# THE EFFECT OF DIFFERENT SOURCES OF DIETARY FIBER ON THE PLASMA TOTAL AND LIPOPROTEIN CHOLESTEROL, LIVER CHOLESTEROL, FECAL NEUTRAL STEROID EXCRETION AND HISTOLOGY OF MAJOR ORGAN TISSUES IN HAMSTERS

by

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## (ABSTRACT)

The effect of diets with various dietary fiber sources on the plasma lipids, liver cholesterol, the histology of the gastrointestinal tract, heart, liver and kidney and the fecal neutral steroid excretion was investigated in hamsters. 155, 9-11 wk old, male Golden-Syrian hamsters were fed a purified basal hypercholesterolemic diet (0.1% cholesterol, 10% fat, 4% dietary fiber) for 5 wk to elevate plasma lipid levels. Based on wk 4 plasma total cholesterol (TC) levels hamsters with elevated levels were randomly assigned, 16 animals/group, into six groups for another 4 wk: control, oat bran, guar gum, cellulose, xylan and sacrifice. After 4 wk of the fiber diets (10% dietary fiber), the plasma TC levels were significantly lowered in the oat bran, guar gum and xylan groups (16%, 12% and 15%, respectively) ($p<.05$). They were also significantly lower than the control and cellulose groups. Plasma HDL-C concentrations tended to be lower in all the treatment groups, but was significantly decreased only in the guar gum group (12%) ($p<.05$). The combined plasma VLDL-C + LDL-C was significantly lowered by the oat bran, cellulose and xylan diets (38%, 40% and 34%, respectively) ($p<.05$). The liver cholesterol concentration increased significantly from 1 mg cholesterol/g liver to 4.1 mg cholesterol/g liver ($p<.05$) after 4 wk of the control diet; this was further increased significantly only in the cellulose group (5.6 mg cholesterol/g liver), while the other treatment groups showed no significant changes or differences compared to the control diet group (wk 4). The total fecal neutral steroid excretion was significantly ($p<.05$) higher in the oat bran group compared to the other treatment groups. No major differences were observed in the tissue histology of the animals in the different treatment groups. In the present study, it appeared that oat bran, guar gum and xylan were effective hypocholesterolemic agents; however, their mechanism of action is still not clear.

**Dedicated to my parents**

**Shri J. Raghunath Rao**

**and**

**Smt. J. Annapurna**

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## TABLE OF CONTENTS

Page

# LIST OF TABLES

# LIST OF APPENDICES

# INTRODUCTION

The post World War II years saw an increase in the number of deaths due to atherosclerosis, a process of accumulation of fat and cell material on the inner walls of the arteries, and it's complications in the United States population (Multiple Risk Factor Intervention Trial (MRFIT) Research Group, 1982). The American Heart Association (AHA) estimated that forty two million Americans, about 20% of the population, had one or more forms of blood vessel diseases and that more than 1.8 million persons were affected by stroke (Posner et al, 1986).

Studies of different population groups have pointed out the contributing roles of diet, mainly through its influence on the plasma lipid levels, hypertension, cigarette smoking, diabetes and other risk factors in the pathogenesis of coronary heart disease (CHD) (MRFIT Research Group, 1982). CHD, a result of atherosclerosis, is responsible for almost 30% of the two million deaths occurring in the United States each year. More than 500,000 people die annually from CHD and more than 6 million Americans have symptomatic CHD. Heart attacks, the leading cause of death in the United States, killed 513,700 persons in 1987 and almost 60% died before they reached the hospital. Approximately 45% of all heart attack victims are under the age of 65 y and 5% are under 40 y (Leaf and Ryan, 1990). In addition to its impact on the nations health, CHD costs the U.S. economy over $ 50 billion annually.

In 1957 it was postulated that diet may play an important role in the pathogenesis of atherosclerosis, especially the fat and total kilocalories (kcal) in the diet. Also the type of fat, i.e., the balance between saturated and unsaturated fat, was thought to be of importance. By 1978 the general consensus was to reduce the total fat in the diet to 30 - 35% of the total kcal, reduce the saturated fat content and substitute with complex carbohydrates and polyunsaturated fatty acids (PUFA), lower the cholesterol intake to less than 300 mg/day and to reduce the salt intake (Consensus Development Conference, 1985). Grundy et al (1982) recognized the major risk factors to be: elevated plasma total cholesterol (TC), elevated plasma low-density lipoprotein cholesterol (LDL-C), lowered plasma high-density lipoprotein cholesterol (HDL-C), an increase in blood pressure, smoking, obesity, diabetes, age, male gender, family history of CHD and physical inactivity.

Raised plasma TC (>240 mg/dl), and more importantly raised plasma LDL-C levels (>160 mg/dl), are causally related to an increase in the risk of CHD. It has been projected that for individuals with initial plasma TC in the range of 240 - 300 mg/dl, a 1% reduction in plasma cholesterol level would result in a 2% reduction in CHD rates (Lipid Research Clinics Program, 1984).

Diet has been the cornerstone for the treatment of high risk cholesterol levels, the aim of which was to lower the elevated cholesterol levels while maintaining a nutritionally adequate eating pattern. Research in this area has progressed extensively over the past two decades. The use of diets consisting of a low total fat (<30%) content, with saturated fat contributing less than 10% of the total kcal, PUFA up to 10% and monounsaturated fat (MUFA) 10-15% of the total kcal, along with a low dietary cholesterol (<300 mg/day) and increased complex carbohydrate (55 - 60%) content have been the bases of most of the research in this area.

The role of complex carbohydrate, including dietary fiber, has received a great deal of attention for more than a decade in Western societies. Epidemiological evidence on the protective role of dietary fiber against diseases such as constipation, diverticular disease, CHD, diabetes mellitus and some forms of cancer, led to the recommendations to increase the dietary fiber intake in the Western world (Vorster et al, 1986).

Dietary fiber is a component of plant materials which is resistant to digestion by the human intestinal enzymes. Of the two types of dietary fiber, the soluble fraction that is found in pectin, guar gum, barley and oat bran, seems to have the potential for lowering plasma TC and LDL-C levels while the insoluble fraction which is a major component of wheat bran, has negligible effects on plasma TC levels (Council on Scientific Affairs, 1989). Several mechanisms for this cholesterol lowering effect by the soluble fraction have been proposed (Anderson, 1987):

1. The soluble fraction binds with the bile acids and other lipids thus interfering with the formation of micelles in the small intestine. This results in alterations in the amount of cholesterol and/or fat absorbed and alters the size and number of the lipoprotein particles formed by the intestinal mucosa.

2. The soluble fraction increases the fecal bile acids excretion leading to an interference of the cholesterol and bile acid homeostasis thereby affecting the hepatic secretion of lipoproteins.

3. Action of colonic bacteria on the soluble fibers results in the production of short chain fatty acids which are absorbed into the portal circulation and can inhibit the hepatic cholesterol synthesis.

Thus it has been of great interest in recent years to establish the role of dietary fiber in reducing the plasma TC, LDL-C and triglycerides (TG) and possibly raising the plasma HDL-C in order to lower the incidence of CHD and associated mortality.

Animal models have been used a great deal in this area of research since it is easier to manipulate and control their lifestyles, environment and diet, perhaps with new and purified sources of dietary fiber. Humans and hamsters have a similar rate of whole body cholesterol synthesis; 1 mg cholesterol/day/100 g body weight and 2.2-3.4 mg cholesterol/day/100 g body weight respectively, as compared to other species such as the rat (12 mg cholesterol/day/100 g body weight) (Spady and Dietschy, 1985). Due to the apparent limited capacity of the liver of man and hamster, the liver is slow to readily adapt to changes in cholesterol flux and thus alter the LDL transportation in response to diet (Spady and Dietschy, 1985). In a study on the effects of adding 0.25% cholesterol to the diet of hamsters, it was observed that their plasma TC increased in a fashion similar to that seen in humans fed a high saturated fat, high cholesterol diet (Singhal et al, 1983). A similar response in plasma TC was reported when 0.1% cholesterol was added to the diet containing 5% fat in hamsters (Sicart et al, 1986). In rats, the more commonly used experimental animal model for cholesterol studies, the plasma HDL-C accounts for as much as 90% of the plasma TC while the hamsters were shown to have relatively abundant LDL with the ratio of cholesterol between the HDL and LDL fractions being 1:1, which is more similar to that of humans (Tsuda et al, 1983). Thus, hamsters were the preferred experimental animal model for this experiment when studying the effect of new purified sources of dietary fiber on plasma cholesterol fractions.

## Research Hypothesis

Ho : There will be no differences in response between treatment groups of hamsters receiving a. control diet, b. control + cellulose diet, c. control + oat bran diet, d. control + guar gum diet and e. control + xylan diet :

1. In the plasma TC, VLDL-C + LDL-C, HDL-C and TG levels.
2. In the liver cholesterol concentration.
3. In the fecal neutral steroid excretion.
4. In the histology of the gastrointestinal tract, heart, kidney and liver.

## Significance of the Study

Numerous studies have examined the hypocholesterolemic effect of high fiber diets. A major drawback has been that it was not possible to establish whether this effect was due solely to a dietary fiber component (xylan or guar gum) or due to a combination of dietary factors that were usually altered such as the level and type of fat or calories, when fiber was added to the diet. This study will enable us to determine if the hypocholesterolemic effect of the dietary fiber was due solely to these particular dietary fiber sources and not due to alteration of other dietary components. It will also enable us to help establish a possible mechanism for the hypocholesterolemic effect of the dietary fiber components.

# Review of Literature

## Introduction

Cardiovascular disease (CVD) is one of the leading causes of death in Western industrialized nations and accounts for nearly half of all the deaths in the United States. As the understanding of coronary heart disease (CHD) improves, it becomes clear that many factors are involved in its development, regulated in part by a variety of genes. These intrinsic factors in turn interact with a variety of environmental factors to cause the disease. An elevated plasma cholesterol level is one of the major risk factors of CHD and is significantly influenced by diet. Epidemiological studies and controlled clinical trials have shown that a causal relationship exists between diet and plasma cholesterol levels (Kris-Etherton et al, 1988). The role of diet in the prevention and treatment of elevated plasma cholesterol has therefore received a great deal of attention.

## The Pathogenesis of Atherosclerosis

Atherosclerosis is a lifelong disease process that begins as early as in childhood and culminates in clinical disease in middle age or later. It involves the formation of localized thickening of arterial walls with the formation of plaques of fatty and fibrous material. This results in the progressive narrowing of the vessel lumen, eventually leading to blockage and a heart attack or stroke. (Brown and Goldstein, 1984)

Fatty streaks are the earliest evidence of atherosclerosis commonly found in children. They are flat lipid rich lesions consisting of both macrophages and some smooth muscle cells. The advanced stage of atherosclerosis is characterized by a fibrous plaque made up of increased intimal smooth muscle cells surrounded by connective tissue matrix and containing varying amounts of intracellular and extracellular lipid. At the lumen of the artery these lesions are covered by a dense fibrous cap of smooth muscle and connective tissue. By ten years the lesions consist of lipid laden macrophages with underlying lipid laden smooth muscle cells. With increasing age advanced fibromuscular lesions are seen at these sites. (Ross, 1986)

Specific types of cells have been found to participate in atherogenesis, which include endothelial cells, platelets, monocytes or macrophages, vascular smooth muscle cells, T lymphocytes and fibroblasts. These cells secrete a variety of growth factors and chemoattractants that cause increased accumulation or proliferation of cells at the site of injury. (Ross, 1986)

Injury to the endothelial cells of the arterial wall increases their permeability thereby allowing the entry of excess cholesterol, mainly derived from the circulating LDL-C. This LDL molecule must undergo structural modifications before it can be taken up by the macrophages. Oxidatively modified LDL has been observed to be taken up by the monocyte/macrophages three to ten times more rapidly than the native LDL. This oxidized form is chemotactic for circulating monocytes and therefore can lead to the build up and accumulation of monocytes in an area. Also this oxidized LDL inhibits the motility of tissue macrophages thus leading to the trapping of macrophages once they have entered the endothelial space. They are also cytotoxic and therefore could contribute to atherogenesis by causing cell injury and death (Steinberg and Witztum, 1990). Therefore the more circulating LDL-C, the more rapidly atherosclerosis develops (Brown and Goldstein, 1984).

The thickening of the arterial wall due to proliferation of the smooth muscle cells can also occur due to injury. The damaged cells become leaky and allow the LDL particles and other blood particles to penetrate. Desquamation due to injury can also result in adhering of the platelets to the exposed surface resulting in the release of the platelet derived growth factor, which induces migration of the smooth muscle cells to the intima, while also stimulating LDL receptor activity. Simultaneously the white blood cell, monocytes, are also attaching to the area and are being activated into macrophages, which act as scavenger cells. These accumulated cells, atheromas, eventually lead to the narrowing of the arterial lumen and finally lead to blockage of the artery. (Brown and Goldstein, 1984)

A direct correlation might exist between the rate of increase in hypercholesterolemia, the degree of hypercholesterolemia, the duration of hypercholesterolemia and the cellular changes and development of proliferative lesions that occur (Ross, 1986). The Pathobiological Determinants of Atherosclerosis in Youth (PDAY) Research Group (1990) studied the relationship of serum cholesterol levels and smoking to atherosclerosis in 390 males, 14 to 34 years of age, who died of violent causes. A positive correlation was observed between the percent of intimal surface involved with atherosclerotic lesions in both the aorta and the right coronary artery with the combined serum VLDL-C and LDL-C levels and was negatively associated with serum HDL-C. Smoking was also observed to be strongly associated with the prevalence of raised lesions. Similarly in the Cholesterol Lowering Atherosclerosis Study (Blankenhorn et al, 1990) an increase in the percent energy from total fat and polyunsaturated fat (PUFA) and a reduction in the percent energy from protein produced a significant ($p<.05$) increase in the risk of developing new lesions in men aged 40-59 y. Also an increase in the intake of lauric, oleic and linoleic acids were observed to increase the risk of developing new lesions. A combination of low fat meat and low fat dairy products were observed to be protective against lesion development. In subjects who did not develop any new lesions a 7.1% reduction in the plasma LDL-C concentration was observed.

Arterial endothelial cells in adults turn over at a relatively low level and it is these cells that determine the nature of the lipoprotein and the other plasma constituents that reach the subendothelial space. They bind with LDL through specific high affinity receptors and modify the LDL so that it is recognized and ingested by cells like macrophages. The number of LDL receptors displayed by a cell varies with the need for cholesterol by the cell, thus protecting the cell from excess cholesterol. However this can lead to a decrease in the rate of removal of LDL from the circulation resulting in an increase in plasma LDL levels thus accelerating atherosclerosis (Brown and Goldstein, 1984).

Information on how each of the various risk factors affect the process of plaque formation at the cellular level is not yet available. Also the long term influence of various dietary modifications to reduce the formation of atherosclerotic lesions, to lower the risk of CHD, is not clear. Further research is required in this area.

### Cholesterol and Lipoproteins

Lipoproteins are particles found in the blood, that play a major role in the transportation of lipids. The relationship between plasma TC and CHD is best understood through the lipoprotein transport system.

Lipoproteins have a hydrophobic core of nonpolar lipids, such as triglycerides (TG) and cholesterol esters, which is surrounded by surfactants, such as phospholipids and unesterified or free cholesterol, and proteins (referred to as apolipoproteins). The lipoproteins are classified on the basis of their chemical and physical properties, which in turn are determined by the relative proportions of lipid to protein in the particles. The five major classes of lipoproteins are chylomicrons (rich in TG), very-low-density lipoprotein (VLDL), which are TG rich particles, intermediate-density lipoprotein (IDL), LDL, the major cholesterol transport protein consisting of nearly 70% cholesterol and HDL, a protein rich particle (Table 1).

Table 1: Lipoprotein Composition

| Lipoprotein | Origin | Density (g/ml) | Size A° | Major Lipid Component |
|---|---|---|---|---|
| Chylomicron | Intestine | < 0.95 | 1200-11000 | TG(dietary) |
| VLDL | Liver<br>Intestine | 0.95 - 1.006 | 250-800 | TG (endogenous) |
| IDL | Circulation<br>Liver | 1.006 - 1.019 | | Cholesterol |
| LDL | Circulation<br>Liver | 1.019 - 1.063 | 210-250 | Cholesterol |
| HDL | Liver<br>Intestine | 1.063 - 1.21 | 45-110 | Phospholipid |

(From: Kris-Etherton et al, 1988)

HDL is not a single molecular entity but a class of particles containing nearly equal amounts of lipid and protein, varying in size and density. There are two major sub-classes of HDL depending on their size and lipid content - the larger more lipid rich $HDL_2$ and the smaller, denser $HDL_3$ which is predominant in human plasma. The $HDL_2$ concentration is thought to be a better predictor of CHD risk than either total HDL-C or $HDL_3$ fractions (Gwynne, 1989; Kris-Etherton et al, 1988).

The lipoprotein, lipid transport system, is divided into two pathways : an exogenous pathway for the cholesterol and TG absorbed from the intestine and an endogenous pathway for the cholesterol and TG entering the blood stream from the liver and other non-intestinal tissues (Brown and Goldstein, 1984).

In the exogenous pathway the dietary cholesterol and TG are packaged into chylomicrons in the intestine, after the TG combines with apolipoprotein (apo) B48 and some apo A, which are transported in the lymphatic system to the thoracic duct into the blood. Chylomicrons receive apo CII, needed for the activation of the lipoprotein lipase found on the cell membrane, from HDL. Also apo E is added onto the chylomicron, which is required for the recognition by the hepatic receptors. Chylomicron TG is hydrolyzed to fatty acids and glycerol, catalyzed by lipoprotein lipase, an enzyme situated in part at the luminal surface of the endothelium but also present on the fat cells. The greater the concentration of this lipase, the more rapidly these chylomicrons are removed from the blood. The liver also contains a lipoprotein lipase but it has different properties from the lipoprotein lipase on the endothelial and adipose tissue cells. These TGs are delivered to the adipose tissue for storage or to the muscle and other organs for oxidation to supply energy. The chylomicron remnant, containing some TG, cholesteryl esters, fat soluble vitamins, phospholipids and some apolipoproteins, is removed from the circulation by receptors, which recognize the apo E present in the remnant, found only on the liver cells. The rate of removal of the chylomicron remnant depends on the subfraction of apo E present i.e. particles containing apo E IV are removed quicker than those containing apo E II. This uptake and removal process is important as it is believed to raise the liver cholesterol levels, which in turn down regulate the level of hepatic LDL receptors, thus increasing circulating LDL levels (Castelli, 1986; Gotto, 1990).

The endogenous pathway begins with the secretion of VLDL into the blood stream by the liver. This VLDL is mainly composed of TG synthesized in the liver, with a small proportion of cholesteryl esters. At the site of adipose tissue and muscle the VLDL-TG is hydrolysed by lipoprotein lipase with the release of fatty acids and glycerol, resulting in the production of the IDL particle, rich in cholesteryl esters. About half of these IDL particles are removed quickly from the circulation by the liver, which uses the cholesterol to produce new VLDL and bile acids. The other half is converted, in the blood stream, into LDL which is removed from the circulation mainly by receptors on the liver (75%) (Brown and Goldstein, 1984). The LDL remaining in the plasma is modified by scavenger receptors on the macrophages and endothelial cells. The cholesterol in atherosclerotic plaques is derived from the LDL circulating in the bloodstream. The amount of LDL in circulation is regulated by LDL receptors which bind with these LDL particles and remove them from the circulation. Once within the cell they are broken down releasing free cholesterol, for utilization by the cells. The number of receptors on the cell surface varies with the cells demand for cholesterol. The liver, adrenal glands and ovary have the highest concentration of these receptors. About 75% of this receptor mediated removal of LDL occurs in the liver. This free cholesterol in the adrenal glands and ovary is converted into the steroid hormones cortisol and estradiol, respectively, and in the liver it is transformed into bile acids (Brown and Goldstein, 1984).

Within the cell, cholesterol accumulation regulates three main processes. Firstly it decreases the cells' ability to produce cholesterol by shutting off the HMGCoA reductase activity. Secondly, the LDL derived cholesterol promotes storage of cholesterol in the cell by activating an enzyme called Acyl CoA Cholesterol Acyl Transferase (ACAT), which esterifies the cholesterol deposited in storage droplets. Lastly, and most significantly, the accumulation of cholesterol within the cell drives a feedback mechanism that makes the cell stop synthesizing new LDL receptors and thus the uptake of LDL-C into the cell is reduced while the circulating LDL-C concentration increases. This has been hypothesized to be one of the main causes for the development of atherosclerosis in the general population (Brown and Goldstein, 1984; Kris-Etherton et al, 1988).

HDL is secreted by the liver and intestine and is discoidal, has no core lipids and is called nascent HDL. Once this nascent HDL enters the circulation, it picks up unesterified cholesterol from the extrahepatic tissues, which is esterified by Lecithin Cholesterol Acyl Transferase (LCAT), thus increasing the size of the HDL particle. This cholesterol ester from the HDL particle is transferred to the liver either by the uptake and catabolism of the HDL particle or by the transfer of cholesterol ester without uptake. The HDL concentration in the circulation is influenced by a number of factors such as the rate of synthesis of apo A-1 by the liver and intestine, a major structural component of the HDL particle, and which is a cofactor of LCAT; the fractional catabolic rate of VLDL and chylomicrons by LPL; and by LCAT concentrations (Miller, 1990).

The plasma HDL fraction, secreted by the liver, is involved in the reverse cholesterol transport. This process involves the transport of cholesterol, after it has been esterified by LCAT, by HDL from the peripheral tissues back to the liver, either directly or through the IDL molecule, which is removed from the circulation by the liver, thus leading to the excretion of cholesterol found in the peripheral tissues (Kris-Etherton et al, 1988). In this process cholesterol is removed from the macrophages and other cells within the arterial wall by an HDL-mediated process and the cholesterol taken up by the HDL is prevented from re-entering the cell by its esterification. Thus the plasma HDL-C concentration is inversely correlated with risk for CHD. An increase in plasma HDL-C by one percent translates to a two to three percent reduction in CHD risk (Gordon et al, 1989). It has been reported that the circulating plasma HDL-C concentrations are increased by behavioral/hygienic factors, such as leanness, alcohol intake and exercise, as well as estrogen replacement. Factors that lower the plasma HDL-C levels include obesity, cigarette smoking, androgens, inactivity and the use of thiazide diuretics. Genetic factors and the use of cardiovascular medications such as alpha and beta blockers can also affect HDL concentrations (Wilson,1990).

## Plasma Total Cholesterol and Coronary Heart Disease

The risk of developing CHD is causally related to raised plasma cholesterol levels specifically raised LDL-C. The risk increases progressively with increasing cholesterol levels, particularly when the plasma total cholesterol (TC) levels rise above 200 mg/dl (Goodman, 1989). Elevated plasma TC, i.e., greater than 240 mg/dl for those over 40 years, is the main cause of CHD and requires aggressive treatment (Lipid Research Clinics Program, 1984). Studies have shown that when about 60% of the arterial surface is covered with raised plaque a critical phase of increased risk of CHD is entered (Grundy, 1986).

In the Framingham study, 5209 men and women were studied over a period of 20 years (Castelli, 1983). A linear relationship between plasma TC and the risk of CHD was seen at the end of the study. It was estimated that for each 1% rise in cholesterol, the incidence of CHD increases by 2% to 3% although it was not possible to establish a cholesterol threshold for the disease (Castelli, 1983). As the subjects grew older the plasma TC lost its significance as a predictor of CHD risk, while the VLDL, LDL and HDL cholesterol gained more predictive power.

In the MRFIT trial (1982) a 2% difference in cholesterol levels between the treatment and control groups saw a 7% difference in the CHD rates. In this trial it was found that the risk of CHD continuously increased with increasing plasma TC levels. Of all the deaths due to CHD, 46% were attributed to plasma TC levels greater than 180 mg/dl. Individuals with a plasma TC of 264 mg/dl had a death rate four times higher than individuals with a plasma TC of 167 mg/dl (Multiple Risk Factor Intervention Trial Research Group, 1982).

Thus, the lipid hypothesis states that lowering the blood cholesterol levels should significantly reduce the incidence of CHD. This hypothesis has been supported by a number of epidemiological and clinical studies. The Wadsworth Veterans Administration Study (Dayton et al, 1969) determined whether a diet high in unsaturated fat would prevent complications of atherosclerosis in a group of 846 men living in Los Angeles Domicile. After eight years, a 13% difference in the serum TC levels between the control and experimental groups was observed. Although both diets were similar in total calories and fat content, the experimental diet was low in cholesterol, saturated fat and higher in polyunsaturated fat (PUFA).

