THE EFFECTS OF A SOLUBLE FIBER (ß-GLUCAN) ON FECAL ACIDIC STEROID EXCRETION AND SERUM LIPIDS IN NORMOLIPIDEMIC YOUNG MEN

by

Kelley Colleen Fitzpatrick

A thesis
presented to the University of Manitoba
in fulfillment of the
thesis requirement for the degree of
Master of Science
in
Foods and Nutrition

Winnipeg, Manitoba

ISBN 0-315-44152-6

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A thesis submitted to the Faculty of Graduate Studies of the University of Manitoba in partial fulfillment of the requirements of the degree of

MASTER OF SCIENCE

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ABSTRACT

A 30-day metabolic study was designed to investigate the effects of a water-soluble fiber, ß-glucan, on fecal acidic steroids, fecal lipids and serum lipids in healthy normolipidemic men. The subjects were eight men, ages 18 to 36 years. Approximately 9 g/day of soluble fiber (ß-glucan) was consumed daily as barley flour incorporated into a mixed diet (BD). The control diet (WD), which excluded ß-glucan, contained wheat flour. Diets contained approximately 36% of total energy as fat and were similar in fatty acids, cholesterol, carbohydrates and protein. Body weights of the subjects were maintained throughout the study. Diets met the nutrient requirements (R.N.I.) for all subjects. All subjects consumed each of the BD and the WD treatments for a 15-day period in a cross-over design. Polyethylene glycol (PEG) served as an internal marker of intestinal transit. Fecal samples were collected during the last five consecutive days in each dietary period and were analyzed for total PEG, total bile acid and total lipid concentrations. Fasting venous blood samples were collected at the beginning of the study and at the conclusion of each dietary period. Serum was analyzed for total cholesterol, LDL cholesterol, HDL cholesterol, triglyceride, and VLDL cholesterol concentrations. Mean fecal output (wet weight) for the BD was 123 ± 14[1] g/day and 101 ± 11 g/day for the WD. Subjects who consumed the BD followed by the WD had a significantly ($p<0.05$) higher mean total fecal output (130 ± 8 g/day) compared to those subjects

[1] Standard Error

following the WD to BD sequence (82 ± 14 g/day). Fecal recoveries PEG were highly variable, ranging from 19% to 129%. Fecal acidic steroid excretion, corrected by PEG recovery values, was not significantly ($p<0.05$) different between the BD (988 ± 126 μmoles/day) and the WD (1155 ± 230 μmoles/day) treatments. Total fecal lipid excretion was also not significantly ($p<0.05$) different when either the BD (4.1 ± 0.5 g/day) or the WD (4.9 ± 1.1 g/day) treatments were consumed by the subjects. A significant ($p<0.05$) difference in mean serum total cholesterol concentrations was observed between the BD (154 ± 7 mg/dL) and the WD (165 ± 9 mg/dL) treatments. Mean serum LDL-C levels were significantly ($p<0.05$) different between the BD (104 ± 6 mg/dL) and the WD (107 ± 8 mg/dL) treatments. Mean serum HDL-C levels were 43 ± 3 mg/dL for the BD and 45 ± 4 mg/dL for the WD, which were not significantly ($p<0.05$) different. No significant ($p<0.05$) differences were noted between the levels of mean serum triglycerides for the BD (70 ± 7 mg/dL) and the WD (78 ± 10 mg/dL) treatments. Also, no significant ($p<0.05$) differences in the levels of mean serum VLDL-C for the BD (8 ± 2 mg/dL) and the WD (10 ± 3 mg/dL) were found. Soluble fiber in the form of ß-glucan did not have any effect on fecal acidic steroid and fat excretion or serum lipids in these subjects.

ACKNOWLEDGEMENTS

I wish to express my deep appreciation to my advisor, Dr. Vivian Bruce, for her support, guidance, and unending assistance in this research, in the preparation of this manuscript, and throughout my academic career at the U of M. I would also like to thank Dr. Bruce McDonald for his helpful suggestions with this thesis and, along with Dr. Ron Marquardt, for serving as committee members.

Sincere thanks go to the eight University students who participated in this study. Their cooperation, interest, and wit helped to make this research an enjoyable success.

I would like to acknowledge and express my gratitude to Marilyn Latta and Stacy Johnson for their excellent technical assistance, guidance and patience in the lab. The statistical advise of Linda Neden was also greatly appreciated.

An important part of this experience for me has been the great friends that I have made. Special thanks go to my fellow graduate students Lynn Wilsack, Chris Picken, Judith Britten, Leesa Chan, Liza Zacharias, Abdullah Hussien and Sheri Betker, whose constant support, humour and encouragement I gratefully appreciated.

I am extremely grateful for the love, encouragement, and continuous support that I received from Darryl Pal, my parents, Pat and Helen, and my sister, Noreen. I could not have completed this thesis without them.

The financial support of the University of Manitoba Graduate Studies Department, the Manitoba Health Research Council and the National Health Research Development Programs is gratefully acknowledged.

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LITERATURE REVIEW

1.1 INTRODUCTION

The Report of the 1985 Consensus Conference of the National Institute of Health (NIH) reviewed the evidence related to the effect of serum cholesterol on the development of atherosclerosis and concluded that the 'elevation of blood cholesterol levels is a major cause of coronary heart disease'. Experimental support for the benefits of maintaining serum cholesterol within physiological limits was provided by the Lipid Research Clinics Primary Prevention Trial (1984). This report, in addition to several other trials , led the NIH committee to conclude that the risk of coronary heart disease was related to the degree of elevation of serum cholesterol. Researchers have also established that serum levels of LDL-C are correlated with increased risk of atheroclerosis (Goldstein and Brown, 1977; Brown et al., 1981), while elevated levels of HDL-C are associated with decreased risk (Miller and Miller, 1975; Miller, 1978). It is, therefore, important to identify constituents in the human diet that will maintain serum cholesterol and lipoprotein levels within recommended physiological limits.

Several dietary factors have been studied, but most emphasis has been directed to dietary cholesterol and fatty acids. However, recent evidence in experimental animals and human clinical studies suggest that

dietary fiber intakes reduce serum cholesterol concentrations. Although a great deal of controversy exists, soluble fibers, specifically, have been shown to be more hypocholesterolemic than insoluble dietary fibers. Effects on serum lipid and bile acid homeostasis have been suggested as possible mechanisms. Most of the research in this area has concentrated on the hypocholesterolemic effects of isolated soluble fiber sources such as pectin and guar gum. Little attention has been given to the effects of soluble fibers contained in natural foods commonly consumed in the human diet.

Soluble forms of dietary fibers differ from insoluble forms both in chemical composition and in their physiological effects on human and animal metabolism, primarily due to the distinctive functions of these fibers in plant tissues. The soluble fiber component includes noncellulosic hemicelluloses, pectic substances (polygalacturonic acid compounds), gums and mucilages. Hemicelluloses such as ß-glucan, act as storage polysaccarides and are found in endosperm cell walls. Pectic substances function as intercellular cementing materials and are dispersed throughout the outer cell wall regions. Gums and mucilages, found in the endosperm of plants, act to prevent dehydration and as protective capsules at sites of plant injury. Whereas the soluble fibers are essentially nonstructural components, insoluble fibers such as cellulose and lignin, are important in the structural integrity of plant cell walls (Kay and Strasberg, 1978). Cellulose and lignin are insoluble in concentrated alkali, and tend to have tight-fitting molecular configurations. In contrast, soluble dietary fibers are composed of more loosely structured linear monosaccharide chains which may be

unbranched, as found in ß-glucan, or more complex due to the presence of several monosaccharide branches, as occur in the structures of the pectic substances.

The physiological properties of dietary fibers reflect both their chemical structures and their functional roles in plant tissues. In the gut, water soluble fibers form viscous, gel-like structures upon hydration. These gels can increase intestinal transit time as well as trap and prevent the absorption of nutrients. This property is believed to be responsible for reducing serum cholesterol levels (Kay and Truswell, 1980). Although inconsistencies exist, present data indicate that the insoluble particulate fibers are ineffective hypocholesterolemic agents but function to decrease intestinal transit time and increase fecal mass.

1.2 ANIMAL STUDIES

Research related to the hypocholesterolemic effects of soluble fibers in animals has concentrated on chemically isolated forms of pectin and guar gum, rather than natural sources present in the diet. Generally, a lack of agreement exists on the role of soluble fiber in the metabolism of cholesterol in different animal models. Inconsistent results have been found in hamsters, rabbits, and swine fed diets supplemented with pectin. However, the hypocholesterolemic action of isolated pectin and guar gum have been frequently demonstrated in rats and poultry. Pectin, in amounts of 2.5% and 5.0% by weight, has been shown to reduce serum cholesterol levels by 15 to 20% (Kirimaya et al., 1969 ; Tsai et al., 1976) in rats. Similar reductions in serum cholesterol levels have been

noted with the incorporation of 10.0% guar gum into rat diets (Ershoff and Wells, 1962; Riccardi and Fahrenbach, 1967). These fiber sources appeared to be effective only when fed in conjuction with dietary cholesterol. Soluble fiber sources, therefore, appear to counteract the effects of dietary cholesterol on serum cholesterol levels in the rat.

In poultry, the addition of similar amounts of isolated pectin or guar gum to cholesterol containing diets produced equivalent reductions in serum cholesterol levels as those seen in rats (Fisher et al., 1966 ; Fahrenbach et al., 1966 ;Griminger and Fisher, 1966). As well, guar gum has also been shown to reduce serum cholesterol levels in poultry fed cholesterol-free diets (Fahrenbach et al., 1966). These fibers appear to act within the animal gut by producing thick, viscous gels capable of trapping dietary cholesterol and fat and therefore reducing absorption (Judd and Truswell, 1985).

It is difficult to postulate the effects that soluble fibers have on cholesterol metabolism in humans based upon animal models, which in themselves differ in response to dietary alterations. Data from human subjects in epidemiological studies have attempted to identify relationships between fiber consumption, blood cholesterol levels and coronary heart disease.

1.3 EPIDEMIOLOGIC DATA

Walker and Arvidson (1954) were among the first investigators to suggest that coronary heart disease was very rare in populations consuming large amounts of dietary fiber. They used the South African Bantu as an example of a group who consumed 60 to 80 g/day of crude fiber, four times the Western norm, and also had very low levels of ischemic heart disease.

Reports of another isolated population, Tarahumara Indians, did not confirm this report (Connor et al., 1978). Crude fiber intakes, approximately 19 g/day, were negatively correlated with serum cholesterol levels. Mean serum cholesterol values of adults were 125 mg/dl. Ninety percent of the energy intake in this group was contributed by corn and beans. However, diets were typically very low in cholesterol (71 mg/day), fat (12% of total energy) and saturated fat (2% of total energy) and were high in plant sterols (400 mg/day). These dietary factors could influence blood cholesterol to greater degrees than the fiber content of the diets. Due to many confounding dietary and lifestyle factors, it is not possible to determine precisely what role crude fiber intake had in reducing serum cholesterol levels in this population.

As diet complexity increases, it becomes more difficult to identify dietary components which are associated with risk of coronary heart disease. In a survey of nutrient intake and serum lipids by Keys and coworkers (1955), the mean plasma cholesterol concentrations for men in Naples, Italy was 170 mg/dL. This was lower than the mean of 233 mg/dL

for an age-matched group in Minnesota. The observed difference was unexpected in view of the fact that the diets of the Italian participants contained approximately 38% of energy as fat, which is equivalent to typical North American diets. Trowell (1972) suggested that the consumption of fruits, vegetables, legumes, and pasta-cereal products, typical in the diets of the Italian subjects, was primarily responsible for the lower blood cholesterol levels. However, recent research by Grundy (1986) suggests that consumption of the monounsaturated fatty acid, oleic acid, may be a more important hypocholesterolemic factor than the dietary fiber intake of this area.

When populations who naturally restrict animal proteins and fats in their diets were investigated, there was no agreement about the effects of dietary fiber. In strict and lacto-ovo vegetarians, Hardinge and Stare (1954) noted that serum cholesterol levels were approximately 11% lower than a non-vegetarian group. The vegetarian subjects consumed, on average, 50% more crude fiber (Hardinge et al., 1958). As well, the diets of this population contained less total and saturated fat, more polyunsaturated fat and significantly less cholesterol (approximately 300 mg/day and 0 mg/day in the lacto-ovo and pure vegetarians, respectively) than the diets of the non-vegetarians. However, detailed analysis by Hardinge and coworkers (1962) showed that a better correlation existed between fiber intake and serum cholesterol levels than the correlation between the intakes of total fat, saturated fat, or the P:S ratio and blood cholesterol concentrations.

Relationships between fiber consumption and coronary heart disease are not consistently reported. Burr and Sweetnam (1982) noted a signif-

icant negative correlation between vegetarianism and mortality from ischemic heart disease (IHD). However, a significant association between overall fiber intake and IHD was not found. The fiber source studied was wholemeal bread which contains primarily insoluble forms of fiber. Fiber intakes of male vegetarians were 50% higher ($p<0.01$) than non-vegetarians, due primarily to a higher consumption of non-cereal fiber. Among female vegetarians fiber intakes were 41% higher ($p<0.01$). Statistical correlations, however, were not examined between intakes of non-cereal fiber, which may consist primarily of soluble forms, and IHD.

In addition, environmental factors may confound dietary effects in epidemiological studies. Morris and associates (1977) attempted to link fiber intake to the development of coronary heart disease within a Western population. Food intakes for 337 middle-aged men in England were recorded between 1956 to 1966 and reexamined in 1976. The data indicated that men with the highest intake of cereal fiber had a lower rate of coronary heart disease than other members of the group. The proposed relationship could not be explained by differences in known medical risk factors such as lower serum cholesterol levels. However, the participants in the high-fiber group smoked fewer cigarettes, and appeared to have consumed a superior diet and exercised more frequently. These differences in environmental variables, plus other dietary variables indicated to Kay and Truswell (1980) that a direct relationship between fiber intake and clinical coronary heart disease could not be established conclusively.

Recent epidemiologic research in Western societies has illustrated that methodolgy and data analysis are important features in the interpretation of dietary data. Kay and associates (1980) noted, in a study of 200 Canadian males, that those with the lowest serum cholesterol and triglyceride levels consumed an average of 5 g of crude fiber per day more than other participants with higher lipid concentrations. Univariate statistical analysis revealed a weak, but significant, correlation between dietary fiber intake and serum lipids. However, multivariate analysis of the data indicated that the relationship was greatly influenced by co-existing differences in other variables. Negative relationships were noted, for example, between fiber intake and each of fat intake and of adiposity. The authors concluded that, in a free-living Western population, dietary fiber may exert an indirect effect on plasma lipids by displacing more lipid-dense nutrients. This study indicates that the variables affecting serum lipids are dependent on, among other factors, the statistical methods used to analyze the data.

Epidemiologic research has failed to prove a direct positive correlation between high dietary fiber intakes and reduced serum cholesterol levels. It appears difficult to conduct an epidemiologic study to determine relationships between soluble dietary fiber intakes and serum lipid levels. The variety and composition of foods consumed by populations who are at risk of developing coronary heart disease makes it difficult to isolate specific dietary variables.

1.4 HUMAN STUDIES

1.4.1 Fiber Containing Foods

Few studies have examined the hypocholesterolemic effects of the soluble fibers which occur naturally in common foods when dietary variables are restricted. In those studies which have concentrated on feeding fiber containing foods, Table 1 shows that fruits, vegetables and legumes are frequently associated with decreases in serum cholesterol.

In controlled experiments conducted by Keys and associates (1960), the substitution of 17% of total energy from sucrose and milk carbohydrates by 45 g of dietary fiber from fruits, vegetables and legumes resulted in reductions of 16-19 mg/dL in serum cholesterol levels. It is not possible to attribute the observed hypocholesterolemic effect to any particular substance as a number of natural fiber-rich foods were fed to the subjects. Similarly, serum cholesterol levels were reduced by a mean of 20 mg/dL with the substitution of either bread or sucrose by a mixture of vegetables containing 40 g of dietary fiber (Grande et al., 1974).

Serum cholesterol was also reduced when 200 g of raw carrots, which contain 6 g dietary fiber, were added to the self- selected diets of five subjects for a three week period (Robertson et al., 1979). Serum cholesterol levels decreased 11% ($p<0.05$), fecal bile acid and fat excretion increased by approximately 31% and 23%, respectively. Neutral steroid excretion remained unchanged. The fiber in carrots is approximately 50% soluble pectin (Eastwood, 1983). In contrast to these

TABLE 1

The Effects of Foods Containing Fiber on Serum Lipids, Fecal Steroid and Fat Excretion in Humans

FOODS	FIBER	AMOUNT (g/day)	DURATION (days)	SUBJECTS[1]	SERUM[2] CHOLESTEROL			FECAL[3] STEROIDS			Reference
					TC	LDL-C	HDL-C	B.A.	N.S.	FAT	
					(%)[4]			(%)			
APPLES	ND[5]	20	21	6N	-7	–[6]	0	–	–	–	Jenkins *et. al.*, 1979
APPLES, VEGETABLES	PECTIN	7.5	35	15N	-4	–	0	0	+50	+32	Stasse-Wolthius et. al.,1980
CARROTS	ND	20	21	6N	0	–	-11	–	–	–	Jenkins *et. al.*, 1979
CARROTS	ND	200	21	5N	-11	–	–	+31	0	+23	Robertson *et. al.*, 1979
CHICKPEAS	ND	–	385	30N	-22	–	–	+23	–	–	Mathur *et. al.*, 1968
PINTO, NAVY BEANS	ND	100(10)[7]	10	10HC	-19	-24	-13	-30	0	–	Anderson *et. al.*, 1984

[1]N = Normolipidemic; HC = Hypercholesterolemic

[2]TC = Total Cholesterol; LDL-C = Low Density Lipoprotein Cholesterol; HDL-C = High Density Lipoprotein Cholesterol

[3]B.A. = Bile Acids; N.S. = Neutral Steroids

[4] % Change from control

[5] Specific source of fiber not determined.

[6]Not Measured.

[7]Amount of soluble fiber present in the bean supplement is indicated in ( ).

results, 20 g of concentrated dietary fiber from carrot, added to controlled diets of six subjects for three weeks had no effect on total serum cholesterol levels, but decreased HDL-C concentration by 11% ($p<0.01$) (Jenkins et al., 1979).

