal Standard:

   a. 100 mg methyl glucopyranoside in 10.0 ml 1-methylimidazole
   b. 0.1 ml methyl glucopyranoside solution (a) above
      0.5 g Hydroxylamine
      19.9 ml 1-methylimidazole

   Internal Standard = Equivalent 0.1 mg/ml when used in derivatization system

3. Standard Solutions:

   a. Arabinose-xylose-glucose-galactose: 0.5 mg/ml in 1N H_2SO_4
   b. Rhaminose-fructose-mannose: 0.2 mg/ml in 1N H_2SO_4

Procedure:

Standard solutions are derivatized by the same procedure

1. Weigh 10.0 mg sample into a 5.0 ml React-Vial.
2. Add 3.0 ml 1N H_2SO_4.
3. Heat on a heating-stirring module at 100°C for 4 hours, stirring slowly. Remove from heat and cool.
4. To derivatize, remove 0.2 ml from hydrolyzed sample into another 5.0 ml React-Vial with stir bar.
5. Add 0.4 ml of GC Reagent (b).
6. Heat on module at 80°C for 10 minutes stirring slowly. Remove and cool.
7. Add 1.0 ml acetic anhydride (in hood) carefully.
8. Mix and hold at room temperature for 5 minutes.
9. Add 1.0 ml chloroform, mix well.

10. Add 1.0 ml water, mix, remove water layer with Pasteur pipet and discard. Repeat water wash.
11. Add 0.4 g sodium sulfate and mix.
12. Remove stir bar and with glass rod, push sodium sulfate into cone of React-Vial.
13. Inject 2 microliters of standard solutions at least once.
14. Inject 2 microliters of the test sample at least 3 times.

Calculations:

1. Calculate the Area Ratio for each sugar in the standard and test solution:

$$\text{Area Ratio} = \frac{\text{counts for sugar}}{\text{counts for internal standard}}$$

2. Calculate the concentration of each identifiable sugar in the test solution:

$$\text{mg/ml} = \frac{\text{area ratio for sugar in sample}}{\text{area ratio for sugar in standard}} \times \text{mg/ml sugar in standard}$$

3. Calculate the percent of each sugar in original plant material:

$$\% \text{ sugar} = \frac{\text{mg/ml sugar}}{3.33^*} \times 100$$

* 3.33 represents the mg/ml of sample in the original acid hydrolysate. A sample of 10 mg of cell wall containing material was hydrolyzed in 3.0 ml of 1N sulfuric acid, as described above.

DETERMINATION OF CELLULOSE:

Reagents:

1. Acetic Nitric Reagent: 150 ml 80% acetic acid
   15 ml concentrated nitric acid
2. 67% H_2SO_4
3. Standard: 0.1 mg/ml Glucose
4. Cellulose Standard: a 100 ml sample containing from 0.5 to 2 grams of cellulose is ordinarily used.
5. Anthrone Reagent: 0.2 g Anthrone
   100 ml concentrated H_2SO_4
   Prepared fresh daily. Chill about 2 hours in refridgerator prior to use.

Procedure:

1. Weigh samples into 15.0 ml clinical centrifuge tubes. Run in duplicate: blank (0 micrograms cellulose), 10, 20, 40 microgram glucose standards, and cellulose standard.
2. Add 3.0 ml acetic nitric reagent one ml at a time, vortexing after each addition.
3. Stopper tubes with plastic volumetric flask stoppers.
4. Place in boiling water bath 30 minutes. Maintain bath water level at the same level as the liquid in the tubes. Cool.
5. Centrifuge 5 minutes at high speed. Decant and discard supernatant.
6. Add 10 ml distilled water wash in a manner similar to step 2.
7. Centrifuge 5 min at high speed. Decant and discard supernatant.
8. Repeat water wash.
9. Add 10 ml 67% H_2SO_4 (v/v) in a manner similar to step 2.
10. Let stand one hour.
11. Dilute samples 1 ml to 10 ml with distilled water. Centrifuge if any precipitate or turbidity is present.
12. Use 5.0 ml to run blanks and glucose standards. Use 2.5 ml to run Pinto Bean sample, 5.0 ml to run Plain Oat Bran sample.
13. Place tubes in an ice bath to cool.
14. Slowly add 10 ml cold anthrone reagent by layering with pipet.
15. Mix well on vortex mixer (parafilm cover). Return to ice bath until all tubes are mixed.
16. Place a marble on top of each tube to reduce evaporation and create a refluxing action. Place tubes in a boiling water bath for 16 minutes.
17. Cool in ice bath 2-3 minutes. Let stand at room temperature 5 to 10 minutes.
18. Read absorbance at 620nm against a reagent blank.

Calculations:

1. Prepare a standard curve from glucose standard solutions (concentration vs Absorbance 620nm).
2. Determine concentration of samples from standard curve.
3. Correct for dilution factors.
4. Correct for cornstarch recovery.