Similarly the Oslo study (Hjermann et al, 1981) showed diet counselling, to lower circulating lipid levels, and smoking cessation, along with regular monitoring, produced a 13% reduction in the serum TC in men aged 40-49 y, who had initial cholesterol levels in the range of 290-380 mg/dl. The incidence of CHD, both fatal and nonfatal myocardial infarction and sudden death, was 3% in the intervention group compared to 6% in the control group ($p<.03$). In addition to a decrease in serum TC, an increase in the ratio of serum HDL-C to the other cholesterol fractions was 66% higher in the good diet responders. Also life style changes, such as exercising and smoking cessation, observed in these individuals, could have contributed to the reduction in the incidence of CHD. Similar results were obtained by the World Health Organization Primary Prevention Trial (Oliver et al, 1978) where a 9% reduction in the serum TC levels with clofibrate treatment was associated with a 20% reduction in the first clinical episodes of myocardial infarction and in the Coronary Drug Project (Canner et al, 1986) where a 10% reduction in the serum TC with niacin treatment produced an 11% decline in the mortality rates over 15 y.

A significant linear relationship between low plasma TC levels and low incidence of peripheral vascular disease and the incidence of CHD in countries like Asia, Africa and

South America, where the plasma TC levels are usually below 150 mg/dl, have led to the recommendation that plasma TC below 200 mg/dl are desirable (Grundy, 1986).

The average North American who suffers from a heart attack has a plasma TC level of 244 mg/dl (Castelli, 1983). It was recommended that the ratio of the plasma TC to HDL-C be used as a better risk indicator to identify those at high risk of CHD. A ratio of 4.5 or higher is thought to indicate high risk and it was suggested that by lowering this ratio the risk of CHD can be decreased. A plasma TC / HDL-C ratio of 4.4 for females and 5.1 for males without CHD would appear to represent standard risk levels in the Framingham population (Castelli, 1983).

In view of the existing evidence, current recommendations are to lower the total fat intake from 35-40% of calories to a maximum of 30%, with the amount of saturated and PUFA less than 10% of total kcal and the dietary cholesterol intake less than 300 mg/day (National Cholesterol Education Program, 1988). For individuals at high risk of CHD based on the presence of elevated plasma lipids and lipoprotein levels or the presence of other risk factors, it is recommended that the total fat intake should not be greater than 20% of kcal (American Heart Association, 1982). Ornish et al (1990) studied the effects of diet and lifestyle changes on CHD. The 28 patients were prescribed a life style modification program for a year, which included a low fat (10%) vegetarian diet, moderate aerobic exercise, stress management training, smoking cessation and group support. The intervention started with a week long retreat at a hotel to teach the lifestyle intervention to the experimental group. Patients then attended regular group support meetings for 4 h twice a week. In the experimental group the serum TC fell by 24.3% and serum LDL-C by 37.4%. Analysis of coronary angiography showed that average percent diameter stenosis regressed from 40 to 37.8% in the experimental group, while it progressed from 42.7 to 46.1% in the control group. 82% of the experimental group patients had an average change towards regression, while in 53% of the control subjects a progression of lesions was observed. Thus, comprehensive life style changes even over a short period appear to bring about regression of severe coronary atherosclerosis. However, whether these effects can be sustained or improved over a longer period of time needs to be evaluated.

Ginsberg et al (1990) studied the effect of a 30% fat Step 1 diet with 10% each from saturated fat, monounsaturated fat (MUFA) and polyunsaturated fat (PUFA) and of a MUFA enriched (18% MUFA, 10% saturated, 10% PUFA and 38% total fat) diet, on the plasma levels of lipids and lipoproteins in 36 normal young men. After ten weeks, significant (p<.025) reductions in the plasma TC levels were observed in the group on the Step 1 diet (-0.37 mmol/l) and in the group on the MUFA diet (-0.46 mmol/l) as compared to those on the average American diet (-0.05 mmol/l). Plasma LDL-C levels also fell significantly (p<.025) in the MUFA diet group (-0.35 mmol/l) and nonsignificant reductions were observed in the Step 1 diet group (-0.25 mmol/l). Although these diets have been proven to be beneficial in normal healthy young men, their effect in an older population or those with CHD needs to be evaluated.

With the recommendations to lower the total fat and saturated fat intakes, in order to lower the plasma TC levels, the fatty acid composition of the fat consumed becomes important. The reduction in saturated fat results in replacement of the energy source by a mixture of PUFA, MUFA and carbohydrates. Wardlaw and Snook (1990) examined the effect of PUFA and MUFA on the serum cholesterol concentrations in 86 male subjects above 25 y, consuming diets with 37-43% of energy as fat. After five weeks of a high oleic acid sunflower oil diet (41% total fat energy of which 28% was from MUFA), the mean serum TC was 9% lower (p<.05) than the baseline values. For the corn oil PUFA diet (41% total fat energy of which 19% was from PUFA) the mean serum TC concentrations were 14% lower (p<.01) compared to baseline values. Similarly 8% and 13% reductions were observed in serum LDL-C concentrations after the MUFA and PUFA diets when compared to baseline values ($p<.001$). The mean serum HDL-C concentrations were not affected by either diet. Compared to a diet where 85% of the dietary fat was provided by butter, which the subjects consumed for 2 wk prior to each treatment phase, these vegetable oil based diets decreased serum TC by 16-21% ($p<.001$), LDL-C by 21-26% ($p<.001$) and TG by 10-21% ($p<.01$), thus suggesting that the first treatment choice for individuals with high serum TC concentrations on Western diets, should be a decrease in the intake of saturated fat.

The mechanism by which fatty acids regulate the plasma cholesterol levels is thought to possibly be a result of a redistribution of cholesterol between the plasma and tissue pools, a change in the lipoprotein composition, change in the LDL production or a change in the clearance rate of LDL. It is, thus, suggested that the liver may play an important role in changing the plasma LDL-C level induced by alteration of the fatty acid content in the diet (Ohtani et al, 1990).

McNamara et al (1987) studied the effects of dietary fat and cholesterol on the cholesterol homeostasis in men. A total of 75 studies were carried out in 50 individuals asymptomatic for CVD and free of secondary causes of hyperlipidemia with mean age of 47 y, comparing a low cholesterol (250 mg/dl) and a high cholesterol (800 mg/dl) diet with an intake of 35% of kcal as either PUFA (P/S = 1.5) or saturated fat (P/S = 0.3). On a low cholesterol diet, consumed for 6 wk, the exogenous cholesterol absorption was 61% which decreased to 55% during the high cholesterol diet, consumed for 6 wk. The increased dietary cholesterol intakes failed to cause any significant increase in the mean plasma TC, LDL-C or HDL-C levels in either the PUFA or saturated fat group. A significant ($p<.05$) increase in the plasma TC (218 vs 243 mg/dl in the low cholesterol period and 224 vs 248 mg/dl in the high cholesterol period, respectively) and LDL-C levels (131 vs 144 mg/dl in the low cholesterol period and 138 vs 150 mg/dl in the high cholesterol period, respectively) was observed when the subjects shifted from a PUFA to a saturated fat diet. Marked heterogeneity was observed among these individuals in their response to an increase in cholesterol intake.

Several sterol balance studies have shown that the major metabolic changes in response to a dietary cholesterol challenge are the suppression of the endogenous cholesterol synthesis and/or an increase in re-excretion of the absorbed dietary cholesterol as fecal neutral steroids. McNamara et al (1987) reported that increases in absorbed dietary cholesterol were accompanied by significant decreases in the mononuclear leukocytes (MNL) sterol synthesis rates. However, no relationship was found between the baseline plasma TC level and sensitivity to a dietary cholesterol challenge, regardless of the type of the dietary fat. With either the PUFA or saturated fat diets, the rate of sterol synthesis in MNL, a negative correlation ($r = 0.931$) ($p<.001$) with the absorbed dietary cholesterol was expressed, thus indicating that the changes in MNL sterol synthesis in response to the dietary cholesterol challenge were independent of the type of fat fed (McNamara et al, 1987).

Miettinen and Kesaniemi (1989) studied the cholesterol absorption and its association with dietary factors, cholesterol synthesis and serum lipoprotein levels in 50 y old men. Body mass index was found to be negatively associated with the fractional cholesterol absorption ($r = -0.358$) ($p<.01$) and absorbed dietary cholesterol ($r = -0.489$) ($p<.001$), suggesting that obesity decreases cholesterol absorption efficiency. No association was found between dietary plant sterols and the P/S ratio of the dietary fat and the amount of cholesterol absorbed, whereas a positive correlation was found between the amount of dietary cholesterol absorbed and the fat intake ($r = 0.544$) ($p<.001$), cholesterol intake ($r = 0.836$) ($p<.001$), linoleic acid ($r = 0.321$) ($p<.05$) and the fiber consumed ($r = 0.266$)

($p<.05$). The total amount of cholesterol absorbed was positively correlated ($r = 0.308$) ($p<.05$) with the fecal neutral sterols of endogenous origin and was significantly negatively associated with cholesterol synthesis ($r = -0.643$) ($p<.001$). They also observed that fractional cholesterol absorption was positively correlated with serum TC ($r = 0.307$) ($p<.05$), serum LDL-C ($r = 0.277$) ($p<.05$) and serum HDL-C ($r = 0.339$) ($p<.001$) levels. Also high serum HDL-C levels were associated with low synthesis and fecal elimination of cholesterol. Similar results were observed by Quintao and Sperotto (1987). Thus, the cholesterol absorption efficiency and absorption of dietary cholesterol significantly regulate cholesterol synthesis and elimination and are important determinants of within population variation in the serum levels of TC, LDL-C and HDL-C. However, until a better understanding of these regulatory mechanisms occurs, prudence concerning cholesterol intake would appear in order.

## Dietary Fiber

Fiber has attracted a great deal of attention over the last two decades initiated by the observations of Burkitt and Trowell in the 1970's that the diseases common in the Western world, were low in incidence or non-existent in rural African populations. These differences were attributed to the consumption of diets high in refined carbohydrates and low in fiber (< 25 g/day by the Western populations vs 100-170 g/day by the African populations) by the Western populations (Selvendran, 1984).

Dietary fiber is a heterogenous material and no universally recognized definition exists. A definition based on its physical properties was proposed by Trowell in 1974, and is generally accepted by many researchers, who defined it as "remnants of plant cells resistant to hydrolysis by the alimentary enzymes of man, the group of substances that remain in the ileum, but are partly hydrolyzed by bacteria in the colon" (Council on Scientific Affairs, 1989). This term refers mainly to the non-starch polysaccharides and lignin in the diet. The main sources of dietary fiber are plant cell walls composed of a variety of polysaccharides including cellulose, hemicellulose, pectic substances, gums, mucilages, algal polysaccharides and lignin.

Depending on their water solubility, dietary fibers are either water insoluble or water soluble. The structural fibers, cellulose, lignin and some hemicellulose, are insoluble and the natural gel forming fibers, pectin, gums, mucilages and remaining hemicelluloses, are water soluble. Most plant foods contain both soluble and insoluble dietary fiber, but may be rich in one type of fiber or another

(Council on Scientific Affairs, 1989). The type of sugars (glucose, galactose, xylose, arabinose, rhamnose, fucose), sugar acids (mannuronic, galacturonic, glucuronic, guluronic and 4-O-methyl-glucuronic acid) and the functional groups (hydrogen, hydroxyl, carbonyl, carboxyl, sulfate, methyl) affect the functional properties of dietary fiber. The relative solubility and functionality of soluble dietary fiber are affected by their polymer size and molecular distribution while the functionality of insoluble dietary fiber is affected by particle size and distribution (Gordon, 1989).

Dietary fiber is thought to be necessary to maintain normal functioning of the gastrointestinal tract. In the small intestine, fiber alters the intestinal transit time, absorption rates of nutrients, action of digestive enzymes and the secretion of intestinal and pancreatic enzymes. Water soluble fibers, such as pectin, form gels and increase the viscosity and stickiness of stomach contents, thus, delaying gastric emptying and, thereby, affecting the rate of digestion of foods and absorption of nutrients. These soluble dietary fibers convert a liquid meal to a solid one by soaking up the water, forming gels, which in turn delay gastric emptying.

Thus, meals high in soluble dietary fiber fill the stomach and provide a feeling of satiety. On the other hand the insoluble dietary fibers cause intestinal hurry by accelerating the intestinal transit time (Council on Scientific Affairs, 1989).

Another important physiological effect of dietary fiber is its ability to increase stool weight and water content. An increase in stool volume and weight with fiber diets occurs through an increase in undigested and unfermentable material, bound water and/or bacterial cell mass and gas production during fermentation of soluble fiber. The water binding capacity of different sources of dietary fiber are inversely related to their ability to increase fecal mass and this property prevents water from being absorbed through the colonic mucosa (Gordon, 1989; Council on Scientific Affairs, 1989).

These physiological properties of fiber have led to the hypothesis that high fiber diets could help prevent certain diseases, such as heart disease, colon cancer, obesity, diabetes, gastrointestinal disorders, to name a few, and the stimulation of a great deal of research.

## Dietary Fiber and Plasma Cholesterol

Dietary modification is the first step in lowering high risk blood cholesterol levels, since it is relatively less expensive than the use of lipid lowering drugs and is non-toxic with minimal side effects. Of the many dietary components, soluble dietary fiber has gained considerable significance for its role in lowering high risk blood cholesterol levels. Experimental studies have shown that soluble fiber fractions, pectin, guar gum, barley and oat bran, lower blood cholesterol levels when given in large amounts (Anderson and Gustafson, 1988).

Oat and bean products contain large amounts of water soluble fiber and seem to be effective hypocholesterolemic agents. Oat bran, the ground inner husk of the grain, is rich in oat gum, a B-glucan, that seems to be the major cholesterol lowering component in oat products (Anderson and Gustafson, 1988). B-glucan is a long glucose based molecule that resembles a kinked cellulose fiber molecule. It is similar to cellulose except that cellulose contains only B,1-4 glucosidic bonds, while B-glucan in addition contains B,1-3 glucosidic bonds, at approximately every third and fourth glucose unit. This B-glucan molecule soaks up water, becomes viscous and decreases the diffusion of bile acids to the intestinal wall. These captured bile acids are excreted, thus making the body draw on its cholesterol pool to replace the excreted bile acids, thus causing a lowering of the circulating cholesterol (Raloff, 1990).

### a. Human Studies

Anderson et al (1980) studied the effect of a control diet containing 43% carbohydrate (11 g plant fiber/1000 kcal), 18% protein and 38% fat (P/S = 0.5) and a high fiber diet (HCF) containing 70% carbohydrate (total plant fiber 34 g/1000 kcal), 18% protein and 12% fat (P/S = 1.1) on the plasma TC and TG in diabetic males. On the HCF diet a linear decline in the fasting plasma TC was achieved during the first seven days, which was 25% lower than the control. A slower decline was seen subsequently up to 18 days when the values averaged 30% below (65 mg/dl lower) control values (206 vs 141 mg/dl). Fasting plasma TG values at the completion of the HCF diet period were 11% lower (18 mg/dl lower) than the control values (168 vs 150 mg/dl). The postprandial TG values were also lowered. These reductions were similar to those observed when dietary cholesterol and saturated fat are reduced. However, it is not clear whether these changes could be attributed solely to the high fiber intake since the total fat intake was also considerably reduced in the HCF diet. Also dietary cholesterol restriction, changes in fatty acid content of the diet and the use of vegetable protein in the HCF diets could contribute to the lowered cholesterol levels observed in these subjects, thus indicating that all these factors could be acting synergistically to lower serum cholesterol levels.

Similar results were shown by Anderson et al (1984) in hypercholesterolemic males, using oat bran or bean supplemented diets which provided 48 g total plant fiber and 18 g of soluble fiber per day from either oat bran or beans. In these subjects serum TC was lowered by 23% (p<.001) (294 to 226 mg/dl) within three weeks. The LDL-C was also lowered by 23% (216 to 167 mg/dl) over the three week period. A 20% reduction in HDL-C (from 37.2 to 29.8 mg/dl) was also observed. However, the HDL-C/LDL-C ratio was raised by 22% on the oat bran diet and by 17% on the bean diet.

Wahenfried et al (1990) also reported the hypocholesterolemic effects of unprocessed oat bran and processed oat bran supplemented (50 g/day) diets in normolipidemic individuals which produced significant reductions in serum TC (p<.05) with an average reduction of 10 - 17% (277.8 to 236.4 mg/dl and 284.5 to 248.9 mg/dl, respectively). The serum TC/HDL-C ratio also decreased significantly over the same period (6.9 to 6.6 and 7 to 6.5, respectively).

Anderson et al (1990) examined the effect of ready to eat oat bran cereal (25 g oat bran, 8.8 g total dietary fiber and 3.5 g soluble fiber / 56 g serving) on the serum lipid levels in hypercholesterolemic men. The total dietary fiber from the oat bran cereal was 21 g of which 7.4 g was soluble dietary fiber compared to 15 g total dietary fiber and 4.5 g soluble dietary fiber from the corn flakes control diet (p<.001). After two weeks of the oat bran diet the serum TC levels decreased by 5.4% (p<.05), the serum LDL-C levels decreased by 8.5% (p<.025) and the serum HDL-C decreased by 3.3% while the serum TG increased by 8.7%. Thus the ready-to-eat oat bran cereal could provide a practical means to incorporate soluble fiber into the diet. Similar results were observed when Van Horn et al (1988) reported that the inclusion of oatmeal (2 oz/day) in conjunction with an American Heart Association fat modified diet in normal individuals, produced a mean reduction in serum cholesterol levels of 2.7% (p<.008), over the 5.2% decrease observed with just the American Heart Association diet. Thus the inclusion of oat products in a prudent diet may have a substantial impact in lowering the risk for CHD.

Kestin et al (1990) compared the effects of non-starch polysaccharides obtained from three cereal brans, namely, wheat, rice and oat, added to a low fiber diet of 24 hypercholesterolemic men. These diets provided 11.8 g dietary fiber / day from either wheat, rice or oat. After four weeks of these diets, wheat and rice bran produced little effect on plasma cholesterol levels, whereas the oat bran, in comparison with wheat and rice bran, significantly lowered plasma TC by 5.6% (p<.001) and 3.8% (p<.01), respectively. This reduction was observed to be mainly in the LDL-C fraction (6.6%). However, rice bran and oat bran increased plasma HDL-C/TC ratio in comparison to wheat bran (2.9% and 4% , respectively) ($p<.05$). A significant 6% ($p<.05$) decrease in the plasma TG was observed with the rice bran supplementation compared to wheat bran. In addition to these effects on plasma lipids, these brans increased stool frequency, with significant increases observed with the rice and wheat bran (9 and 8%, respectively) ($p<.005$). Thus, an increase in intake of fiber rich foods from several sources may be advisable. Similar results were reported by Vorster et al (1986) who observed a significant correlation between the change in serum cholesterol and the dose of bran.

Viscous gel forming soluble fibers, like pectin (found in fruits and vegetables, especially peels of citrus fruits) and guar gum (galactomannan), may also be effective in lowering cholesterol. Another similar fiber is psyllium, which is a highly branched arabino xylan, derived from the seed of Plantago ovata. Abraham and Mehta (1988) reported that psyllium supplementation (21 g/day) in healthy males lowered plasma TC by 35 mg/dl, plasma LDL-C by 15 mg/dl and plasma HDL-C by 4 mg/dl. An increase in fecal mass and dry matter was reported along with a non-significant increase in cholesterol excretion. Psyllium supplementation tended to delay lipid absorption as well (Abraham and Mehta, 1988). Similar results were shown with soy cotyledon fiber supplements (13 to 25 g fiber/day) which resulted in a 6%

reduction in plasma TC, comparable to that seen with oat bran and oat meal consumption (Lo, 1989; Lo et al,1986).

Bell et al (1990) studied the effect of ready-to-eat breakfast cereals containing soluble fiber (pectin or psyllium) on the serum cholesterol levels in 60 male hypercholesterolemic patients. After following the Step 1 AHA diet for six weeks (30% total energy as fat, 55% carbohydrate, 15% protein and < 300 mg dietary cholesterol/day) patients were asked to consume one 57 g package of the supplemented cereal per day as part of breakfast in the Step 1 diet, for six weeks. Pectin and psyllium each contributed approximately 50% of total soluble fiber in the respective cereals. During the diet-only-phase serum TC decreased by 3.8% and serum LDL-C decreased by 4%. With the addition of pectin enriched cereal to the diet, an additional 2.1% decrease in serum TC ($p<.0234$) and 3.9% decrease in serum LDL-C ($p<.16$) was observed; and in the psyllium enriched cereal group the corresponding values decreased by 5.9% ($p<.005$) and 5.7% ($p<.34$), respectively. Overall the pectin enriched cereal lowered the serum TC and LDL-C by 6.4% and 8.4%, while the psyllium enriched cereal decreased these values by 9.2 and 9.7%, respectively. Both these fibers had no significant effect on the serum HDL-C and TG concentrations. Thus, for those individuals who have difficulties in incorporating a variety of foods high in different fibers, the use of foods high in soluble fiber, such as the soluble fiber enriched cereals used in this study, in conjunction with a prudent diet could be a reasonable alternative.

Similarly Levin et al (1990) compared the effect of the administration of 5.1 g of psyllium (as Metamucil) or cellulose twice daily for 16 weeks as adjuncts to a prudent diet in the management of moderate hypercholesterolemia in 96 male and female subjects. After 16 weeks of treatment, the plasma TC in the psyllium group decreased by 5.6% compared with 0.1% increase in the cellulose group (p<.01). Also the plasma LDL-C levels were 8.6% below that observed after 8 wk of the Step 1 AHA diet in the psyllium group and 2.2% below post-diet levels in the cellulose group (p<.05). Overall the psyllium group witnessed a 9.5% (p<.01) reduction in the plasma LDL-C levels. Similar results were reported by Neal and Balm (1990) who observed that psyllium supplementation (20.4 g/day) could effectively enhance the cholesterol lowering effect of the Step 1 AHA diet.

Another water soluble fiber with cholesterol lowering properties is guar gum, a galactomannan storage polysaccharide, which forms a viscous gel in aqueous solutions. Simons et al (1982) supplemented diets of hypercholesterolemic individuals with 6 g of active guar formulations over a one year period. The initial three months saw a significant ($p<0.001$) 15% reduction in plasma TC (7.8 to 6.7 mmol/l) while the plasma TG was not affected. This decrease in the plasma TC was associated with a 20% reduction in plasma LDL-C ($p<0.001$) and with reduced cholesterol absorption. Similar results were reported by Tuomilehto et al (1988) when hypercholesterolemic subjects were supplemented with 30 g of granulated guar gum. An 18% reduction in plasma TC was observed during the first eight weeks of treatment (10.03 vs 8.22 mmol/l) ($p<0.001$), which after 50 wk of treatment was 12% lower than baseline (8.97 mmol/l) ($p<0.001$) and plasma LDL-C levels fell by 14.9% by 34 weeks of treatment (8.06 vs 6.86 mmol/l) ($p<0.001$). However guar gum supplementation did not help to normalize the highly elevated plasma cholesterol levels, commonly encountered in these individuals.

Men with moderately elevated cholesterol levels were also reported to respond in a similar manner (Superko et al, 1988). These subjects were supplemented with 15 g of guar gum/day for eight weeks in one of the three forms - medium viscosity solid or liquid form and a high viscosity liquid form. After four weeks of therapy on guar gum of medium viscosity (solid and liquid), a 10.4% reduction in plasma TC (241 vs 216 mg/dl) ($p<0.035$) and a 14.1% reduction in plasma LDL-C (163 vs 140 mg/dl) ($p<0.15$) were observed. In the high viscosity guar gum group, plasma TC was lowered from

252 to 215 mg/dl ($p<0.003$) and plasma LDL-C was lowered from 171 to 141 mg/dl ($p<0.02$). Plasma TG and HDL-C were not significantly altered.

Another soluble fiber component that could play a significant role in lowering cholesterol would be xylan, which constitutes the major hemicellulose in the primary cell walls of monocots. Xylan is also a component of oat bran. It has been shown to improve the protein efficiency ratio, to decrease the intestinal transit time in the rat (Fleming and Lee, 1983), to maintain the absorption and tissue concentrations of zinc and copper in rats (Jiang, 1986), and to be easily fermentable (Nyman and Asp, 1988). Using soluble fibers, such as oat bran, for lowering elevated blood cholesterol levels has been shown to be cost effective when compared to cholestyramine and colestipol (Kinosian and Eisenberg, 1988) as cholesterol lowering agents.