A lack of agreement also exists on the hypocholesterolemic effects of diets supplemented with fresh fruit. No alteration in serum lipids was noted when 20 g of dietary fiber from fruit sources was substituted for bread and sucrose (Grande et al., 1974). A number of different types of fresh fruit were incorporated into the diet. In contrast, Jenkins and co-workers (1979) observed a 7% reduction ($p<0.02$) in serum cholesterol levels when 20 g of concentrated dietary fiber from apples was fed as a supplement to six subjects for three weeks. Apples, like carrots, contain water soluble pectins which may have been responsible for the lipid-lowering results observed (Kay and Truswell, 1977).

Conflicting results have been reported on the hypocholesterolemic effects of legumes added to human diets. Grande and associates (1965) observed that the substitution of a mixture of lima beans and split peas, which were substituted for an amount of sucrose and soybean protein equivalent to 500 kilocalories, lowered serum cholesterol levels by 9% for twelve middle-aged men. The legumes were consumed for three weeks. However, these results were not confirmed when the same researchers fed the bean mixture to a group of younger men. Likewise, no effects on blood lipids were noted when chickpeas were substituted for 500 kilocalories of sucrose for two weeks in the same study. This latter result is in contrast with the report of Mathur et al. (1968), who substituted chickpeas for wheat flour at the level of 38% of total

energy. The exact amount of the chickpea supplement was not reported. At the end of fifty-five weeks, serum cholesterol levels had decreased an average of 22% ($p<0.01$) in the thirty institutionalized males. Total bile acid excretion increased significantly by 23% ($p<0.01$). It is possible that the hypocholesterolemic effects of chickpeas and other legumes, may not be observed during short-term dietary periods or in amounts equivalent to 500 kilocalories.

Previous investigators did not identify the type or the amount of the specific soluble fiber(s) that may have been responsible for observed hypocholesterolemic effects. Dietary soluble fiber was quantitated and identified by Anderson and co-workers (1984) in a study examining the lipid-lowering effects of pinto and navy beans in ten hypercholesterolemic male subjects. One hundred g of dried beans, containing approximately 10 g of soluble fiber, were included in a basal diet which provided 37% of energy as fat and approximately 420 mg of cholesterol daily. The diet was fed for ten days. Serum cholesterol, LDL-C, and HDL-C levels decreased significantly by 18.6% ($p<0.0005$), 24.4% ($p<0.0025$), and 12.7% ($p<0.025$), respectively. There was an overall decrease in triglycerides. However, the body weights of the subjects were erratic. In nine of the ten subjects, body weights were reduced during the test diet. Fecal excretion of total bile acids was 30% lower ($p<0.01$) when the bean diet was consumed compared to the control diet. No significant changes occurred in fecal neutral steroid excretion. The bean diet was slightly lower in energy and had slightly higher amounts of polyunsaturated fatty acids than the control diet. It also contained 6 g of unspecified soluble fiber, that may have enhanced the hypocholesterolemic effects of the bean supplement.

Many of the foods incorporated into the diets in these investigations contain appreciable amounts of insoluble fibers. Only recently has the analysis of soluble fiber fractions been possible and thus in many of these studies it is difficult to conclusively identify the portion of the fiber that may be responsible for the hypocholesterolemic effects noted.

1.4.2 Isolated Fiber Sources

1.4.2.1 Pectin

In an attempt to better define the specific hypocholesterolemic actions of soluble fiber components, investigators have utilized chemically isolated sources of fiber. Pectin and guar gum have received the most attention in clinical studies on normolipidemic and hyperlipidemic individuals. A summary of the effects of pectin on cholesterol metabolism is found in Table 2.

Dietary pectin was first recognized as a hypocholesterolemic agent by Keys and coworkers (1961). The ingestion of 15 g of pectin resulted in a significant decrease of 5% in serum cholesterol levels. The pectin was incorporated into a controlled diet and fed for a three week period. Six, 8, and 10 g supplements of pectin, consumed by sixteen subjects for three four-week consecutive periods, resulted in reductions of 4% ($p<0.05$), 6% ($p<0.01$) and 6% ($p<0.01$) in serum cholesterol levels, respectively (Palmer and Dixon, 1966). Pectin fed at 2 or 4 g daily failed to significantly lower serum cholesterol. Negative correlation coefficients indicated that as the daily pectin dose increased, the corresponding serum cholesterol level decreased in twelve of the sixteen

TABLE 2

The Effects of Soluble Fibers on Serum Lipids, Fecal Steroid and Fat Excretion in Humans

FIBER	AMOUNT (g/day)	DURATION (days)	SUBJECTS[1]	SERUM[2] CHOLESTEROL			FECAL[3] STEROIDS			Reference
				TC	LDL-C	HDL-C	B.A.	N.S.	FAT	
				(%)[4]			(%)			
PECTIN	15	21	24N	-5	–[5]	–	–	–	–	Keys *et. al.*, 1961
	10	28	16N	-6	–	–	–	–	–	Palmer & Dixon, 1966
	36	14	7N	-13	–	–	–	–	–	Jenkins *et. al.*, 1975
	12	21	12N	-8	-17	–	–	–	–	Durrington *et. al.*, 1976
	15	21	9N	-13	–	–	+33	+17	+44	Kay & Truswell, 1977
	9	35	15N	-10	–	0	+51	+18	+75	Stasse-Wolthius *et. al.*,1980
	15	18	8N	–	–	–	+6	0	0	Ross & Leklem, 1981
	40-50	10-14	2N, 7HC	-13	–	–	+57	+10	+26	Miettinen & Tarpila, 1977
	9	14	12HC	-10	–	0	+9	+8	–	Nakamura *et. al.*, 1982
GUAR GUM	17	21	3N	-12	–	0	–	–	–	Jenkins *et. al.*, 1979
	9	28	24N	-16	-26	0	–	–	–	Khan *et. al.*, 1981
	5.7	14	6N	-16	–	–	–	–	–	Penagini *et. al.*, 1986
	10-18	56	7HC	-13	-16	-3	–	–	–	Jenkins *et. al.*, 1980
	18	356	13HC	-13	-20	–	–	–	–	Simons *et. al.*, 1982
	15	84	14HC	-8	-14	0	–	–	–	Aro *et. al.*, 1984
ROLLED OATS	140	21	21N	-11	–	–	–	–	–	deGroot *et. al.*, 1963
	125	21	10N	-8	–	0	+35	0	+48	Judd & Truswell, 1981
OAT BRAN	50	42	12N	-11	–	–	–	–	–	Storch *et. al.*, 1984
	94(14)[6]	10	8HC	-18	-14	0	+54	-18	–	Kirby *et. al.*, 1981
	98(14.5)	21	10HC	-19	-23	-6	+40	0	–	Anderson *et. al.*, 1984

[1]N = Normolipidemic; HC = Hypercholesterolemic
[2]TC = Total Cholesterol; LDL-C = Low Density Lipoprotein Cholesterol; HDL-C = High Density Lipoprotein Cholesterol
[3]B.A. = Bile Acids; N.S. = Neutral Steroids
[4] % Change from control
[5]Not Measured
[6]Amount of soluble fiber present in the oat bran supplement is indicated in ( ).

subjects. Other studies have reported reductions in serum cholesterol levels of 8% to 13% with the incorporation of 12 to 36 g of isolated pectin into the diets of normolipidemics (Jenkins et al., 1975 ; Durrington et al., 1976). However, in these studies, all participants followed self-selected diets supplemented with pectin and no information on dietary composition was reported.

The reduction in serum cholesterol may be explained by a concurrent increase in fecal fat and bile acid excretion. In a controlled metabolic study, Kay and Truswell (1977) fed 15 g of pectin to nine healthy subjects for a twenty-one day period, preceded and followed by fourteen day basal diet periods. The basal diet contained 30% of total energy as fat and 425 mg/day of cholesterol. Serum cholesterol levels decreased significantly ($p<0.001$) by an average of 13% (range 5% to 26%). Plasma triglycerides did not change. Fecal fat excretion increased significantly ($p<0.001$) by 44%. Daily excretion of the neutral sterols, cholesterol and coprostanol, increased by 17% ($p<0.001$) and bile acid excretion increased by 33% ($p<0.02$).

Fecal steroid excretion in males and females was also studied in relation to foods containing pectin and isolated commercial pectin supplements (Stasse-Wolthius et al., 1980). The same amount of pectin was fed either as that found naturally in apples and cooked vegetables (potatoes, green cabbage, beans, carrots, endive, beetroot, and tomatoes) or as commercial pectin. Two groups of fifteen healthy subjects followed controlled diets for five weeks. Diets contained either 7.5 g of pectin from the fruits and vegetables (NP) or 9 g of commercial pectin (CP). The diets comprised 19% of energy from saturated fatty

acids (SFA); 12% of energy from monounsaturated fats (MUFA); 3% of energy from polyunsaturated fats (PUFA) and approximately 375 mg of cholesterol. Serum cholesterol concentrations for both males and females were reduced 4% ($p<0.02$) with the NP and 7% to 10% ($p<0.01$) with the CP diets after the first 2 weeks. When the NP diet was consumed, cholesterol levels began to increase at week three and were no longer significant at week five. Cholesterol levels remained significantly ($p<0.01$) below baseline at week five when the CP diet was ingested. HDL-C levels did not change during either diet treatment. In the male subjects, fecal neutral steroid excretion increased 50% ($p<0.01$) during the NP dietary period and 18% ($p<0.05$) during the CP dietary period. The CP treatment resulted in a 51% ($p<0.05$) increase in fecal bile acid excretion whereas the NP treatment had minimal effects. No changes in fecal steroid excretion were noted in the female subjects. Fecal fat excretion increased by 75% ($p<0.01$) during the CP treatment but did not increase significantly during the NP diet.

Fecal neutral and acid steroid excretion associated with pectin supplemention were also reported by Ross and Leklem (1981). A 15 g pectin supplement was added to a low fiber, basal diet consisting of 33% of energy as fat, 260 mg of cholesterol and 4.79 g of dietary fiber. Eight male subjects began one of two dietary periods. Each diet was fed for eighteen days. The diets were fed in a crossover design as pectin-nonpectin-pectin or nonpectin-pectin-nonpectin preceded by a four day control period. Fecal fat output was inconsistent in both feeding patterns. Total neutral steroid excretion was not significantly different between the pectin diet and the basal diet. Subjects fed the

pectin diet had a mean excretion of bile acids of 454 mg/day, which was 6% higher than when the basal diet was consumed. This result was not significant nor were significant time effects revealed, indicating that dietary sequence was not a factor in the results. Although these subjects consumed the same amount of pectin as fed by Kay and Truswell (1977), fecal acidic steroid and fat excretion was not similar. This may be due to the variability in the fecal outputs of the participants in the study by Ross and Leklem (1981).

Research has also been focused on the hypocholesterolemic effects of pectin in hyperlipidemic subjects. Generally, the results have been similar to those of normolipidemics as shown in Table 2. Seven hypercholesterolemic and two healthy male subjects, 33 to 60 years of age, consumed a control diet for two weeks followed by the same diet supplemented with 40 to 50 g of pectin for a further two weeks (Miettinen and Tarpila, 1977). The results observed were similar to those of Kay and Truswell (1977). Serum cholesterol levels decreased an average of 13% ($p<0.02$) while triglyceride concentrations remained at baseline. Excretion of fecal bile acids increased in both groups by 57% ($p<0.005$) and neutral steroid excretion increased by 10% ($p<0.05$) due primarily to an increased cholesterol excretion of 27% ($p<0.02$). Fecal fat increased in seven of the nine subjects, but was not significant. The changes observed in all parameters studied were greater for the hypercholesterolemic patients than for the healthy volunteers. However, the results are confounded by the heterogeniety of the subjects. The greatest reductions in serum cholesterol were noted in the older, hyperlipidemic patients. Keys and coworkers (1950) have reported that serum choles-

terol levels tend to increase with age. As well, the basal diet that was fed is described as a standardized low cholesterol (120 mg/2400 kilocalories), solid food diet. The low cholesterol content may be responsible, in part, for the results noted. As well, information is not included on the amount or type of fat nor on the total energy intakes which may have been reduced due to the large amount of pectin ingested.

The results of Meittinen and Tarpila (1977) were confirmed however, in a study by Nakamura and co-workers (1982). Twelve institutionalized male subjects with mild hypercholesterolemia consumed 9 g of pectin added to their normal diets for two weeks. Total serum cholesterol concentrations were reduced 10%, while HDL-C and triglycerides remained unchanged. Fecal excretion was measured in two of the subjects. Bile acid and neutral steroids increased 9% and 8%, respectively. The conclusions are limited by the lack of dietary control. As well, no statistical analyses were performed on the data.

1.4.2.2 Guar Gum

Guar gum supplements have been shown to be equivalent to pectin in hypocholesterolemic properties in normolipidemics (Table 2). Six g of guar gum, fed to seventy institutionalized male subjects for sixty-six days resulted in a 12 mg/dL reduction in serum cholesterol concentrations (Fahrenbach et al., 1965). In contrast, a 6 g pectin supplement did not have any effect in the same study.

Seventeen g of guar gum fed to three male subjects for three weeks reduced serum cholesterol levels 12% ($p<0.01$) (Jenkins et al., 1979). No changes were noted in serum HDL-C or triglycerids levels. A basal diet, consisting of 35% of total energy as fat, was fed for three weeks preceding the addition of the guar supplement. The basal diet contained 22 g of fiber from unspecified sources which may have contributed to reductions in serum cholesterol concentrations. As well, no information is given on the amount of cholesterol in the diets. The small number of subjects who participated makes it diffucult to draw conclusions from this study.

A double-blind trial studied the effects of a 9 g supplement of guar gum compared to a placebo on blood lipids of twenty-four volunteers (Khan et al., 1981). Guar gum was added to the self-selected diets of the subjects for four weeks. Serum cholesterol and LDL-C levels decreased significantly, 16% ($p<0.05$) and 25.6% ($p<0.05$), respectively. These decreases were greater during the first two weeks. Triglycerides, VLDL-C, and HDL-C levels did not change.

A recent study reported that 5.7 g of guar lowered serum cholesterol levels 16% ($p<0.05$) in six healthy male volunteers (Penagini et al., 1986). Triglycerides did not change. A control diet was consumed for two weeks followed by a two week "free" period. The treatment period of two weeks was then followed. The basal diet contained an apple and 100 g of carrots, both good sources of pectin, as well as 30 ml of olive oil, which may have contributed to the lipid-lowering effects observed due to the presence of oleic acid (Grundy, 1986).

Like pectin, guar gum has also been shown to be effective in reducing blood lipid levels to various degrees in hyperlipidemics (Table 2). Seven hypercholesteremic patients consumed controlled diets to which an average of 13 g of hydrated guar was incorporated into a crispbread for eight weeks (Jenkins et al., 1980). Serum cholesterol and LDL-C levels decreased significantly ($p<0.002$) by 13% and 16%, respectively. The greatest decreases were evident during the initial two and three weeks of supplemention. There were no changes in serum HDL-C levels. Triglyceride levels decreased 13%, although the reductions were not significant. The decrements in triglycerides noted may be partly due to changes in body weights (Olefsky et al., 1974), which decreased significantly ($p<0.02$) by 1.5 kg during the study.

Thirteen hypercholesterolemic subjects, who had followed a low fat, low cholesterol diet for a minimum of one year ingested a supplement of 18 g of guar gum for one year (Simons et al., 1982). After an initial three months, serum cholesterol levels had decreased 15% ($p<0.01$) and remained significantly lower for the duration of the study. Serum LDL-C levels decreased 20% ($p<0.01$) during the first three months and remained at this level for the year. No changes occurred in triglyceride levels. The observed changes may have been due to a combination of the lipid-lowering diets and the guar supplementation.

In a similar study, serum cholesterol levels were reduced 8% ($p<0.001$) and LDL-C levels 14% ($p<0.001$) when a 15 g guar supplement was fed to fourteen hyperlipidemic males for twelve weeks (Aro et al., 1984). Triglycerides, VLDL-C and HDL-C levels remained unchanged. However, all blood lipids were also significantly reduced during a four

week control period which preceded the test diet. This observation suggests that other dietary constituents, such as fat, cholesterol or energy intake, may have also been altered during the study.

Chemically isolated pectin and guar gum may have pharmacological uses as hypocholesterolemic agents in disease-states. However, their practicality for normolipidemics is questionable as pectin is present in natural foods in small concentrations and guar, obtained from the cluster bean, is not commonly consumed in the diet. Another source of soluble fiber in the Canadian diet is provided by cereals, rather than pectin. Oats and barley contain a 'gummy' water soluble fiber known as ß-glucan which has been investigated with respect to hypocholesterolemic properties in animals and humans.

1.5 ß-GLUCAN

The water soluble polysaccharide (1-3)(1-4)-ß-D-glucan has been identified in a number of tissues in the plant family Gramineae, including barley (Hordeum vulgare) and in whole and dehulled oats (Avena sativa). The structure of oat and barley ß-glucan is shown in Figure 1. Because of its importance in the brewing industry, the ß-glucans in barley have been a focus of research. Barley kernels contain 7-10% ß-glucan by weight and oats approximately 2-7% (Wood, 1984). Oat and barley ß-glucan, found primarily in endosperm cell walls, is a linear molecule consisting of unbranched ß-(1-4) and (1-3)-glucopyranosyl units in the proportion of approximately 7:3 (Wood, 1984). Because of the mixture of glucosyl bonds, oat and barley ß-glucans are loosely folded and form gelatinous, highly viscous solutions upon hydration.