DETERMINATION OF PECTIN (HEXURONIC ACID):

Reagents:

1. Chilled concentrated H_2SO_4
2. 2% NaCl
3. Dimethyl Phenol: 0.1 g 3,5-dimethylphenol in 100 ml glacial acetic acid

Procedure:

1. Place 10 ml beakers in ice-filled container.
2. Weigh 5.0 mg sample into beaker.
3. Add 2.0 ml chilled H_2SO_4 to sample in beaker. Stir 2 minutes.
4. Add 0.5 ml water slowly and stir 5 minutes. Repeat 2 times.
5. Carefully transfer sample to a 10 ml volumetric flask, rinsing out beaker several times with water and pouring rinse into volumetric flask.
6. Bring 10 ml volumetric flask up to volume.
7. Make up test tubes for the following samples and standards using 0.2 mg/ml galacturonic acid in duplicate (measure accurately):

   a. Blank - 0 micrograms galacturonic acid
   b. Standards - 4, 8, 12, 16 micrograms galacturonic acid
   c. Samples - 125 microliters sample

8. To above test tubes add 125 microliters 2% NaCl.
9. Add 2.0 ml H_2SO_4. Vortex.
10. Heat 10 minutes at 70°C. Cool in room temperature water bath 2 minutes.
11. Add 0.1 ml Dimethyl Phenol (in glacial acetic acid). Vortex slowly.
12. Let samples sit 20 minutes before reading.
13. Read absorbance at 450nm and 400nm.

Calculations:

Abs 450nm - Abs 400nm = Abs to be read from curve

1. Create a standard curve
2. Find concentration of samples from Absorbance on curve (see above)
3. Correct for dilutions and multiply by 100 to obtain % in sample.

DETERMINATION OF SOLUBLE SUGARS BY HPLC:

Reagents:

80% EtOH

Procedure:

1. Weigh 2.0 grams sample in duplicate into round-bottom 100 ml volumetric flask.
2. Add 25.0 ml 80% EtOH, a few boileezers, and a magnetic stirring bar.
3. Reflux 30 minutes using hotplate and stirring slowly.
4. Remove and cool.
5. Filter through #541 (hardened ashless) filter paper.
6. Add 5.0 ml 80% EtOH to wash residue into a 100 ml beaker. Repeat 4 times to equal a total of 25 ml EtOH.
7. Dry down in hood using N_2; continue drying overnight without N_2.
8. Heat water to 80°C. Add 5 ml to beaker.
9. Transfer to 10 ml volumetric flask using hot water to rinse out beaker.
10. Allow to cool, make up to volume with water.
11. Remove 1 ml and deionize with 0.2 ml old Resin. Filter.
12. If samples are still cloudy, remove 4.0 ml and centrifuge 20,000G for 10-15 minutes.
13. Run on HPLC.
14. Run standards for glucose, fructose, maltose, and sucrose on HPLC.

Calculations:

1. Create a standard curve for each sugar.
2. Determine concentrations of sugars by comparing on standard curve.
3. Correct for dilutions and multiply by 100 to obtain % sugar in sample.

DETERMINATION OF INSOLUBLE AND SOLUBLE DIETARY FIBER:

Reagents:

1. Heat Stable alpha amylase
2. Protease
3. Amyloglucosidase
4. Petroleum Ether - reagent grade (if necessary)
5. Ethanol 95% (v/v) technical grade
6. Ethanol 78% (v/v): 207 ml H_2O + 793 ml 95% ethanol
7. Phosphate buffer - 0.08 M, pH=6.0:
   1.40 g Na_2HPO_4 + 9.86 g NaH_2PO_4 + 700 ml H_2O
   Dilute to 1 liter with H_2O and adjust pH to 6.0 with either NaOH or H_3PO_4
8. NaOH - 0.275 N
   275 ml 1.0 N NaOH diluted to 1 liter with H_2O
9. HCl - 0.325 N
   325 ml 1.0 N HCl diluted to 1 liter with H_2O
10. Celite 545 - acid washed
11. Acetone - reagent grade

Procedure:

1. Run all samples in duplicate.
2. Homogenize and dry sample overnight in a 70°C vacuum oven, cool in dessicator and grind to 0.3-0.5 mm mesh.
3. Clean crucibles thoroughly, heat at 525°C for one hour, cool to room temperature, soak and rinse in water.
4. Air dry crucibles, add 0.5 g celite to each crucible and dry at 130°C to constant weight (about 1 hour). Cool and store in dessicator until needed.
5. Run blanks through the entire procedure (two for soluble residue, two for insoluble residue) along with samples to measure any contribution from reagents to residue. Mix 1.0 g sample and 50 ml Phosphate Buffer, pH=6.0 in a 400 ml tall form beaker.
6. Add 50 microliters heat stable alpha-amylase, mix well, cover with aluminum foil and incubate in boiling water bath for 30 minutes. The incubation time starts when the beaker contents reach 90°C.
7. Cool to room temperature (approx. 20°C) and adjust pH to 7.5 with 0.275 N NaOH (approx. 10 ml).
8. Add 50 microliters protease, mix well and incubate at 60°C for 30 minutes with continuous agitation. The incubation time starts when beaker contents reach 60°C.
9. Cool to room temperature and adjust pH to 4.5 with 0.325 N HCl (approx 10 ml).