### b. Animal Studies

Ney et al (1988) examined the effect of 6% dietary fiber from oat fiber, obtained either from oat bran, high fiber oat flour or processed oat product, on the lipoprotein composition in rats fed diets, supplemented with 1% cholesterol and 0.2% cholic acid, for twenty days. Compared to the cholesterol fed cellulose group, the oat fiber diets altered the response to dietary cholesterol feeding as seen by a 25-45% lower plasma TC, 40-60% lower plasma VLDL-C + LDL-C and 25-40% higher plasma HDL-C concentrations ($p<.01$). The lowered combined plasma VLDL-C and LDL-C concentrations could be due to the 40-60% lower plasma apo B concentrations observed with oat fiber ingestion in these animals. The processed oat product which contained 40% more soluble fiber than the oat bran or oat flour normalized the lipoprotein profile associated with the ingestion of the atherogenic diet, significantly more than the oat bran or oat flour. This study further highlights the role of soluble fiber, present in oat fiber, in the prevention of atherosclerosis by regulating levels of VLDL-C and LDL-C concentrations in the circulation.

Similar results were observed by Ranhotra et al (1990) who examined the effect of oat bran and oat bran concentrate on the serum lipid levels in rats fed purified diets containing 1% cholesterol. These diets contained 7.68% total dietary fiber, over one-third of which was soluble dietary fiber. Cholesterol feeding significantly ($p<.05$) increased serum TC levels by week two in the cellulose group (142 mg/dl) compared to the oat bran (116 mg/dl) and oat bran concentrate (121 mg/dl) diets. The oat bran concentrate also significantly ($p<.05$) increased the serum HDL-C concentration after four weeks, in the hypercholesterolemic rats when compared to the oat bran and cellulose diets (85 mg/dl vs 66 and 67 mg/dl, respectively).

Kahlon et al (1990) evaluated the influence of full-fat and defatted rice bran, oat bran and reduced levels of rice bran in combination with wheat bran, on the plasma TC and TG levels in hamsters. All diets were isonitrogenous and contained 0.5% cholesterol, 10% total dietary fiber and 10.7% fat. After three weeks, the rice bran and oat bran diets significantly ($p<.05$) lowered plasma TC values compared to the control diet ( 274.2 and 294 mg/dl vs 401.8 mg/dl, respectively). Liver cholesterol values were also significantly ($p<.05$) lowered in these two treatment groups compared to the control group (31.2 mg/g, 39.8 mg/g vs 56.6 mg/g, respectively). Defatting the rice bran resulted in a loss of its cholesterol lowering properties. Replacing one third of the stabilized rice bran fiber with wheat bran fiber also resulted in lower plasma and liver cholesterol. The results of this study are consistent with those reported so far.

This hypocholesterolemic effect of the various soluble fiber sources could be due to a number of possible mechanisms which follow (Anderson, 1987):

1. Soluble fibers bind with bile acids and cholesterol in the intestine, decreasing their absorption. This could

result in alterations in the amount of cholesterol and fatty acids absorbed and in the size of the lipoprotein particles formed by the intestinal mucosa.

2. Soluble fibers increase the fecal excretion of bile acids and, thus, less bile acids return to the enterohepatic circulation. This could either be due to the accumulation of the soluble dietary fiber in the ileum, resulting in an ileal brake, which in turn would slow and/or reduce the amount of bile acids reabsorbed. Also, soluble dietary fiber could form a viscous mass in the ileum thus affecting the reabsorption of bile acids. This in turn could lead to an increase in hepatic cholesterol production to meet the need for bile acid synthesis and lower the cholesterol availability for the lipoprotein synthesis.

3. In the colon, the soluble fibers are fermented by the colon bacteria to short chain fatty acids (SCFA) (acetate, propionate and butyrate) which are absorbed into the portal circulation and can inhibit hepatic cholesterol synthesis. These SCFA can also inhibit cholesterol synthesis in the peripheral tissues resulting in an increase in peripheral LDL receptors and an increase in LDL clearance.

These hypothesis seem promising, but more work needs to be done to identify the exact route of action.

It is also possible that the beneficial effects observed with oat bran may be due to the displacement of foods in the diet that contain saturated fat rather than the soluble fiber in the oat bran. This was investigated by Swain et al (1990) who compared the effects of a high fiber diet containing oat bran (38.9 g/day) and a low fiber diet containing refined wheat (18.4 g/day). With this supplementation a decrease in the daily saturated fat (11.6 to 9.8 % of kcal) and cholesterol intake (274 to 184 mg/day) and an increase in PUFA (6.1 to 11.1 % of kcal) intake was observed for both diets. The high fiber diet lowered plasma TC by 7.5% (4.8 to 4.44 mmol/dl) and the low fiber diet by 7.1% (4.8 to 4.46 mmol/dl) ($p<.05$) when compared to baseline. The plasma LDL-C was also significantly lowered, compared to baseline, by both treatments, 9.1% and 6.4%, respectively ($p<.05$). A trend of lower plasma HDL-C levels on the low fiber diet was observed. The investigators concluded that oat bran had little cholesterol lowering effect and that high fiber and low fiber dietary grain supplements reduce plasma cholesterol levels about equally, probably because they replace dietary fats. However, subjects on the high fiber diets consumed significantly more total fat, saturated fat, MUFA and PUFA than those on the low fiber diet and these differences could contribute to the changes observed in the plasma cholesterol levels in the low fiber group. This study suggested that normal individuals, or those with elevated serum cholesterol levels, would benefit from diets that are rich in oat bran if their dietary fat intake is lowered to compensate for the kcal coming from oat bran.

The use of dietary fiber as a hypocholesterolemic agent raises a number of questions which need to be answered. What is the minimum daily dose of these soluble fibers that would have a positive effect, the frequency of consumption of these fiber sources, the optimal mode of administration in relation to meals, the long term effectiveness, side effects and how to overcome them?

### Dietary Fiber and Liver Cholesterol

Several mechanisms have been suggested by which dietary fiber may affect plasma cholesterol levels. These involve modifications in bile acid metabolism, inhibition of cholesterol absorption due to the binding of bile acids by fiber or an enhanced excretion of bile acids due to binding with fiber resulting in an increased cholesterol turnover. The short chain fatty acids produced as a result of fermentation of the soluble dietary fiber in the colon could influence hepatic cholesterol synthesis, which in turn could contribute to the cholesterol lowering effect observed with the feeding of soluble dietary fiber (Chen et al, 1984). Thus dietary fiber could influence the hepatic cholesterol concentrations.

Kritchevsky et al (1988) examined the effect of different dietary fibers on the serum and liver lipids in rats. The animals were fed semi-purified diets (14% fat) for 28 d, containing either particulate fibers (alfalfa, 10%; cellulose, 10%; wheat bran, 10%), soluble ionic fiber (pectin, 5%), soluble nonionic fibers (guar gum, 5%; metamucil, 10%), a mixed fiber preparation (Fibyrax, 10%), or an insoluble, ionic bile acid binding resin (cholestyramine, 2%). Compared to the fiber free control diet guar gum, Fibyrax and cholestyramine lowered serum TC levels by more than 10% (110 mg/dl vs 85 mg/dl, 79 mg/dl, 93 mg/dl, respectively) ($p<.05$). Rats fed alfalfa or wheat bran were observed to have the highest serum TC levels (127 mg/dl and 121 mg/dl, respectively). The liver total cholesterol levels were significantly elevated in all the fiber treatment groups, except in the wheat bran and cholestyramine groups, compared to the control group (450 mg/100 g) ($p<.05$). Among the fiber groups, the liver total cholesterol levels were highest in the Fibyrax and metamucil groups (616 mg/100 g, 552 mg/100 g, respectively) ($p<.05$), while they were lowest among the cholestyramine and wheat bran groups (462 mg/100 g, 449 mg/100 g, respectively)

($p<.05$). The alfalfa, cellulose, pectin and guar gum groups had intermediary values (541 mg/100 g, 523 mg/100 g, 539 mg/100 g and 513 mg/100 g, respectively) ($p<.05$). No correlation was observed between the total liver cholesterol and the fecal bile acid or the neutral steroid excretion.

Similarly Beynen et al (1989) observed that the liver cholesterol concentrations in mice were affected by the composition of the diet, with the type of carbohydrate and fat (5%) being the major determinants, when using a cholesterol free semi-purified diet. Olive oil (5% of total kcal) was observed to increase the liver cholesterol when compared to tallow, sunflowerseed oil and cocoa fat (19.1 umol/g vs 13.3 umol/g, 10.5 umol/g, 9.8 umol/g, respectively) ($p<.05$). Among the carbohydrate sources tested, fructose and sucrose produced significantly higher liver cholesterol concentrations compared to galactose and lactose (12.5 umol/g, 11.3 umol/g vs 6.4 umol/g, 9.8 umol/g, respectively) ($p<.05$). The addition of pectin (12%), to these cholesterol free diets, when compared to cellulose (12%) did not have any significant influence on the liver cholesterol concentration in mice (7.2 umol/g vs 6.9 umol/g, respectively).

Soluble dietary fibers have been shown to have hypocholesterolemic effects while the insoluble dietary fibers are relatively ineffective in lowering serum cholesterol concentrations. Chen et al (1981) studied the effect of feeding diets containing cholesterol (1%) (fat 6%) supplemented with either cellulose, pectin, oat gum or oat bran fiber (10%) for 21 d, on the plasma and liver cholesterol levels in rats. Plasma TC levels were 40% lower in the pectin and oat gum group (78 mg/dl and 83 mg/dl, respectively) and 24% lower in the oat bran group (107 mg/dl), compared to the cellulose group (140 mg/dl) ($p<.05$). The plasma HDL-C levels were significantly higher in the pectin, oat gum and oat bran groups compared to the cellulose group (34 mg/dl, 37 mg/dl, 34 mg/dl vs 21 mg/dl, respectively) ($p<.05$). The liver cholesterol concentrations were significantly lower in the pectin, oat gum and oat bran groups compared to the cellulose group (8 mg/g, 15 mg/g, 31 mg/g vs 57 mg/g, respectively) ($p<.05$). The relative liver size was observed to be bigger in the cellulose and oat bran groups compared to the other two groups. Story et al (1981) examined the effect of the addition of dietary fiber from either alfalfa, cellulose or lignin (5%) to diets containing either 0.5% or 1% cholesterol and 10% fat, fed to male rats for 28 d. With cholesterol feeding (0.5% or 1%) the liver total cholesterol levels were observed to increase significantly compared to the cholesterol free diet (17.88 mg/g and 31.28 mg/g vs 3.63 mg/g, respectively) ($p<.05$). The addition of either alfalfa, cellulose or lignin to the 1% cholesterol diet did not have any significant effect on the liver total cholesterol concentration (30.41 mg/g, 35.96 mg/g and 25.21 mg/g, respectively). However, the addition of dietary fiber to the 0.5% cholesterol diet resulted in significant reductions in the liver total cholesterol concentration in the lignin and pectin groups (6.07 mg/g and 4.53 mg/g, respectively) ($p<.05$) and insignificant reductions in the cellulose group (12.55 mg/g), compared to the 0.5% cholesterol diet (17.88 mg/g). Similarly Kritchevsky et al (1982) observed in rats, consuming diets (31% of kcal from fat) containing either 10% guar gum, mannan or pectin for 23 d, that the liver total cholesterol concentration was lowered significantly compared to the cellulose diet (177 mg/100 g, 162 mg/100 g and 169 mg/100 g vs 235 mg/100 g, respectively) ($p<.05$).

Although the research in this area is not conclusive it could be hypothesized that this increase in the liver total cholesterol concentration with soluble dietary fiber supplementation, seen in some of the studies reviewed, could be a result of decreased cholesterol absorption and an increased bile acid and neutral steroid excretion as a result of dietary fiber intake. The increase in liver cholesterol synthesis could be to compensate for these losses via the gut and for the increased utilization of this cholesterol for bile acid synthesis. This suggests that the synthesis, catabolism and release of cholesterol from the liver may be directly or indirectly altered by dietary fiber.

## Dietary Fiber, Bile Acids and Neutral Steroids

An increase in fecal bile acid excretion could lead to a decrease in the intestinal cholesterol absorption, one of the mechanisms proposed for the hypocholesterolemic effect of soluble dietary fiber. An increase in bile acid excretion in turn could lead to lowered fat absorption, both (i.e. bile acid excretion and fat absorption) of which are inversely related, thus affecting the body's cholesterol and bile acid homeostasis.

Reddy et al (1988) studied the influence of a low fat (<10% of total kcal), high dietary fiber (mixed sources, 37 g/day) and high complex carbohydrate (75-80%) diet on the fecal bile acids and neutral steroid excretion pattern in normal females. The high fiber diet increased the fecal weight 1.7 to 2.2 fold. The excretion of cholesterol and its bacterial products, coprostanol and coprostanone, were lower during the test diet when compared to the pre-test diet. Similar results were achieved with respect to the excretion of the total bile acids and secondary bile acids, deoxycholic acid and lithocholic acid. This could be due to reduced biliary production of bile acids and endogenous cholesterol as a result of the low fat, high fiber diet. The serum TC levels were significantly ($p<.01$) lower during the test period and the follow-up period ($p<.05$) than during the pre-test period (234 mg/dl, 181 mg/dl and 254 mg/dl, respectively). Reddy et al (1989) also reported that a 10 g supplement of wheat bran or cellulose significantly decreased the concentration of fecal neutral sterols (individual and total) daily, while oat bran raised the fecal concentration and total excretion of these neutral sterols. The excretion of secondary bile acids and total bile acids were also significantly ($p<.05$) lower during the cellulose and wheat bran periods while they were unaffected by the oat bran. The effect seen with neutral steroids could have been due to the binding of these substances by oat bran fiber, thus, decreasing absorption and increasing excretion.

On the other hand Salvoli et al (1985) reported that 60 g of insoluble wheat bran fiber lowered the cholesterol absorption from 50.1% to 42%. Wheat bran increased the excretion of neutral sterols compared to the pre-test diet (681.7 vs 629.6 mg/day, respectively) ($p<.05$) and also the fecal bile acid output increased from 353 mg/day to 411 mg/day ($p<.05$).

Abraham and Mehta (1988) examined the effect of a three week supplementation of the diets with 21 g/day of psyllium husk, on the plasma lipid levels and fecal neutral and acidic sterol excretion in seven men. After three weeks of psyllium supplementation the total, low and high density cholesterol levels were significantly lowered ($p<.002$, $p<.01$ and $p<.03$, respectively). The total fecal neutral steroid excretion, determined from five day fecal collections, was not different between the control and supplementation periods (1.39 vs 1.83 mmol/day, respectively). There was a small nonsignificant increase in cholesterol excretion with psyllium supplementation. A similar trend was observed with the total fecal bile acids (0.75 vs 0.828 mmol/day, respectively). These investigators concluded that the cholesterol lowering mechanism of psyllium did not appear to involve the increased excretion of fecal steroids.

Kesaniemi et al (1990) studied the cholesterol metabolism in 34, 50 year old men on high and low fiber mixed diets. The high fiber diet consisted of both gel forming and non gel forming components and the fiber intake during this period was twice that during the low fiber period (26.2 vs 11.6 g/day). After eight weeks of the high fiber diets the serum TC, LDL-C and HDL-C were significantly ($p<.05$) lower than on the low fiber diets by 5, 7 and 8 %, respectively. Serum lathosterol, which was used as an indicator of cholesterol synthesis, was significantly ($p<.05$) increased by the high fiber diet as compared to the low fiber diet (1.6 mmol/mol of cholesterol vs 1.45 mmol/mol of cholesterol), whereas the concentrations of plant sterols reflecting sterol absorption remained unchanged. Total fecal bile acid excretion was significantly ($p<.05$) increased with the high fiber diet compared to the low fiber diets (1391 vs 1231 umol/day, respectively), while no significant differences were observed in total neutral steroid excretion (2088 vs 2268 umol/day, respectively). A decrease in the bacterial conversion of the fecal sterols to secondary products was observed. The authors concluded that the reduction in serum cholesterol concentration due to high fiber diets, could be due to the enhanced fecal output of bile acids resulting in enhanced cholesterol synthesis, as indicated by increased serum lathosterol concentrations. However, more evidence is required to substantiate this claim and before definite conclusions can be drawn.

Fecal bile acid excretion in rats was also observed to vary according to the type and amount of dietary fiber (Reddy et al, 1980). Bianchini et al (1989) studied the effect of dietary fat, starch and cellulose content of the diet on the individual and total fecal bile acids in mice. The animals were fed diets containing different levels of fat (5 and 29%), starch (3,36 and 57-65%) and cellulose (2 and 10%) for four weeks. An increase in dietary fat (from 5 to 29%) significantly ($p<.001$) increased the amount of deoxycholic acid and total bile acids in the fecal samples, while an increase in starch content (36 and 57-65%) did not have any significant effect. However, an increase in cellulose content (from 2 to 10%) resulted in a marked reduction of the deoxycholic and total bile acids in the fecal samples. These results further suggest that diet could influence bile acid excretion.

So far the literature suggests that fecal bile acid and neutral steroid excretion could be regulated by the nature and amount of dietary fiber consumed. This, in turn, could lead to the lowering of tissue and plasma cholesterol levels in order to provide the liver with adequate amounts of cholesterol for bile acid synthesis (Kritchevsky, 1987).

### Hamster as an Animal Model

The use of animals in research provides a great advantage, in terms of control and compliance, while trying to study the effects of different variables such as diet, environment and activity on the different physiological and metabolic functions. However, a direct extrapolation of the results with animals to human problems has to be dealt with very carefully.

Singhal et al (1983) reported that the addition of 0.25% cholesterol to the hamster diet produced an increase in plasma TC similar to that seen in humans fed a high saturated, high cholesterol diet. Addition of cholesterol (0.15 or 1%) to the chow diet, fed for a month, produced a dose related increase in the plasma and liver cholesterol levels and lowered hepatic microsomal HMG-CoA reductase activity by 90% in both the 0.15 and 1% cholesterol diets. Supplementation of the diet with 2% cholestyramine lowered the plasma TC level by 20% and caused a five fold increase in the HMG-CoA reductase activity. Addition of dietary bile acid (0.1%) to a stock diet containing 0.01% cholesterol raised the plasma TC levels, with the most significant increase seen with cholic acid (200 mg/dl vs 119 mg/dl in the no dietary bile acid group) ($p<.01$). Also the liver cholesterol concentration doubled in the cholic acid group (4.05 mg/g vs 1.91 mg/g) ($p<.01$); this was associated with a 90% reduction in the HMG-CoA reductase activity. However, none of the dietary bile acids affected the endogenous steroid synthesis in the cholesterol fed animals and, thus, the action of bile acids on cholesterol metabolism in hamsters appears to depend on the cholesterol content of the diet.

Similar results were reported by Sicart et al (1984). In a strain of hamsters that developed spontaneous hypercholesterolemia with a standard commercial diet and with aging, the level of cholesterol in the circulating plasma VLDL, LDL and HDL fractions was observed to increase by 40, 84 and 69%, respectively. However, the percent of plasma cholesterol transported by each lipoprotein fraction in these hypercholesterolemic hamsters was similar to those carried by the VLDL, LDL and HDL fractions in the normal hamsters (17, 32 and 51%, respectively). The high plasma HDL levels could be responsible for the lack of cholesterol deposit in the aortic tissue and, to some extent, for the accumulation of lipids in the liver, which was 1213 mg/100 g in the hypercholesterolemic hamster compared to 335 mg/100 g in the normal hamster ($p<.01$). In these hypercholesterolemic hamsters the percent of cholesterol ester in the VLDL fraction doubled, compared to that in the normal hamster (21 vs 11%, respectively) ($p<.01$). A close relationship was observed between the enrichment of VLDL with cholesterol ester and the liver cholesterol levels ($r=0.7$) ($p<.01$). This increase in cholesterol ester fraction in the VLDL was observed to occur at the expense of TG which was lowered to 50% compared to 66% in the normal VLDL ($p<.01$).

In a similar study Sicart et al (1986) investigated to what extent, the hypercholesterolemic hamsters could maintain the distribution of plasma cholesterol among the lipoprotein fractions, when they developed further hypercholesterolemia, in response to a high cholesterol diet (0.1 and 0.5%). A marked increase was observed in the plasma TC levels in both groups, with the 0.5% cholesterol diet producing a marked effect compared to the 0.1% cholesterol diet (327 mg/dl vs 196 mg/dl) ($p<.05$). The increase in plasma cholesterol with the high cholesterol diet was accompanied by a redistribution of the sterol among the various lipoproteins, with a significant increase observed in the cholesterol content of the VLDL fraction. Up to 200 mg/dl of the TC in the plasma, the cholesterol transported in the IDL and HDL fractions increased significantly ($p<.05$), but above this, the excess exogenous cholesterol was found only in the VLDL, which was five fold higher than that in control animals (144 mg/dl vs 25.9 mg/dl) ($p<.05$). Thus, the effect of cholesterol feeding on the distribution of plasma cholesterol among the circulating lipoproteins varied depending on the level of cholesterol in the plasma.

Similar results were obtained by Ohtani et al (1990), on feeding 0.1% cholesterol diets to hamsters for two weeks. They also observed the effect of the addition of palmitic and linoleic acids (5% w/w) to the 0.1% cholesterol diets. On addition of palmitic acid the plasma TC increased significantly, compared to the base 0.1% cholesterol diet (224.8 vs 178.2 mg/dl) ($p<.05$) while the addition of linoleic acid lowered the plasma TC levels (160.9 vs 178.2 mg/dl). The plasma VLDL-C and LDL-C fractions were affected by the cholesterol and fatty acid feeding, while the plasma HDL-C fraction was unaffected. The plasma LDL-C was lowest in the linoleic acid group (32.3 mg/dl) compared to the palmitic acid (63.7 mg/dl) ($p<.05$) and the 0.1% cholesterol diet (50.1 mg/dl) ($p<.05$). This could be a reason for the lower plasma TC levels observed in this group. Cholesterol feeding dramatically increased the hepatic cholesterol ester levels and suppressed HMG-CoA reductase activity compared to the control diet (0.05% cholesterol) (13067 vs 4968 ug/g liver, respectively and 1.92 vs 4.17 pmol/min/mg protein, respectively) ($p<.05$). This accumulation of cholesterol could be through the chylomicron remnant pathway. However, fatty acid feeding did not affect cholesterol accumulation or HMG-CoA reductase activity. Hepatic LDL receptor activity was considerably suppressed by the control diet containing 0.05% cholesterol; a further small suppression was induced by a diet enriched with 0.1% cholesterol with or without the 5% palmitic acid. However, dietary linoleic acid diminished the effect of dietary cholesterol on the suppression of hepatic LDL receptor activity which could have, thus, resulted in the lowered plasma LDL-C level seen in the linoleic acid group.

## Cholesterol Distribution and Metabolism in the Apoproteins in Small Animal Species

Much of the cholesterol research has been done with the rat as the experimental model. However, the use of hamsters as a model is becoming more common due to a number of factors. One factor is the cholesterol distribution in the different lipoprotein fractions. Tsuda et al (1983) observed high plasma HDL-C levels in hamsters and mice (164 to 389 mg/dl) (2.4 fold) on a normal laboratory diet. The hamsters had equally high amounts of plasma LDL-C levels, while in the mice and rat these levels were observed to be low. After one week of cholesterol feeding, the plasma TC in the rat increased by 2.7 fold (63 to 162 mg/dl) while the plasma HDL-C decreased and the B-VLDL fraction increased. In the hamster the increase in plasma TC was related to an increase in the levels of all the lipoprotein fractions, LDL-C and HDL-C; the HDL to LDL cholesterol ratio was observed to be around 1:1, which is very similar to that seen in humans. Further, the absence of a gall bladder in rats could lead to changes in the type and amount of bile acids synthesized in the liver, due to the lack of the feedback control mechanism. On the other hand, the individual bile acid composition in the hamster is similar to that in humans (Kuroki et al, 1983).