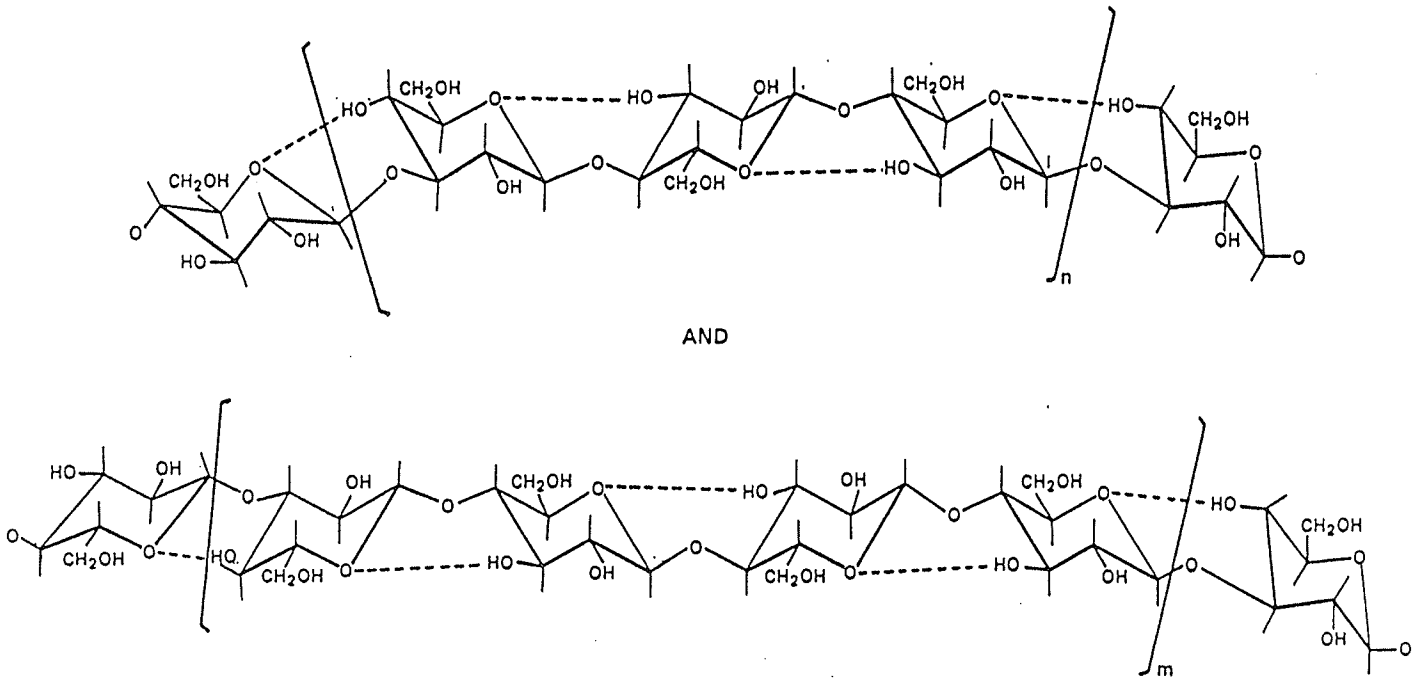

Figure 1: The Structure of Cereal ß-Glucan

Source: Wood, P.J. 1984. Physicochemical Properties and Technological and Nutritional Significance of Cereal ß-glucans. In Cereal Polysaccharides in Technology and Nutrition. ed.Rasper, V.F. Amer. Assc. Cereal Chem.,Inc., St. Paul, Minnesota. pp. 35-78.

Barley ß-glucan accounts for approximately 75% of the endosperm cell wall where it forms a matrix with pentosans (20%), starch (1-2%), and protein (5%) (Forrest and Wainwright, 1977). Limited information is available about the chemical and physical properties of oat endosperm ß-glucan. However, Chen and Anderson (1980) reported that oat bran contains 14.8% water soluble fiber which is primarily in the gum component and contains approximately 66% ß-glucan.

Depending on the culitivar and growth conditions, between 30-60% of the total ß-glucan in barley is soluble in water at 65°C (Anderson et al., 1978). There are varying reports concerning the molecular weight of ß-glucan. Using water extraction, Forrest and Wainwright (1977) found that ß-glucan had a molecular weight of approximately $4x10^7$ Daltons, which is in agreement with values reported by Bourne and Pierce (1970) who used a gel filtration system. A number of enzymes are required for complete hydrolysis of ß-glucan to glucose (Prentice et al., 1980).

Oat and barley ß-glucan is selectively stained by the dye Congo Red and by Calfluor, a florescent whitening agent (Wood, 1984). Staining is essentially due to the ability of the glucans to adopt configurations in which surfaces are present along the chain that are favorable to dye adsorption. This high affinity for aromatic dyes suggests that ß-glucan may interact in a similar fashion with metabolites such as bile acids (Wood, 1984).

Oats and barley appear to be unique with respect to the large amounts of ß-glucan in the grain. Wheat kernels, for example, contain only 0.5

to 1% ß-glucan (Beresford and Stone, 1983). Only 15% of ß-glucan is present in wheat endosperm walls (Mares and Stone, 1973) and the majority is in the bran fraction (Wood, 1984). This suggests that ß-glucan may be a storage or reserve polysaccaride rather than a required structural component (Fincher, 1975).

1.5.1 Animal Studies

ß-glucans are both desirable and undesirable cereal components. The presence of ß-glucan in animal feed decreases the feed efficiency of barley and thus limits its use (Classen et al., 1985). Oat bran and barley have been reported to be hypocholesterolemic in the rat and chicken, essentially due to ß-glucan. However, studies in animal models have utilized primarily oat bran ß-glucan.

1.5.1.1 Rats

In an early study, serum cholesterol levels were reduced to approximately 45% of control values when rolled oats were substituted for wheat starch at a level of 25% of energy in rat diets. The oats were added to a hypercholesterolemic diet containing 1% cholesterol, 0.2% cholic acid and 15% hydrogenated fat (deGroot et al., 1963). However, the rolled oat supplement contained approximately 2.5% linoleic acid (Bruins et al., 1965) which was not present in the wheat diet and may have contributed to the cholesterol-lowering activity observed.

The hypocholesterolemic effects of oat bran appears to be of less significance in the rat than either of the water soluble fibers, pectin

or guar gum. Ten percent pectin or guar gum reduced serum cholesterol levels by 37% and 21%, respectively, when incorporated into a basal diet containing 1% cholesterol, 0.2% cholic acid and 6% fat (23% SFA, 27% MUFA, and 46% PUFA) (Chen and Anderson, 1979). Oat bran added at 36.5% by weight and providing 10% 'plant fiber', reduced serum cholesterol by 15%. HDL-C levels were increased by 31% (pectin), 72% (guar gum), and 39% (oat bran). Liver cholesterol levels were reduced by 74%, 55%, and 47%, respectively. It should be noted that in a later study, Chen and Anderson (1986) failed to demonstrate any changes in HDL-C when oat bran was fed to rats in a hypercholesterolemic diet.

In a similar study, diets containing 10% oat gum, by weight, resulted in a reduction in serum cholesterol levels of 41% ($p<0.05$) compared to a control diet which contained 10% cellulose (Chen et al., 1981). Plasma HDL-C levels were increased 43% ($p<0.05$) while triglycerides were reduced 49% ($p<0.05$). Liver total lipid and liver cholesterol decreased 51% and 74%, respectively. The oat gum proved to be as effective as pectin and guar gum in reducing plasma and liver cholesterol levels. Such observations are most likely due to ß-glucan which comprised 66% of the gums extracted from the oats.

ß-glucan in barley has not been fed to the same extent as oats as a hypocholesterolemic agent in the rat. However, deGroot and coworkers (1963) noted a 30% reduction in serum cholesterol levels when barley was substituted for 25% wheat starch.

1.5.1.2 Poultry

The cholesterol-lowering effects of whole ground oats and oat fractions have been reported in chicks fed diets containing 0.65% cholesterol (Fisher and Griminger, 1967). Serum cholesterol concentrations were significantly ($p<0.01$) decreased by 35% and 30% when diets contained 43% whole oats and 15% oat hulls, respectively. Diets containing 28% rolled oats resulted in a reduction of 24% in serum cholesterol while 27% oat oil did not significantly affect plasma cholesterol levels. Liver cholesterol and total lipid concentrations were reduced slightly with ground oats, oat hulls and rolled oats and increased slightly with oat oil. Excretion of fecal lipids was higher in chickens fed rolled oats compared to birds fed control diets. These results indicate that the hypocholesterolemic effects of oats do not appear to be due to high linoleate content, as the oat oil had no lipid lowering effects. Fatty acid analysis indicated that the oat oil contained 48% linoleate.

Bile acid excretion was postulated as the mechanism responsible for the hypocholesterolemic effects of oats in chicks fed a diet containing 0.6% cholesterol for 4 weeks (Chenoweth and Benninck, 1976). Compared to control animals who received corn starch diets, serum cholesterol levels were reduced 18% when defatted oats were consumed; 29% with ground rolled oats and 32% with defatted-defibered oats. In contrast, serum cholesterol levels were unchanged when defatted- defibered-degummed oats were fed. Increases in bile acid excretion were observed with corresponding reductions in serum cholesterol levels. These data indicate that the gum component of the oats, comprised primarily of

ß-glucan, appears to be responsible for the increase in fecal bile acid excretion and the concurrent reductions in serum cholesterol levels.

Barley ß-glucan appears to have similar hypocholesterolemic effects as oats and pectin in poultry. Serum cholesterol levels were reduced by 21% in chicks fed a diet containing 43% barley by weight, 1% cholesterol and 0.2% cholic acid for 2 weeks (Fisher and Griminger, 1967). In the same study, diets containing 43% oats and 3% pectin by weight resulted in reduced serum cholesterol levels of 23% and 29%, respectively.

When soluble fibers are fed, 'gels' may appear within the small intestine which may reduce lipid absorption and inhibit bile acid reabsorption thereby reducing serum cholesterol levels (Kay and Truswell, 1980). Research findings suggest that barley ß-glucan exhibits similar viscous properties in the gut as pectin and guar gum and may have hypocholesterolemic properties due to its effects on fat absorption.

In young chicks, dehulled barley depressed growth significantly ($p<0.01$) compared to corn when fed as 67.4% of a diet containing 16% soybean oil, for four weeks (Fry et al., 1958). The addition of an 8.7% tallow supplement to the barley ration gave the same growth results as those obtained on the unsupplemented corn ration.

Burnett (1966) observed poor digestion, reduced energy levels, and lower weight gains in chicks fed a basal diet containing barley for five days. The chicks excreted sticky feces which adhered to the metal cages and also exhibited stable, viscous intestinal contents. The addition of ß-glucanase alleviated the negative effects of the barley. Burnett attributed the results to the presence of ß-glucan in the barley.

Similar observations on growth and excreta were made in chicks fed 10% pectin in the same study. The addition of a pectozyme enzyme, which hydrolyzes pectin in the gut and reduces its gel-forming abilities, significantly ($p<0.05$) improved weight gains of the birds. These findings suggest that barley reduces fat absorption and impairs growth in poultry.

Dry matter content of excreta has been shown to be related to the viscosity of a barley sample (Gohl et al., 1978). As the viscosity of the barley fed to the chicks increased, excreta appeared more fluid and the consistency changed from particulate to pasty. Feces became more sticky as the dry matter decreased. These effects were noted several weeks after withdrawal of the barley diet.

Chicks fed a diet containing 74% barley exhibited greater intestinal viscosities of 9.3 ± 1.63[2] compared to 2.3 ± 0.13 ($p<0.05$) for birds fed a corn diet (White et.al, 1981). The addition of cellulase 4000, a ß-glucanase enzyme, significantly ($p<0.05$) improved the average weight gain by 20% in the chicks fed the barley diet. In the same study, the addition of isolated barley ß-glucan (10 g/kg) to a basal diet containing 57% ground corn significantly ($p<0.05$) increased the viscosity of the intestinal fluid in chicks fed for three days. This research indicates that isolated ß-glucan is capable of increasing intestinal fluid viscosity as was reported for barley diets.

Barley ß-glucan modifies the viscosity of the gut and fat excretion in animals. It must be emphasized, however, that the gastrointestinal tract of animals differs from that of humans. In rats and poultry, the

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[2] Standard Error

gastrointestinal tract is shorter and thus ß-glucan may have an impact on lipid and bile acid metabolism which may not be measureable in humans. However, ß-glucan has been shown to affect fat excretion as well as bile acid excretion in humans.

1.5.2 Human Studies

In normolipidemic humans, the hypocholesterolemic properties of ß-glucans have been studied using rolled oats and oat bran supplements. The results are summarized in Table 2.

The incorporation of 140 g of rolled oats into the diets of twenty-one male volunteers for a three week period resulted in a mean reduction of 11% in serum cholesterol levels (deGroot et al., 1963). Three hundred g of oat bread was substituted for regular bread in the self-selected diets of the subjects. However, the consumption of such large intakes of bread products may have changed the composition of the diet (Judd and Truswell, 1981), as food records and body weights are not reported. Normal fat or energy intakes of the subjects may have decreased with corresponding reductions in serum cholesterol levels.

In contrast, serum cholesterol levels were not significantly reduced when 125 g of rolled oats was incorporated into a basal diet fed to ten healthy volunteers for a twenty-one day period (Judd and Truswell, 1981). HDL-C and triglycerides did not change. Fecal fat excretion increased significantly ($p<0.005$) by 48% when the oats were consumed. Fecal bile acid excretion increased 35% ($p<0.005$) compared to the control wheat diet. The largest increase, 41%, occurred in deoxycholic

acid excretion. Neutral steroid excretion did not change. To compensate for larger amounts of polyunsaturated fats found in rolled oats, an artificial 'oat oil' was made to match the fatty acid composition of the oats and was added to the control diet. The authors attribute the observed changes in blood lipids and fecal excretion partly to the ß-glucan found in the gum of the rolled oats and to the lignin component present in whole oats. Lignin has been shown to lower plasma cholesterol levels in rats (Judd et al., 1976) but its effects in humans have been inconsistent (Kay, 1982). Unfortunately, an analysis of the fiber components of the rolled oat supplement was not performed and it is therefore difficult to speculate as to the specific type and amount of fiber that appears to be responsible for the observed effects.

A preliminary study by Anderson (1980) reported a reduction of 36% in serum LDL-C concentrations and an 82% increase in HDL-C levels when 100 g of oat bran was added to a basal diet for 10 days. However, the diet consumed contained 11% of energy as fat, 70% as carbohydrate and was cholesterol-free. A low fat, low cholesterol diet such as this would result in reductions in serum cholesterol. The subject size was small (n=4 males) and no details were given on their health status. The observed increases in HDL-C are inconsistent with previous reports quoted which have generally found no changes in this lipoprotein fraction with soluble fiber consumption.

The influence of oat bran intake on blood lipids has been assessed in free-living populations. The effects of the addition of 50 g of oat bran to the daily diets of twelve healthy subjects for six weeks was studied by Storch and co-workers in 1984. After a two week control

period, the volunteers were randomly assigned to each of the oat bran or wheat bran muffin diets for the treatment period of six weeks, followed by a seven week 'washout' period. Serum cholesterol levels were reduced significantly by 11% ($p<0.001$) from 185 ± 7 mg/dl with the control diet to 164 ± 7 mg/dl with the oat bran diet. Wheat bran had no effect on cholesterol levels. Serum triglycerides and body weights did not change significantly.

The additional hypocholesterolemic benefits of two types of oat products, oat bran and oatmeal, added to fat-modified diets of 208 healthy adults were investigated by Van Horn and co-workers (1986). The volunteers consumed diets containing 30 to 34% of total energy as fat and 250 mg/day of cholesterol for six weeks, after which they were divided into three groups. Groups 1 and 2 consumed 36 g of oat bran and 34 g of oatmeal, per day, respectively. The oat bran supplement contributed 5.6 g of soluble fiber and the oatmeal, 2.7 g. Group 3 served as the control and continued to follow the fat-modified diet without added soluble fiber. After consuming the low fat, low cholesterol diets for 6 weeks, serum cholesterol levels had decreased an average of 5.2% ($p<0.001$) from baseline for the entire group. At the end of twelve weeks, serum cholesterol levels were reduced a further 2.7% and 3.3% in groups 1 and 2, respectively, while group 3 experienced no further reductions. A comparison of initial values with those at week twelve indicated that the 3 groups had significantly ($p<0.02$) different reductions in serum cholesterol levels of 8.0%, 9.3% and 4.5% for groups 1, 2, and 3, respectively. Although no data is provided, the authors report that LDL-C, triglycerides, VLDL-C, and HDL-C levels did not

differ between the participants consuming the oat products or the control diet. Reported dietary composition was similar between the groups during the 12 weeks. The results of this study indicated that oat bran and oatmeal enhanced the serum cholesterol lowering effect of a fat-modified diet to approximately the same degree. The reduction in serum cholesterol of control group subjects may reflect the decrease in fat and cholesterol intakes. This effect would supplement the hypocholesterolemic affects of the oats.

Oat bran has been shown to exhibit similar cholesterol lowering properties in hyperlipidemics as have been observed in normolipidemic subjects (Table 2). Ninety-four g of oat bran, containing approximately 14 g of soluble fiber, reduced serum cholesterol levels by 18% ($p<0.01$) in a group of subjects consisting of six hyperlipidemics and two normolipidemic males (Kirby et al., 1981). Subjects consumed either the oat bran diet or a control diet in a randomized crossover design for 10 days. Two patients continued the oat bran diet for a total of 14 days and experienced a further reduction of 8% in serum cholesterol levels. A reduction in LDL-C concentrations of 14% ($p<0.05$) was reported. In contrast to earlier observations by Anderson (1980), there were no changes in HDL-C levels. Triglycerides decreased 7% with oat bran and 5% with the controls diets, partly due to reductions in body weights of 1.5% and 1.9% during each respective diet period. Total bile acid excretion increased significantly by 54% ($p<0.001$) when the oat bran diet was consumed due primarily to increases in the excretion of cholic and chenodeoxycholic acids. Daily fecal cholesterol excretion was significantly ($p<0.025$) lower for the oat bran diet. Fecal fat was not

quantitated. The control and oat bran diets were similar in fat, insoluble fiber, and energy composition and therefore observed alterations in lipid metabolism appear to be due to the inclusion of the soluble fiber, which is primarily ß-glucan, found in the oat bran.

In a later study by the same researchers, 98 g of oat bran, containing 14.5 g of soluble fiber, reduced serum cholesterol levels 19% ($p<0.0005$) when incorporated into a basal diet and consumed for 21 days (Anderson et al., 1984). Concentrations of LDL-C and HDL-C decreased 23% ($p<0.0025$) and 5.6%, respectively. The diets contained 37% of total energy as fat and 436 mg/day of cholesterol. The subjects were ten hypercholesterolemic males, eight of whom were in excess of 10% of ideal body weight. Seven of the ten subjects lost more than 1 kg of body weight during the oat bran period which may have contributed to the significant ($p<0.025$) reductions in triglycerides that were noted. Total fecal bile acid excretion increased by 40% with the oat bran diet while no differences occurred in neutral steroid excretion. No data was reported for fat excretion. Although, Anderson and coworkers (1984) attribute the hypocholesterolemic effects of oat bran supplements to the ß-glucans, the control diet contained 6 g of undefined soluble fiber. This may have enhanced the hypocholesterolemic effects of the oat supplement to result in a 23% reduction in serum cholesterol.