10. Add 150 microliters amyloglucosidase, mix well, cover with aluminum foil and incubate at 60°C for 30 minutes with continuous agitation. The incubation time starts when beaker contents reach 60°C.
11. Cool to room temperature.
12. Filter contents (enzyme digest) through Pyrex Astm 40-6- filter containing 0.5 g celite which has been pre-wetted with 78% EtOH. (see step 23).
13. Save supernatant (contains soluble residue).
14. Wash precipitate (insoluble residue) with 2 x 5 ml water and add to supernatant.
15. Wash precipitate with: 3 x 20 ml 78% EtOH
    2 x 10 ml 95% EtOH
    2 x 10 ml acetone
    DO NOT add these washes to supernatant.
16. Dry precipitate in air oven overnight at 105°C.
17. Cool in dessicator and weigh.
18. Save for kjeldahl and ash assays.
19. Heat supernatant (containing soluble fiber fraction) to 60°C.
20. Add 325 ml 95% EtOH which has been pre-heated to 60°C (measure before heating) to supernatant.
21. Let precipitate form at room temperature (approx. 20°C) overnight.
22. Weigh crucible containing glass fiber filter paper and 0.5 g Celite to nearest 0.1 mg. Wet and redistribute bed of Celite by using stream of 78% EtOH from wash bottle. Apply suction to draw Celite onto fritted glass as an even mat. Maintain suction and quantitatively transfer precipitate to crucible.
23. Wash residue with: 3 x 20 ml 78% EtOH
    2 x 10 ml 95% EtOH
    2 x 10 ml acetone
24. Dry crucible overnight in 105°C air oven.
25. Cool in dessicator and weigh.
26. Save to perform Kjeldahl and ash.

Calculations:

weight dry residue (insoluble) =

weight (crucible + Celite + residue) - weight (crucible + Celite)

weight dry residue (soluble) =

weight (crucible + Celite + filter paper + residue) -

weight (crucible + Celite + filter paper)

Subtract weight of blank residue from both measurements to determine actual weight residue.

Determination of Fiber Ash:

Procedure:

1. Incinerate one sample and one blank from each of soluble and insoluble procedures for 5 hours at 525°C in muffle furnace.
2. Cool in dessicator and weigh to nearest 0.1 mg.

Calculations:

weight ash (insoluble) =

weight (crucible + Celite + ash) - weight (crucible + Celite)

weight ash (soluble) =

wt (crucible + Celite + filter + ash) - wt (crucible + Celite + filter)

Determination of Fiber Protein (Kjeldahl):

Reagents:

1. 0.1 N HCl
2. 0.05 N NaOH
3. Concentrated sulfuric acid
4. Tashiro Indicator: 0.1% methyl red
   0.05% methylene blue
   Solvent: alcohol

   Transition pH=5.4

   Acid color: red-violet
   Transition color: dirty blue
   Basic color: green

Procedure:

1. Run the following samples:

   a. Kjeldahl blank
   b. Glycine compound (to determine recovery)
   c. Fiber blank: - insoluble
      - soluble (include filter paper)
   d. Fiber sample: - insoluble
      - soluble (include filter paper)

2. To all add: 40 mg $CuSO_4$
   11 g K_2SO_4
   Stir bar
   5 glass beads

   To Glycine compound add: 0.5 G Celite + 25 mg glycine compound

   To Kjeldahl blank add: 0.5 g Celite

3. After adding all dry ingredients, add 15 ml concentrated sulfuric acid to all.
4. Mix immediately so Celite does not clump. Keep mixing 5-10 minutes.
5. Put in block digestor 410°C (no lid) in hood for 45 minutes - make sure mixing continues.
6. Take out of block digestor and set on stir plate so does not crystallize.
7. Cool 10 minutes.
8. Add 100 ml distilled water very slowly with intermittant stirring. Allow to cool while stirring, until just warm to the touch.
9. Add 50 ml 50% NaOH and distill with steam distiller to 100-120 ml into Ehrlenmeyer flask containing:

   4 ml 0.1 N HCl
   16 ml dH_2O
   10 drops Tashiro Indicator

10. Titrate to endpoint with 0.05 N NaOH

Calculations:

1. Calculate for all blanks, glycine cpd and samples:

   net ml for sple = ml HCl needed for titration (fiber sample)
   - ml HCl needed for titration (kjeldahl blank)

2. Calculate for fiber samples and fiber blanks:

   a. $\%\ \text{Nitrogen} = \dfrac{(\text{net ml for sple})(0.1\ \text{N})(14\ \text{g/mole})}{\text{sample weight}} \times 100$

   b. % Protein = (6.25)(% Nitrogen)