Spady and Dietschy (1983) tried to quantitate and characterize the sterol synthesis in vivo in the organs of different animal species such as the squirrel monkey, hamster, rabbit and guinea pig using [$^3$H] water. It was observed that the plasma TC in the rat was lower than in the hamster (56 mg/dl and 124 mg/dl, respectively), with a major proportion of the rat TC being present in the HDL (approximately 60%) while in the hamster it was equally distributed within the LDL and HDL fractions. Also, in the rat the major site for sterol synthesis was the liver (51%), while in the hamster the liver contributed towards 27% of the total sterol synthesis with the gastrointestinal tract, skin and carcass being the major sites of synthesis. Similarly, in man the liver makes relatively small contributions to the total body sterol synthesis. The rate of whole body sterol synthesis in the rat was four fold that in the hamster (16.1 umol/hr/100 g body weight vs 2.9-4.6 umol/hr/100 g body weight) which corresponds to approximately 12 mg cholesterol/day/100 g body weight in the rat and 2.2-3.4 mg cholesterol/day/100 g body weight in the hamster. Humans have a rate similar to that seen in the hamster, 1 mg cholesterol/day/100 g body weight. Due to these low hepatic cholesterol synthesis rates, the liver in humans and hamsters cannot readily adapt to changes in the cholesterol flux and, therefore, alter the rate of LDL uptake in response to changes in diet (Spady and Dietschy, 1985). The conclusion of these researchers was that the hamster was a good model for cholesterol research since the hamster plasma LDL-C fraction responds to changes in dietary intake in a manner similar to that seen in human (Spady and Dietschy, 1988).

Spady and Dietschy (1985) reported that in hamsters saturated fat intake (20%) was associated with a significant ($p<.05$) four fold increase in plasma LDL-C (175 mg/dl) within a month. A significant reduction in the receptor dependent LDL uptake by the liver was observed when 0.12% cholesterol was added to the saturated fatty acid diet. The addition of only 0.12% cholesterol to the diet for a month increased the plasma cholesterol concentrations from 84 mg/dl to 234 mg/dl ($p<.05$), along with an increase in plasma LDL-C levels from 25 mg/dl to 82 mg/dl ($p<.05$). Simultaneously, a marked suppression in hepatic cholesterol synthesis and an increase in the level of cholesteryl esters in the liver were observed, which was associated with a decrease in receptor dependent hepatic LDL clearance. In a later study, Spady and Dietschy (1988) investigated the effects of dietary cholesterol and saturated or unsaturated fatty acids on the rates of receptor-dependent and receptor-independent LDL transport in the hamster liver. With 0.1, 0.2 and 1% cholesterol feeding for a month, the receptor dependent LDL transport was significantly suppressed, 43, 63 and 77% respectively, with a reciprocal increase in plasma LDL-C concentration. The addition of 20% coconut oil further increased the plasma LDL-C levels, while the addition of 20% PUFA (safflower oil) led to the lowering of the plasma LDL-C levels in all the three cholesterol enriched diets.

Thus, the hamster model seemed to respond like humans to the various dietary modifications. The soluble dietary fiber seems to be an effective hypocholesterolemic agent although the exact mechanism of action it still not clear; it is also not clear whether the observed effect is entirely due to the soluble dietary fiber component or due to a combination of dietary factors that are altered with the addition of dietary fiber. Review of the existing literature shows that the hamster could be a better animal model to study the effect of soluble fiber on the plasma total and lipoprotein cholesterol levels.

# The effect of different dietary fiber sources on the plasma total and lipoprotein cholesterol, liver cholesterol, fecal neutral steroid excretion and histology of major organ tissues in hamsters [1-3]

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# ABSTRACT

The effect of diets with various dietary fiber sources on the plasma lipids, liver cholesterol, the histology of the gastrointestinal tract, heart, liver and kidney and fecal neutral steroid excretion was investigated in hamsters. 155, 9-11 wk old, male Golden- Syrian hamsters were fed a purified basal hypercholesterolemic diet (0.1% cholesterol, 10% fat, 4% dietary fiber) for 5 wk to elevate plasma lipid levels. Based on wk 4 plasma total cholesterol (TC) levels, hamsters with elevated levels were randomly assigned, 16 animals/group, into six groups for another 4 wk: control, oat bran, guar gum, cellulose, xylan and sacrifice. After 4 wk of the fiber diets (10% dietary fiber), the plasma TC levels were significantly lowered in the oat bran, guar gum and xylan groups (16%, 12% and 15%, respectively) ($p<.05$). They were also significantly lower than the control and cellulose groups. Plasma HDL-C concentration tended to be lower in all the treatment groups, but was significantly decreased only in the guar gum group (12%) ($p<.05$). The combined plasma VLDL-C + LDL-C levels were significantly lowered by the oat bran, cellulose and xylan diets (38%, 40% and 34%, respectively) ($p<.05$). The liver cholesterol concentration increased significantly from 1 mg cholesterol/g liver to 4.1 mg cholesterol/g liver

(p<.05) after 4 wk of the control diet; this was further increased significantly only in the cellulose group (5.6 mg cholesterol/g liver), while the other treatment groups showed no significant changes or differences compared to the control diet group (wk 4). The total fecal neutral steroid excretion was significantly (p<.05) higher in the oat bran group compared to the other treatment groups. No major differences were observed in the tissue histology of the animals in the different treatment groups. In the present study, it appeared that oat bran, guar gum and xylan were effective hypocholesterolemic agents; however, their mechanism of action is still not clear.

## INTRODUCTION

The post World War II years saw an increase in the number of deaths due to atherosclerosis and its complications in the United States population (1). Coronary heart disease (CHD), a result of atherosclerosis, is responsible for almost 30% of the two million deaths occurring in the United States each year (1). More than 500,000 people die annually from CHD and more than six million Americans have symptomatic CHD. Diet has been identified as playing an important role in the treatment of high risk plasma total cholesterol (TC) levels, an important risk factor associated with CHD. The use of diets with low total fat (< 30% of the total kcal), low dietary cholesterol intake (< 300 mg/day) and increased complex carbohydrates (55 - 60%) forms the basis of the latest dietary guidelines from the National Cholesterol Education Program (2).

In addition to other dietary components, dietary fiber, has received a great deal of attention for its protective role against high plasma TC. The soluble fraction of various dietary fiber sources, as found in oat bran and barley, appears to have the potential for lowering plasma TC and low-density lipoprotein cholesterol (LDL-C) levels (3-5). Viscous gel forming soluble fibers, like pectin and guar gum, have also been observed to be effective hypocholesterolemic agents (6,7). Research has suggested that high fiber diets (50 g/day), similar to the American Heart Association (AHA) diets, reduce plasma TC by 11-32%; long term use of these high fiber diets may sustain these reductions up to seven years (5). A soluble fiber which has not received much attention, that could play a significant role in lowering plasma cholesterol, would be xylan, which is a component of oat bran and constitutes the major hemicellulose in the primary cell walls of monocots. Purified xylan (10% of the diet) has been shown to decrease the intestinal transit time and to be easily fermentable in rats (8).

Although numerous studies have examined the hypocholesterolemic effect of high fiber diets, a major drawback has been that it has not been possible to clearly establish whether this effect was due solely to the dietary fiber component or due to the alteration of dietary factors such as the level and type of fat when fiber was added to the diet. Also the mechanism by which the soluble dietary fiber fractions bring about their hypocholesterolemic effect is still not clear although many hypothesis have been put forward (4). One effect of dietary fiber that has gained some attention is the ability to increase the fecal bile acid and neutral steroid excretion. This is thought to interfere with the cholesterol and bile acid homeostasis, thereby, affecting the hepatic secretion of lipoproteins (4).

The purpose of this study was to investigate the effect of four different dietary fiber sources, namely, oat bran, guar gum, xylan and cellulose, in hamsters fed purified diets with the same nutrient composition except for the source of dietary fiber on plasma total and lipoprotein cholesterol levels. The influence of these dietary fiber sources on the liver cholesterol concentration, fecal neutral steroid excretion and histology of the heart, liver, kidney and gastrointestinal tract of these animals were also examined.

## METHODS

### Animal Model

One hundred and fifty five, 9-11 week old, Golden Syrian male hamsters (approximately 120 g) were purchased from Harlan Sprague-Dawley Inc. (Haslett, MI). They were housed at the university vivarium in a temperature-humidity controlled room with equal 12 h periods of light and dark cycles. Upon arrival at the vivarium, the hamsters were quarantined for a week and fed a standard chow diet. The hamsters were then transferred to the research room, housed individually in metal cages and continued on the standard chow diet. Food consumption and body weight were recorded every other day. All protocols were approved by the Virginia Tech Animal Care Committee.

## Diet

At the end of the second week, all the hamsters were switched to a purified, basal hypercholesterolemic diet (control diet) for five weeks. The control diet contained 68.5% carbohydrate (of which 4% was alphacel), 16% crude protein, 10% fat and 0.1% cholesterol as shown in Table 2. The mixture of three sources of fat ( butter, puritan oil and mazola oil) was designed to provide a P/S ratio of 0.5. At wk 5 the animals were assigned (16 animals / group) to the sacrifice group, the control diet group or one of the four experimental diet groups containing 10% dietary fiber from either cellulose (Alphacel, non-nutritive bulk, ICN Biomedicals, Costa Mesa, CA), guar gum (TIC Guar Gum, Belcamp, MD) (positive control), oat bran (Quaker Oats CO., Chicago, IL) (positive control), or xylan (isolated from steam exploded peanut hulls, Biobased Materials Technology Development Center, Virginia Polytechnic Institute and State University, Blacksburg, VA). The composition of the experimental diet is shown in Table 3.

Table 2. Composition of control diet

| Component | % Dry Weight | % Of Fat Mix |
|---|---|---|
| Casein[1] | 16 | - |
| Carbohydrate | | |
| Cornstarch | 36.5 | - |
| Dextrose[2] | 28 | - |
| Alphacel[3] | 4 | - |
| Fat Mix | 10 | - |
| Butter | - | 52.6 |
| Puritan oil | - | 31.6 |
| Mazola oil | - | 15.8 |
| Cholesterol[4] | 0.1 | - |
| DL-Methionine[5] | 0.1 | - |
| Vitamin Mix[6] | 1 | - |
| Mineral Mix[7] | 4 | - |
| Choline Chloride[8] | 0.3 | - |

[1-5,8] 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.

[6] Vitamin Mix composed of (per kg mix): Thiamine Hydrochloride 600 mg; Riboflavin 600 mg; Pyridoxine Hydrochloride 700 mg; Nicotinic Acid 3 g; D-Calcium Pantothenate 1.6 g; Folic Acid 200 mg; D-Biotin 20 mg; Cyanocobalamin 1 mg; Retinyl Palmitate 1.6 g; DL -alpha-Tocopherol Acetate 20 g; Cholecalciferol 250 mg; Menaquinone 5 mg; Sucrose 972.9 g; ICN Biomedicals Inc., Costa Mesa, CA.

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

Table 3. Composition of experimental diet

| Components | % Dry Weight | % Of Fat Mix |
|---|---|---|
| Casein[1] | 16 | - |
| Carbohydrate | | |
| Cornstarch | 30.5 | - |
| Dextrose[2] | 28 | - |
| Fiber[*] | 10 | - |
| Fat Mix | 10 | - |
| Butter | - | 52.6 |
| Puritan oil | - | 31.6 |
| Mazola oil | - | 15.8 |
| Cholesterol[3] | 0.1 | - |
| DL-Methionine[4] | 0.1 | - |
| Vitamin Mix[5] | 1 | - |
| Mineral Mix[6] | 4 | - |
| Choline Chloride[7] | 0.3 | - |

[1-4,7] Casein, Vitamin Free; Dextrose; Cholesterol U.S.P.; DL-Methionine U.S.P.; Choline Chloride; ICN Biomedicals Inc., Costa Mesa, CA.

[5] Vitamin Mix composed of (per kg mix): Thiamine Hydrochloride 600 mg; Riboflavin 600 mg; Pyridoxine Hydrochloride 700 mg; Nicotinic Acid 3 g; D-Calcium Pantothenate 1.6 g; Folic Acid 200 mg; D-Biotin 20 mg; Cyanocobalamin 1 mg; Retinyl Palmitate 1.6 g; DL-alpha-Tocopherol Acetate 20 g; Cholecalciferol 250 mg; Menaquinone 5 mg; Sucrose 972.9 g; ICN Biomedicals Inc., Costa Mesa, CA.

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

[*] From Oat Bran (Quaker Oats Co., Chicago, IL) or Guar Gum (TIC Guar Gum, Belcamp, MD) or Xylan (Steam exploded peanut hulls, Biobased Materials Technology Development Center, Virginia Polytechnic Institute and State University, Blacksburg, VA) or Cellulose (Alphacel non-nutritive bulk, ICN Biomedicals, Costa Mesa, CA)

## Design

The experimental design is shown in Table 4. At wk 0 (after 2 wk on laboratory chow), 16 animals were sacrificed for baseline histology of the gastrointestinal tract, heart, kidney and liver. Similarly at wk 4, 16 animals were sacrificed to determine the effect of the hypercholesterolemic control diet on the histology of the above tissues. At the end of the study the remaining animals were sacrificed and similar histological examinations were done to determine the effect of the experimental diets.

Fasting blood samples were taken, prior to the introduction of the basal hypercholesterolemic diet, to establish baseline values for the different plasma parameters. After four weeks of feeding the hypercholesterolemic control diet, fasting blood samples were again taken to determine the new levels of plasma total and lipoprotein cholesterol levels. Based on these values the animals were ranked by plasma TC and assigned by randomized block design to one of the five experimental diet groups (n=16), namely, control diet, cellulose, guar gum, oat bran or xylan diets and the sacrifice group. Animals were not started on the experimental diets until wk 5, in order to assign the animals to treatments and to stabilize their weight after the blood collection done at

Table 4. Experimental design

| | | | |
| --- | --- | --- | --- |
| Chow n=155<br>Quarantine | Control (C) n=139<br>Hypercholesterolemic diet (4% cellulose) | | C + Xylan 10%,n=16<br>C + Cellulose 10%,n=16<br>Control,continued,n=16<br>C + Oat Bran 10%,n=16<br>C + Guar Gum 10%,n=16 |
| Weeks | 0 | 4 | 9 |
| Blood samples | x | x | x |
| Liver cholesterol | x | x | x |
| Fecal collection | | | ¦----¦ |
| Histo-logical examination | x | x | x |

wk 4. After 4 wk of the experimental diets, at wk 9, fasting blood samples were collected from the animals before they were sacrificed with sodium pentobarbital.

Fecal samples were collected over a four day period during the last week of the experimental diets for each animal for steroid analysis.

## Analytical Procedures

### Blood Samples

The blood samples (approximately 2 ml) at each sampling period were drawn from the orbital sinus of the 12 h fasted hamsters after the administration of the anesthetic ketamine (0.2 - 0.3 ml/animal) (9). Plasma samples were obtained by centrifugation at 3000 rpm for 30 min. Aliquots of 100 ul of each plasma sample were taken for the analysis of the high-density lipoprotein cholesterol (HDL-C) fraction by ultracentrifugation. The plasma TC and triglyceride (TG) were analyzed with a modification of the enzymatic method of Allain et al (10) and Wahlefeld (11), respectively. The plasma HDL-C fraction (d > 1.063 g/ml) was separated from the very-low-density lipoprotein cholesterol (VLDL-C) and LDL-C fractions with a 16.7% saline solution, using the Beckman TL-100 Table Top Ultracentrifuge method (12). The plasma HDL-C in the lower fraction was determined by the enzymatic method of Allain et al (10). The combined plasma

VLDL-C and LDL-C concentrations were calculated as follows:

VLDL-C + LDL-C = TC - HDL-C

## Liver Cholesterol Analysis

At the time of sacrificing, a portion of the liver from each animal was taken, before the rest of the liver was placed in formalin, and frozen. The cholesterol in these liver samples was extracted using the chloroform : methanol (2:1, v/v) extraction procedure of Folch et al (13); the cholesterol concentration was determined using the modified enzymatic cholesterol procedure of Allain et al (10).

## Fecal Samples

Fecal samples collected over the four day period for each animal were pooled and frozen at -20°C until analysis. These samples were freeze dried, ground and then analyzed for neutral steroid content using a toluene and petroleum ether extraction procedure (14); the steroid concentration was quantitatively determined on the Shimadzu Gas Chromatograph GC-9A using a glass column packed with 3% SP 2250 on 100/120 SUPELCOPORT, 3′ x 2 mm ID. The following conditions were used : temperature : isothermal analysis with a column temperature of 275°C, detector and injector temperature of 295° C; flow rate : FID air - 400 ml/min, $H_2$ - 40 ml/min, carrier gas through column - 50 ml/min;

sensitivity : FID - 4 x $10^{-11}$ AFS.

The percent water content of the fecal matter was calculated as the percent difference between the wet and dry fecal weights (15).

### Tissue Samples

At the beginning, middle and end of the study (0,4 and 9 weeks) animals were sacrificed using sodium pentobarbital (40 - 60 mg/kg) and the gastrointestinal tract, heart, kidney and liver from each animal were removed for histological examinations. All the tissues were placed in 10% buffered formalin until further processing. Later these tissues were trimmed, dehydrated in a graded series of alcohol and infiltrated with paraffin. These paraffin embedded tissues were sectioned on a microtome, rehydrated and stained with aqueous dyes (hematoxylin and eosin) to accentuate the cellular details. The slides were again dehydrated and a coverslip was fixed with mounting medium and examined by the pathologist.

### Diet Analysis

The control and experimental diets were analyzed for macronutrients by proximate analysis (16) and total dietary fiber (17).

## Statistical Analysis

A general linear model (GLM) with repeated measures (wk 0, 4 and 9) was used to test for overall differences among the treatment groups ($p<.05$) using the SAS system (SAS Institute Inc., Cary, NC). A Dunn t test was used to locate differences among the means of the groups.

## RESULTS

### Proximate Analysis of the Diets

The proximate analysis of the five treatment diets are presented in Table 5. The nutrient compositions of these semi-purified diets were very similar to each other and were similar to the calculated formulations (Table 2 and 3). The experimental diets had a P/S ratio of 0.5 and a total fiber content of approximately 10%. Acid hydrolysis of xylan, for individual sugars, showed that it was made up of 34% xylose, 6.5% glucose, 3% galactose and 2% arabinose; guar gum consisted of 45% mannose, 27% galactose, 2% glucose and 1% arabinose.

### Food Intake and Body Weight

The mean food intake and final body weights for animals in each of the five groups over the nine week study period are shown in Table 6. No significant differences were observed in the average food intake and final body weights across the groups. Although the feed intake was lowest in the oat bran diet group (13 g/day), the mean body weight of this group was towards the upper range of weights (145 g). The control group had the highest mean final body weight (148 g); the xylan group had the lowest (136 g).

Table 5. Proximate analysis of the diets

| Diet | Absolute Dry Matter (%) | Protein (%) | Fat (%) | Ash (%) | Dietary Fiber Soluble (g/100 g) | Dietary Fiber Insoluble (g/100 g) |
|---|---|---|---|---|---|---|
| Control | 93.9 | 14.6 | 10.2 | 3.1 | 1.7 | 7.6 |
| Oat Bran | 94.3 | 13.0 | 8.9 | 4.5 | 4.2 | 5.7 |
| Guar Gum | 93.8 | 14.8 | 10.3 | 3.1 | 8.9 | 2.9 |
| Xylan | 94.6 | 14.0 | 8.6 | 4.7 | 10.9 | 2.9 |
| Cellulose | 94.4 | 14.1 | 9.7 | 3.3 | 0.9 | 12.7 |

Table 6. Mean food intake and final body weight *

| Group | Food Intake (g/day) | Final Body Weight (g) |
|---|---|---|
| Control | 15 ± 4 | 148 ± 21 |
| Oat Bran | 13 ± 4 | 145 ± 21 |
| Guar Gum | 16 ± 5 | 141 ± 16 |
| Xylan | 15 ± 4 | 136 ± 18 |
| Cellulose | 18 ± 5 | 141 ± 19 |

Mean ± SD

* n per group = 16

## Plasma Total Cholesterol

Plasma TC levels (Table 7) were significantly increased in all groups from wk 0 to wk 4 (p<.05), after consuming a control diet consisting of 10% fat and 0.1% cholesterol. At wk 9, the plasma TC levels in the oat bran group (212 mg/dl), guar gum group (219 mg/dl) and xylan group (210 mg/dl) were significantly lower than the plasma TC levels in the control (234 mg/dl) and cellulose (228 mg/dl) groups (p<.05). After four weeks of the fiber diets, the oat bran group had a significant reduction in plasma TC, from 253 mg/dl to 212 mg/dl (-41 mg/dl, 16%) (p<.05). The guar gum group also showed a significant reduction in plasma TC, from 249 mg/dl to 219 mg/dl (-30 mg/dl, 12%) (p<.05); similar reductions were observed in the plasma TC levels in the xylan group, which were lowered from 248 mg/dl to 210 mg/dl (-38 mg/dl, 15%) (p<.05). The plasma TC levels at wk 9 in the control and cellulose groups were not significantly different from those at wk 4, although there was a downward trend from the levels at wk 4 (234 vs 245 mg/dl and 228 vs 249 mg/dl, respectively).

## Plasma High-Density Lipoprotein Cholesterol

The changes in plasma HDL-C levels at the three time periods (week 0, 4 and 9) are presented in Table 8. The baseline plasma HDL-C levels ranged from 75 to 82 mg/dl,

Table 7. Mean plasma total cholesterol levels *

| Group | Week 0 | Week 4 | Week 9 |
|---|---|---|---|
| | (mg/dl) | | |
| Control | 107 ± 12$^{a}$ | 245 ± 27$^{b}$ | 234 ± 43$^{b}$ |
| Oat Bran | 100 ± 10$^{a}$ | 253 ± 27$^{b}$ | 212 ± 47$^{c}$ |
| Guar Gum | 113 ± 14$^{a}$ | 249 ± 24$^{b}$ | 219 ± 33$^{c}$ |
| Xylan | 110 ± 12$^{a}$ | 248 ± 24$^{b}$ | 210 ± 52$^{c}$ |
| Cellulose | 107 ± 13$^{a}$ | 249 ± 21$^{b}$ | 228 ± 36$^{b}$ |

Mean ± SD

$^{a-c}$ values within a row or column with different letters are significantly different ($p<.05$)

* n per group = 16

which increased significantly after four weeks of the control diet, ranging from 162 mg/dl to 172 mg/dl ($p<.05$). After four weeks of experimental diets, the plasma HDL-C level was significantly lowered only in the guar gum group, from 168 mg/dl to 148 mg/dl (-20 mg/dl, 12%) ($p<.05$). The oat bran and xylan groups experienced slight reductions in the plasma HDL-C levels by wk 9; these changes were not significant. The control and cellulose groups showed slight increases. In spite of these changes the TC/HDL-C ratios across the groups remained the same across the three time periods (1.4, 1.5 and 1.3, respectively).

### Plasma Very-Low and Low-Density Lipoprotein Cholesterol

The combined plasma VLDL-C + LDL-C (Table 9) were significantly increased after four weeks of the control diet ($p<.05$). After four weeks of the experimental diets, this combined cholesterol fraction decreased significantly, compared to the level at wk 4, by 38% in the oat bran group (82 to 51 mg/dl) ($p<.05$), by 34% in the xylan group (78 to 52 mg/dl) ($p<.05$) and by 40% in the cellulose group (80 to 48 mg/dl) ($p<.05$). Changes from 81 to 70 mg/dl in the guar gum group and 83 to 66 mg/dl in the control group were also observed, although they were not significant.