Studies in animals and humans show that ß-glucan reduces serum cholesterol levels as do pectin and guar gum. The effects of soluble fiber on blood lipids and fat and bile acid excretion appear to be similar in normolipidemics and hyperlipidemics. Several hypotheses have been suggested to explain the hypocholesterolemic properties of these

fibers. Many investigators attribute these activities to the ability of soluble fibers to reduce fat and cholesterol absorption and bile acid reabsorption in the gut.

It appears that soluble fibers can significantly lower serum cholesterol concentrations. However, the responsible mechanisms remain inadequately defined. The literature is incomplete with respect to the response of fecal bile acids and fecal fat to soluble fiber supplements. As well, there is little information available concerning the effects of soluble fibers on lipoprotein fractions and triglycerides. However, based on a limited number of studies, it appears that soluble fibers increase fecal bile acids and fat excretion. Neutral steroid excretion, in most cases, remains unchanged. Soluble fibers appear also to decrease LDL-C in similar proportions as total cholesterol levels. No changes in HDL-C levels have been noted. In the majority of reports, VLDL-C has not been measured. A decrease in triglycerides has been identified only in hypercholesterolemic subjects where significant reductions in body weights have also been observed.

Investigations that have implicated soluble fiber sources as hypocholesterolemic agents have involved pharmacological amounts of commercially isolated pectin and guar gum. Although their use in certain disease states is promising, these sources have limited application in free-living populations. The amounts of soluble fiber supplements that have been utilized would, in most cases, be difficult to reproduce in the average Canadian diet. The inconsistent results found in studies utilizing fiber containing foods and in those using isolated fibers indicates the problem in clarifying the role of soluble fiber as it

exists naturally in the diet. It is not known conclusively whether a small but realistic amount of soluble fiber, derived from a natural food, would have any effect on serum cholesterol levels or bile acid excretion.

Many of the experimental diets utilized in studies on soluble fibers have included unidentified sources of fibers which may have enhanced lipid lowering effects. Also, dietary constituents known to affect blood lipids, such as cholesterol and composition of dietary fat, have not been properly monitored or controlled in many diets. Thus, it has not always been possible to attribute alterations in blood lipids to soluble fiber intakes. It is , therefore, desirable that all dietary intakes in the control and test periods are the same except for the fiber source being studied. As well, in many of the studies, subjects have not acted as their own controls and thus inter-subject variability may be high and may bias the results. There are no published reports that have examined the effects of barley ß-glucan on serum lipids and bile acid excretion in humans.

The objective of the research project reported here was to investigate the effects of the water soluble fiber, barley ß-glucan, on serum lipids and fecal bile acid excretion in healthly normolipidemic young men. The test diet contained one source of soluble dietary fiber, ß-glucan. Both the test diet and the control diet, which excluded ß-glucan, were low in other sources of soluble fiber as well as insoluble forms of fiber. Both diets also contained a constant amount of fat, approximately 36% of total energy, and cholesterol. Protein and carbohydrates were similarily controlled. Both fecal acidic sterols and

lipids were measured. Serum cholesterol, triglycercides, LDL-C, HDL-C, and VLDL-C were also quantitated. The aim of the present study was to ascertain whether ß-glucans, found naturally in barley flour, have the potential to favorably alter cholesterol metabolism in normolipidemics. The data collected from this study will help to further define the effects of soluble fibers on acidic sterol and serum lipid metabolism in man.

Chapter II

METHODS AND MATERIALS

2.1 EXPERIMENTAL DESIGN AND STATISTICAL ANALYSIS

The experimental design is illustrated in Figure 2. The 30 day metabolic study was divided into two-fifteen day periods; the Wheat Diet (WD) and the Barley Diet (BD) periods. Each subject consumed the WD and the BD in a crossover design in order to reduce inter-subject variability and to overcome the effect of diet order. Subjects were assigned randomly to the diets. Four subjects were assigned to the WD treatment for the first 15 days and then the BD treatment for the remaining 15 days; while four other subjects were assigned to the diets in the reverse order. One subject, SS, left the study midway through the first dietary period due to illness. This subject was replaced by subject AL, who started the study during the second dietary period (BD) and continued to the WD for an additional 15 days.

Data were analyzed by univariate and multivariate analysis using the General Linear Model of the Statistical Analysis System (SAS) (1983). Analysis of variance for a split-plot design (Steel and Torrie, 1980) was performed to identify any effects of diet treatment, person within sequence variability, dietary sequence, and sequence and diet interaction. Evaluation of fecal bile acids, lipids, and total fecal output were performed on the differences between values for each subject for

SUBJECT	DIET PERIOD I (15 Days)	DIET PERIOD II (15 Days)
	BARLEY	WHEAT
BI SM MM GW AL		
	WHEAT	BARLEY
HP RW BK SS	(Discontinued)	

FIGURE 2 **Distribution of Subjects in Crossover Experimental Design**

both diets. Evaluation of serum cholesterol, triglycerides, LDL-C, HDL-C, and VLDL-C were determined on the differences between values for each subject for each diet treatment period corrected by initial values. The mean differences for each group of subjects assigned a given dietary sequence were also analyzed for each of the fecal and serum variables using SAS. Paired two-tailed Student's t-tests were used to analyze differences in serum lipids between baseline values and each of the dietary periods, BD and WD. Differences between treatment means for sequence and for diets were compared using Scheffe's Multiple Comparison test statistics.

2.2 SUBJECTS

The eight male subjects were healthy normolipidemic University students, ages 18 to 36 years (mean age 25 years). They were selected from approximately twenty volunteers who responded to notices posted at various locations on the University campus. The experimental protocol was carefully explained to each subject and written consent was obtained after a personal interview was conducted. A physician at the Student Health Center conducted physical examinations and screened the subjects for cardiovascular disease and diabetes. All subjects were within normal weight ranges. None of the subjects ingested lipid-altering drugs or other medications.

All subjects were normolipidemic. The initial mean serum cholesterol (± SD) for all subjects was 174 ± 20 mg/dL; the initial mean serum triglyceride was 83 ± 21 mg/dL; the initial mean serum LDL-C was 118 ± 24 mg/dL; the initial mean serum HDL-C and VLDL-C were 45 ± 10 mg/dL and

12 ± 8 mg/dL, respectively. Individual and mean serum lipid values are shown in Tables 10 through 14.

All subjects resided in their own homes during the study and maintained their normal activity. SM and RW participated in a number of athletic activities while the other subjects were basically sedentary throughout the study. All subjects, with the exception of SS, maintained good health during the duration of the two feeding trials. Table 3 indicates the age and weight changes of the subjects throughout the study.

2.3 DIETS

2.3.1 Description

Two experimental diets were designed which were similar except for the inclusion of plant fiber. The source of water-soluble fiber, ß-glucan, was barley endosperm flour. Wheat pastry flour in the control diet contained negligible amounts of ß-glucan and was substituted for the barley flour. The diets were designated the Barley Diet (BD) and the Wheat Diet (WD). The menus are shown in Appendix A. The diets consisted of natural foods and were designed to meet the average daily Recommended Nutrient Intakes (1983) for Canadian males, 19 to 35 years of age.

The mean energy intakes from fat and carbohydrate for the two diets in this study were 36% and 50%, respectively. These figures are slightly different from the average nutrient intakes of Canadian males who, according to the Nutrition Canada Survey carried out between 1970

and 1972, consume approximately 42% and 43% of total energy from fat and carbohydrate, respectively (Nutrition Canada, 1977). The higher carbohydrate and lower fat intakes in the present research are due to above average amounts of flour incorporated into the diets. Protein contributed approximately 15% of the total energy intake which is equivalent to the amount reported to be consumed, on average, by Canadian males (Nutrition Canada , 1977).

Carbohydrate energy was equally divided between cereal and non-cereal components. Both diets contained 25% of total energy from Tupper hull-less barley endosperm flour or wheat pastry endosperm flour which were incorporated into bread products. Barley biscuits were formulated from 236.5 g of barley flour which provided approximately 8.75 g of ß-glucan daily. Barley flour was prepared by Dave Martin of the Grain Research Laboratory (Winnipeg, Manitoba). Similar biscuits, prepared with 120 g of wheat pastry flour, plus six slices of white bread, were included in the WD. The bread products were served at meals and throughout the day as snacks during each diet period. The remaining 25% of total energy from carbohydrate included cereals, canned fruits, fresh and canned vegetables, and milk. Fruits and vegetables chosen for the diets contained negligible amounts of the water-soluble fiber, pectin.

Insoluble fiber was approximately equivalent in the two diets. In order to compensate for the amount of insoluble fiber found in 236.5 g of barley flour, 3.8 g of wheat bran which contained 1.65 g of insoluble fiber (Handbook #8, 1963) was incorporated into the WD. Fiber data for barley were based upon analysis by Bhatty (1986) for flour of 50% extraction. The WD contained a total of 9 g of crude fiber. The barley

TABLE 3

AGE AND WEIGHT CHANGES OF SUBJECTS
DURING DIETARY PERIODS

Subject	Age	Initial Weight (kg)	BARLEY DIET PERIOD		WHEAT DIET PERIOD	
			Mean Weight[1] (kg)	Mean Change[2] in Weight (kg)	Mean Weight (kg)	Mean Change in Weight (kg)
BI	31	60.9	61.7 ± 0.6	+ 0.4	61.8 ± 0.4	+ 0.1
SM	24	80.5	80.0 ± 0.6	- 0.2	79.0 ± 0.6	+ 0.1
MM	24	64.7	64.9 ± 0.2	- 0.2	65.2 ± 0.5	+ 0.1
GW	18	75.7	76.0 ± 0.5	+ 0.3	75.3 ± 0.6	- 0.1
AL	23	54.9	55.5 ± 0.4	+ 0.1	55.9 ± 0.4	- 1.0
HP	27	81.8	80.9 ± 0.5	- 0.1	81.1 ± 0.4	- 0.1
RW	19	73.2	73.0 ± 0.5	0.0	72.7 ± 0.5	- 0.1
BK	36	82.3	82.1 ± 0.2	0.0	82.1 ± 0.3	0.0

[1]Mean ± SD for daily weighings.

[2]Mean change between daily weighings.

flour contained 6 g of crude dietary fiber plus approximately 8.75 g of soluble ß-glucan, for a total of 14.75 g of dietary fiber. These fiber intakes compare well with the average dietary fiber consumption of Canadians which was reported to be 14.6 ± 9.8[3] g/day (Nutrition Canada, 1977).

Protein from plant and animal sources comprised approximately 15% of total energy. Lean ground beef provided 38% of the protein, while approximately 25% was supplied by bread products. Other sources of protein included peanut butter and milk, and to a lesser extent, fruits and vegetables.

The fatty acid composition of the diets was similar (Table 4). Fats contributed approximately 35% of total energy. Total fat was analzyed to be 120.5 g and 120.2 g for the BD and WD, respectively. A commercial corn oil margarine comprised approximately 22% of the total fat on both diets. The barley biscuits contributed 36% of the total fat in the BD while the wheat products contributed 30% of the total fat in the WD. The analyzed fat content of the ground beef was 9.3% by weight and contributed 17% of the total fat on both diets. Peanut butter and 2% fat milk contributed 21% and 6%, respectively, of the total fat during the BD and WD periods.

The diets were supplemented with one vitamin and mineral capsule daily[4] in order that the recommendations of the R.N.I.(1983) for Canadian males, 19 to 35 years, of age were met.

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[3] Standard Deviation

[4] Multivitamin and Mineral Tablets, Westcan Pharmaceuticals, Winnipeg, Manitoba.

Polyethylene glycol,[5] a non-toxic, non-absorbable substance, served as an internal marker of intestinal transit. Subjects received three capsules, each containing 100 mg of PEG, daily five days prior to and for the first three days during the fecal collection period.

All food supplies were purchased in bulk, with the exception of milk, tomatoes and lettuce, in order to minimize variability. Barley and wheat biscuits were prepared in advance using a standardized recipe and frozen in daily serving size portions. Likewise, beef patties were weighed, individually packaged and frozen prior to the commencement of the study. Foods were weighed on a Sartorius balance or measured prior to each meal. Foods were prepared, heated and served in the same dishes to minimize losses. Limited amounts, 15 grams of ketchup and 45 grams of diet jam, as well as spices, such as onion flakes and chili powder, were allowed to improve the palatability of certain menu items. Clear tea, coffee, and calorie-free carbonated beverages were allowed ad libitum. All foods provided during both dietary treatments were consumed by the subjects and tolerated well.

The director of the study supervised all food consumed by the subjects. Meals were prepared and eaten in the Metabolic Laboratory. Between-meal snacks were packaged. As a convenience to subjects, breakfasts and some weekend meals were pre-packaged in reheatable containers for consumption at home.

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5 PEG 3350, Fisher Scientific Co., Ltd., N.J.

Individual body weights were maintained by adjusting energy intake from carbohydrate and fat sources in the proportions of 50% and 36% of total energy in the diet. The adjustments did not significantly affect the nutrient density of the diets for any individual. Mean daily energy, protein, carbohydrate, and fat intakes of the subjects for both treatments are shown in Appendix B.

2.3.2 Analysis of Diets

Fatty acid analysis for both diets is shown in Table 4. Diets were analysed for total fat and total ß-glucan content as indicated in Table 5. Energy was estimated from tables of food composition (Handbook #8, U.S.D.A., 1963).

Aliquots representing one-fifth of each diet were weighed using a Sartorius top-loading balance (Model 2353).[6] Food items containing greater than one gram of fat per serving, as per tables of food composition (Handbook #8, U.S.D.A., 1963), were included in both samples. Each aliquot was homogenized with 200 ml glass distilled water in a one gallon Waring commercial blender (Model CB-5).[7] Weighed aliquots of homogenate were lypophilized in a Virtis Freeze Dryer (Model 10-145 MR-BA).[8] The lypophilized samples were ground, using a pestle and mortar, into a fine homogenous mixture and stored in plastic containers at -10 ^{0}C until required for analysis.

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[6] Sartorius-Werke AG, Gottingen, Germany.

[7] Waring Products Co., Winsted, Connecticut.

[8] Virtis Co., Inc., Gardiner, N.Y. 12525.

TABLE 4

FATTY ACID COMPOSITION OF DIETS[1]

Fatty Acid	Barley Diet	Wheat Diet
10:0[2]	tr[3]	tr
12:0	tr	tr
14:0	1.14	1.60
14:1	tr	tr
16:0	16.92	16.91
16:1	1.34	1.59
16:2	tr	tr
17:0	tr	tr
18:0	7.66	7.78
18:1	45.29	45.27
18:2	23.85	22.11
18:3	1.02	0.85
20:0	0.76	0.82
20:1	0.52	0.63
20:4	tr	tr
22:0	tr	tr
22:1	tr	tr

[1]Values expressed as % of total methyl esters. All values are the mean of duplicate analysis.

[2]Carbon #: number of double bonds.

[3]Assigned to any value less than 0.5%

TABLE 5

COMPOSITION OF BASAL DIETS

DIET	METHOD	ENERGY (KJ)	PROTEIN (g)	CEREAL CARBOHYDRATES (g)	OTHER CARBOHYDRATES (g)	β-GLUCAN (g)	FAT (g)	CHOLESTEROL (mg)
BARLEY	Calculation[1]	12770	108.5 (14.2)[3]	187.7 (25.0)	201.0 (26.3)	NC[4]	123.5 (36.4)	210.0
	Analysis[2]	ND[5]	ND	ND	ND	8.75 (1.2)	120.5 (35.5)	ND
WHEAT	Calculation	12602	116.1 (15.4)	189.0 (25.1)	198.6 (26.4)	NC	123.6 (36.9)	225.0
	Analysis	ND	ND	ND	ND	ND	120.2 (35.9)	ND

[1]Unless otherwise noted, energy, protein, carbohydrate and fat values calculated using U.S.D.A. Handbook #8, *Composition of foods* (Watt and Merrill, 1963). Energy, protein, and fat values for barley flour are based on those listed in U.S.D.A. Handbook #8, *Composition of Foods* (Watt & Merrill, 1963), for pastry flour. Protein value for barley flour is from Bhatty, 1986. Cholesterol values calculated from U.S.D.A. Handbook #8, *Composition of Foods* (Watt and Merrill, 1975).

[2]Mean value of triplicate analysis for β-glucan; values averaged within 3% of mean. Mean value of duplicate analysis for fat; values averaged within 3% of mean.

[3]% of total energy.

[4]NC = No calculations performed.

[5]ND = No analytical data available.

Total lipid was extracted from approximately four grams of freeze-dried food sample according to the method of Bligh and Dyer (1959) using a monophasic mixture of chloroform, methanol and water (1:2:0.8 v/v/v/). The chloroform layer was isolated overnight using separatory funnels, then evaporated and flushed thoroughly with nitrogen. Total lipid was determined gravimetrically using an electronic analytical top-loading balance (Mettler AE200)[9] following drying in a dessicator overnight.

An aliquot of the lipid, dissolved in hexane, was transferred to a screw-top vial, flushed with nitrogen and stored at -10 ^{0}C overnight prior to fatty acid analysis. The lipid extract was saponified and then esterified using a method adapted from Metcalfe et al. (1966). Two ml of 0.5 N methanolic NaOH, 2.5 ml of BF_3-methanol and 2.5 ml of hexane were used in this adaptation rather than 4.0 ml, 5.0 ml, and 20.0 ml of petroleum ether, respectively. Fatty acid methyl esters, dissolved in hexane, were analyzed with a Varian Aerogragh gas chromatograph (Model 1740-10)[10] containing dual flame ionizer detectors and a Hewlett-Packard recording integrator (Model 3380-S).[11] The chromatograph was fitted with dual stainless steel columns 6 feet long x 0.125 inch O.D. (medium wall thickness) packed with 10% EGSS-Y on 100/120 mesh GAS CHROM Q.[12] The columns were set isothermically at 190 ^{0}C with an injector temperature of 230 ^{0}C and a detector temperature of 250 ^{0}C. Gas flow rates were 25 ml/minute for hydrogen, 30 ml/minute for helium, and 250 ml/minute for air. Individual fatty acids were identified for each chromatograph by

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[9] Mettler Instruments Corp., Hightstown, N.J. 08520.

[10] Varian Aerograph, Malton, Ontario.

[11] Hewlett-Packard Ltd., Canada.

[12] Applied Science Laboratories, Inc., State College, Pa. 16801.

comparison of retention times to standardized fatty acid references.[13]

Total ß-glucan content of the barley flour was analyzed using a phenol-sulfuric procedure by the Plant Science Department, University of Manitoba.