3. Calculate for each fiber sample:

   % Protein (fiber sample) = % protein (sample) - % protein (fiber blank)

4. Calculate recovery from glycine compound results
5. Correct % Protein (fiber sample) for recovery:

actual % Protein (sample) = % protein (fiber sple)(% recovery)

Calculations for Insoluble and Soluble Fiber in Samples:

actual % fiber in samples = % fiber in sample (from fiber procedure)
- % ash (from fiber ash procedure)
- % protein (from fiber Kjeldahl)

LABORATORY METHODOLOGY - BLOOD ANALYSIS

ORBITAL SINUS BLEEDING PROCEDURE:

1. Allow 8 hour fast and then administer 15cc ketamine to hamster, allow 5-10 minutes to take effect. Halothane can also be used - use enough in ventilator for hamster to become unconscious, continue procedure immediately.
2. Holding animal on flat surface, the operator's thumb is used to apply pressure to the external jugular vein. The forefinger of the same hand is used to pull the dorsal eyelid back.
3. A heparinized capillary tube is then used to penetrate the orbital conjunctiva and rupture the orbital sinus.
4. Collect blood into a test tube containing EDTA to avoid coagulation.
5. Blood flow will cease when the capillary tube is released and pressure is removed from the external jugular vein.
6. Animals completely recover within 24 to 48 hours, allowing for serial sampling on a routine basis.

DETERMINATION OF PLASMA TOTAL CHOLESTEROL (TC):

Reagents:

1. Cholesterol reagent - mix under hood in an ice bath the day before needed. Combine in the following order and add slowly to the reagent bottle - DO NOT MIX - very strong reaction possible - USE CAUTION:

   1245 ml acetic anhydride
   1245 ml glacial acetic acid
    408 ml sulfiric acid
    102 ml phosphoric acid

   put reagent bottle in ice overnight in the refridgerator. After solution has cooled, mix gently before using. Allow to come to room temperature before using to assure accurate measurements, and keep refridgerated after use.

2. Cholesterol working standards: (for standard curve)

    50 mg/dl
   100 mg/dl
   200 mg/dl
   400 mg/dl

3. Plasma cholesterol standard

Procedure:

1. Accurately transfer 0.025 ml of distilled water (blank), 0.025 ml of each of the four cholesterol working standards - 50, 100, 200, 400 mg/dl (standards), 0.025 ml of plasma standard (plasma standard), 0.025 ml of unknown plasma from hamster (sample) in duplicate, into appropriately labelled test tubes.
2. Add 1.5 ml Cholesterol Reagent to each test tube and vortex well.
3. Place all test tubes in a 37°C water bath for 20 minutes.
4. Remove all test tubes from the water bath and vortex thoroughly.
5. Transfer contents of test tubes into microcuvettes and read standards and samples against the reagent blank at 625 nm within 30 minutes.

Calculations:

1. Plot optical density versus concentration of working standards on graph paper or calculator
2. Read values of plasma control and unknowns directly from the standard curve

DETERMINATION OF PLASMA TRIGLYCERIDES (TG):

Kit: Stanbio kit for Quantitative Fully Enzymatic Colorimetric Determination of Serum or Plasma Triglycerides by Glyceryl phosphate Oxidase - Procedure #2000.

Reagents:

1. Enzymatic Triglycerides Reagent GPO (lyophilized)
2. Enzymatic Triglycerides Buffer GPO
3. Enzymatic Triglycerides Standard GPO, 200 mg/dl

Procedure:

1. Reconstitute reagents as in kit directions.
2. Pipet accurately into test tubes the following volumes in duplicate:

   Blank - add 1 ml of TG Reagent
   Standard - add 1 ml of TG Reagent + 0.01 ml of TG Standard (200 mg/dl)
   Sample - add 1 ml of TG Reagent + 0.01 ml of unknown plasma sample

3. Incubate all test tubes at room temperature for 10 minutes.
4. Use Reagent Blank to zero the spectrophotometer at 500nm

5. Read absorbance of Standard and Samples at 500nm within 60 minutes.

Calculations:

Values are derived by the following equation:

plasma TG (mg/dl) = Au/As x 200

where Au and As are the absorbance values of unknown and standard, respectively, and 200 is the concentration of the standard (mg/dl).

DETERMINATION OF PLASMA HDL-CHOLESTEROL (HDL-C):

Reagents:

1. Manganese Chloride Solution 1.06M: ($MnCl_2 \cdot 4H_2O$ MW=197.91) Weigh out 209.78g $MnCl_2 \cdot H_2O$ and dissolve in a small amount of distilled water. Dilute to 1 liter volume with distilled water. This solution is thought to be stable indefinitely.
2. Sodium Chloride 0.15M (saline): Weigh out 8.77g NaCl and dissolve in about 500 ml water. Bring up to 1 liter volume with distilled water.
3. Heparin 40,000 units/ml: Commercial heparin preparations contain different strengths of heparin by weight (units per mg). CHECK LABEL ON HEPARIN VIAL BEFORE PREPARATION:

   If heparin preparation contains 140 units/mg, weigh out 0.286g heparin and dissolve in 1 ml 0.15M saline. Since the volume is so slight, use a very small glass vial to prepare solution. The heparin is hard to dissolve so it will be necessary to vortex the solution vigorously. Before removing an aliquot for the combined reagent, let the heparin solution set awhile and check to make sure all the heparin is in solution. Prepare fresh for each run.