Table 8. Mean plasma high-density lipoprotein cholesterol levels *

| Group | Week 0 | Week 4 | Week 9 |
|---|---|---|---|
| | (mg/dl) | | |
| Control | 78 ± 9$^a$ | 162 ± 15$^b$ | 174 ± 36$^b$ |
| Oat Bran | 76 ± 8$^a$ | 172 ± 16$^b$ | 162 ± 39$^b$ |
| Guar Gum | 82 ± 13$^a$ | 168 ± 15$^b$ | 148 ± 32$^c$ |
| Xylan | 79 ± 7$^a$ | 170 ± 15$^b$ | 158 ± 46$^b$ |
| Cellulose | 75 ± 13$^a$ | 169 ± 14$^b$ | 181 ± 43$^b$ |

Mean ± SD

$^{a-c}$ values within a row or column with different letters are significantly different ($p<.05$)

* n per group = 16

Table 9. Mean plasma very-low and low-density lipoprotein cholesterol levels *

| Group | Week 0 | Week 4 | Week 9 |
|---|---|---|---|
| | (mg/dl) | | |
| Control | $28 \pm 11^{a}$ | $83 \pm 23^{b}$ | $66 \pm 32^{b}$ |
| Oat Bran | $24 \pm 10^{a}$ | $82 \pm 20^{b}$ | $51 \pm 28^{c}$ |
| Guar Gum | $31 \pm 10^{a}$ | $81 \pm 14^{b}$ | $70 \pm 25^{b}$ |
| Xylan | $29 \pm 13^{a}$ | $78 \pm 23^{b}$ | $52 \pm 32^{c}$ |
| Cellulose | $33 \pm 12^{a}$ | $80 \pm 16^{b}$ | $48 \pm 32^{a}$ |

Mean ± SD

$^{a-c}$ values within a row or column with different letters are significantly different ($p<.05$)

* n per group = 16

## Plasma Triglycerides

The changes in the plasma TG levels during the study period are shown in Table 10. A significant increase was observed in the plasma TG levels after four weeks of the control diet, which ranged from 181 mg/dl (xylan group) to 256 mg/dl (oat bran group) ($p<.05$). After four weeks of the experimental diets, only the guar gum group had a significant reduction in the plasma TG levels, from wk 4 to wk 9 (234 to 123 mg/dl, 47%) ($p<.05$). The oat bran and cellulose groups showed apparent reductions in plasma TG levels at wk 9 compared to wk 4, but they were not significant (256 to 194 mg/dl; 222 to 183 mg/dl, respectively). However, considerable inter-animal variation was observed in the plasma TG levels in all the groups resulting in a large standard deviation which very likely affected the statistical results.

## Liver Cholesterol

The baseline average liver cholesterol concentration was 1 mg cholesterol/g liver. After 4 weeks of the 0.1% cholesterol diet, the liver cholesterol concentration increased significantly to 4.1 mg cholesterol/g liver ($p<.05$). At wk 9, after 4 weeks of the treatment diets, the cholesterol concentration in the liver was observed to rise significantly only in the cellulose group

Table 10. Mean plasma triglyceride levels *

| Group | Week 0 | Week 4 | Week 9 |
|---|---|---|---|
| | (mg/dl) | | |
| Control | 68 ± 25[a] | 249 ± 136[b] | 234 ± 139[b] |
| Oat Bran | 65 ± 29[a] | 256 ± 160[b] | 194 ± 110[b] |
| Guar Gum | 70 ± 13[a] | 234 ± 133[b] | 123 ± 74[c] |
| Xylan | 53 ± 18[a] | 181 ± 90[b] | 183 ± 156[b] |
| Cellulose | 65 ± 32[a] | 222 ± 98[b] | 183 ± 127[b] |

Mean ± SD

[a-c] values within a row or column with different letters are significantly different ($p<.05$)

* n per group = 16

(5.6 mg cholesterol/g liver) ($p<.05$), while the other fiber treatments showed no significant differences when compared to the control group or to the wk 4 values (Table 11), though there was a trend for lower values in the oat bran, guar gum and xylan groups.

## Fecal Data

The mean fecal weights, moisture content and percent water content are presented in Table 12. The cellulose diet produced greater fecal mass, both on the wet and dry weight basis. The control and oat bran diets produced intermediate amounts of wet and dry feces. The fecal matter produced by the cellulose group had significantly higher water content than that of the guar gum group (0.2 g) ($p<.05$). The percent water content of the fecal matter was highest in the cellulose group (14%) and lowest in the guar gum group (8%), with no significant differences between groups.

## Fecal Neutral Steroid Excretion

The neutral steroid excretion (Table 13) expressed as mg/g fecal dry weight was significantly ($p<.05$) higher in the oat bran and guar gum groups (1.33 and 1.0 mg/g fecal dry weight, respectively) compared to the xylan and cellulose groups (0.44 and 0.49 mg/g fecal dry weight).

Table 11. Mean liver cholesterol concentration *

| Group | Week 9<br>(mg/g) |
|---|---|
| Control | 3.9 ± 1.5[a] |
| Oat Bran | 3.2 ± 1.2[a] |
| Guar Gum | 3.0 ± 1.1[a] |
| Xylan | 2.8 ± 1.0[a] |
| Cellulose | 5.6 ± 2.0[b] |

Mean ± SD

[a-b] values with different letters are significantly different (p<.05)

* n per group = 16

Table 12. Fecal weights and moisture content and water *

| Group | Wet Fecal Weight (g/4 d) | Dry Fecal Weight (g/4 d) | Moisture Content (g) | Percent Water Content (%) |
|---|---|---|---|---|
| Control | $4.6 \pm 1.1^{b,c}$ | $4.1 \pm 0.8^{b,c}$ | $0.5 \pm 0.5^{a,b}$ | $10.9 \pm 8.4^{a}$ |
| Oat Bran | $4.8 \pm 1.0^{b,c}$ | $4.2 \pm 0.8^{b,c}$ | $0.5 \pm 0.6^{a,b}$ | $12.4 \pm 17.2^{a}$ |
| Guar Gum | $3.4 \pm 0.6^{c}$ | $3.2 \pm 0.6^{c}$ | $0.2 \pm 0.1^{b}$ | $8.1 \pm 2.8^{a}$ |
| Xylan | $6.0 \pm 1.6^{a,b}$ | $5.3 \pm 1.1^{b}$ | $0.6 \pm 0.4^{a,b}$ | $10.7 \pm 6.3^{a}$ |
| Cellulose | $7.9 \pm 4.0^{a}$ | $6.8 \pm 2.7^{a}$ | $1.1 \pm 1.4^{a}$ | $13.6 \pm 10.5^{a}$ |

Mean ± SD

$^{a-c}$ values within a column with different letters are significantly different (p<.05)

* n per group = 16

In the control group the neutral steroid excretion was intermediate at 0.87 mg/g fecal dry weight, and was not significantly different from the other diet groups. The total neutral steroid excretion based on the total fecal dry weight for the four day collection period was significantly higher in the oat bran group (5.37 mg/total fecal dry weight) compared to the other fiber diets ($p<.05$), but was not significantly different from the control diet (3.56 mg/total fecal dry weight).

### Histological Examination

Microscopic examination at wk 9 of the heart, liver, kidney and the gastrointestinal tract (stomach, duodenum, jejunum, ileum, cecum and colon) showed no major abnormalities in the animals fed the different treatment diets. Autolysis was frequently observed in the tissues examined. Since no specific abnormalities were observed in these tissues at wk 9, microscopic examination of the tissues obtained at weeks 0 and 4 was not considered necessary.

Table 13. Fecal neutral steroid excretion *

| Group | Neutral Steroid Excretion (mg/g) | Total Neutral Steroid Excretion (mg/total fecal dry weight) |
|---|---|---|
| Control | 0.87 ± $0.36^{a,b}$ | 3.56 ± $1.42^{a,b}$ |
| Oat Bran | 1.33 ± $0.63^{a}$ | 5.37 ± $2.16^{a}$ |
| Guar Gum | 1.00 ± $0.60^{a}$ | 3.11 ± $1.75^{b}$ |
| Xylan | 0.44 ± $0.29^{b}$ | 2.29 ± $1.73^{b}$ |
| Cellulose | 0.49 ± $0.26^{b}$ | 3.30 ± $2.17^{b}$ |

Mean ± SD

$^{a-b}$ values within a column with different letters are significantly different ($p<.05$)

* n per group = 16

## DISCUSSION

The hypocholesterolemic effect of soluble fiber has gained a great deal of attention in Western societies, as one of several possible means of lowering the incidence of CHD. When evaluating a new soluble fiber source, the use of a small animal model is logical for testing the material with possible unknown side effects, in a controlled environment. Among the rodents, hamsters have the most abundant plasma LDL-C levels, with the ratio of cholesterol content between plasma HDL-C and LDL-C being 1:1 (18). However, the use of the hamster as a small animal model for cholesterol related research has been limited. Several studies have reported that the plasma TC concentrations in hamsters respond to hypercholesterolemic diets in a manner similar to that seen in humans fed high saturated fat and high cholesterol diets (19-21). Due to the low hepatic cholesterol synthesis rates in hamsters, the liver in these animals cannot readily adapt to changes in the cholesterol flux and, thus, alters the rate of LDL-C uptake in response to changes in diet, which is similar to the response seen in humans (22,23). It was, thus, concluded by this author and others (19-23) that the hamster was a good model to study the hypocholesterolemic effect of different dietary fiber sources.

In the present study, after 4 wk on the

hypercholesterolemic diet (0.1% cholesterol), an elevated response was seen in all the plasma cholesterol fractions (Tables 7-10). This response was similar to the elevated responses seen in hamsters fed diets with varying amounts of cholesterol (0.1%, 0.15%, 0.5% and 1%) (19,21). Singhal et al (19) observed that feeding hamsters diets containing 0.15% cholesterol for thirty days increased the serum cholesterol levels from 114 mg/dl to 215 mg/dl (p<.01) and a diet containing 1% cholesterol increased the serum cholesterol from 114 to 342 mg/dl (p<.01). Similarly Sicart et al (21) observed in hamsters consuming a diet containing 0.5% cholesterol for 4 wk, that the plasma cholesterol levels were 327 mg/dl compared to 196 mg/dl in hamsters fed a diet containing 0.1% cholesterol (p<.01). Tsuda et al (18) also reported similar results in hamsters fed a 1% cholesterol diet for a week. The plasma cholesterol levels were observed to increase from 164 mg/dl to 389 mg/dl, a 2.4 fold increase. They also observed that an increase in plasma TC levels was associated with an increase in all the lipoprotein fractions with a HDL-C to LDL-C ratio of 1:1.

After 4 wk of consuming diets containing 10 to 14% dietary fiber with significant amounts of soluble fiber from oat bran, guar gum and xylan, the plasma TC was significantly lowered by 16%, 12% and 15%, respectively (p<.05) from levels at wk 4 of the study. The cellulose and control groups did not show any significant change. This reduction in plasma TC could be attributed largely to the dietary fiber component present in the diet, since these diets had similar amounts of all other nutrients, as seen by their proximate analysis (Table 5). The amounts of soluble and insoluble fiber did vary, however, between diets (Table 5). The lower plasma TC levels could not be attributed to the lower body weights or food intake as body weights of the animals at nine weeks and average food intakes in the experimental groups were not different (Table 6).

Similar results were observed by Kahlon et al (25) in hamsters fed diets containing 10% total dietary fiber, from either full-fat or defatted rice bran, oat bran or rice bran + wheat bran, for 3 wk. The rice bran and oat bran diets significantly lowered the plasma TC levels compared to the control diet (274.2 mg/dl and 294 mg/dl vs 401.8 mg/dl, respectively) ($p<.05$).

The results of the present study are also similar to those reported by Ney et al (24) who observed a 25-45% lower plasma TC in hypercholesterolemic rats fed diets for 20 d containing either oat fiber, high fiber oat flour or processed oat products (all containing 6% dietary fiber). The soluble fiber content in processed oat products was 40% greater than that in the untreated oat bran and this, plus being a different species, could have accounted for the greater reduction observed in the plasma TC with these processed oat products. Ranhotra et al (26) also observed that cholesterol feeding (1%) significantly ($p<.05$) increased serum TC levels in rats by wk 2 in the cellulose group (142 mg/dl) compared to oat bran (116 mg/dl) and oat bran concentrate (121 mg/dl) groups. These fiber diets provided 7.68% total dietary fiber, over one-third of which was soluble dietary fiber.

Although plasma HDL-C levels were observed to be somewhat lower after feeding the three soluble fiber diets, in the present study, no significant reductions were observed except with guar gum (12%) ($p<.05$). These reductions in the plasma HDL-C levels were less than the reductions observed in the plasma TC and thus the TC/HDL-C ratio remained the same. At the same time the combined plasma VLDL-C + LDL-C levels decreased in all the fiber treatments (soluble and insoluble) with significant reductions in the oat bran (38%), xylan (34%) and cellulose (40%) groups ($p<.05$). Thus, the proportion of cholesterol in this atherogenic fraction was significantly influenced by these dietary fiber treatments. This greater reduction in the combined plasma VLDL-C + LDL-C fraction in comparison to the reduction in plasma HDL-C could, thus, theoretically reduce the risk of CHD, if the same effect holds true in the human.

Ney et al (24) observed a 40-60% lower plasma VLDL-C + LDL-C and a 25-40% higher plasma HDL-C concentration ($p<.01$) in rats fed diets containing 6% oat fiber for 20 d. Similarly Ranhotra et al (26) observed that the serum HDL-C levels, as a percent of TC, were significantly increased in the hypercholesterolemic rats fed oat bran concentrate for 4 wk, compared to the cellulose group (85% vs 67%) ($p<.05$).

The effect of these dietary fiber treatments on the plasma TG levels appeared to vary considerably, with only guar gum producing a significant reduction ($p<.05$). Except for xylan, there was a lowering trend for the other three groups. Considerable variation was observed with the plasma TG values and the large standard deviations obtained influenced the statistical results. The influence of fiber on this lipid fraction has not been studied extensively, probably due to its variability.

Similar effects of oat bran and guar gum on the plasma TC have been reported in humans (5). Simons et al (27) observed a 15% reduction, similar to the 12% decrease observed in this study, in plasma TC in individuals supplemented with 6 g of active guar formulation for over a year. The 15% reduction in plasma TC levels observed in the xylan group indicate that this fiber source could also be an effective hypocholesterolemic agent.

Anderson et al (28) observed that feeding an oat bran cereal diet for two weeks (25 g/d) to hypercholesterolemic males, lowered the serum TC and LDL-C levels significantly by 5.4% ($p<.05$) and 8.5% ($p<.025$), respectively. Similar results were documented with guar gum which was observed to produce 15-20% reductions in the plasma LDL-C in hypercholesterolemic individuals (27,29).

Oat products are rich in B-glucan, which could be the major lipid lowering component. It is hypothesized that this B-glucan molecule soaks up water, becomes viscous and reduces the diffusion of bile acids through the intestinal wall. These captured bile acids are excreted, thus making the body draw on its cholesterol pool to replace the excreted bile acids, leading to a reduction in the circulating cholesterol (30). The viscosity of guar gum appears to play a major role in lowering plasma cholesterol levels (3). The exact properties of xylan that could play a role in its hypocholesterolemic effect is not clear at this point and further chemical and physical characterization of this soluble fiber is required. Also these soluble fibers could interfere with the micelle formation in the proximal small intestine, which could decrease the absorption of cholesterol or fatty acid or alter the lipoprotein particle formed by the intestinal mucosa (4). One of the commonly hypothesized theories for the hypocholesterolemic effect of these soluble fibers is their ability to increase fecal excretion of bile acids and/or neutral steroids leading to lesser amounts of bile acids and cholesterol returning to the enterohepatic circulation. This in turn could lead to an increase in hepatic cholesterol production to meet the need for bile acid synthesis and thus lower the cholesterol available for lipoprotein synthesis (31).

Although high fiber diets have been shown to increase fecal bile acid excretion, their influence on fecal neutral steroid excretion is still not clear (6,32). In the present study animals on oat bran and guar gum treatments had significantly higher fecal neutral steroid excretion (1.33 and 1.00 mg/g fecal dry weight, respectively) than did those in the xylan and cellulose groups (0.44 and 0.49 mg/g fecal dry weight, respectively) with the control group (0.87 mg/g fecal dry weight) intermediate (Table 13), suggesting that neutral steroid excretion did not explain the significantly lower plasma TC for the xylan group. The total neutral steroid excretion was significantly higher in the oat bran group (5.4 mg/ total fecal dry weight) compared to the other fiber treatment groups ($p<.05$) and, therefore, might be part of the mechanism of lowering plasma TC by the oat fiber.

In humans, psyllium husk supplementation (21 g/d) did not increase fecal neutral steroid excretion compared to a low fiber control diet (1.39 vs 1.83 mmol/d, respectively) (6), while 60 g of wheat bran increased fecal neutral steroid excretion compared to the pre-test diet (681.7 vs 629.6 mg/d, respectively) ($p<.05$) (32). Reddy et al (33) reported that 10 g of oat bran supplement increased the fecal neutral steroid concentration, which could be due to the binding or entrapment of these substances by the oat fiber thus decreasing absorption and increasing excretion. This possible mode of action could be partially responsible for the results observed in the present study with the oat bran and guar gum treatments, but apparently not, for the xylan treatment.

Most fibers increase the stool volume and weight through an increase in undigested and unfermented material, bound water and bacterial cell mass. This property of dietary fiber can, thus, interfere with the absorption of nutrients and other compounds in the small intestine. The cellulose diet produced greater dry fecal mass compared to the other fiber groups ($p<.05$) (Table 12). Fecal water content was greatest for cellulose diets (Table 12), indicating that high water content could be responsible for the increased fecal weights in these fiber fed hamsters.

In the present study, feeding a 0.1% cholesterol diet for 4 wk significantly increased the liver cholesterol concentration ($p<.05$). However, the soluble fiber treatments did not have any significant effect on the liver cholesterol concentrations, although a lowering trend was observed. However the cellulose fiber treatment was observed to significantly increase the liver cholesterol concentration compared to the control and the three soluble fiber treatments ($p<.05$) (Table 11).

The results of the present study are similar to those observed by Kahlon et al (25) in hamsters fed diets containing 0.5% cholesterol and 10% total dietary fiber, from either full-fat or defatted rice bran, oat bran or rice bran + wheat bran, for 3 wk. In these hypercholesterolemic hamsters the liver cholesterol was significantly lowered by the oat bran and full-fat rice bran treatment compared to the cellulose diet (39.8 and 31.2 mg/g vs 56.6 mg/g, respectively) ($p<.05$).

Chen et al (34) observed that feeding rats with diets containing cholesterol (1%) supplemented with either cellulose, pectin, oat gum or oat bran fiber (10%) for 21 d, produced a 40% reduction in the plasma TC in the pectin and oat gum groups and a 24% reduction in the oat bran group compared to the cellulose group ($p<.05$). The liver total cholesterol concentrations were also significantly lower in the pectin, oat gum and oat bran groups compared to the cellulose group (8 mg/g, 15 mg/g, 31 mg/g vs 57 mg/g, respectively) ($p<.05$). Similarly Story et al (35) observed that feeding rats with diets containing 0.5% cholesterol, supplemented with either cellulose, lignin or pectin for

3 wk resulted in significantly lower liver total cholesterol in the lignin and pectin groups compared to the control diet (6.07 mg/g and 4.53 mg/g vs 17.88 mg/g, respectively) ($p<.05$) while an insignificant reduction was observed in the cellulose group (12.55 mg/g). Story et al (35) hypothesize that this alteration in the liver cholesterol concentration with fiber containing diets could be due to the influence on the synthesis, catabolism and release of cholesterol from the liver either directly or indirectly by the dietary fiber, which in turn could influence the plasma cholesterol levels and bile acid metabolism.

The results of this study show that the soluble dietary fibers oat bran, guar gum and xylan were effective in lowering plasma TC levels in hypercholesterolemic hamsters, while the insoluble fiber had no effect on the plasma TC levels. Although the plasma HDL-C levels were also observed to be lowered with these fiber treatments, no significant reductions were observed, except in the guar gum group. In addition to lowering the plasma TC levels, the xylan and oat bran treatments significantly lowered the combined plasma VLDL-C + LDL-C levels. The cellulose fiber treatment significantly and unexpectedly decreased this combined lipoprotein fraction as well. In trying to establish the mechanism of action of the soluble fibers, the total fecal neutral steroid excretion was highest in the oat bran treatment, while the other treatments did not exhibit any significant effect. This suggested that the hypocholesterolemic effects of oat bran could be mediated through binding with steroids, thus, interrupting the enterohepatic pathway. This was apparently not the case with guar gum or xylan. A trend towards lowering the liver cholesterol concentration was observed with the soluble fiber treatments.

Thus, in the present study, it appeared that the cholesterol lowering effect observed with the addition of 10% soluble dietary fiber could be attributed to this dietary component. These dietary fiber sources had a significant lowering effect on the plasma TC and the combined plasma VLDL-C + LDL-C levels in the hamster. However, the mechanism of their hypocholesterolemic effect is still not clear. Thus, the use of the hamster as a small animal research model provided some valuable information relative to the effect of soluble dietary fiber, and helped to establish that xylan isolated from steam exploded peanut hulls may be a useful soluble dietary fiber source to lower plasma total cholesterol and some lipoprotein levels. However, more research is needed to substantiate these results and to establish a mechanism of action.

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# SUMMARY AND CONCLUSIONS

Coronary heart disease (CHD) is the leading cause of death in Western industrialized nations and accounts for nearly half of all the deaths in the United States. More than 500,000 people die annually from CHD and more than 6 million Americans have symptomatic CHD. Several risk factors have been independently associated with CHD, such as cigarette smoking, high blood pressure and elevated plasma lipid levels. An elevated plasma cholesterol level is one of the major risk factor of CHD and is significantly influenced by diet.

Complex carbohydrates, including dietary fiber, have received a great deal of attention for their protective role against some of the diseases prevalent in Western society. Soluble dietary fiber, such as guar gum and pectin, and found in oat bran, barley and legumes appears to have the potential for lowering the plasma TC and LDL-C levels. However, it is not clear whether this effect is due solely to the dietary fiber component per se or due to a combination of dietary factors that are usually altered, such as the level and type of fat or calories, when fiber is added to the diet. Also the mechanism by which dietary fibers produce their hypocholesterolemic effect is still not clear although several theories have been hypothesized.

The hamster was used in this study because of similarities in their rates of cholesterol synthesis, and the ratios of plasma HDL-C to LDL-C with that seen in humans. When consuming a diet with added cholesterol, the hamster has shown an elevated plasma TC and LDL-C response as seen in humans consuming a diet high in saturated fat.

The present study was conducted to evaluate the effects of dietary fiber from either oat bran, guar gum, xylan or cellulose, at a level of 10% of the diet, on the plasma TC, HDL-C, combined VLDL-C + LDL-C and TG levels, in hamsters that were made hypercholesterolemic by feeding a 0.1% cholesterol, 10% fat (P/S = 0.5) diet. The effect of these dietary fibers on the liver cholesterol concentration and on the fecal neutral steroid excretion was examined to determine a possible mechanism for the hypocholesterolemic effects of dietary fibers. The influence of cholesterol feeding and dietary fiber on the histology of the heart, liver, kidney and gastrointestinal tract were also examined.

155 hamsters were fed a hypercholesterolemic diet (0.1% cholesterol and 10% fat) for 4 wk. Blood samples (2 ml) were collected from the orbital sinus of the fasted hamsters, prior to the introduction of the basal hypercholesterolemic diet, then again at wk 4, after consuming the hypercholesterolemic diet and at wk 9, after 4 wk of consuming the treatment fiber diets. The plasma

samples were analyzed for TC, HDL-C, combined VLDL-C + LDL-C and TG. Based on wk 4 plasma TC levels hamsters that responded to the hypercholesterolemic diet with elevated plasma TC levels were ranked and using a randomized block design were assigned to six groups of 16 hamsters each; control (4% cellulose), oat bran, guar gum, xylan (from steam exploded peanut hulls), cellulose (at 10% dietary fiber) and sacrifice group. One week was used to stabilize the hamsters weights, after drawing blood, at wk 4, before putting them on five the treatment diets. Fasting blood samples were drawn at wk 9, after 4 wk on the treatment diets, and the hamsters were sacrificed and tissue samples collected. Fecal samples were collected over a four day period during the last week (wk 9) for each hamster and was analyzed for fecal neutral steroid concentration.

After 4 wk of the hypercholesterolemic diets, the plasma TC, HDL-C, combined VLDL-C + LDL-C and TG levels were significantly raised ($p<.05$). After 4 wk of the fiber diets, the plasma TC levels were reduced by 16%, 12% and 15% in the oat bran, guar gum and xylan groups, respectively ($p<.05$). The plasma HDL-C levels also tended to be lower, but significantly reduced only in the guar gum group (12%, $p<.05$). The combined plasma VLDL-C + LDL-C concentrations were altered significantly by the fiber diets, with oat bran and xylan producing the greatest reductions (38% and 34%, respectively) ($p<.05$). Even the cellulose group showed a 40% reduction in this combined lipoprotein fraction while the guar gum produced a 13% reduction as well. The percent reduction in the combined plasma VLDL-C + LDL-C was greater than that observed in the plasma HDL-C. The plasma TG in all the groups were lowered compared to those obtained at wk 4, but no significant changes were obtained, except in the guar gum group. This could probably be attributed in part to the great variability observed in this plasma lipid among the animals in each treatment group.