2.4 FECAL SAMPLES

Fecal samples were collected during the last five consecutive days in each dietary period (days 11 to 15 and 26 to 30, respectively) and frozen at -10 °C until required for analysis. Frozen samples were composited, weighed using a Sartoruis top-loading balance (Model 2254)[14] and allowed to thaw overnight. Glass distilled water, approximately equal to the total weight of each fecal sample, was added; the samples were weighed and shaken, in a closed one litre paint can, on a paint-shaker for 30 minutes. Approximately 300 grams of homogenate were transferred to plastic containers, weighed, frozen and lypophilized in a Virtus Freeze Dryer (Model 10-145 MR-BA).[15] The freeze-dried samples were ground to a fine homogenous mixture using a Wiley Mill (#20 Mesh)[16] and stored in glass containers at -10 °C until required for analysis. Fecal materials were analyzed for total PEG, total bile acids, and total lipid concentrations.

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[13] Hormel Institute, Lipids Preparation Laboratory, Austin, Minnesota.

[14] Sartorius-Werke AG, Gottingnen, Germany.

[15] Virtus Co., Inc., Gardiner, N.Y. 12525.

[16] Arthur H. Thomas Co., Philadelphia, Pa.

2.5 POLYETHYLENE GLYCOL DETERMINATION

Polyethylene glycol (PEG) concentration for each sample was determined using a modification of the method of Hyden (1955). PEG was extracted, in triplicate, from approximately 300 mg of freeze- dried material following the addition of 20 ml of glass distilled water, subsequent heating and centrifugation (Model CPS)[17] for 20 minutes at 2500 rpm. Proteins were precipitated with 0.3 N barium hydroxide, 5% zinc sulfate and 10% barium chloride reagents and removed by centrifuging for 2-3 minutes followed by filtration through a Whatman #5 filter paper. Two ml of filtrate were pipetted into test tubes, in duplicate, to which 2 ml of 30% (w/v) TCA containing 5·9% $BaCl_2 \cdot 2H_2O$ was added. The concentration of PEG was measured by reading the absorbance at an optical density of 525 nm using a Model SP6-300 spectrophotometer[18] exactly 15 minutes after the addition of the TCA. Duplicate filtrate blanks were read for each sample without the addition of color reagent to correct for absorbance due to the fecal filtrate itself. PEG extraction, in mg/day, was derived from the following equation:

$$\text{Sample Absorbance} \times \frac{\text{Standard Concentration (mg/ml)}}{\text{Standard Absorbance (nm)}} \times 30 \times \frac{\text{24 hour Fecal Dry Weight (g)}}{\text{Fecal Sample Weight (g)}} = \text{PEG (mg/day)}$$

(where 30 refers to the dilution factor).

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[17] International Centrifuge, International Equipment, Co., Boston, Mass.

[18] Pye Unicam, Ltd., Cambridge, England.

2.6 BILE ACID DETERMINATION

2.6.1 Extraction of Bile Acids

Total bile acid concentration was determined following the principle of DeWael et al. (1977) but several modifications were made to obtain satisfactory results. Approximately 100 mg of a freeze-dried fecal sample was dissolved, in triplicate, in 1.0 ml of potassium hydroxide (KOH) in glycerol, rather than ethylene glycol, and heated for 15 minutes at 220 ^{0}C. Glycerol, unlike ethylene glycol, does not boil at 220 ^{0}C and therefore fecal material was less likely to adhere to the test tubes in this medium. After cooling and the addition of NaCl, 20 glass beads were placed in each test tube to improve mixing and ensure that the fecal material was finely dispersed. Samples were next acidified with concentrated HCl, followed by the extraction of the bile acids with four-6.0 ml aliquots of diethyl ether. After evaporation of the ether, the dry residue from each sample was dissolved in 2.0 ml of ethanol rather than in 1.0 ml of methanol. The dry residue from the fecal samples dissolved better in ethanol and yielded more consistent results during pre-testing than did those samples dissolved in methanol. Samples, in ethanol, were stored at -10 ^{0}C until required for enyzme analysis for bile acids.

2.6.2 Enzyme Determinations of Bile Acid Concentrations

100 ul (0.10 ml) of ethanol or 100 ul of cold lithocholic acid (5-ß-cholanic acid-3-α-ol) standard[19] was added to cuvettes containing solutions of 2 ml 0.10 M sodium pyrophosphate buffer (pH 9.4) and 0.50

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[19] Sigma Chemical Co., St. Louis, Mo. 63178

ml hydrazine hydrate. Likewise, 50 ul (0.050 ml) aliquots of extracted fecal samples were added to cuvettes containing the same solution plus 50 ul of ethanol. Unless otherwise specified, all solutions were at room temperature. Subsequently, 0.30 ml containing 0.45 I.U., of cold 3α- hydroxysteroid dehydrogenase, hydrated initially in 1.0 ml 0.01 M sodium phosphate (pH 7.2) and dissolved in 6.70 ml 0.10 M sodium pyrophosphate (pH 9.4), was added to each cuvette, mixed and allowed to sit for 10 minutes at room temperature. An initial absorbance measurement was taken using a UV/VIS spectrophotometer (Model SP8-400)[20] at an optical density of 340 nm against water. Following this reading, 100 ul (0.10 ml) of a 6.0 mM ß-NAD solution (10.0 mg ß-NAD in 2.0 ml sodium pyrophosphate) was added to each cuvette, mixed, and allowed to incubate for 45 minutes. The reaction that occurred involved the oxidation of the 3α- hydroxy group of the C-24 bile acid steroid molecule, catalyed by the enzyme 3α-hydroxysteroid dehydrogenase, in the presence of ß-NAD. The endproduct, NADH, was directly proportional to the concentration of bile acids containing the 3α-hydroxy group in the fecal sample. NADH was measured at the completion of the reaction after 45 minutes using a UV/VIS spectrophotometer at an optical density of 340 nm. Both conjugated and unconjugated bile acids are assayed enzymatically (Weber et al.,1972). The 3α-hydroxysteroid dehydrogenase preparation is specific for the 3α-hydroxy group which is possessed by greater than 90% of fecal bile acids (Ali et al.,1966). The bile acid concentration was derived using the following equation:

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[20] Pye Unicam Ltd., Cambridge, England

$$\text{nMoles} \times \frac{2.0 \text{ ml}}{0.05 \text{ ml}} \times \frac{\text{24 hour Fecal Dry Weight (g)}}{\text{Sample Weight (g)}} \times 1/1000$$

=μMoles of Bile Acids excreted/day

where:

nMoles are the number of bile acids derived from the standard curve.

2.0 ml is the volume of ethanol used to dissolve the sample.

0.05 ml is the volume of sample used in the enzyme assay.

Using this method, the recovery of standards[21] added to the samples before extraction were: deoxycholic acid (5-ß-cholanic acid-3α-12α-diol) 99.6 ± 5.1%[22] cholic acid (3α,7α, 12α-trihydroxy-5ß-cholan-24oic acid) 71.8 ± 4.2%; and lithocholic acid (5ß-cholanic acid-3α-ol) 84.9 ± 7.8%.

2.7 TOTAL LIPID EXTRACTION

Total lipid was extracted, in triplicate, from a 1.0 gram sample of lypophilized feces using a modification of the method of Sobel (1974). Initially, samples were acidified with concentrated HCL to ensure a consistent pH of 1.0 for all samples. The lipid was extracted into weighed tared Soxhlet flasks using an ethanol-petroleum ether solvent mixture (1:4 v/v), followed by two 20 ml aliquots of petroleum ether. Following evaporation of the solvents on a steam bath for approximately 30 minutes, the flasks were placed in a dessicator overnight and weighed the following morning. The lipid was determined by difference.

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[21] Sigma Chemical Co., St. Louis, Mo.63178

[22] Standard Deviation

2.8 BLOOD ANALYSIS

Venous blood samples were taken by a medical technologist after a 12 hour fast on day one of the study and at the conclusion of each dietary period. Approximately 10 ml of blood was drawn from each subject into Becton Dickinson vacutainer tubes (#6432). This blood was allowed to clot for 30 minutes at room temperature and then centrifuged (Model CS)[23] for approximately five minutes at 2000 rpm to precipitate the clot. Sera were pipetted into two screw-top vials, flushed with nitrogen and stored at -5 ^{0}C until required for analysis.

2.8.1 Total Cholesterol Determination

Sera for each subject taken on days 1, 16, and 31 were analyzed enzymatically for total cholesterol using an analytical kit prepared by Fisher Diagnostics (Reference DK-1225-26).[24] This kit is based upon a modification of the method of Allain et al. (1974). Free cholesterol, saponified from cholesterol esters by cholesterol- esterase, was oxidized in the presence of cholesterol-oxidase to yield cholesten-3-one and hydrogen peroxide. The hydrogen peroxide produced reacted with 4-aminoantipyrine and phenol, catalyzed by peroxidase, to yield a colored quinoneimine dye. The intensity of the color, which is directly proportional to the concentration of total cholesterol in the sample, was measured at an optical density of 505 nm on a spectrophotometer (Model SP6-300).[25]

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[23] IEC International Centrifuge, International Equipment Co., Boston, Massechusets.

[24] Fisher Scientific, Ottawa, Ontario.

[25] Pye Unicam Ltd., Cambridge, England.

The mean absorbance for duplicate measurements of total serum cholesterol concentration for each sample were derived by comparison with the absorbance of a cholesterol standard of known concentration.

2.8.2 Serum Lipoprotein Cholesterol Determination

The serum lipoproteins LDL-C, HDL-C and VLDL-C for each sample, were fractionated by ultracentrifugation (rotor type 40.3) at 18 ^{0}C and 34,000 rpm for 18 hours (Beckman L5-50B Ultracentrifuge)[26] based upon a modification of the method of Bronzert and Brewer (1977). A NaCl solution of density 1.0063 g/ml was used to separate VLDL (top layer) from the HDL and LDL (bottom layer) when ultracentrifugation as described above was performed on all samples. Similarily, a NaCl solution of density 1.0630 g/ml separated the VLDL and LDL (top layer) from the HDL (bottom layer) during ultracentrifugation. The bottom layers consisting of (a) HDL and LDL and (b) HDL were used for the enzyme assay to quantitate the cholesterol concentrations of the fractions. The method described for total serum cholesterol was employed to obtain a mean absorbance for duplicate measurements of the concentrations of LDL-C and HDL-C for each sample. VLDL-C was determined by difference. These values were then compared with the absorbance of a cholesterol standard of known concentration.

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[26] Beckman Instruments, Palo Alto, California 94304.

2.8.3 Triglyceride Determination

Serum triglyceride for each subject on days 1,16 and 31 were measured using an enzyme kit developed by Fisher Diagnostics (Reference DK-1210-75)[27] and based upon a modification of the methods described by Fossati and Lorenzo (1982) and McGowan et al. (1983). The procedure involved the hydrolysis of the triglycerides to glycerol and free fatty acids catalyzed by lipase and the subsequent conversion of glycerol to glycerol-1-phosphate in the presence of glycerol kinase. The glycerol-1-phosphate was then oxidized in the presence of glycerol-phosphate -oxidase to yield hydrogen peroxide. The hydrogen peroxide produced condenses with DHBS (3,5-dicloro-2-hydroxy-benzenesulfonic acid) and 4-aminoantipyrene, catalyzed by peroxidase to yield a red colored quinoneimine dye. The optical density was measured at 515 nm on a spectrophotometer (Model SP6-300).[28] The formation of the colored complex was directly proportional to the concentration of triglycerides in the sample. The mean absorbance for duplicate measurements of serum triglyceride concentrations for each sample was obtained by comparison with the absorbance of a triglyceride standard of known concentration.

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[27] Fisher Scientific, Ottawa, Ontario.

[28] Pye Unicam Ltd., Cambridge, England.

Chapter III

RESULTS

3.1 SUBJECTS

All subjects, with the exception of SS, completed the thirty day study and remained in good health. Individual body weights were recorded daily before breakfast. As Table 3 indicates, body weights were very stable throughout the study. During the BD period subjects experienced a mean increase of 0.04 kg in body weight, while a mean reduction of 0.13 kg occurred during the WD period. For all subjects, mean fluctuations in body weights were 1.0 kg or less throughout both dietary periods.

The mean daily energy, protein, carbohydrate and fat intakes of subjects during the WD and the BD periods is shown in Appendix B. Subjects reported greater feelings of satiety when the BD was ingested in comparison to the WD. One subject (AL) required slight reductions in energy intake during both dietary periods in order to maintain his weight. Energy intake for another subject (SM) was increased during the WD period in response to slight reductions in his weight and personal reports of hunger.

3.2 FECAL EXCRETION

3.2.1 Total Bile Acids

Fecal recovery of the marker, polyethylene glycol (PEG), was used to correct individual data for fecal bile acids and fecal lipids. Total PEG excretion and recovery for all subjects during both dietary treatments are indicated in Table 6. It can be noted that recovery was highly variable between subjects and less so within subjects consuming the different diets. Recoveries of PEG for subjects HP and BK were very low for both periods, less than 50.0%, while the remaining subjects had more acceptable recoveries of 78.0% and above.

Analysis of variance indicated that fecal bile acid excretion for all subjects, corrected by PEG recovery values, was not significantly ($p<0.05$) different between the BD and WD treatments (Appendix C Table 1). Individual and mean data for total bile acid excretion and PEG corrected values are found in Table 7. It can be noted that there was inconsistency and variability between individual subjects. Total fecal output and PEG recoveries were very low for subjects HP and BK resulting in large correction values. Hence, PEG recovery data for fecal bile acids were much higher for these two subjects than for the others. Mean fecal bile acid excretion for the BD and WD periods was 988 ± 126[29] μmoles/day and 1155 ± 230 μmoles/day, respectively. No significant ($p<0.05$) effects of variability due to sequence or diet were found by either analysis of variance or by Scheffe's tests for differences between treatment means. Inter-subject variability and sequence and diet interactions were not found to be significantly ($p<0.05$) different

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[29] Standard Error

TABLE 6

INDIVIDUAL AND MEAN TOTAL POLYETHYLENE GLYCOL (PEG) EXCRETION OF SUBJECTS ACCORDING TO DIETARY SEQUENCE.[1]

SUBJECT	DAY 16 BARLEY DIET		DAY 31 WHEAT DIET	
	Total Excretion	% Recovery	Total Excretion	% Recovery
BI	309.0	103.0	376.0	125.0
SM	303.0	101.0	386.0	129.0
MM	271.0	91.0	281.0	94.0
GW	267.0	89.0	233.0	78.0
AL	268.0	89.0	189.0	84.0
MEAN	284.0	95.0	293.0	102.0
± SE[2]	9.0	3.0	39.0	11.0
SUBJECT	DAY 16 WHEAT DIET		DAY 31 BARLEY DIET	
	Total Excretion	% Recovery	Total Excretion	% Recovery
HP	71.0	24.0	177.0	59.0
RW	238.0	79.0	177.0	92.0
BK	57.0	19.0	87.0	29.0
MEAN	122.0	41.0	147.0	60.0
± SE	58.0	19.0	30.0	18.0

[1]Values are the mean of triplicate analysis expressed in mg/day.

[2]Standard Error

between the two treatments.

Analysis of variance was also performed using the PEG corrected bile acid excretion data of subjects GW, SM, MM, AL, and RW, plus the uncorrected results of subjects HP and BK. It was hypothesized that the PEG corrected data from the latter two subjects may have skewed the statistical results and hidden possible significant relationships between parameters. Total bile acid excretion was found to differ significantly ($p<0.05$) between the two dietary periods as indicated by analysis of variance (Appendix C Table 2). However, this result was due almost entirely to a highly significant ($p<0.05$) difference found in the variability of the subjects. No significant ($p<0.05$) differences were noted between the two treatments for the effects of sequence or diet treatment by either the analysis of variance or by Scheffe's tests. As well, no significant ($p<0.05$) interaction was demonstrated between the sequence and diet by analysis of variance. Mean bile acid excretion for all subjects was reduced to 832 ± 148[30] μmoles/day when the BD was consumed and 755 ± 143 μmoles/day for the WD, when uncorrected values for subjects HP and BK were used in the analysis of the data. Therefore, the analysis of the data in this format indicated that the PEG corrected results for subjects HP and BK, although much higher than those of the other subjects, did not affect the statistical results of the data.

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[30] Standard Error

TABLE 7

INDIVIDUAL AND MEAN TOTAL FECAL BILE ACID EXCRETION OF SUBJECTS ACCORDING TO DIETARY SEQUENCE.[1]

SUBJECT	DAY 16 BARLEY DIET		DAY 31 WHEAT DIET	
	Total Excretion	PEG CORRECTED	Total Excretion	PEG CORRECTED
BI	532.0	512.0	653.0	522.0
SM	736.0	728.0	594.0	462.0
MM	1146.0	1266.0	993.0	1062.0
GW	1375.0	1545.0	1235.0	1591.0
AL	530.0	594.0	667.0	796.0
MEAN	864.0	929.0	828.0	887.0
± SE[2]	170.0	202.0	123.0	206.0
SUBJECT	**DAY 16 WHEAT DIET**		**DAY 31 BARLEY DIET**	
	Total Excretion	PEG CORRECTED	Total Excretion	PEG CORRECTED
HP	493.0	2081.0	579.0	979.0
RW	581.0	733.0	1004.0	1089.0
BK	379.0	1983.0	343.0	1189.0
MEAN	484.0	1599.0	642.0	1086.0
± SE	59.0	434.0	194.0	61.0

[1]Values are the mean of triplicate analysis expressed in μmoles/day.

[2]Standard Error

3.2.2 Total Fecal Lipid

Total fecal lipid excretion, corrected for PEG recovery values for all subjects, was not significantly ($p<0.05$) different when the BD or the WD was fed, as demonstrated by analysis of variance (Appendix C Table 3). Total lipid excretion and PEG corrected lipid values for all individuals are given in Table 8.

The corrected fecal lipid values were similar for all subjects, with the exception of subjects HP and BK. As was indicated with fecal bile acids, PEG recovery data for these two subjects are much higher than those of the other six subjects. The mean fecal lipid value for all subjects who consumed the BD was 4.1 ± 0.5[31] g/day compared to 4.9 ± 1.1 g/day for the WD. No significant ($p<0.05$) differences in fecal lipid excretion were found due to variability attributed to sequence or diet treatment, either by analysis of variance or by Scheffe's tests. Likewise, analysis of variance demonstrated no significant ($p<0.05$) differences due to inter-person variability or sequence and diet interaction between the two treatment periods.