4. Combined heparin-manganese reagent: Add 0.6 ml 40,000 units heparin/ml to 10 ml 1.06M $MnCl_2 \cdot 4H_2O$. Prepare fresh for each run.

Procedure:

1. Remove plasma samples from refrigerator and allow to warm to room temperature.
2. Mix plasma samples well. Using a calibrated micropipet, transfer 0.1 ml plasma into a small plastic conical centrifuge tube. Run in duplicate, if possible.
3. Vortex combined heparin-manganese reagent well. Transfer 0.01 ml combined reagent into each centrifuge tube. A precipitate will form immediately.
4. Vortex each tube lightly and allow the tubes to stand at room temperature for 10 minutes.
5. Centrifuge tubes in refrigerated centrifuge (4°C) at 3000 rpm for 10 minutes. A hard pellet will form on the bottom of the tube.

   NOTE: It is necessary to use a swinging bucket rotor for this procedure in order to form a pellet which is lodged in the bottom of the conical centrifuge tube. If a fixed-angle rotor is used, a firm pellet will not form and the precipitate will smear along the sides of the tube.

6. Supernatants may be directly decanted, but to avoid possible contamination, remove most of the supernatant with a disposable pipet and store in a 7 ml vial.
7. HDL are suspended in the supernatant and are determined the same as the plasma TC, using the Cholesterol Reagent as described earlier. Prepare cholesterol working standard solutions of 25, 50, 100, and 200 mg/dl. Analyze in duplicate.

Calculations:

Calculate HDL-C the same as in the determination of TC.

DETERMINATION OF LOW DENSITY LIPOPROTEIN CHOLESTEROL (LDL-C) AND VERY LOW DENSITY LIPOPROTEIN CHOLESTEROL (VLDL-C) IN POOLED PLASMA SAMPLES:

LRC Quantitation of Lipoproteins Using Ultracentrifuge: ultra-centrifugal separation should be carried out as soon as the samples are taken, but samples may be stored at 4°C for up to 5 days.

Equipment:

1. Beckman Preparative Ultracentrifuge, Model L2-65 (or later equipment).
2. Model 50.3 Ti rotor - store in refridgerator.

Reagents:

1. LRC Saline: 0.15M saline containing 0.02% EDTA (pH=7.4):

   8.77g NaCl (reagent grade)
   0.10g EDTA

   Bring up to 1 liter with distilled water. Make to pH=7.4 with 2N HCl. Cool to 4°C for use.

Procedure:

1. Precool rotor to 10°C in ultracentrifuge.
2. Label centrifuge tubes with subject number and mark a line for the saline overlay such that the overlay reaches a mark that is just below tha base of the caps. Next, measure 1/2-5/8 inches below the first line and make a line here also. This denotes the VLDL-C fraction.
3. Place the labeled tubes in a rack.
4. Assemble the centrifuge tube caps. Check for defective O-rings, and replace if they appear to be worn or cracked.
5. Allow samples to warm to room temperature (approximately 23°C) and pipet 3.0 ml pooled plasma + 2.0 ml saline (or other known volume to equal up to 5 mls) into the appropriately labeled centrifuge tube.
6. Carefully overlay the plasma with the cooled LRC Saline such that the overlay reaches the designated mark. Use a pasteur pipet for the overlay and be careful not to disturb the plasma layer.
7. Cap the tubes.
8. Place the tubes in the pre-cooled rotor such that the rotor is precisely balanced.
9. Seal the rotor tightly and load into the ultracentrifuge.
10. Run ultracentrifuge at 40,000 rpm at 10°C for 18 hours.
11. When spin is complete, remove tubes from rotor.

12. Carefully remove the caps from the tubes, allowing any liquid in the cap to run into the tube. Using a Pasteur pipet, rinse cap with 1.0 ml LRC Saline, allowing solution to drip into a 5 ml volumetric flask (one for each sample).
13. Using a Pasteur pipet, remove the lipid layer at the top. Go down to the second mark (1/2-5/8 inch from the cap mark) on the tube. All that you remove here should go into the 5 ml volumetric flask that has the cap washing in it. When removing this layer carefully guide the pipet around the tubes liquid surface.
14. Bring the liquid in the 5 ml volumetric up to volume with the chilled LRC Saline. Stopper the flask and invert to mix. Transfer the liquid to an appropriately labelled storage vial. This is the VLDL-C fraction. The VLDL-C fractions should be removed within 1 hour of stopping the ultracentrifuge.
15. To prepare the LDL-C and HDL-C fraction:

    Using a glass stirring rod, loosen the material from the bottom and sides of the ultracentrifuge tube and pour the fluid into a 10 ml graduated cylinder. Wash the tube with approximately 1 ml of the cooled LRC Saline a couple of times, using the glass stirring rod for mixing in the tube. Add these washings to the graduated cylinder. Bring the volume up to 5 ml with the LRC Saline. Mix the contents of the graduated cylinder with the glass stirring rod and pour the contents into a labeled storage vial.