After 4 wk of the hypercholesterolemic diet, the liver cholesterol concentration was significantly raised ($p<.05$). After 4 wk of the treatment fiber diets no significant effect was observed in the oat bran, guar gum and xylan diet groups, although a lowering trend was observed. Thus these soluble dietary fibers did not seem to have a significant influence on the hepatic cholesterol synthesis, catabolism and release within the four week time frame, thereby suggesting that they did not considerably influence the cholesterol absorption and the bile acid and steroid excretion. The cellulose diet was observed to increase the liver cholesterol concentration even more during the experimental period compared to the other fiber treatment groups ($p<.05$).

Only the oat bran group significantly increased the total fecal neutral steroid excretion ($p<.05$) compared to the other fiber treatment groups. This suggested that the hypocholesterolemic effects of oat bran could be through the binding with neutral steroids and thus interrupting the enterohepatic pathway. The guar gum and cellulose groups had intermediate excretion values while the xylan group had the lowest neutral steroid excretion rate, indicating that the lower plasma TC observed in the xylan group could not be explained on the basis of steroid excretion. Based on the results of the liver cholesterol analysis and the fecal neutral steroid excretion it is not entirely clear if the hypocholesterolemic effects of the soluble dietary fibers used in the present study could be explained on the basis of the increased bile acid and neutral steroid excretion hypothesis. The cellulose treatment significantly ($p<.05$) increased the fecal mass and water holding capacity compared to the other groups, thus proving to be an effective bulking agent.

Histological examination of the heart, liver, kidney and gastrointestinal tract did not show any significant changes in the histology of these tissues in any of the animals in the different treatment groups. This suggested that the different fiber sources used at 10% of the diet, had no adverse effect on these tissues.

In conclusion, the addition of 10% soluble dietary fiber from either oat bran, guar gum or xylan proved to be effective hypocholesterolemic agents. These soluble dietary fibers might be effective in altering this risk factor of CHD, since they had a significant influence on lowering the atherogenic lipoprotein cholesterol fractions (VLDL-C + LDL-C) along with the reductions observed in the plasma TC. The hypocholesterolemic effects observed with these fiber treatments could be attributed solely to their inherent properties since the semi-purified diets used in this study were very similar in nutrient composition except for their soluble and insoluble dietary fiber content. All the diets provided approximately 10% fat with a P/S ratio of 0.5 and had a total dietary fiber content of approximately 10%. These similarities in the composition of the diets further highlights that the hypocholesterolemic effects observed with the various treatments in the present study could be attributed to the dietary fiber source and not due to the alterations of the other dietary components. These fiber diets, except for the increases with the cellulose diet, had no significant influence on the liver cholesterol concentration. However, it was not possible to establish a mechanism for the hypocholesterolemic effects of these soluble dietary fibers. Since no major histological changes were observed in the tissues examined it could be concluded that these dietary fibers are apparently safe to use for this length of time to help lower plasma cholesterol levels. Of the three soluble dietary fibers sources used oat bran and xylan had the most desirable effects on the lipoprotein cholesterol levels in lowering the plasma VLDL-C + LDL-C levels. If this effect is similar in the humans then this would reduce the risk of CHD.

The use of the hamster in the present study provided some valuable information on the effect of soluble dietary fiber on the plasma total and lipoprotein cholesterol levels and the value of a new xylan source from steam exploded plant material (peanut hulls). However, more research is required to establish the prolonged effects of using these dietary fiber sources to lower plasma cholesterol levels and also to more firmly establish possible mechanisms for the hypocholesterolemic effect of these dietary fibers. Also, whether a similar response would be seen in borderline hypercholesterolemic and normal animals should be investigated. In the present study, 9 wk old hamsters were used which were growing steadily during the course of the study. The influence of this growth and maturity on the plasma parameters needs to be examined carefully since this could also play an important role in influencing the plasma variables. In the present study 4 wk of the soluble fiber treatments produced significant reductions in the plasma cholesterol concentrations. However, future research should determine whether this positive effect is sustained over a prolonged period.

These dietary fibers could influence the viscosity of the gut contents which in turn could influence the absorption process. Also dietary fiber influences the intestinal transit time which could influence both the digestion and absorption processes which in turn can influence the plasma levels of various nutrients. It would thus be useful to study the influence of these dietary fiber sources on the viscosity of the gut contents and intestinal transit time in the hamster to see if this is a possible mechanism for their cholesterol lowering action.

In the present study a lowering of the plasma HDL-C was observed after the fiber treatment. The significance of this lowering effect would be more relevant if it were possible to determine which one of the HDL fractions was affected by the fiber treatment. Also the influence of the fiber treatments on the various apoprotein fractions needs to be given more consideration since this could also possibly help in understanding the mechanism of action.

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# Appendix A

## Orbital sinus bleeding procedure

1. Allow 12 hour fast and then administer ketamine (0.2 to 0.3 ml/ hamster) to the hamster. Allow adequate time to make animal unconscious, but not into deep stages of unconsciousness.

2. Holding the animal on a 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. Use a heparin capillary tube (used for hematocrit determination) to penetrate the orbital conjunctiva and rupture the orbital sinus. Blood is collected into 2 ml polyethylene tubes containing EDTA (1.5 mg / ml blood).

4. Blood flow will cease when the tube is released and pressure is removed from the external jugular vein.

# Appendix B

## Determination of plasma total cholesterol (TC)

TC was determined using a quantitative, fully enzymatic colorimeteric method using cholesterol oxidase supplied by Stanbio TC Kit #1010 (Stanbio Laboratory, Inc., San Antonio, Texas) (Allain et al, 1974).

1. To a test tube add 1 ml reagent for the blank.
2. To the standard test tubes add 1 ml reagent, 5, 10 and 15 ul of the 200 mg/dl standard, provided in the kit,in duplicate to get three standard concentrations of 100, 200 and 300 mg/dl to make a standard curve.
3. To the unknown test tube and control test tube add 1 ml reagent and 10 ul of the unknown sample and control in duplicates.
4. Vortex all tubes at slow speed and incubate at room temperature for 10 mins.
5. Zero spectrophotometer with reagent blank at 500 nm.
6. Read absorbance of the standards and samples at 500 nm within 60 mins.
7. Plasma TC concentrations (mg/dl) were calculated using a standard curve.

# Appendix C

## Determination of plasma triglycerides (TG)

Plasma TG was determined using a quantitative, fully enzymatic colorimeteric method using glycerophosphate oxidase supplied by Stanbio TG kit #2000-715 (Stanbio Laboratory , Inc., San Antonio, Texas) (Wahlefeld, 1974).

1. To a test tube add 1 ml reagent for the blank.
2. To the standard test tubes add 1 ml reagent, 5, 10 and 15 ul of the 200 mg/dl standard, provided in the kit, in duplicate to get three standard concentrations of 100, 200 and 300 mg/dl to make a standard curve.
3. To the unknown test tube and control test tube add 1 ml reagent and 10 ul of unknown samples and control in duplicate.
4. Vortex all tubes at low speed and incubate all test tubes at room temperature for 10 mins.
5. Zero spectrophotometer with reagent blank at 500 nm.
6. Read absorbance of the standards and samples at 500 nm within 60 mins.
7. Plasma TG concentrations (mg/dl) were calculated using a standard curve.

# Appendix D

## Determination of the plasma HDL-C and VLDL-C + LDL-C

The TL-100 Table top Ultracentrifuge (Natio, 1986) was used to separate the different lipoprotein fractions based on the differences in their hydrated densities. Using salt solutions of various densities in a series of differential flotation runs, these fractions can be obtained. The plasma VLDL-C and LDL-C fractions were separated from the HDL-C fraction using a saline solution at a density of 1.063 g/ml.

1. Pipet 100 ul of 16.7% saline solution into 0.2 ml thick walled, polycarbonate tubes. Add 100 ul of plasma, allowing plasma and saline to mix.
2. Load the tubes into the TLA-100.1 fixed angle rotor and close lid tightly. Place the loaded rotor into the TL-100 and set the run parameters as follows:

| | |
|---|---|
| Speed: | 100,000 rpm |
| Temperature: | 16°C |
| Time: | 2.5 h |
| Acceleration: | 5 |
| Deceleration: | 7 |

3. Upon completion of the run carefully remove the rotor.
4. Remove each tube from the rotor, using a forceps, being careful not to disturb the layers and set the tubes on the stand. Using a 100 ul pipet remove the top layer which will

contain chylomicrons, VLDL-C and LDL-C fractions.

5. The remaining bottom layer is stirred using a thin steel rod. This fraction which contains the HDL-C fraction was used to determine the plasma HDL-C concentration, using the procedure outlined for the determination of plasma TC (Appendix B).

6. The combined VLDL-C and LDL-C fraction was calculated as the difference between the TC and HDL-C levels.

VLDL-C + LDL-C = (TC - HDL-C)

# Appendix E

## Fecal Neutral Steroid Analysis

### A.Fecal collection and preparation

1. Fecal samples were collected over four days during the last week of the study, weighed and stored in the freezer.
2. Prior to analysis the fecal samples were freeze-dried and reweighed to determine dry weight.

### B. Fecal extraction

1. Grind the sample to a fine powder using small Wiley mill or a coffee grinder. Weigh out 0.5 g and place in a 25 ml (20 x 125 mm) screw top culture tube.
2. Add 3 ml acetic acid, 1 ml of an internal standard solution for acidic steroids (5B-cholanic acid, 0.50 mg/ml in ethyl acetate) and 1 ml of an internal standard solution for neutral steroids (5a-cholestane, 0.25 mg/ml in ethyl acetate).
3. Add 2 boiling beads, cover with loosely placed caps, mix and place in heating block at 110° C for 1.5 hours; mix every thirty minutes using a vortex at low speed.
4. Cool in an ice bath, add 7 ml of toluene, and mix well.
5. Allow phases to separate overnight (approximately 12 hrs) and then pipet 7 ml of supernatant into a large 25 ml culture tube. Evaporate solvent under $N_2$ at 60° C being

careful not to "overdry".

## C. Enzymatic hydrolysis of conjugates

1. Add 2 ml chologlycine hydrolase (3 mg/ml) in acetate buffer, 1 ml EDTA solution, and 1 ml dithiothreitol solution (Clelands reagent).

2. Cap and vortex until suspended; incubate at 37°C for 4 hrs with mixing every 15 mins on a vortex at low speed.

## D. Neutral steroid extraction and analysis

1. Add 1 ml deionized water and 0.25 ml of 30% NaOH and mix.

2. Add 4 ml methanol and mix thoroughly.

3. Extract neutral steroids with 4 x 7 ml petroleum ether. Transfer the organic layer after each extraction to a 15 ml (16 x 125 mm) culture tube and dry down each time under $N_2$ at 40°C. Wash down sides of tube with petroleum ether after final drying to wash all steroids to bottom of tube.

4. Resuspend neutral steroids in 1 ml of petroleum ether.

5. Transfer 100 ul of the above suspension, to a small culture tube and add 200 ul silylating reagent (hexamethyldisilazane: trimethylchlorosilane:pyridine (3:1:9), Supelco).

6. Cap, mix, and let the solution stand for 30 mins at room temperature.

7. Dry under $N_2$, resuspend silylated neutral steroid in 100

ul of hexane, and let stand for 10 mins.

8. Inject approximately 1-2 ul using a Hamilton syringe into the Gas Chromatograph GC-9A (Shimadzu, Columbia, MD) using the following conditions:

   a. glass column packed with 3% SP 2250 on 100/120 SUPELCOPORT, 3'x 2 mm ID.

   b. temperature: isothermal analysis with column temperature at 275°C, detector and injector temperature at 295°C.

   c. flow rate: FID detector air - 400 ml/min; $H_2$ - 40 ml/min; carrier gas through column - 50 ml/min.

   d. sensitivity: FID - 4 x $10^{-11}$ AFS.

## E. Reagents

1. Acetate buffer (0.1 M, pH 5.6) is made by making a solution containing 91% 0.1 N sodium acetate and 9% 0.1 N acetic acid. (Combine 910 ml sodium acetate solution (13.61 g/l) with 90 ml acetic acid solution (2.21 ml/500 ml).

2. EDTA : 0.93 g/50 ml or 1.86 g/100 ml (tetrasodium salt).

3. Dithiothreitol (prepare fresh for each run) : 2.5 mg/l deionidized water.

4. NaOH (30% w/v) : 3 g NaOH/10 ml deionized water.

## F. Calculation

$$\frac{\text{Area of internal standard}}{\text{Conc. of internal standard}} = \frac{\text{Area of unknown}}{\text{Conc. of unknown}}$$

where

internal standard = cholestane with a retention time approximately 3.5 min

conc. of internal standard = 0.25 mg/ml

unknown = cholesterol with a retention time of approximately 6.5 min

# Appendix F

## Extraction of Liver Cholesterol

1. Weigh approximately 1 g of frozen unthawed liver and place sample in a 50 ml glass tube.

2. Add 20 ml of chloroform : methanol solution (chloroform : methanol are in the ratio of 2 : 1 by volume).

3. Homogenize the liver sample using a Brinkman Polyton homogenizer for approximately 3 min. Rinse homogenizer with the chloroform : methanol solvent before homogenizing the next sample.

4. Filter the homogenate through a fat free filter paper (Whatman filter no. 1) into a 50 ml screw top glass test tube and adjust the volume to 20 ml with the chloroform : methanol solution.

5. Add 2 ml distilled water to the crude extract.

6. Mix the two liquids with a glass stirring rod and rinse the rod with a minimal amount of pure lower phase solvent solution (chloroform : methanol : water are in the ratio of 86 : 14 : 1 by volume).

7. Cap the tube and centrifuge at 260 g in a Beckman centrifuge for 20 min.

8. Remove the upper layer as completely as possible with a transfer pipette. This layer contains the non lipid fraction and can be discarded.

9. Rinse the inside of the test tube with 1.5 ml of the pure solvent upper phase solution (chloroform : methanol : water are in the ratio of 3 : 48 : 47 by volume).

10. Rotate the tube gently to insure mixing of the rinsing fluid with the remaining original upper layer and remove the upper layer. Repeat this step one more time.

11. Take 1 ml of the bottom layer in a small glass bottle and remove chloroform completely by evaporation.

12. To the lipid residue in the glass bottle add 1 ml of 0.9% saline solution and 10 ul of TritonX-100. This solution was then used to determine the liver cholesterol content.

13. Liver cholesterol content was determined by the procedure outlined for the determination of plasma TC (Appendix B).

## Appendix G

### Protocol for necropsy of hamsters and preservation of tissues and for the preparation of slides for histopathologic examination

Necropsy of the animals is done in a standardized manner.

1. After each animal is humanely killed (40 - 60 mg/kg sodium pentabarbitol), the animal is thoroughly examined grossly. Any external abnormalities, including growths, injuries, or discharges should be noted.
2. The animal is then opened up from the point of the mandibles to the genital opening. The skin is reflected back, and the subcutaneous tissues examined. The major organs should be examined in situ.
3. The gastrointestinal tract should then be removed. The stomach and gut should be opened and the mucosa examined.
4. Tissue samples of the representative areas should then be collected in 10% neutral buffered formalin.
5. The mesenteric nodes, the liver, pancreas and spleen should be sampled.
6. When the gut is removed, the kidneys and adrenals should be examined and then sampled, along with the renal artery and vein and the abdominal aorta. The urinary bladder should be saved in a fixative.
7. The dissection of the thoracic viscera should then be

done.

8. The heart, lungs, trachea, and esophagus should be removed and examined, and then sampled.

9. The major salivary glands should be sampled before the trachea and thyroids are examined and sampled.

10. Finally, the skull should be removed and the brain examined and then removed and sampled. Care should be taken to remove the pituitary and place it in a capsule in fixative.

11. Following an adequate period of fixation, tissues are trimmed, dehydrated in a graded series of alcohols and infiltrated with paraffin.

12. These paraffin-embedded tissues are then sectioned on a microtome, rehydrated, and stained with aqueous dyes like hematoxylin and eosin, which accentuate various cellular details.

13. The slides are then dehydrated again, and a coverslip is fixed with mounting medium.

14. The tissues can then be examined by an individual familiar with the normal and pathologic microanatomy.

# Appendix H

Individual plasma total cholesterol values at weeks 0, 4 and 9 (mg/dl)

| I.D | Group | 0 | 4 | 9 |
|---|---|---|---|---|
| 845 | C | 92 | 295 | 225 |
| 838 | C | 104 | 283 | 266 |
| 865 | C | 105 | 277 | 199 |
| 899 | C | 126 | 262 | 214 |
| 894 | C | 107 | 262 | 219 |
| 810 | C | 130 | 256 | 197 |
| 911 | C | 107 | 254 | 344 |
| 938 | C | 96 | 245 | 222 |
| 862 | C | 121 | 239 | 230 |
| 851 | C | 107 | 236 | 275 |
| 915 | C | 107 | 232 | 249 |
| 806 | C | 107 | 227 | 283 |
| 875 | C | 115 | 225 | 245 |
| 854 | C | 90 | 221 | 213 |
| 856 | C | 101 | 210 | 195 |
| 867 | C | 93 | 195 | 168 |
| 903 | OB | 115 | 309 | 352 |
| 900 | OB | 111 | 291 | 178 |
| 925 | OB | 86 | 282 | 215 |
| 917 | OB | 106 | 275 | 223 |
| 934 | OB | 114 | 269 | 228 |
| 873 | OB | 98 | 259 | 181 |
| 837 | OB | 110 | 257 | 177 |
| 879 | OB | 92 | 252 | 186 |
| 863 | OB | 100 | 245 | 264 |
| 869 | OB | 103 | 244 | 166 |
| 881 | OB | 100 | 235 | 174 |
| 928 | OB | 90 | 232 | 235 |
| 912 | OB | 87 | 231 | 225 |
| 941 | OB | 85 | 225 | 227 |
| 836 | OB | 102 | 221 | 208 |
| 829 | OB | 109 | 214 | 161 |

Plasma total cholesterol values contd.

| I.D. | Group | 0 | 4 | 9 |
|---|---|---|---|---|
| 858 | GG | 132 | 297 | 185 |
| 940 | GG | 109 | 282 | 205 |
| 839 | GG | 81 | 277 | 193 |
| 885 | GG | 107 | 265 | 245 |
| 897 | GG | 108 | 261 | 279 |
| 800 | GG | 124 | 258 | 255 |
| 907 | GG | 109 | 252 | 202 |
| 804 | GG | 129 | 246 | 240 |
| 888 | GG | 119 | 242 | 208 |
| 831 | GG | 102 | 241 | 281 |
| 872 | GG | 104 | 240 | 198 |
| 848 | GG | 132 | 236 | 181 |
| 896 | GG | 110 | 234 | 222 |
| 842 | GG | 130 | 221 | 197 |
| 864 | GG | 102 | 216 | 180 |
| 841 | GG | 114 | 210 | 231 |
| 920 | X | 121 | 300 | 176 |
| 815 | X | 125 | 281 | 186 |
| 947 | X | 119 | 270 | 299 |
| 902 | X | 124 | 269 | 210 |
| 871 | X | 88 | 262 | 155 |
| 809 | X | 128 | 257 | 304 |
| 926 | X | 104 | 253 | 191 |
| 909 | X | 116 | 247 | 243 |
| 905 | X | 98 | 242 | 173 |
| 803 | X | 118 | 240 | 255 |
| 826 | X | 107 | 234 | 187 |
| 893 | X | 101 | 233 | 148 |
| 933 | X | 96 | 227 | 189 |
| 843 | X | 113 | 225 | 145 |
| 818 | X | 100 | 219 | 219 |
| 834 | X | 100 | 214 | 279 |

**Plasma total cholesterol values contd.**

| I.D. | Group | 0 | 4 | 9 |
|---|---|---|---|---|
| 844 | CE | 115 | 287 | 289 |
| 884 | CE | 98 | 285 | 192 |
| 892 | CE | 95 | 272 | 252 |
| 886 | CE | 114 | 266 | 213 |
| 825 | CE | 139 | 258 | 200 |
| 914 | CE | 99 | 258 | 221 |
| 950 | CE | 104 | 253 | 188 |
| 801 | CE | 113 | 248 | 194 |
| 901 | CE | 107 | 244 | 274 |
| 889 | CE | 99 | 241 | 216 |
| 953 | CE | 128 | 236 | 285 |
| 802 | CE | 115 | 233 | 249 |
| 833 | CE | 96 | 231 | 180 |
| 910 | CE | 102 | 230 | 210 |
| 930 | CE | 107 | 225 | 267 |
| 832 | CE | 86 | 219 | 217 |

C - Control diet
OB - Oat bran diet
GG - Guar gum diet
X - Xylan diet
CE - Cellulose diet

# Appendix I

Individual plasma high-density lipoprotein cholesterol values at weeks 0, 4 and 9 (mg/dl)

| I.D. | Group | 0 | 4 | 9 |
|---|---|---|---|---|
| 845 | C | 82 | 177 | 197 |
| 838 | C | 94 | 173 | 232 |
| 865 | C | 85 | 174 | 148 |
| 899 | C | 79 | 192 | 188 |
| 894 | C | 66 | 159 | 163 |
| 810 | C | 92 | 139 | 183 |
| 911 | C | 77 | 164 | 227 |
| 938 | C | 77 | 149 | 185 |
| 862 | C | 82 | 160 | 191 |
| 851 | C | 75 | 170 | 189 |
| 915 | C | 66 | 155 | 175 |
| 806 | C | 77 | 172 | 169 |
| 875 | C | 87 | 158 | 158 |
| 854 | C | 62 | 161 | 183 |
| 856 | C | 75 | 134 | 115 |
| 867 | C | 77 | 150 | 89 |
| 903 | OB | 73 | 196 | 235 |
| 900 | OB | 72 | 175 | 119 |
| 925 | OB | 71 | 183 | 191 |
| 917 | OB | 72 | 190 | 202 |
| 934 | OB | 84 | 173 | 177 |
| 873 | OB | 72 | 152 | 118 |
| 837 | OB | 82 | 166 | 102 |
| 879 | OB | 82 | 193 | 134 |
| 863 | OB | 86 | 179 | 189 |
| 869 | OB | 93 | 166 | 138 |
| 881 | OB | 71 | 183 | 145 |
| 928 | OB | 75 | 152 | 175 |
| 912 | OB | 69 | 173 | 196 |
| 941 | OB | 64 | 164 | 204 |
| 836 | OB | 72 | 140 | 125 |
| 829 | OB | 80 | 163 | 136 |

Plasma high-density lipoprotein cholesterol values contd.

| I.D. | Group | O | 4 | 9 |
|---|---|---|---|---|
| 858 | GG | 98 | 187 | 118 |
| 940 | GG | 86 | 177 | 153 |
| 839 | GG | 56 | 183 | 118 |
| 885 | GG | 74 | 184 | 163 |
| 897 | GG | 73 | 188 | 187 |
| 800 | GG | 75 | 161 | 149 |
| 907 | GG | 78 | 168 | 140 |
| 804 | GG | 92 | 168 | 200 |
| 888 | GG | 85 | 171 | 182 |
| 831 | GG | 77 | 161 | 182 |
| 872 | GG | 71 | 181 | 164 |
| 848 | GG | 113 | 150 | 85 |
| 896 | GG | 80 | 166 | 164 |
| 842 | GG | 81 | 154 | 105 |
| 864 | GG | 95 | 144 | 123 |
| 841 | GG | 78 | 143 | 142 |
| 920 | X | 86 | 167 | 153 |
| 815 | X | 73 | 196 | 139 |
| 947 | X | 68 | 167 | 182 |
| 902 | X | 83 | 174 | 118 |
| 871 | X | 85 | 178 | 114 |
| 809 | X | 88 | 194 | 231 |
| 926 | X | 70 | 165 | 170 |
| 909 | X | 80 | 146 | 151 |
| 905 | X | 85 | 183 | 152 |
| 803 | X | 79 | 172 | 215 |
| 826 | X | 73 | 171 | 111 |
| 893 | X | 73 | 182 | 135 |
| 933 | X | 84 | 143 | 169 |
| 843 | X | 92 | 162 | 64 |
| 818 | X | 76 | 167 | 197 |
| 834 | X | 74 | 157 | 233 |

Plasma high-density lipoprotein cholesterol values contd.