Since the corrected values for subjects HP and BK were large, the data was also analyzed using the uncorrected data for these two subjects plus the PEG corrected data for the other six subjects. Analysis of variance of the data in this format showed no significant ($p<0.05$) differences between the two dietary periods for the effects of sequence, inter-person difference, diet or sequence and diet interactions (Appendix C Table 4). It therefore appeared that the PEG corrected data for the subjects HP and BK did not conceal possible differences in fecal

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[31] Standard Error

TABLE 8

INDIVIDUAL AND MEAN TOTAL FECAL LIPID EXCRETION OF SUBJECTS ACCORDING TO DIETARY SEQUENCE.[1]

SUBJECT	DAY 16 BARLEY DIET		DAY 31 WHEAT DIET	
	Total Excretion	PEG CORRECTED	Total Excretion	PEG CORRECTED
BI	2.9	2.8	3.3	2.6
SM	4.7	4.7	4.5	3.5
MM	3.0	3.4	5.1	5.4
GW	3.5	3.9	2.5	3.3
AL	4.0	4.5	3.5	4.1
MEAN	3.6	3.9	3.8	3.8
± SE[2]	0.3	0.4	0.5	0.5
SUBJECT	**DAY 16 WHEAT DIET**		**DAY 31 BARLEY DIET**	
	Total Excretion	PEG CORRECTED	Total Excretion	PEG CORRECTED
HP	1.5	6.5	1.2	2.0
RW	1.8	2.3	4.8	5.3
BK	2.2	11.5	1.8	6.2
MEAN	1.8	6.8	2.6	4.5
± SE	0.2	2.7	1.1	1.3

[1]Values are the mean of triplicate analysis expressed in g/day.

[2]Standard Error

lipid between the two dietary treatments.

Scheffe's Multiple Comparison tests also revealed no significant ($p<0.05$) differences between the means of respective dietary sequences or diet treatments. However, the mean fecal lipid output for all subjects was reduced to 3.4 ± 0.5 g/day when the BD was consumed and 3.1 ± 0.4 g/day with the WD, when the uncorrected data for subjects HP and BK was used.

Multivariate analysis was used to test for correlations between mean fecal fat and bile acids for both dietary treatments. No significant ($p<0.05$) relationships were found between these parameters due to variability attributable to sequence, dietary treatment or sequence and diet interaction.

3.2.3 Fecal Output

Total wet weight of feces is given for all subjects in Table 9. Total fecal output was not significantly ($p<0.05$) different between the two dietary periods as indicated by analysis of variance (Appendix C Table 5). Mean total fecal weights between the two dietary sequences were found to be significantly ($p<0.05$) different by analysis of variance and by a Scheffe's test. In sequence one, subjects BI, SM, MM, GW, and AL ingested the BD for the initial 15 day period, followed by the WD. The subjects who followed sequence two, consumed the diets in the opposite order. Mean total fecal wet weight for the five subjects following sequence one during both diets was 130 ± 8[32] g/day compared to 82 ± 14 g/day for subjects HP, RW, and BK, who followed sequence two.

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[32] Standard Error

However, no significant ($p<0.05$) difference was found between dietary treatments either by analysis of variance or by a Scheffe's test. Mean total fecal wet weight was 123 ± 14 g/day when the BD was fed compared to 101 ± 11 g/day for the WD. No significant ($p<0.05$) differences were indicated by analysis of variance due to inter-subject variability or to sequence and diet interaction.

TABLE 9

INDIVIDUAL AND MEAN TOTAL FECAL OUTPUT (WET WEIGHT) OF SUBJECTS ACCORDING TO DIETARY SEQUENCE

SUBJECT	DAY 16 BARLEY DIET (g/day)	DAY 31 WHEAT DIET (g/day)
BI	108.0	108.5
SM	138.6	123.8
MM	162.7	131.8
GW	170.4	92.3
AL	118.9	142.5
MEAN	139.7	119.8
± SE[1]	12.1	8.8
SUBJECT	**DAY 16 WHEAT DIET (g/day)**	**DAY 31 BARLEY DIET (g/day)**
HP	80.8	80.0
RW	85.2	145.8
BK	45.0	56.8
MEAN	70.3	94.2
± SE	12.7	26.7

[1]Standard Error

3.3 SERUM LIPIDS

3.3.1 Serum Total Cholesterol

Mean total serum cholesterol levels, for all subjects, were significantly ($p<0.05$) lower than baseline values when subjects consumed the BD, but not the WD, as determined by Student's paired t-test (Appendix D). Individual and mean values for serum cholesterol for each subject are shown in Table 10. Serum cholesterol levels decreased from baseline values by a mean of 19 mg/dL for the BD compared to 9 mg/dL for the WD. The overall mean serum cholesterol value for all subjects during the BD period was 154 ± 7[33] mg/dL compared to 165 ± 9 mg/dL during the WD period.

Analysis of variance indicated an overall significant ($p<0.05$) difference between the two treatment periods (Appendix E Table 6). However, when the data was analyzed for diet treatment alone no significant ($p<0.05$) difference between the BD and WD periods was found. In addition, there was no significant ($p<0.05$) difference in serum cholesterol levels related to the sequence followed by the two groups of subjects (Figure 3). Sequence and diet interaction was also shown to be non-significant ($p<0.05$). However, the variability due to 'person effect' was found to be significantly ($p<0.05$) different between the two dietary treatments. Figure 4 presents the serum cholesterol levels for the individual subjects throughout the study. Serum cholesterol levels were decreased in all subjects, except BK and GW, during the first experimental period, regardless of the dietary treatment. Reductions were greatest for subject HP with the WD and subject SM with the BD.

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[33] Standard Error

During experimental period two, serum cholesterol levels increased to slightly below baseline values in subjects BI, MM, SM, AL, and RW. Subject BK experienced a reduction, and subject GW a very slight increase in serum cholesterol levels during this time.

Scheffe's Multiple Comparison test for differences between treatment means also indicated no significant ($p<0.05$) differences in serum cholesterol levels due to dietary sequence or diet treatment.

TABLE 10

INDIVIDUAL AND MEAN SERUM TOTAL CHOLESTEROL OF SUBJECTS ACCORDING TO DIETARY SEQUENCE[1]

SUBJECT	INITIAL	DAY 16 BARLEY DIET	DAY 31 WHEAT DIET
BI	185	165	184
SM	160	118	135
MM	179	151	178
GW	147	156	157
AL	178	169	192
MEAN	170.0	152.0	169.0
± SE[2]	7.0	9.0	10.0
SUBJECT	**INITIAL**	**DAY 16 WHEAT DIET**	**DAY 31 BARLEY DIET**
HP	204	154	159
RW	149	125	140
BK	187	191	177
MEAN	180.0	157.0	159.0
± SE	16.0	19.0	11.0

[1]Values are the mean of duplicate analysis expressed in mg/dl serum.

[2]Standard Error

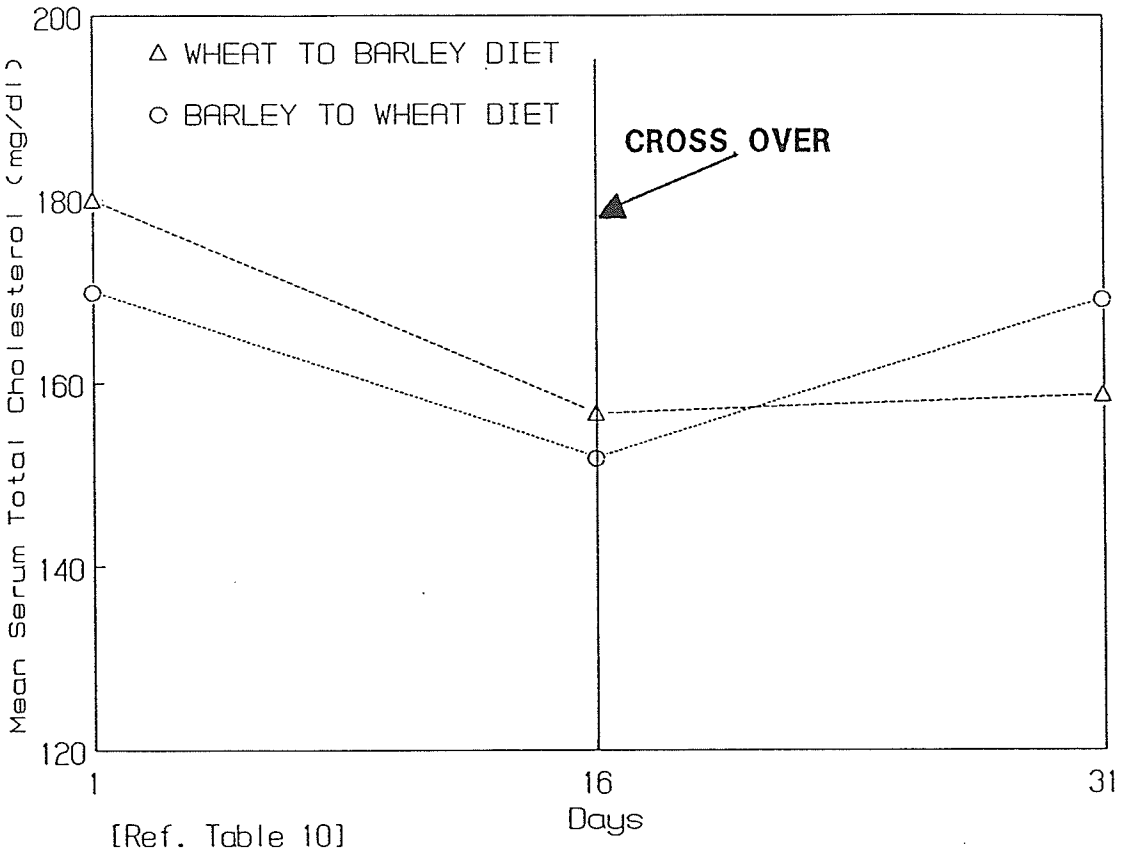
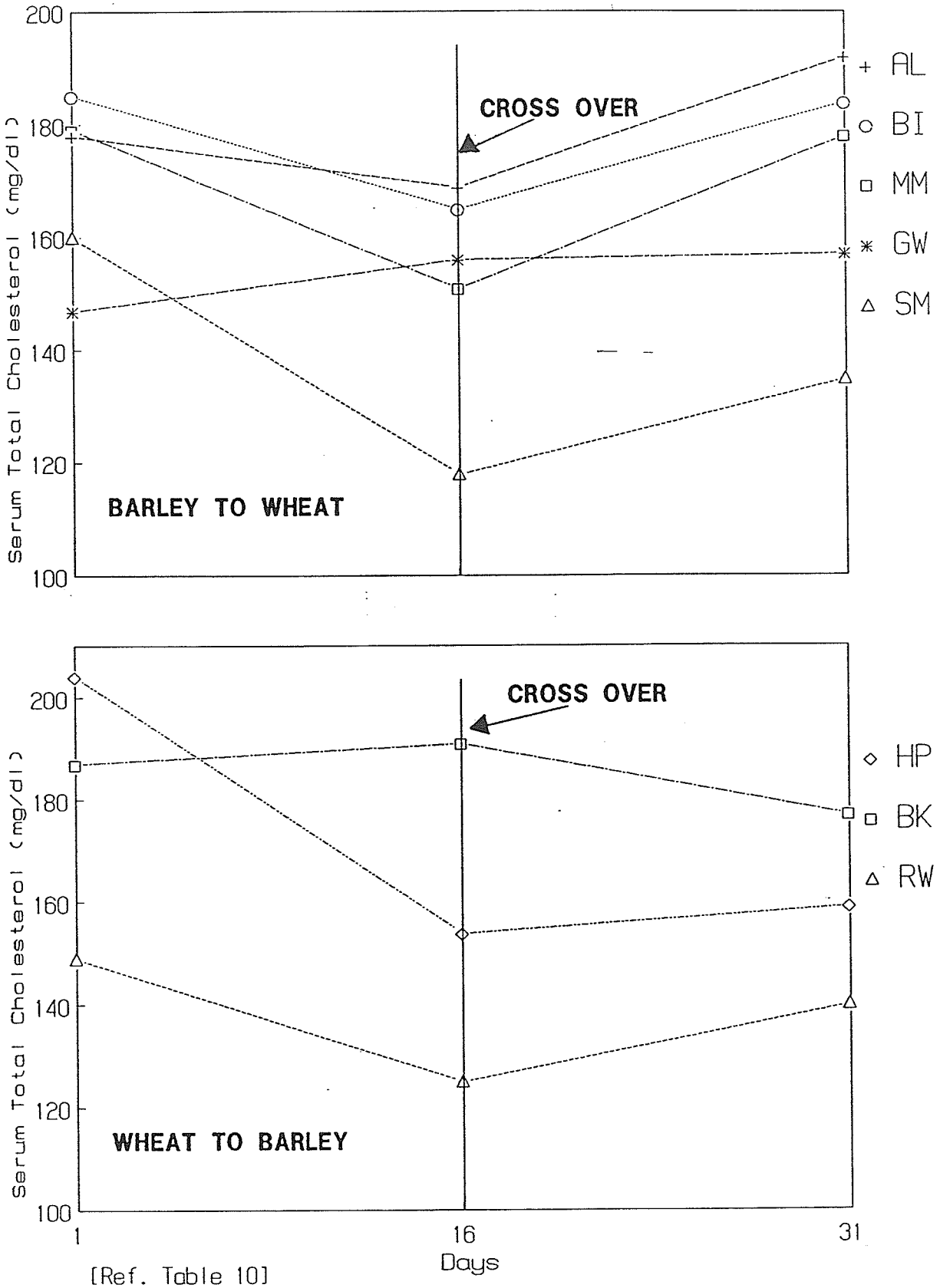


FIGURE 3 **MEAN SERUM TOTAL CHOLESTEROL OF SUBJECTS ACCORDING TO DIETARY SEQUENCE**



[Ref. Table 10]

FIGURE 4 **INDIVIDUAL SERUM TOTAL CHOLESTEROL OF SUBJECTS ACCORDING TO DIETARY SEQUENCE**

3.3.2 Serum Low Density Lipoprotein Cholesterol

Serum LDL-C levels did not differ significantly ($p<0.05$) from initial levels when either the BD or WD was ingested when analyzed by the Student's paired t-test (Appendix D). The mean serum LDL-C for all subjects fed the BD was 104 ± 6 mg/dL,[34] a reduction of 13 mg/dL from baseline, compared to 107 ± 8 mg/dL for the WD, a decrease of 10 mg/dL from initial values. Table 11 shows individual and mean serum LDL-C values for each subject throughout the study.

Analysis of variance indicated that the serum LDL-C levels were significantly ($p<0.05$) different between the two treatment periods for all subjects (Appendix E Table 7). However, consistent with the results for serum cholesterol, no significant ($p<0.05$) differences were found between the effects of the BD and the WD treatments. As well, the sequence in which the diets were fed had no significant ($p<0.05$) effect. No interaction was found between dietary sequence and diet treatment ($p<0.05$). However, a significant ($p<0.05$) difference was noted in the effects on serum LDL-C levels of person-to-person variability between the two treatments. Mean serum LDL-C concentrations for subjects according to dietary sequence are indicated in Figure 5. Figure 6 represents schematically, the individual changes in serum LDL-C values over the 30-day study period. Individual serum LDL-C levels were highly variable and generally had patterns similar to those of serum cholesterol for all subjects except for AL, BK, and HP, where inconsistencies were noted.

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[34] Standard Error

TABLE 11

INDIVIDUAL AND MEAN SERUM LOW DENSITY LIPOPROTEIN CHOLESTEROL OF SUBJECTS ACCORDING TO DIETARY SEQUENCE[1]

SUBJECT	INITIAL	DAY 16 BARLEY DIET	DAY 31 WHEAT DIET
BI	125	113	114
SM	102	76	78
MM	125	101	117
GW	92	102	105
AL	100	106	118
MEAN	109.0	100.0	106.0
± SE[2]	7.0	6.0	7.0
SUBJECT	**INITIAL**	**DAY 16 WHEAT DIET**	**DAY 31 BARLEY DIET**
HP	153	123	114
RW	94	71	91
BK	149	131	132
MEAN	132.0	108.0	112.0
± SE	19.0	19.0	12.0

[1]Values are the mean of duplicate analysis expressed in mg/dl serum.