16. Store the samples at 4°C until analysis (fractions may be frozen at -20°C).
17. Analyze samples using the Cholesterol Reagent as with the TC and HDL-C samples.

Calculations:

1. Calculate the mg/dl of (HDL-C + LDL-C) by correcting the measured Absorbance for the volume of plasma used:

$$\text{mg/dl (HDL-C + LDL-C)} = \frac{\text{Abs (HDL+LDL) fraction}}{\text{volume plasma used (ml)}} \times 5 \text{ ml}$$

2. Calculate mg/dl LDL-C as follows:

$$\text{mg/dl LDL-C} = \text{mg/dl (HDL-C + LDL-C)} - \text{mg/dl HDL-C}^*$$

* determined by direct precipitation of pooled plasma sample as in "Determination of plasma HDL-C".

3. Calculate VLDL-C as follows: (VLDL-C was calculated by difference because concentrations were so low that detection by spectrophotometry was inaccurate)

mg/dl VLDL-C = mg/dl TC - mg/dl (HDL-C + LDL-C)

APPENDIX B

Individual Average Feed Intake (grams/day) of Hamsters for Weeks 0-3 and Weeks 4-6

SUBJECT	GROUP	WEEKS 0-3	WEEKS 3-6
59	HC	7.5	6.7
19	HC	7.8	6.5
50	HC	7.7	6.8
64	HC	7.7	7.8
70	HC	9.2	8.0
57	HC	8.0	7.9
29	HC	9.0	9.3
74	HC	9.6	7.8
12	HC	9.9	8.2
8	HC	8.1	8.7
73	HC	7.3	6.0
42	HC	7.3	7.0
65	HC	7.9	6.8
47	HC	6.8	---
15	FC	8.0	7.2
52	FC	14.6	11.6
6	FC	11.1	8.3
7	FC	7.5	7.0
71	FC	7.2	5.6
58	FC	7.7	7.2
21	FC	7.7	6.3
18	FC	6.5	6.9
16	FC	7.8	6.7
1	FC	8.1	7.1
17	FC	8.8	7.3
5	FC	8.9	6.9
30	FC	9.0	7.8
22	FC	13.0	---

HC = Hypercholesterolemic control
FC = Fiber control
OB = Oat bran
PB = Pinto bean
-- = expired hamster

APPENDIX B (continued)

Individual Average Feed Intake (grams/day) of Hamsters for Weeks 0-3 and Weeks 4-6

SUBJECT	GROUP	WEEKS 0-3	WEEKS 3-6
62	OB	8.3	7.0
49	OB	8.0	6.3
68	OB	9.7	7.9
24	OB	6.8	6.5
25	OB	8.0	6.9
66	OB	6.4	6.9
26	OB	8.0	7.0
27	OB	8.4	7.8
38	OB	8.2	6.4
69	OB	6.9	6.6
61	OB	7.2	6.6
37	OB	7.8	7.4
72	OB	5.5	6.1
45	OB	8.0	7.7
53	PB	7.0	6.9
67	PB	8.5	7.3
10	PB	8.9	7.7
60	PB	7.4	7.1
40	PB	9.0	8.7
20	PB	7.2	7.0
32	PB	9.2	7.9
4	PB	8.1	7.5
33	PB	12.1	9.1
2	PB	7.4	6.6
43	PB	8.7	8.3
51	PB	7.6	7.0
36	PB	8.7	7.2
54	PB	8.3	---

HC = Hypercholesterolemic control
FC = Fiber control
OB = Oat bran
PB = Pinto bean
-- expired hamster

APPENDIX C

Average Weight (grams) of Hamsters at Weeks 0, 3, 6

Group*	Week 0	Week 3	Week 6
		Mean ± SEM	
HC (n=13)	116 ± 3.2	133 ± 3.1	137 ± 3.1[ab]
FC (n=13)	113 ± 3.2	131 ± 3.1	132 ± 3.1[a]
OB (n=14)	114 ± 3.0	130 ± 2.9	140 ± 2.9[ab]
PB (n=13)	110 ± 3.3	129 ± 3.2	142 ± 3.2[b]

* HC = Hypercholesterolemic control
FC = Fiber control
OB = Oat bran
PB = Pinto bean

[ab] means within columns with different superscripts are significantly different at $p<0.05$