| I.D. | Group | 0 | 4 | 9 |
|---|---|---|---|---|
| 844 | CE | 78 | 196 | 268 |
| 884 | CE | 67 | 167 | 139 |
| 892 | CE | 55 | 186 | 167 |
| 886 | CE | 83 | 183 | 175 |
| 825 | CE | 92 | 163 | 109 |
| 914 | CE | 74 | 174 | 164 |
| 950 | CE | 82 | 163 | 178 |
| 801 | CE | 63 | 168 | 183 |
| 901 | CE | 74 | 184 | 231 |
| 889 | CE | 78 | 180 | 216 |
| 953 | CE | 84 | 155 | 195 |
| 802 | CE | 99 | 149 | 191 |
| 833 | CE | 81 | 152 | 109 |
| 910 | CE | 50 | 156 | 161 |
| 930 | CE | 69 | 160 | 176 |
| 832 | CE | 64 | 166 | 242 |

C - Control diet
OB - Oat bran diet
GG - Guar gum diet
X - Xylan diet
CE - Cellulose diet

# Appendix J

Individual plasma combined very-low and low-density-lipoprotein cholesterol values at weeks 0, 4 and 9 (mg/dl)

| I.D. | Group | 0 | 4 | 9 |
|---|---|---|---|---|
| 845 | C | 10 | 118 | 28 |
| 838 | C | 10 | 110 | 34 |
| 865 | C | 20 | 103 | 51 |
| 899 | C | 47 | 70 | 26 |
| 894 | C | 41 | 103 | 56 |
| 810 | C | 38 | 117 | 14 |
| 911 | C | 30 | 90 | 117 |
| 938 | C | 19 | 96 | 37 |
| 862 | C | 39 | 79 | 39 |
| 851 | C | 32 | 66 | 86 |
| 915 | C | 41 | 77 | 74 |
| 806 | C | 30 | 55 | 114 |
| 875 | C | 28 | 67 | 87 |
| 854 | C | 28 | 60 | 30 |
| 856 | C | 26 | 76 | 80 |
| 867 | C | 16 | 45 | 79 |
| | | | | |
| 903 | OB | 42 | 113 | 117 |
| 900 | OB | 39 | 116 | 59 |
| 925 | OB | 15 | 99 | 24 |
| 917 | OB | 34 | 85 | 21 |
| 934 | OB | 30 | 96 | 51 |
| 873 | OB | 26 | 107 | 63 |
| 837 | OB | 28 | 91 | 75 |
| 879 | OB | 10 | 59 | 52 |
| 863 | OB | 14 | 66 | 75 |
| 869 | OB | 10 | 78 | 28 |
| 881 | OB | 29 | 72 | 29 |
| 928 | OB | 15 | 80 | 60 |
| 912 | OB | 18 | 58 | 29 |
| 941 | OB | 21 | 61 | 23 |
| 836 | OB | 30 | 81 | 83 |
| 829 | OB | 29 | 51 | 25 |

Plasma combined very-low and low-density lipoprotein cholesterol values contd.

| I.D. | Group | 0 | 4 | 9 |
|---|---|---|---|---|
| 858 | GG | 34 | 110 | 67 |
| 940 | GG | 23 | 105 | 52 |
| 839 | GG | 25 | 94 | 75 |
| 885 | GG | 33 | 81 | 82 |
| 897 | GG | 35 | 73 | 92 |
| 800 | GG | 49 | 97 | 106 |
| 907 | GG | 31 | 84 | 62 |
| 804 | GG | 37 | 78 | 40 |
| 888 | GG | 34 | 71 | 26 |
| 831 | GG | 25 | 80 | 99 |
| 872 | GG | 33 | 59 | 34 |
| 848 | GG | 19 | 86 | 96 |
| 896 | GG | 30 | 68 | 58 |
| 842 | GG | 49 | 67 | 92 |
| 864 | GG | 7 | 72 | 57 |
| 841 | GG | 36 | 67 | 89 |
| 920 | X | 35 | 133 | 23 |
| 815 | X | 52 | 85 | 47 |
| 947 | X | 51 | 103 | 117 |
| 902 | X | 24 | 95 | 92 |
| 871 | X | 3 | 84 | 41 |
| 809 | X | 40 | 63 | 73 |
| 926 | X | 34 | 88 | 21 |
| 909 | X | 36 | 101 | 92 |
| 905 | X | 13 | 59 | 21 |
| 803 | X | 39 | 68 | 40 |
| 826 | X | 34 | 63 | 76 |
| 893 | X | 28 | 51 | 13 |
| 933 | X | 12 | 84 | 20 |
| 843 | X | 21 | 63 | 81 |
| 818 | X | 24 | 52 | 22 |
| 834 | X | 26 | 57 | 46 |

Plasma combined very-low and low-density lipoprotein cholesterol values contd.

| I.D. | Group | 0 | 4 | 9 |
|---|---|---|---|---|
| 844 | CE | 37 | 91 | 21 |
| 884 | CE | 31 | 118 | 53 |
| 892 | CE | 40 | 86 | 85 |
| 886 | CE | 31 | 83 | 38 |
| 825 | CE | 47 | 95 | 91 |
| 914 | CE | 25 | 84 | 57 |
| 950 | CE | 22 | 90 | 10 |
| 801 | CE | 50 | 80 | 11 |
| 901 | CE | 33 | 50 | 43 |
| 889 | CE | 21 | 61 | 0 |
| 953 | CE | 44 | 81 | 90 |
| 802 | CE | 16 | 84 | 58 |
| 833 | CE | 15 | 79 | 71 |
| 910 | CE | 52 | 74 | 49 |
| 930 | CE | 38 | 65 | 91 |
| 832 | CE | 22 | 53 | 0 |

C - Control diet
OB - Oat bran diet
GG - Guar gum diet
X - Xylan diet
CE - Cellulose diet

# Appendix K

Individual plasma triglyceride values at weeks 0, 4 and 9 (mg/dl)

| I.D. | Group | 0 | 4 | 9 |
|---|---|---|---|---|
| 845 | C | 48 | 365 | 362 |
| 838 | C | 105 | 383 | 516 |
| 865 | C | 80 | 521 | 142 |
| 899 | C | 32 | 475 | 327 |
| 894 | C | 95 | 322 | 110 |
| 810 | C | 49 | 27 | 134 |
| 911 | C | 79 | 166 | 347 |
| 938 | C | 65 | 311 | 260 |
| 862 | C | 46 | 138 | 126 |
| 851 | C | 63 | 282 | 362 |
| 915 | C | 64 | 175 | 337 |
| 806 | C | 75 | 160 | 225 |
| 875 | C | 98 | 154 | 315 |
| 854 | C | 29 | 132 | 30 |
| 856 | C | 53 | 181 | 81 |
| 867 | C | 106 | 190 | 63 |
| 903 | OB | 54 | 148 | 178 |
| 900 | OB | 113 | 658 | 195 |
| 925 | OB | 42 | 429 | 146 |
| 917 | OB | 58 | 329 | 182 |
| 934 | OB | 50 | 191 | 234 |
| 873 | OB | 57 | 447 | 284 |
| 837 | OB | 74 | 143 | 99 |
| 879 | OB | 40 | 417 | 132 |
| 863 | OB | 59 | 174 | 434 |
| 869 | OB | 48 | 250 | 98 |
| 881 | OB | 149 | 271 | 202 |
| 928 | OB | 55 | 151 | 199 |
| 912 | OB | 44 | 93 | 229 |
| 941 | OB | 49 | 78 | 404 |
| 836 | OB | 68 | 181 | 36 |
| 829 | OB | 75 | 143 | 55 |

Plasma triglyceride values contd.

| I.D. | Group | 0 | 4 | 9 |
|---|---|---|---|---|
| 858 | GG | 68 | 519 | 69 |
| 940 | GG | 77 | 322 | 51 |
| 839 | GG | 93 | 400 | 192 |
| 885 | GG | 72 | 286 | 189 |
| 897 | GG | 52 | 408 | 250 |
| 800 | GG | 77 | 151 | 48 |
| 907 | GG | 75 | 182 | 154 |
| 804 | GG | 84 | 134 | 181 |
| 888 | GG | 83 | 259 | 202 |
| 831 | GG | 53 | 181 | 176 |
| 872 | GG | 63 | 328 | 125 |
| 848 | GG | 84 | 39 | 42 |
| 896 | GG | 51 | 204 | 171 |
| 842 | GG | 64 | 114 | 66 |
| 864 | GG | 55 | 116 | 23 |
| 841 | GG | 72 | 98 | 29 |
| 920 | X | 47 | 238 | 90 |
| 815 | X | 64 | 75 | 54 |
| 947 | X | 75 | 230 | 355 |
| 902 | X | 83 | 175 | 119 |
| 871 | X | 54 | 250 | 106 |
| 809 | X | 55 | 255 | 325 |
| 926 | X | 31 | 260 | 215 |
| 909 | X | 55 | 92 | 541 |
| 905 | X | 36 | 410 | 283 |
| 803 | X | 53 | 139 | 407 |
| 826 | X | 91 | 166 | 24 |
| 893 | X | 52 | 87 | 44 |
| 933 | X | 26 | 131 | 134 |
| 843 | X | 53 | 162 | 39 |
| 818 | X | 34 | 65 | 156 |
| 834 | X | 38 | 156 | 30 |

Plasma triglyceride values contd.

| I.D. | Group | 0 | 4 | 9 |
|---|---|---|---|---|
| 844 | CE | 41 | 341 | 410 |
| 884 | CE | 49 | 277 | 46 |
| 892 | CE | 70 | 353 | 272 |
| 886 | CE | 24 | 173 | 145 |
| 825 | CE | 86 | 163 | 59 |
| 914 | CE | 63 | 64 | 127 |
| 950 | CE | 67 | 284 | 61 |
| 801 | CE | 51 | 211 | 115 |
| 901 | CE | 46 | 430 | 399 |
| 889 | CE | 58 | 266 | 182 |
| 953 | CE | 169 | 250 | 334 |
| 802 | CE | 56 | 108 | 119 |
| 833 | CE | 42 | 142 | 75 |
| 910 | CE | 85 | 163 | 353 |
| 930 | CE | 70 | 162 | 147 |
| 832 | CE | 69 | 169 | 86 |

C - Control diet
OB - Oat bran diet
GG - Guar gum diet
X - Xylan diet
CE - Cellulose diet

# Appendix L

## Weekly individual mean body weights (g)

| I.D. | Group | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|---|---|---|---|---|---|---|---|---|---|
| 845 | C | 146 | 151 | 164 | 172 | 172 | 178 | 183 | 188 |
| 838 | C | 134 | 141 | 156 | 161 | 158 | 170 | 186 | 195 |
| 865 | C | 128 | 139 | 154 | 163 | 151 | 150 | 154 | 161 |
| 899 | C | 129 | 140 | 149 | 151 | 140 | 146 | 156 | 166 |
| 894 | C | 155 | 161 | 174 | 179 | 178 | 178 | 186 | 191 |
| 810 | C | 112 | 108 | 110 | 116 | 113 | 121 | 126 | 134 |
| 911 | C | 123 | 115 | 125 | 131 | 131 | 137 | 148 | 161 |
| 938 | C | 141 | 148 | 157 | 163 | 165 | 176 | 186 | 197 |
| 862 | C | 105 | 109 | 115 | 120 | 121 | 129 | 141 | 149 |
| 851 | C | 140 | 146 | 158 | 162 | 162 | 172 | 180 | 185 |
| 915 | C | 117 | 123 | 135 | 139 | 140 | 151 | 163 | 167 |
| 806 | C | 123 | 129 | 140 | 148 | 150 | 156 | 167 | 174 |
| 875 | C | 122 | 128 | 142 | 145 | 142 | 153 | 161 | 164 |
| 854 | C | 122 | 126 | 141 | 143 | 140 | 142 | 152 | 161 |
| 856 | C | 132 | 131 | 140 | 141 | 134 | 134 | 137 | 130 |
| 867 | C | 136 | 146 | 152 | 150 | 148 | 154 | 164 | 165 |
| | | | | | | | | | |
| 903 | OB | 119 | 115 | 121 | 129 | 132 | 143 | 160 | 175 |
| 900 | OB | 118 | 123 | 135 | 139 | 134 | 146 | 156 | 163 |
| 925 | OB | 127 | 128 | 136 | 140 | 135 | 154 | 168 | 178 |
| 917 | OB | 133 | 138 | 144 | 151 | 149 | 160 | 171 | 181 |
| 934 | OB | 123 | 131 | 144 | 147 | 144 | 157 | 168 | 175 |
| 873 | OB | 105 | 105 | 117 | 128 | 125 | 131 | 148 | 153 |
| 837 | OB | 129 | 138 | 149 | 153 | 150 | 158 | 167 | 171 |
| 879 | OB | 129 | 141 | 152 | 149 | 142 | 155 | 169 | 178 |
| 863 | OB | 120 | 119 | 128 | 133 | 134 | 146 | 158 | 165 |
| 869 | OB | 117 | 127 | 138 | 141 | 135 | 139 | 146 | 154 |
| 881 | OB | 146 | 157 | 167 | 177 | 187 | 197 | 208 | 215 |
| 928 | OB | 131 | 133 | 144 | 150 | 148 | 160 | 172 | 183 |
| 912 | OB | 137 | 138 | 146 | 155 | 158 | 170 | 183 | 193 |
| 941 | OB | 110 | 114 | 120 | 122 | 119 | 128 | 138 | 147 |
| 836 | OB | 117 | 121 | 130 | 132 | 130 | 136 | 143 | 146 |
| 829 | OB | 128 | 138 | 149 | 153 | 150 | 158 | 167 | 171 |

Weekly mean body weights contd.

| I.D. | Group | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|---|---|---|---|---|---|---|---|---|---|
| 858 | GG | 122 | 132 | 150 | 156 | 154 | 148 | 151 | 158 |
| 940 | GG | 116 | 117 | 125 | 132 | 126 | 128 | 130 | 135 |
| 839 | GG | 148 | 153 | 161 | 165 | 163 | 163 | 154 | 151 |
| 885 | GG | 119 | 128 | 145 | 148 | 146 | 144 | 150 | 159 |
| 897 | GG | 127 | 144 | 158 | 163 | 166 | 168 | 173 | 177 |
| 800 | GG | 129 | 127 | 134 | 142 | 138 | 130 | 111 | 108 |
| 907 | GG | 134 | 138 | 146 | 148 | 147 | 144 | 150 | 155 |
| 804 | GG | 113 | 111 | 116 | 124 | 122 | 129 | 141 | 152 |
| 888 | GG | 116 | 116 | 125 | 131 | 130 | 135 | 143 | 151 |
| 831 | GG | 116 | 127 | 142 | 149 | 144 | 148 | 154 | 159 |
| 872 | GG | 126 | 131 | 138 | 142 | 143 | 144 | 144 | 151 |
| 848 | GG | 144 | 141 | 143 | 141 | 140 | 139 | 145 | 153 |
| 896 | GG | 118 | 116 | 110 | 118 | 118 | 122 | 123 | 133 |
| 842 | GG | 123 | 126 | 132 | 131 | 131 | 137 | 147 | 153 |
| 864 | GG | 147 | 152 | 165 | 171 | 172 | 176 | 182 | 183 |
| 841 | GG | 136 | 136 | 140 | 141 | 138 | 138 | 144 | 153 |
| 920 | X | 121 | 131 | 139 | 146 | 143 | 153 | 160 | 166 |
| 815 | X | 118 | 121 | 129 | 130 | 126 | 140 | 156 | 163 |
| 947 | X | 117 | 121 | 126 | 135 | 137 | 148 | 158 | 162 |
| 902 | X | 102 | 97 | 102 | 113 | 111 | 129 | 138 | 139 |
| 871 | X | 119 | 124 | 133 | 136 | 134 | 145 | 153 | 157 |
| 809 | X | 124 | 129 | 141 | 147 | 148 | 161 | 173 | 183 |
| 926 | X | 113 | 124 | 136 | 141 | 135 | 147 | 158 | 163 |
| 909 | X | 126 | 141 | 148 | 146 | 143 | 152 | 166 | 178 |
| 905 | X | 115 | 123 | 133 | 135 | 127 | 138 | 148 | 155 |
| 803 | X | 123 | 129 | 134 | 134 | 130 | 145 | 159 | 170 |
| 826 | X | 115 | 120 | 125 | 125 | 121 | 132 | 141 | 145 |
| 893 | X | 110 | 112 | 115 | 116 | 117 | 128 | 135 | 141 |
| 933 | X | 106 | 118 | 126 | 128 | 125 | 135 | 146 | 151 |
| 843 | X | 121 | 128 | 139 | 144 | 147 | 157 | 162 | 163 |
| 818 | X | 110 | 115 | 121 | 121 | 119 | 131 | 146 | 161 |
| 834 | X | 115 | 116 | 126 | 128 | 116 | 133 | 155 | 167 |

**Weekly mean body weights contd.**

| I.D. | Group | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|---|---|---|---|---|---|---|---|---|---|
| 844 | CE | 122 | 132 | 145 | 152 | 156 | 162 | 171 | 178 |
| 884 | CE | 120 | 122 | 127 | 128 | 126 | 132 | 141 | 148 |
| 892 | CE | 123 | 131 | 144 | 151 | 153 | 166 | 173 | 173 |
| 886 | CE | 112 | 110 | 115 | 121 | 119 | 131 | 147 | 158 |
| 825 | CE | 151 | 151 | 158 | 162 | 158 | 164 | 173 | 177 |
| 914 | CE | 117 | 120 | 120 | 122 | 111 | 117 | 126 | 135 |
| 950 | CE | 112 | 116 | 129 | 133 | 126 | 132 | 142 | 150 |
| 801 | CE | 102 | 106 | 118 | 121 | 120 | 123 | 123 | 121 |
| 901 | CE | 135 | 143 | 146 | 147 | 143 | 154 | 166 | 177 |
| 889 | CE | 126 | 135 | 142 | 145 | 140 | 149 | 159 | 169 |
| 953 | CE | 152 | 158 | 170 | 175 | 171 | 175 | 183 | 191 |
| 802 | CE | 121 | 116 | 127 | 128 | 126 | 132 | 141 | 148 |
| 833 | CE | 128 | 134 | 145 | 147 | 143 | 147 | 142 | 137 |
| 910 | CE | 109 | 111 | 126 | 129 | 127 | 136 | 148 | 156 |
| 930 | CE | 123 | 128 | 136 | 140 | 140 | 149 | 157 | 165 |
| 832 | CE | 133 | 137 | 148 | 151 | 150 | 152 | 148 | 159 |

C - Control diet
B - Oat bran diet
GG - Guar gum diet
X - Xylan diet
CE - Cellulose diet

# Appendix M

## Weekly individual mean food intake (g/day)

| I.D. | Group | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|---|---|---|---|---|---|---|---|---|---|
| 845 | C | 19 | 30 | 28 | 23 | 18 | 15 | 14 | 15 |
| 838 | C | 17 | 18 | 17 | 12 | 12 | 17 | 11 | 12 |
| 865 | C | 14 | 14 | 14 | 13 | 16 | 20 | 24 | 24 |
| 899 | C | 13 | 18 | 18 | 12 | 12 | 17 | 18 | 15 |
| 894 | C | 17 | 19 | 12 | 11 | 14 | 19 | 17 | 17 |
| 810 | C | 10 | 14 | 13 | 9 | 13 | 17 | 19 | 15 |
| 911 | C | 19 | 20 | 20 | 16 | 16 | 20 | 18 | 17 |
| 938 | C | 16 | 15 | 16 | 12 | 11 | 13 | 11 | 12 |
| 862 | C | 16 | 15 | 13 | 12 | 15 | 18 | 18 | 16 |
| 851 | C | 13 | 16 | 14 | 13 | 12 | 14 | 14 | 13 |
| 915 | C | 15 | 22 | 18 | 191 | 18 | 17 | 13 | 14 |
| 806 | C | 15 | 24 | 22 | 20 | 16 | 20 | 17 | 13 |
| 875 | C | 8 | 12 | 11 | 10 | 11 | 10 | 14 | 12 |
| 854 | C | 9 | 13 | 15 | 11 | 12 | 20 | 23 | 20 |
| 856 | C | 9 | 11 | 10 | 9 | 10 | 12 | 8 | 10 |
| 867 | C | 15 | 22 | 20 | 21 | 17 | 17 | 17 | 12 |
| 903 | OB | 19 | 25 | 26 | 24 | 15 | 13 | 13 | 3 |
| 900 | OB | 17 | 22 | 21 | 17 | 15 | 15 | 15 | 2 |
| 925 | OB | 13 | 17 | 14 | 13 | 11 | 8 | 7 | 7 |
| 917 | OB | 9 | 13 | 12 | 12 | 10 | 11 | 11 | 11 |
| 934 | OB | 17 | 18 | 22 | 16 | 13 | 12 | 12 | 13 |
| 873 | OB | 14 | 19 | 17 | 14 | 12 | 13 | 13 | 11 |
| 837 | OB | 12 | 14 | 14 | 11 | 11 | 15 | 12 | 12 |
| 879 | OB | 13 | 18 | 19 | 14 | 11 | 11 | 12 | 11 |
| 863 | OB | 10 | 11 | 12 | 10 | 10 | 11 | 8 | 8 |
| 869 | OB | 9 | 13 | 12 | 10 | 8 | 9 | 10 | 10 |
| 881 | OB | 14 | 17 | 21 | 17 | 10 | 10 | 7 | 10 |
| 928 | OB | 14 | 20 | 19 | 16 | 12 | 12 | 13 | 12 |
| 912 | OB | 9 | 12 | 15 | 14 | 9 | 11 | 11 | 11 |
| 941 | OB | 14 | 18 | 19 | 19 | 12 | 10 | 11 | 10 |
| 836 | OB | 11 | 15 | 11 | 7 | 9 | 10 | 9 | 9 |
| 829 | OB | 17 | 22 | 15 | 15 | 10 | 11 | 12 | 10 |

Mean weekly food intake contd.

| I.D. | Group | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|---|---|---|---|---|---|---|---|---|---|
| 858 | GG | 15 | 16 | 14 | 12 | 17 | 19 | 16 | 17 |
| 940 | GG | 13 | 15 | 15 | 14 | 15 | 16 | 18 | 16 |
| 839 | GG | 12 | 16 | 16 | 16 | 13 | 16 | 17 | 15 |
| 885 | GG | 15 | 15 | 14 | 12 | 14 | 21 | 19 | 16 |
| 897 | GG | 14 | 13 | 11 | 11 | 10 | 12 | 10 | 5 |
| 800 | GG | 13 | 15 | 15 | 13 | 13 | 16 | 10 | 18 |
| 907 | GG | 14 | 14 | 10 | 7 | 20 | 30 | 29 | 24 |
| 804 | GG | 16 | 20 | 24 | 20 | 21 | 19 | 21 | 17 |
| 888 | GG | 12 | 14 | 16 | 13 | 15 | 22 | 23 | 21 |
| 831 | GG | 11 | 16 | 15 | 11 | 16 | 25 | 24 | 22 |
| 872 | GG | 8 | 10 | 13 | 18 | 15 | 15 | 16 | 13 |
| 848 | GG | 12 | 20 | 17 | 16 | 14 | 19 | 21 | 20 |
| 896 | GG | 18 | 22 | 21 | 15 | 22 | 35 | 36 | 40 |
| 842 | GG | 14 | 21 | 18 | 15 | 16 | 24 | 16 | 14 |
| 864 | GG | 10 | 11 | 12 | 12 | 13 | 17 | 19 | 19 |
| 841 | GG | 14 | 19 | 20 | 16 | 16 | 23 | 13 | 13 |
| 920 | X | 18 | 23 | 19 | 17 | 18 | 19 | 20 | 17 |
| 815 | X | 10 | 16 | 13 | 9 | 12 | 15 | 14 | 11 |
| 947 | X | 10 | 15 | 17 | 15 | 13 | 20 | 15 | 12 |
| 902 | X | 16 | 20 | 17 | 13 | 11 | 12 | 10 | 9 |
| 871 | X | 15 | 22 | 20 | 17 | 17 | 19 | 19 | 16 |
| 809 | X | 18 | 20 | 11 | 14 | 16 | 19 | 17 | 14 |
| 926 | X | 12 | 16 | 13 | 11 | 12 | 14 | 13 | 14 |
| 909 | X | 12 | 19 | 19 | 20 | 17 | 22 | 17 | 14 |
| 905 | X | 14 | 21 | 20 | 17 | 16 | 21 | 18 | 18 |
| 803 | X | 11 | 13 | 18 | 13 | 16 | 25 | 25 | 22 |
| 826 | X | 11 | 12 | 11 | 13 | 10 | 15 | 14 | 10 |
| 893 | X | 10 | 18 | 18 | 11 | 14 | 21 | 23 | 16 |
| 933 | X | 14 | 16 | 17 | 16 | 16 | 14 | 15 | 13 |
| 843 | X | 12 | 15 | 15 | 12 | 11 | 12 | 11 | 10 |
| 818 | X | 10 | 18 | 18 | 12 | 15 | 23 | 24 | 19 |
| 834 | X | 11 | 16 | 20 | 13 | 16 | 17 | 14 | 12 |