[2]Standard Error

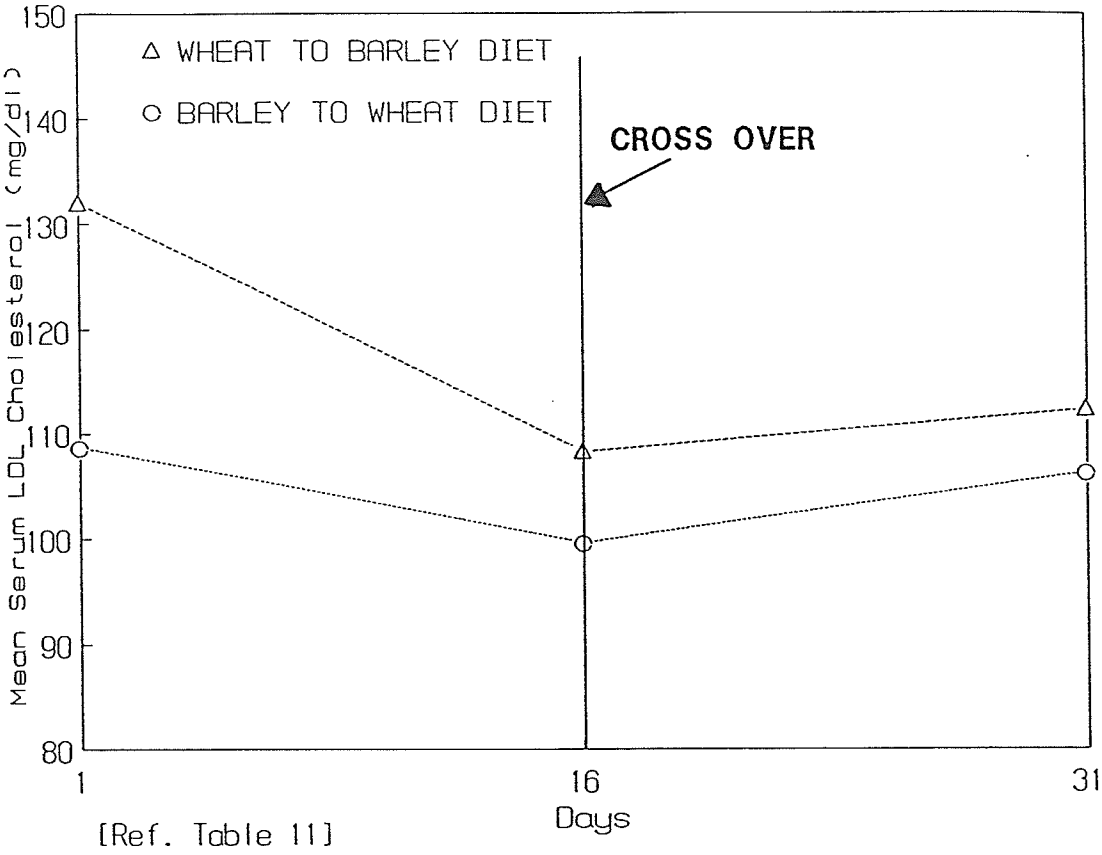
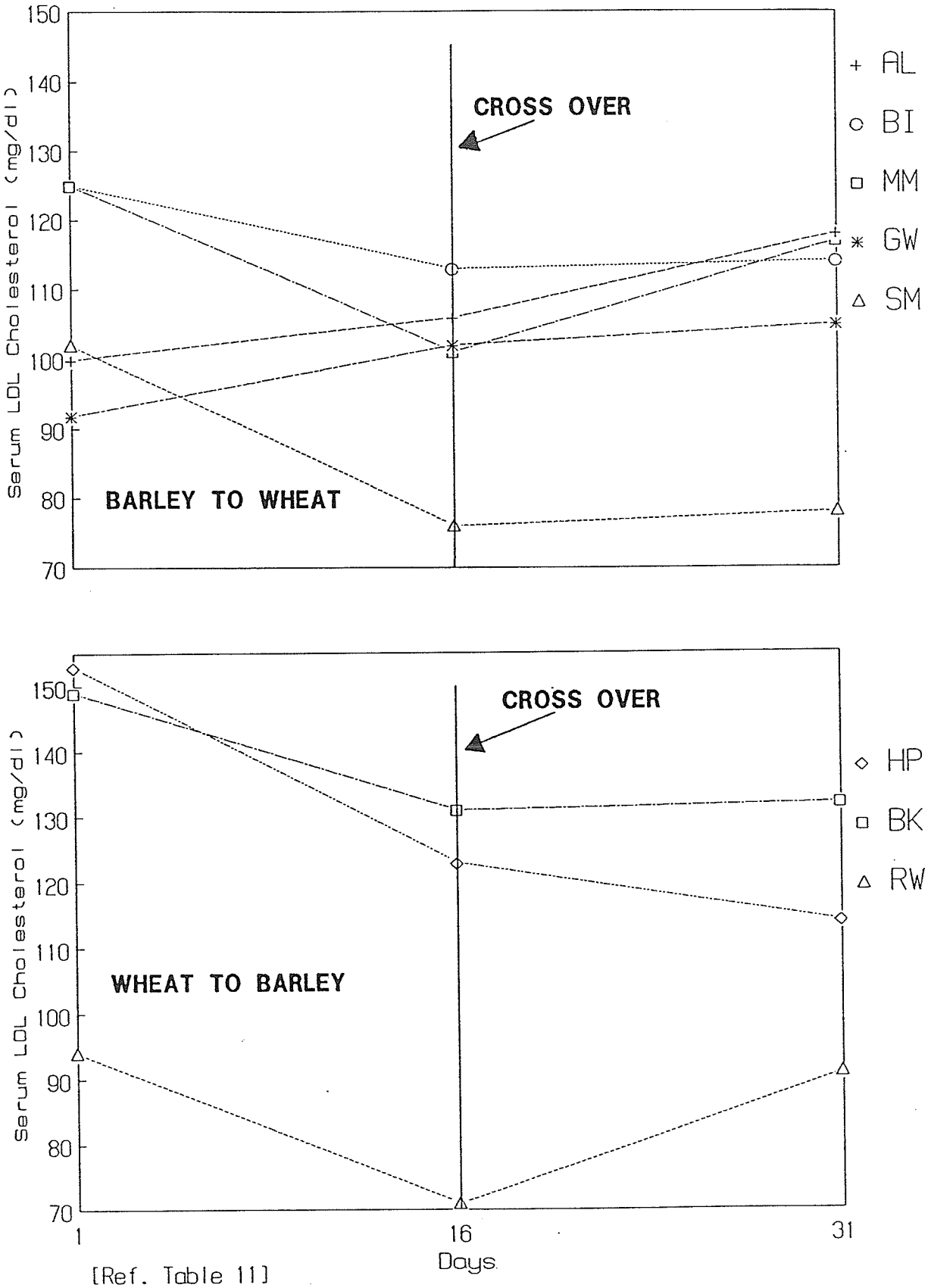


FIGURE 5 **MEAN SERUM LOW DENSITY LIPOPROTEIN CHOLESTEROL OF SUBJECTS ACCORDING TO DIETARY SEQUENCE**



[Ref. Table 11]

FIGURE 6 **INDIVIDUAL SERUM LOW DENSITY LIPOPROTEIN CHOLESTEROL OF SUBJECTS ACCORDING TO DIETARY SEQUENCE**

The two dietary sequences and dietary treatments did not differ significantly ($p<0.05$) in their effect on serum LDL-C values as indicated by Scheffe's tests.

3.3.3 Serum High Density Lipoprotein Cholesterol

Serum HDL-C levels were not significantly ($p<0.05$) different when either the WD or BD were fed as shown by analysis of variance (Appendix E Table 8). Serum HDL-C levels at the end of each experimental period were also not significantly ($p<0.05$) different from baseline as determined by paired t-tests (Appendix D). Table 12 and figures 7 and 8 present individual and mean HDL-C values for each subject according to diet sequence. It can be noted that changes in individual subject responses were inconsistent with the changes in total and LDL-C. Serum HDL-C levels were reduced by a mean of 1.8 mg/dL for all subjects following the BD compared to a mean increase of 0.6 mg/dL following the WD. Mean serum HDL-C levels for all subjects were 43 ± 3 mg/dL[35] for the BD and 45 ± 4 mg/dL for the WD. Figure 8 indicates the mean HDL-C values for the subjects according to dietary sequence.

Analysis of variance showed that dietary sequence, person-to-person variablility, and dietary treatment had no significant ($p<0.05$) effects on serum HDL-C levels during the study. No significant ($p<0.05$) interaction was noted between dietary sequence and diet treatment. Scheffe's tests also indicated no significant ($p<0.05$) differences in serum HDL-C levels due to dietary treatment or sequence.

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[35] Standard Error

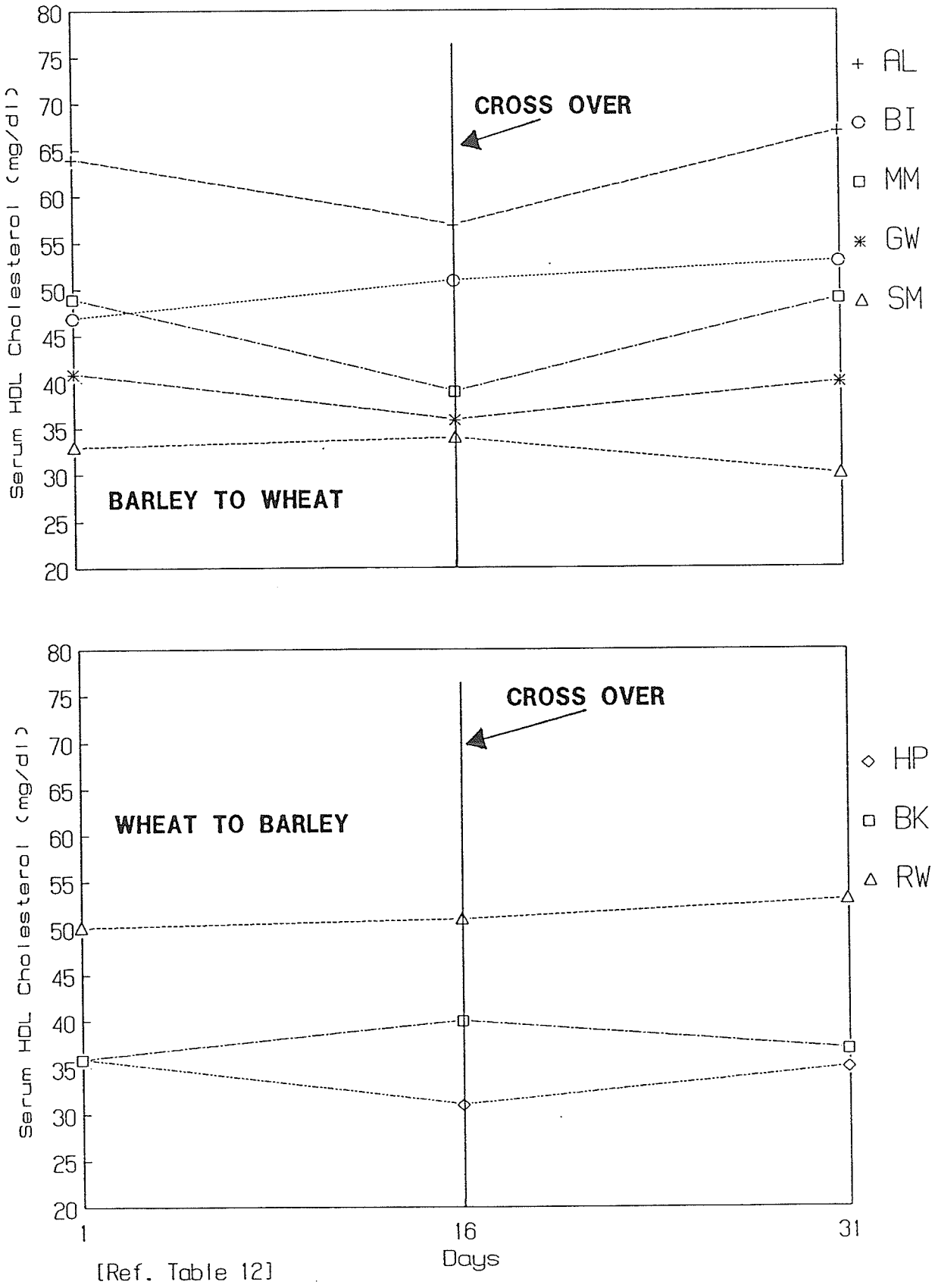
TABLE 12

INDIVIDUAL AND MEAN SERUM HIGH DENSITY LIPOPROTEIN CHOLESTEROL OF SUBJECTS ACCORDING TO DIETARY SEQUENCE[1]

SUBJECT	INITIAL	DAY 16 BARLEY DIET	DAY 31 WHEAT DIET
BI	47	51	53
SM	33	34	30
MM	49	39	49
GW	41	36	40
AL	64	57	67
MEAN	47.0	43.0	48.0
± SE[2]	5.0	5.0	6.0
SUBJECT	**INITIAL**	**DAY 16 WHEAT DIET**	**DAY 31 BARLEY DIET**
HP	36	31	35
RW	50	51	53
BK	36	40	37
MEAN	41. 0	41.0	42.0
± SE	5.0	6.0	6.0

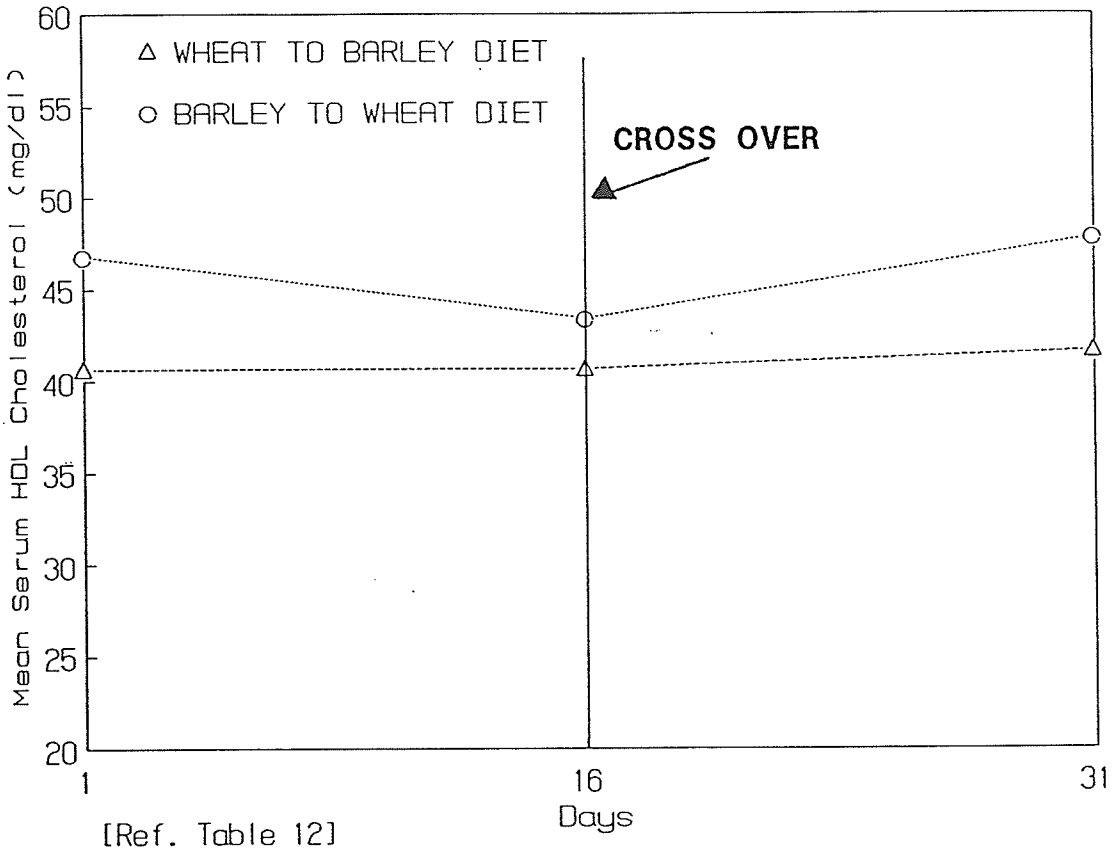
[1]Values are the mean of duplicate analysis expressed in mg/dl serum.

[2]Standard Error



[Ref. Table 12]

FIGURE 7 **INDIVIDUAL SERUM HIGH DENSITY LIPOPROTEIN CHOLESTEROL OF SUBJECTS ACCORDING TO DIETARY SEQUENCE**



[Ref. Table 12]

FIGURE 8 **MEAN SERUM HIGH DENSITY LIPOPROTEIN CHOLESTEROL OF SUBJECTS ACCORDING TO DIETARY SEQUENCE**

Multivariate analysis was used to test for correlations between mean changes in serum cholesterol, LDL-C and HDL-C and each dietary treatment. No significant ($p<0.05$) relationships were found between these variables for sequence, diet or sequence and diet interaction.

3.3.4 Serum Triglycerides

Serum triglyceride levels were not significantly ($p<0.05$) different for the subjects who consumed the BD or WD as shown by analysis of variance (Appendix E Table 9). There were no significant ($p<0.05$) changes from initial values indicated by paired t-tests for either diet (Appendix D). The initial, and day 16 individual and mean values for triglycerides are reported in Table 13. The mean serum triglyceride value for all subjects fed the BD was 70 ± 7[36] mg/dL compared to 78 ± 10 mg/dL for the WD. The mean reductions in serum triglyceride levels from baseline values were 13 mg/dL for the BD and 5 mg/dL for the WD. Mean serum triglycerides for the subjects according to the dietary sequence are shown in Figure 9.

Figure 10 illustrates the individual responses for serum triglycerides during both periods. It can be noted that the individual values are highly variable. Subjects AL and BI demonstrated an initial fall in serum triglycerides when the BD was fed and slight increases during the WD period. Subjects HP and RW were fed the diets in the opposite order but showed similar responses. Subject SM experienced a large reduction in serum triglycerides when fed the BD followed by an increase to a level greater than baseline when the WD was consumed. Subjects GW and

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[36] Standard Error

MM demonstrated increases in serum triglycerides during the BD period. Serum triglycerides decreased for subject GW when the WD was ingested, while subject MM experienced a slight increase. Subject BK showed an increase followed by a decrease in serum triglycerides for each of the WD and BD periods, respectively.

Neither dietary sequence nor diet treatment had a significant ($p<0.05$) effect on serum triglycerides when analyzed by analysis of variance or by Scheffe's tests. Even though the individual subject responses appear to be highly variable, no significant ($p<0.05$) effect of inter-person variability was indicated. As well, sequence and diet interaction did not contribute to variablity ($p<0.05$).