APPENDIX D

Individual Body Weight (grams) of Hamsters Every Three Weeks

SUBJECT	GROUP	WEEK 0	WEEK 3	WEEK 6
59	HC	99	116	114
19	HC	103	128	135
50	HC	117	130	127
64	HC	118	136	142
70	HC	120	145	152
57	HC	125	133	138
29	HC	117	131	139
74	HC	144	153	156
12	HC	126	138	148
8	HC	119	129	136
73	HC	124	136	136
42	HC	85	106	110
65	HC	108	142	150
47	HC	112	124	---
15	FC	122	141	143
52	FC	115	130	127
6	FC	115	124	124
7	FC	119	139	138
71	FC	129	135	131
58	FC	105	124	126
21	FC	99	117	119
18	FC	115	126	135
16	FC	85	119	121
1	FC	115	130	132
17	FC	122	145	144
5	FC	110	133	131
30	FC	112	141	144
22	FC	109	127	---

HC = Hypercholesterolemic control
FC = Fiber control
OB = Oat bran
PB = Pinto bean
-- = expired hamster

APPENDIX D (continued)

Individual Body Weight (grams) of Hamsters Every Three Weeks

SUBJECT	GROUP	WEEK 0	WEEK 3	WEEK 6
62	OB	125	146	150
49	OB	119	136	143
68	OB	130	149	158
24	OB	119	134	142
25	OB	111	130	139
66	OB	108	115	130
26	OB	115	132	146
27	OB	110	129	137
38	OB	118	138	140
69	OB	113	125	136
61	OB	111	124	131
37	OB	116	130	146
72	OB	104	108	123
45	OB	104	128	140
53	PB	114	135	139
67	PB	133	152	164
10	PB	103	125	142
60	PB	104	123	135
40	PB	127	148	158
20	PB	101	119	129
32	PB	113	119	138
4	PB	99	115	129
33	PB	104	126	137
2	PB	101	123	134
43	PB	127	155	165
51	PB	101	117	127
36	PB	105	134	143
54	PB	116	133	---

HC = Hypercholesterolemic control
FC = Fiber control
OB = Oat bran
PB = Pinto bean
-- = expired hamster

APPENDIX E

Average Weight Gain of Hamsters (grams) for Weeks 0-3 and Weeks 4-6

Group*	Week 0-3	Week 3-6
	Mean ± SEM	
HC (n=13)	16.9 ± 1.9	4.4 ± 1.1[a]
FC (n=13)	18.4 ± 1.9	0.9 ± 1.1[b]
OB (n=14)	16.1 ± 1.8	9.8 ± 1.0[c]
PB (n=13)	19.9 ± 1.9	11.4 ± 1.1[c]

* HC = Hypercholesterolemic control
FC = Fiber control
OB = Oat bran
PB = Pinto bean

[abc] means within columns with different superscripts are significantly different at $p<0.05$

APPENDIX F

Individual Plasma Total Cholesterol Levels (mg/dL) of Hamsters Every Three Weeks

SUBJECT	GROUP	WEEK 0	WEEK 3	WEEK 6
59	HC	92	259	233
19	HC	94	246	181
50	HC	109	237	211
64	HC	67	221	204
70	HC	72	179	193
57	HC	85	176	188
29	HC	73	171	212
74	HC	81	213	226
12	HC	109	212	215
8	HC	94	207	255
73	HC	67	202	160
42	HC	88	193	180
65	HC	73	184	188
47	HC	98	190	---
15	FC	93	259	293
52	FC	81	239	206
6	FC	97	227	221
7	FC	103	224	289
71	FC	73	183	235
58	FC	100	177	165
21	FC	48	162	151
18	FC	72	213	238
16	FC	72	213	238
1	FC	119	202	218
17	FC	88	193	220
5	FC	85	189	224
30	FC	99	188	177
22	FC	87	202	---

HC = Hypercholesterolemic control
FC = Fiber control
OB = Oat bran
PB = Pinto bean
-- = expired hamster

APPENDIX F (continued)

Individual Plasma Total Cholesterol Levels (mg/dL) of Hamsters Every Three Weeks

SUBJECT	GROUP	WEEK 0	WEEK 3	WEEK 6
62	OB	77	253	211
49	OB	87	250	211
68	OB	87	230	156
24	OB	101	221	207
25	OB	79	178	164
66	OB	85	171	163
26	OB	50	165	142
27	OB	102	218	223
38	OB	69	207	185
69	OB	80	206	158
61	OB	90	202	174
37	OB	57	193	201
72	OB	92	189	144
45	OB	72	184	173
53	PB	82	185	181
67	PB	76	251	227
10	PB	83	247	218
60	PB	87	235	205
40	PB	66	224	213
20	PB	59	174	199
32	PB	70	163	158
4	PB	85	215	204
33	PB	64	208	227
2	PB	90	203	224
43	PB	98	196	186
51	PB	74	196	196
36	PB	59	190	151
54	PB	70	183	---