**Mean weekly food intake contd.**

| I.D. | Group | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|---|---|---|---|---|---|---|---|---|---|
| 844 | CE | 9 | 12 | 13 | 13 | 14 | 20 | 20 | 7 |
| 884 | CE | 20 | 20 | 18 | 17 | 16 | 15 | 15 | 3 |
| 892 | CE | 17 | 17 | 14 | 13 | 16 | 22 | 14 | 3 |
| 886 | CE | 16 | 17 | 16 | 15 | 15 | 19 | 18 | 12 |
| 825 | CE | 14 | 18 | 18 | 15 | 16 | 23 | 23 | 21 |
| 914 | CE | 16 | 20 | 15 | 16 | 16 | 26 | 27 | 16 |
| 950 | CE | 21 | 27 | 27 | 22 | 22 | 29 | 27 | 22 |
| 801 | CE | 12 | 16 | 16 | 14 | 15 | 14 | 21 | 14 |
| 901 | CE | 17 | 22 | 22 | 21 | 20 | 25 | 27 | 24 |
| 889 | CE | 15 | 23 | 24 | 17 | 19 | 31 | 27 | 28 |
| 953 | CE | 9 | 13 | 13 | 11 | 11 | 15 | 14 | 14 |
| 802 | CE | 14 | 18 | 19 | 14 | 17 | 20 | 28 | 22 |
| 833 | CE | 14 | 13 | 14 | 11 | 12 | 13 | 11 | 12 |
| 910 | CE | 16 | 23 | 19 | 19 | 17 | 21 | 18 | 19 |
| 930 | CE | 10 | 12 | 14 | 13 | 12 | 18 | 15 | 14 |
| 832 | CE | 12 | 21 | 19 | 17 | 17 | 24 | 20 | 26 |

C - Control diet
OB - Oat bran diet
GG - Guar gum diet
X - Xylan diet
CE - Cellulose

# Appendix N

Individual fecal weights and moisture content of fecal samples over four days during week nine

| I.D. | Group | Wet Fecal Weight (g) | Dry Fecal Weight (g) | Moisture Content(g) |
|---|---|---|---|---|
| 845 | C | 6.0 | 5.5 | 0.5 |
| 838 | C | 5.4 | 5.1 | 0.3 |
| 865 | C | 4.5 | 4.2 | 0.3 |
| 899 | C | 4.3 | 4.1 | 0.2 |
| 894 | C | 5.1 | 4.5 | 0.6 |
| 810 | C | 3.8 | 3.6 | 0.2 |
| 911 | C | 4.1 | 3.7 | 0.4 |
| 938 | C | 5.3 | 4.8 | 0.3 |
| 862 | C | 4.3 | 3.8 | 0.5 |
| 851 | C | 4.8 | 4.3 | 0.5 |
| 915 | C | 3.5 | 3.2 | 0.3 |
| 806 | C | 4.1 | 3.7 | 0.4 |
| 875 | C | 4.4 | 4.1 | 0.3 |
| 854 | C | 7.6 | 5.4 | 2.2 |
| 856 | C | 3.2 | 2.9 | 0.3 |
| 867 | C | 3.8 | 3.5 | 0.3 |
| 903 | OB | 5.0 | 4.8 | 0.2 |
| 900 | OB | 5.0 | 4.7 | 0.3 |
| 925 | OB | 4.8 | 4.6 | 0.2 |
| 917 | OB | 4.9 | 4.3 | 0.6 |
| 934 | OB | 5.4 | 5.0 | 0.4 |
| 873 | OB | 4.1 | 3.8 | 0.3 |
| 837 | OB | 5.8 | 5.3 | 0.5 |
| 879 | OB | 4.3 | 4.0 | 0.3 |
| 863 | OB | 4.1 | 3.8 | 0.3 |
| 869 | OB | 3.0 | 2.8 | 0.2 |
| 881 | OB | 6.7 | 3.8 | 2.9 |
| 928 | OB | 5.5 | 5.1 | 0.4 |
| 912 | OB | 5.9 | 5.4 | 0.5 |
| 941 | OB | 4.1 | 3.7 | 0.4 |
| 836 | OB | 3.3 | 3.0 | 0.3 |
| 829 | OB | 4.2 | 3.9 | 0.3 |

Fecal weights and moisture content contd.

| I.D. | Group | Wet Fecal Weight (g) | Dry Fecal Weight (g) | Moisture Content(g) |
|---|---|---|---|---|
| 858 | GG | 3.4 | 3.2 | 0.2 |
| 940 | GG | 3.3 | 3.1 | 0.2 |
| 839 | GG | 2.8 | 2.6 | 0.2 |
| 885 | GG | 3.6 | 3.4 | 0.2 |
| 897 | GG | 3.2 | 3.0 | 0.2 |
| 800 | GG | 2.6 | 2.4 | 0.2 |
| 907 | GG | 4.1 | 3.9 | 0.2 |
| 804 | GG | 3.3 | 3.1 | 0.2 |
| 888 | GG | 2.9 | 2.5 | 0.4 |
| 831 | GG | 2.6 | 2.4 | 0.2 |
| 872 | GG | 3.8 | 3.4 | 0.4 |
| 848 | GG | 4.6 | 4.3 | 0.3 |
| 896 | GG | 4.1 | 3.9 | 0.2 |
| 842 | GG | 3.6 | 3.3 | 0.3 |
| 864 | GG | 3.8 | 3.5 | 0.3 |
| 841 | GG | 3.2 | 2.9 | 0.3 |
| 920 | X | 6.3 | 5.8 | 0.5 |
| 815 | X | 8.6 | 7.0 | 0.6 |
| 947 | X | 4.8 | 4.5 | 0.3 |
| 902 | X | 2.8 | 2.7 | 0.1 |
| 871 | X | 7.8 | 6.1 | 1.7 |
| 809 | X | 6.1 | 5.6 | 0.5 |
| 926 | X | 4.5 | 4.2 | 0.3 |
| 909 | X | 5.4 | 5.1 | 0.3 |
| 905 | X | 7.3 | 6.3 | 1.0 |
| 803 | X | 6.1 | 5.6 | 0.5 |
| 826 | X | 5.0 | 4.6 | 0.4 |
| 893 | X | 6.3 | 5.6 | 0.7 |
| 933 | X | 6.2 | 5.5 | 0.7 |
| 843 | X | 4.8 | 4.5 | 0.3 |
| 818 | X | 8.5 | 7.0 | 1.5 |
| 834 | X | 4.8 | 4.5 | 0.3 |

Fecal weight and moisture content contd.

| I.D. | Group | Wet Fecal Weight (g) | Dry Fecal Weight (g) | Moisture Content(g) |
|---|---|---|---|---|
| 844 | CE | 8.9 | 8.0 | 0.9 |
| 884 | CE | 5.0 | 4.6 | 0.4 |
| 892 | CE | 6.0 | 5.5 | 0.5 |
| 886 | CE | 5.9 | 5.4 | 0.5 |
| 825 | CE | 8.2 | 7.4 | 0.8 |
| 914 | CE | 6.5 | 6.0 | 0.5 |
| 950 | CE | 7.5 | 6.5 | 1.0 |
| 801 | CE | 3.8 | 3.4 | 0.4 |
| 901 | CE | 10.9 | 9.7 | 1.2 |
| 889 | CE | 12.4 | 8.7 | 3.7 |
| 953 | CE | 5.4 | 5.0 | 0.4 |
| 802 | CE | 7.6 | 7.0 | 0.6 |
| 833 | CE | 5.1 | 4.7 | 0.4 |
| 910 | CE | 7.0 | 6.5 | 0.5 |
| 930 | CE | 6.5 | 6.0 | 0.5 |
| 832 | CE | 20.6 | 15.0 | 5.6 |

C - Control diet
OB - Oat bran diet
GG - Guar gum diet
X - Xylan diet
CE - Cellulose diet

# Appendix O

Percent water content of feces collected over four days during week nine, for individual animals

| I.D. | Group | Percent Water Content |
|---|---|---|
| 845 | C | 9.1 |
| 838 | C | 5.9 |
| 865 | C | 7.1 |
| 899 | C | 4.9 |
| 894 | C | 13.3 |
| 810 | C | 5.5 |
| 911 | C | 10.8 |
| 938 | C | 6.2 |
| 862 | C | 13.2 |
| 851 | C | 11.6 |
| 915 | C | 9.4 |
| 806 | C | 10.8 |
| 875 | C | 7.3 |
| 854 | C | 40.7 |
| 856 | C | 10.3 |
| 867 | C | 8.6 |
| 903 | OB | 4.2 |
| 900 | OB | 6.4 |
| 925 | OB | 4.3 |
| 917 | OB | 14.0 |
| 934 | OB | 8.0 |
| 873 | OB | 7.9 |
| 837 | OB | 9.4 |
| 879 | OB | 7.5 |
| 863 | OB | 7.9 |
| 869 | OB | 7.1 |
| 881 | OB | 76.3 |
| 928 | OB | 7.8 |
| 912 | OB | 9.3 |
| 941 | OB | 10.8 |
| 836 | OB | 10.0 |
| 829 | OB | 7.7 |

Percent water content contd.

| I.D. | Group | Percetn Water Content |
|---|---|---|
| 858 | GG | 6.2 |
| 940 | GG | 6.4 |
| 839 | GG | 7.7 |
| 885 | GG | 5.9 |
| 897 | GG | 6.7 |
| 800 | GG | 8.3 |
| 907 | GG | 5.1 |
| 804 | GG | 6.4 |
| 888 | GG | 16 |
| 831 | GG | 8.3 |
| 872 | GG | 11.8 |
| 848 | GG | 7.0 |
| 896 | GG | 5.1 |
| 842 | GG | 9.1 |
| 864 | GG | 8.6 |
| 841 | GG | 10.3 |
| 920 | X | 8.6 |
| 815 | X | 8.6 |
| 947 | X | 6.7 |
| 902 | X | 3.7 |
| 871 | X | 27.9 |
| 809 | X | 9.0 |
| 926 | X | 7.1 |
| 909 | X | 5.9 |
| 905 | X | 15.9 |
| 803 | X | 9.0 |
| 826 | X | 8.7 |
| 893 | X | 12.5 |
| 933 | X | 12.7 |
| 843 | X | 6.7 |
| 818 | X | 21.4 |
| 834 | X | 6.7 |

Percent water content contd.

| I.D. | Group | Percent Water Content |
|---|---|---|
| 844 | CE | 11.2 |
| 884 | CE | 8.7 |
| 892 | CE | 9.1 |
| 886 | CE | 9.3 |
| 825 | CE | 10.8 |
| 914 | CE | 8.3 |
| 950 | CE | 15.4 |
| 801 | CE | 11.8 |
| 901 | CE | 12.4 |
| 889 | CE | 42.5 |
| 953 | CE | 8.0 |
| 802 | CE | 8.6 |
| 833 | CE | 8.5 |
| 910 | CE | 7.7 |
| 930 | CE | 8.3 |
| 832 | CE | 37.3 |

C - Control diet
OB - Oat bran diet
GG - Guar gum diet
X - Xylan diet
CE - Cellulose diet

# Appendix P

Fecal neutral steroid concentration in feces collected over four days during week nine, for individual animals

| I.D. | Group | Neutral Steroid Conc. (mg/g feces) | Total Neutral Steroid Conc. (mg/dry fecal weight) |
|---|---|---|---|
| 845 | C | 0.68 | 3.7 |
| 838 | C | 1.11 | 5.7 |
| 865 | C | 0.69 | 3.0 |
| 899 | C | 1.61 | 6.6 |
| 894 | C | 0.78 | 3.5 |
| 810 | C | 0.36 | 1.3 |
| 911 | C | 1.24 | 4.6 |
| 938 | C | 0.90 | 4.3 |
| 862 | C | 0.76 | 3.0 |
| 851 | C | 0.64 | 2.7 |
| 915 | C | 0.85 | 2.7 |
| 806 | C | 0.28 | 1.0 |
| 875 | C | 0.87 | 3.6 |
| 854 | C | 0.55 | 3.0 |
| 856 | C | 1.43 | 4.1 |
| 867 | C | 1.16 | 4.1 |
| 903 | OB | 1.96 | 9.4 |
| 900 | OB | 1.18 | 5.5 |
| 925 | OB | 0.83 | 3.8 |
| 917 | OB | 1.12 | 4.8 |
| 934 | OB | 1.15 | 5.7 |
| 873 | OB | 2.39 | 9.1 |
| 837 | OB | 0.56 | 3.0 |
| 879 | OB | 2.14 | 8.6 |
| 863 | OB | 0.77 | 3.0 |
| 869 | OB | 2.5 | 7.0 |
| 881 | OB | 0.97 | 3.7 |
| 928 | OB | 0.51 | 2.6 |
| 912 | OB | 0.86 | 4.6 |
| 941 | OB | 1.60 | 6.0 |
| 836 | OB | 1.57 | 4.7 |
| 829 | OB | 1.15 | 4.5 |

Fecal neutral steroid values cont.

| I.D. | Group | Neutral Steroid Conc. (mg/g feces) | Total Neutral Steroid Conc. (mg/dry fecal weight) |
|---|---|---|---|
| 858 | GG | 1.68 | 5.4 |
| 940 | GG | 0.80 | 2.5 |
| 839 | GG | 0.82 | 2.1 |
| 885 | GG | 0.81 | 2.7 |
| 897 | GG | 0.56 | 1.7 |
| 800 | GG | 1.36 | 3.3 |
| 907 | GG | 0.57 | 2.2 |
| 804 | GG | 0 | 0 |
| 888 | GG | 0.35 | 1.0 |
| 831 | GG | 1.87 | 4.5 |
| 872 | GG | 1.15 | 4.0 |
| 848 | GG | 0.86 | 3.7 |
| 896 | GG | 0.72 | 2.8 |
| 842 | GG | 0.56 | 1.8 |
| 864 | GG | 1.67 | 5.8 |
| 841 | GG | 2.19 | 6.3 |
| | | | |
| 920 | X | 0.11 | 0.6 |
| 815 | X | 0.36 | 2.5 |
| 947 | X | 0.65 | 3.0 |
| 902 | X | 0.56 | 1.5 |
| 871 | X | 1.35 | 8.2 |
| 809 | X | 0.43 | 2.4 |
| 926 | X | 0.72 | 3.0 |
| 909 | X | 0.22 | 1.1 |
| 905 | X | 0.28 | 1.8 |
| 803 | X | 0.26 | 1.6 |
| 826 | X | 0.27 | 1.2 |
| 893 | X | 0.26 | 1.5 |
| 933 | X | 0.28 | 1.5 |
| 843 | X | 0.59 | 2.6 |
| 818 | X | 0.40 | 2.8 |
| 834 | X | 0.32 | 1.4 |

Fecal neutral steroid values contd.

| I.D. | Group | Neutral Steroid Conc. (mg/g) | Total Neutral Steroid Conc. (mg/g dry fecal wt.) |
|---|---|---|---|
| 844 | CE | 0.24 | 2.0 |
| 884 | CE | 0.54 | 2.5 |
| 892 | CE | 0.95 | 5.2 |
| 886 | CE | 0.15 | 0.8 |
| 825 | CE | 0.19 | 1.4 |
| 914 | CE | 0.26 | 1.6 |
| 950 | CE | 0.47 | 3.0 |
| 801 | CE | 0.44 | 1.5 |
| 901 | CE | 0.62 | 6.0 |
| 889 | CE | 0.39 | 3.4 |
| 953 | CE | 0.79 | 4.0 |
| 802 | CE | 0.28 | 2.0 |
| 833 | CE | 0.82 | 3.8 |
| 910 | CE | 0.23 | 1.5 |
| 930 | CE | 0.85 | 5.1 |
| 832 | CE | 0.60 | 9.0 |

C - Control diet
OB - Oat bran diet
GG - Guar gum diet
X - Xylan diet
CE - Cellulose diet

## Appendix Q

Mean liver cholesterol values for individual animals at week 9 (mg/g)

| I.D. | Group | Week 9 |
|---|---|---|
| 845 | C | 4 |
| 838 | C | 4 |
| 865 | C | 6 |
| 899 | C | 3 |
| 894 | C | 4 |
| 810 | C | 4 |
| 911 | C | 2 |
| 938 | C | 4 |
| 862 | C | 5 |
| 851 | C | 2 |
| 915 | C | 3 |
| 806 | C | 2 |
| 875 | C | 2 |
| 854 | C | 7 |
| 856 | C | 4 |
| 867 | C | 5 |
| 903 | OB | 4 |
| 900 | OB | 2 |
| 925 | OB | 3 |
| 917 | OB | 5 |
| 934 | OB | 3 |
| 873 | OB | 3 |
| 837 | OB | 2 |
| 879 | OB | 3 |
| 863 | OB | 1 |
| 869 | OB | 2 |
| 881 | OB | 2 |
| 928 | OB | 6 |
| 912 | OB | 4 |
| 941 | OB | 4 |
| 836 | OB | 2 |
| 829 | OB | 5 |

**Mean liver cholesterol values contd.**

| I.D. | Group | Week 9 |
|---|---|---|
| 858 | GG | 2 |
| 940 | GG | 3 |
| 839 | GG | 4 |
| 885 | GG | 3 |
| 897 | GG | 2 |
| 800 | GG | 3 |
| 907 | GG | 5 |
| 804 | GG | 4 |
| 888 | GG | 2 |
| 831 | GG | 3 |
| 872 | GG | 4 |
| 848 | GG | 4 |
| 896 | GG | 4 |
| 842 | GG | 1 |
| 864 | GG | 2 |
| 841 | GG | 2 |
| | | |
| 920 | X | 3 |
| 815 | X | 2 |
| 947 | X | 2 |
| 902 | X | 2 |
| 871 | X | 3 |
| 809 | X | 3 |
| 926 | X | 3 |
| 909 | X | 3 |
| 905 | X | 4 |
| 803 | X | 4 |
| 826 | X | 1 |
| 893 | X | 1 |
| 933 | X | 4 |
| 843 | X | 2 |
| 818 | X | 3 |
| 834 | X | 4 |

**Mean liver cholesterol values contd.**

| I.D. | Group | Week 9 |
|---|---|---|
| 844 | CE | 10 |
| 884 | CE | 5 |
| 892 | CE | 4 |
| 886 | CE | 4 |
| 825 | CE | 4 |
| 914 | CE | 8 |
| 950 | CE | 8 |
| 801 | CE | 5 |
| 901 | CE | 5 |
| 889 | CE | 4 |
| 953 | CE | 8 |
| 802 | CE | 4 |
| 833 | CE | 5 |
| 910 | CE | 3 |
| 830 | CE | 6 |
| 832 | CE | 5 |

C - Control diet
OB - Oat bran diet
GG - Guar gum diet
X - Xylan diet
CE - Cellulose diet

**Mean liver cholesterol values for individual animals after four weeks of the control diet (mg/g)**

| I.D. | Week 4 |
|---|---|
| 820 | 4 |
| 876 | 4 |
| 935 | 4 |
| 936 | 3 |
| 850 | 3 |
| 857 | 4 |
| 861 | 5 |
| 923 | 6 |
| 835 | 3 |
| 840 | 4 |
| 874 | 4 |
| 942 | 7 |
| 805 | 2 |
| 849 | 4 |
| 882 | 4 |
| 929 | 5 |

# Appendix R

Mean plasma total cholesterol levels (mmol/l)[*]

| Group | Week 0 | Week 4 | Week 9 |
|---|---|---|---|
| | ----------------- | (mmol/l) | ----------------- |
| Control | $2.8 \pm .31^{a}$ | $6.3 \pm .7^{b}$ | $6 \pm 1.1^{b}$ |
| Oat Bran | $2.6 \pm .3^{a}$ | $6.5 \pm .7^{b}$ | $5.5 \pm 1.2^{c}$ |
| Guar Gum | 2.9 ± .4a | $6.4 \pm .6^{b}$ | $5.7 \pm .8^{c}$ |
| Xylan | $2.8 \pm .3^{a}$ | $6.4 \pm .6^{b}$ | $5.4 \pm 1.3^{c}$ |
| Cellulose | $2.8 \pm .3^{a}$ | $6.4 \pm .5^{b}$ | $5.9 \pm .9^{b}$ |

Means ± SD
$^{a-c}$ values within a row or column with different letters are significantly different ($p<.05$)

[*] (mg/dl X 0.02586 = mmol/l)

## Appendix S

Mean plasma high-density lipoprotein cholesterol levels (mmol/l)*

| Group | Week 0 | Week 4 | Week 9 |
|---|---|---|---|
| | (mmol/l) | | |
| Control | 2 ± .2$^{a}$ | 4.2 ± .4$^{b}$ | 4.5 ± .9$^{b}$ |
| Oat Bran | 2 ± .2$^{a}$ | 4.4 ± .4$^{b}$ | 4.2 ± 1$^{b}$ |
| Guar Gum | 2 ± .3$^{a}$ | 4.3 ± .4$^{b}$ | 3.8 ± .8$^{c}$ |
| Xylan | 2 ± .2$^{a}$ | 4.4 ± .4$^{b}$ | 4.1 ± 1.2$^{b}$ |
| Cellulose | 2 ± .3$^{a}$ | 4.4 ± .4$^{b}$ | 4.7 ± 1.1$^{b}$ |

Means ± SD
$^{a-c}$ values within a row or column with different letters are significantly different ($p<.05$)

*(mg/ml X 0.02586 = mmol/l)

## Appendix T

Mean plasma very-low and low-density lipoprotein cholesterol levels (mmol/l)*

| Group | Week 0 | Week 4 | Week 9 |
|---|---|---|---|
| | | (mmol/l) | |
| Control | .72 ± .3$^{a}$ | 2.1 ± .6$^{b}$ | 1.7 ± .8$^{b}$ |
| Oat Bran | .62 ± .3$^{a}$ | 2.1 ± .5$^{b}$ | 1.3 ± .7$^{c}$ |
| Guar Gum | .80 ± .3$^{a}$ | 2.1 ± .4$^{b}$ | 1.8 ± .6$^{b}$ |
| Xylan | .75 ± .3$^{a}$ | 2.0 ± .6$^{b}$ | 1.3 ± .8$^{b}$ |
| Cellulose | .85 ± .3$^{a}$ | 2.1 ± .4$^{b}$ | 1.2 ± .8$^{a}$ |

Means ± SD
$^{a-c}$ values within a row or column with different letters are significantly different ($p<.05$)

*(mg/ml X 0.02586 = mmol/l)

## Appendix U

**Mean plasma triglyceride levels (mmol/l)***

| Group | Week 0 | Week 4 | Week 9 |
|---|---|---|---|
| | (mmol/l) | | |
| **Control** | **.77 ± .3$^a$** | **2.8 ± 1.5$^b$** | **2.6 ± 1.6$^b$** |
| **Oat Bran** | **.73 ± .3$^a$** | **2.9 ± 1.8$^b$** | **2.2 ± 1.2$^b$** |
| **Guar Gum** | **.79 ± .1$^a$** | **2.6 ± 1.5$^b$** | 1.4 ± .8$^c$ |
| Xylan | .60 ± .2$^a$ | 2.0 ± 1.0$^b$ | 2.1 ± 1.8$^b$ |
| Cellulose | .73 ± .4$^a$ | 2.5 ± 1.1$^b$ | 2.1 ± 1.4$^b$ |

Means ± SD
$^{a-c}$ values within a row or column with different letters are significantly different ($p<.05$)

*(mg/ml X 0.01129 = mmol/l)

# VITA

Satya was born on December 6, 1966, in Visakhapatnam, India. In 1985 she received her B.Sc. degree in Foods and Nutrition from Maharaja SayajiRao University (M.S.U.) of Baroda, Baroda, India and subsequently earned her M.Sc. in Foods and Nutrition in 1987. In the Fall of 1987, she came to the United States and entered the Masters program at Case Western Reserve University, Cleveland, Ohio and received a M.S. in Nutrition in the Fall of 1988. In January 1989, she enrolled in the doctoral program at Virginia Polytechnic Institute and State University, Blacksburg, Virginia. Satya has been offered a Postdoctoral Fellowship at the Foster Biomedical Research Laboratories, Brandies University, Waltham, MA and will be joining the program in July.

J. Satya Srivathsa

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