HC = Hypercholesterolemic control
FC = Fiber control
OB = Oat bran
PB = Pinto bean
-- = expired hamster

APPENDIX G

Individual Plasma Triglyceride Levels (mg/dL) of Hamsters Every Three Weeks

SUBJECT	GROUP	WEEK 0	WEEK 3	WEEK 6
59	HC	56	269	198
19	HC	62	266	181
50	HC	104	255	279
64	HC	41	299	402
70	HC	70	268	262
57	HC	54	193	278
29	HC	122	149	245
74	HC	76	179	331
12	HC	48	193	471
8	HC	86	164	470
73	HC	91	307	157
42	HC	56	108	202
65	HC	82	222	236
47	HC	45	141	---
15	FC	148	188	603
52	FC	74	369	429
6	FC	106	209	180
7	FC	98	486	1035
71	FC	99	233	380
58	FC	75	336	189
21	FC	102	87	163
18	FC	104	193	265
16	FC	72	166	196
1	FC	135	337	231
17	FC	68	454	339
5	FC	110	171	365
30	FC	158	269	282
22	FC	101	178	---

HC = Hypercholesterolemic control
FC = Fiber control
OB = Oat bran
PB = Pinto bean
-- = expired hamster

APPENDIX G (continued)

Individual Plasma Triglyceride Levels (mg/dL) of Hamsters Every Three Weeks

SUBJECT	GROUP	WEEK 0	WEEK 3	WEEK 6
62	OB	79	803	504
49	OB	62	369	195
68	OB	75	454	260
24	OB	55	212	162
25	OB	47	140	140
66	OB	65	213	142
26	OB	51	192	87
27	OB	86	350	222
38	OB	72	302	237
69	OB	85	326	124
61	OB	57	137	96
37	OB	90	264	389
72	OB	54	242	120
45	OB	134	176	118
53	PB	73	251	254
67	PB	53	551	443
10	PB	59	258	338
60	PB	50	264	215
40	PB	42	347	343
20	PB	95	48	75
32	PB	95	48	75
4	PB	92	172	287
33	PB	121	86	278
2	PB	56	123	246
43	PB	74	261	349
51	PB	61	170	239
36	PB	90	173	165
54	PB	90	248	---

HC = Hypercholesterolemic control
FC = Fiber control
OB = Oat bran
PB = Pinto bean
-- = expired hamster

APPENDIX H

Pooled Plasma Total Cholesterol Levels (mg/dL) of Hamsters for Week 3 and Week 6

GROUP	WEEK 3	WEEK 6
HC	194	218
FC	196	233
OB	195	193
PB	193	212

HC = Hypercholesterolemic control
FC = Fiber control
OB = Oat bran
PB = Pinto bean

Pooled Plasma Triglyceride Levels (mg/dL) of Hamsters for Week 3 and Week 6

GROUP	WEEK 3	WEEK 6
HC	148	220
FC	188	360
OB	184	193
PB	175	279

HC = Hypercholesterolemic control
FC = Fiber control
OB = Oat bran
PB = Pinto bean

APPENDIX H (continued)

Pooled Plasma HDL-Cholesterol Levels (mg/dL) of Hamsters for Week 3 and Week 6

GROUP	WEEK 3	WEEK 6
HC	70	53
FC	72	67
OB	70	78
PB	67	68

HC = Hypercholesterolemic control
FC = Fiber control
OB = Oat bran
PB = Pinto bean

Pooled Plasma LDL-Cholesterol Levels (mg/dL) of Hamsters for Week 3 and Week 6

GROUP	WEEK 3	WEEK 6
HC	89	106
FC	101	86
OB	83	72
PB	89	104

HC = Hypercholesterolemic control
FC = Fiber control
OB = Oat bran
PB = Pinto bean

Pooled Plasma VLDL-Cholesterol Levels (mg/dL) of Hamsters for Week 3 and Week 6

GROUP	WEEK 3	WEEK 6
HC	65	34
FC	62	79
OB	103	17
PB	96	41

HC = Hypercholesterolemic control
FC = Fiber control
OB = Oat bran
PB = Pinto bean

VITA

Teresa J. Cross was born on August 25, 1961, in Lincoln, Illinois. At age five, her family moved to Portland, Oregon, where she graduated from Lincoln High School. She attended Oregon State University for two years, then transferred to the University of Virginia in Charlottesville, Virginia, where she completed a B.A. degree in Biochemistry in 1983. After graduating, she worked in Anesthesiology Research at the University of Virginia Medical School for three years. She then attended the Virginia Polytechnic Institute and State University in Blacksburg, Virginia, and received a Master of Science degree in Human Nutrition and Foods in 1993. During the completion of her thesis, she worked as a Public Health Nutritionist at the Thomas Jefferson Health District in Charlottesville, Virginia. Her future plans include the successful completion of a Certified Dietetic Internship Program, and the establishment of her own Professional Nutrition Counseling and Consulting business.