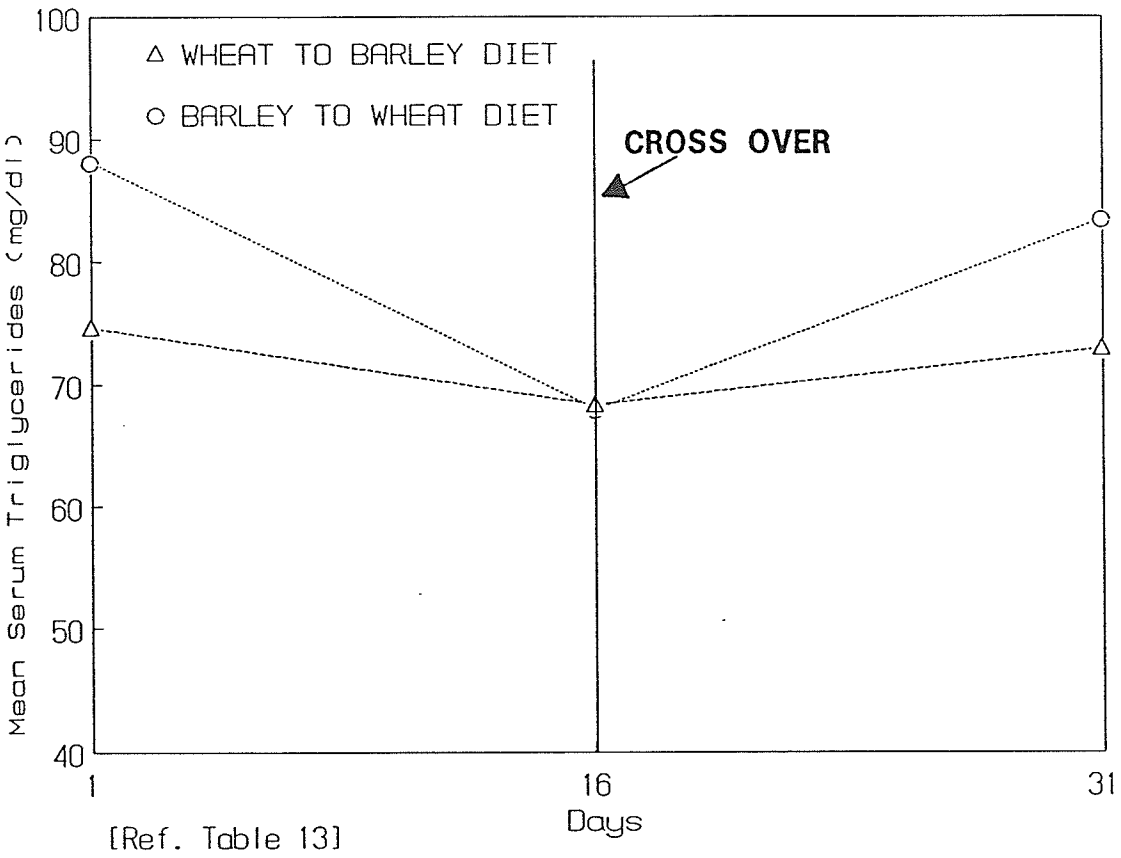
TABLE 13

INDIVIDUAL AND MEAN SERUM TRIGLYCERIDES OF SUBJECTS ACCORDING TO DIETARY SEQUENCE[1]

SUBJECT	INITIAL	DAY 16 BARLEY DIET	DAY 31 WHEAT DIET
BI	96	58	60
SM	112	51	137
MM	49	72	78
GW	85	103	82
AL	99	56	61
MEAN	88.0	68.0	84.0
± SE[2]	11.0	9.0	14.0
SUBJECT	**INITIAL**	**DAY 16 WHEAT DIET**	**DAY 31 BARLEY DIET**
HP	89	84	94
RW	60	42	55
BK	75	79	70
MEAN	75.0	68.0	73.0
± SE	8.0	13.0	11.0

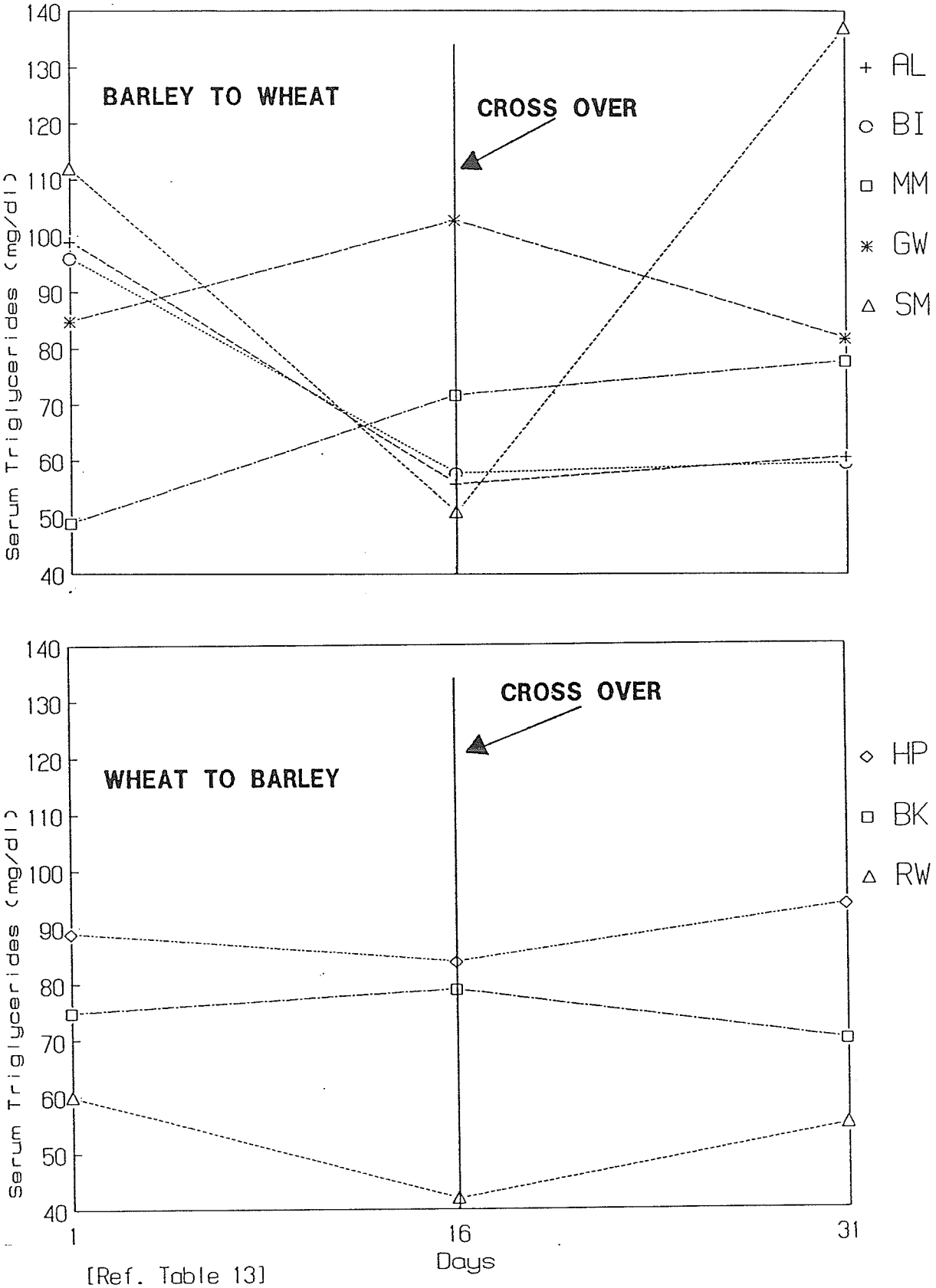
[1]Values are the mean of duplicate analysis expressed in mg/dl serum.

[2]Standard Error



[Ref. Table 13]

FIGURE 9 **MEAN SERUM TRIGLYCERIDES OF SUBJECTS ACCORDING TO DIETARY SEQUENCE**



[Ref. Table 13]

FIGURE 10 **INDIVIDUAL SERUM TRIGLYCERIDES OF SUBJECTS ACCORDING TO DIETARY SEQUENCE**

3.3.5 Serum Very Low Density Lipoprotein Cholesterol

Individual and mean serum VLDL-C values are given in Table 14 for each subject according to diet sequence. Serum VLDL-C levels for either diet were not significantly ($p<0.05$) different from initial values as demonstrated by a paired t-test (Appendix D). Analysis of variance indicated that the two dietary treatments had no significant ($p<0.05$) effect on VLDL-C levels (Appendix E Table 10). Mean reductions of 4 mg/dL and 2 mg/dL for all subjects were observed when the BD and the WD were ingested, respectively. Mean serum VLDL-C levels for all subjects were 8 ± 2[37] mg/dL at the end of the BD period compared to 10 ± 3 mg/dL for the WD. Figure 11 indicates mean serum VLDL-C values for subjects according to dietary sequence.

Individual values for serum VLDL-C responses for each dietary period are shown in Figure 12. Generally, individual subject values reflect serum triglyceride values of the subjects in each treatment group. Similar reductions and increases occurred during the respective dietary periods. However, during the BD period, subjects RW and BK demonstrated opposite responses in serum VLDL-C levels to what occurred in serum triglycerides. No significant ($p<0.05$) effects on serum VLDL-C levels of diet sequence or diet treatment were found by analysis of variance or by Scheffe's tests. Analysis of variance also revealed no significant ($p<0.05$) inter-subject effects or sequence and diet interactions.

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[37] Standard Error

TABLE 14

INDIVIDUAL AND MEAN SERUM VERY LOW DENSITY LIPOPROTEIN CHOLESTEROL OF SUBJECTS ACCORDING TO DIETARY SEQUENCE[1]

SUBJECT	INITIAL	DAY 16 BARLEY DIET	DAY 31 WHEAT DIET
BI	13	0	16
SM	26	8	27
MM	6	11	12
GW	14	18	12
AL	13	6	6
MEAN	14.0	9.0	15.0
± SE[2]	3.0	3.0	3.0
SUBJECT	**INITIAL**	**DAY 16 WHEAT DIET**	**DAY 31 BARLEY DIET**
HP	16	0	10
RW	5	3	0
BK	2	2	8
MEAN	8.0	2.0	6.0
± SE	4.0	1.0	3.0

[1]Values are the mean of duplicate analysis expressed in mg/dl serum.

[2]Standard Error

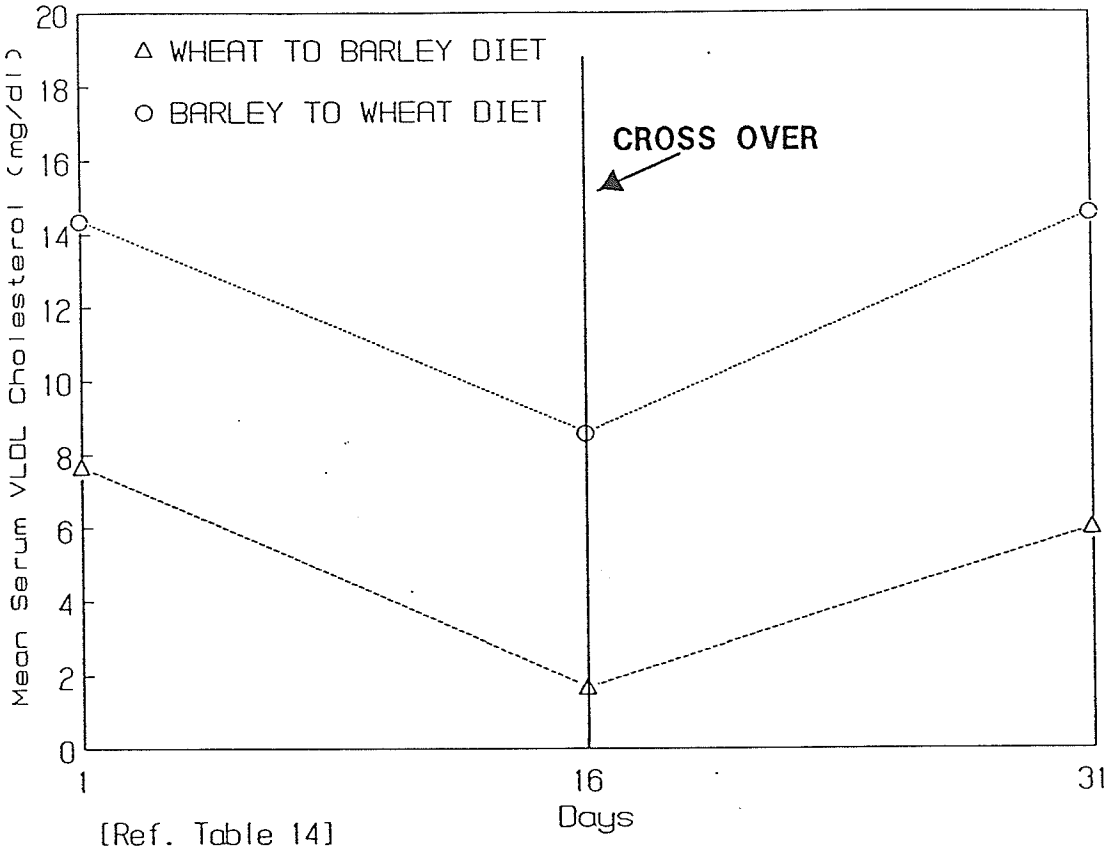
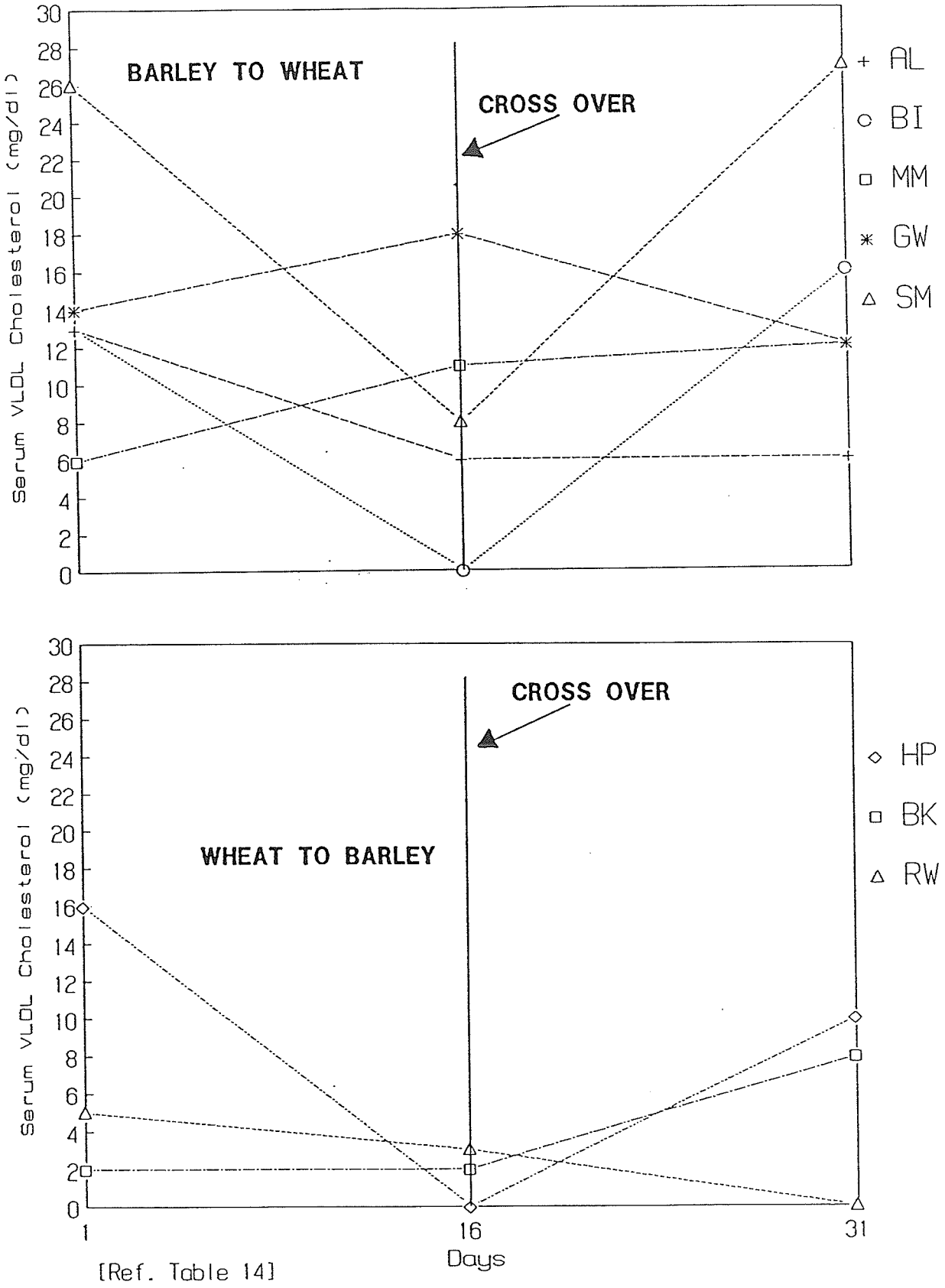


FIGURE 11 MEAN SERUM VERY LOW DENSITY LIPOPROTEIN CHOLESTEROL OF SUBJECTS ACCORDING TO DIETARY SEQUENCE



[Ref. Table 14]

FIGURE 12 INDIVIDUAL SERUM VERY LOW DENSITY LIPOPROTEIN CHOLESTEROL OF SUBJECTS ACCORDING TO DIETARY SEQUENCE

Chapter IV

DISCUSSION

4.1 TOTAL BILE ACIDS AND FECAL LIPIDS

In the present study, it was expected that bile acid and fecal fat excretion would increase when ß-glucan was included in a mixed diet as the only source of soluble fiber. Increased viscosity in the gastrointestinal tract and sequestration of bile acids by the fiber gel were hypothesized to be the mechanisms responsible for these effects (Kay and Truswell, 1980). However, fecal bile acids and fat excretion did not increase significantly in response to the ingestion of barley ß-glucan. In agreement with the present study, Ross and Leklem (1981) did not report any changes in fecal fat excretion when they fed normal subjects a pectin supplement in a mixed diet. However, they did observe an increase in fecal bile acids. No changes in bile acid excretion and an increase in fecal fat were reported by Stasse-Wolthius et al. (1980) for normal subjects consuming a diet which included fruits and vegetables. These results, and those of the research reported here, are in contrast with the reports of Kay and Truswell (1977) and Stasse-Wolthius et al. (1980) who fed pectin to healthy subjects, and Judd and Truswell (1981) who fed normolipidemics rolled oats in a mixed diet. In these dietary trials, fecal bile acid and lipid excretion were significantly increased. The present study differs in a number of experimental factors from published reports.

In the present study, the soluble fiber supplement was fed in its natural form, as barley flour, incorporated into biscuits. Soluble fibers found in natural food sources are usually present as part of protein and starch matrixes within cell walls and therefore may not react as freely with either water, to produce gels, or with bile acids. In contrast to the present research, chemically isolated pectins were used in the studies of Kay and Truswell (1977), Stasse-Wolthius and coworkers (1980) and Ross and Leklem (1981). Isolated soluble fibers may hydrate to a greater degree in the gut, thus increasing their capacity to trap bile acids and dietary lipid (Kay and Truswell, 1980). Stasse-Wolthius and associates (1980) noted that bile acid excretion increased 51% and fecal fat 75% when purified pectin was incorporated into a basal diet. In comparison, the addition of pectin in the form of fresh fruits and vegetables increased bile acid excretion only slightly in the same subjects. Fecal fat was increased 32% but the result was not significant.

Clinical studies utilizing ß-glucans found naturally in barley have not been reported. However, other sources of ß-glucan in natural foods fed to human subjects have resulted in increases in bile acid and fat excretion. Judd and Truswell (1981) observed increases of 35% and 48% in bile acid and fat excretion, respectively, when ß-glucan was consumed as rolled oats. Similarily, Kirby and coworkers (1981) and Anderson and associates (1984) fed oat bran, incorporated into cereal and muffins in a mixed diet, to hyperlipidemics. Bile acid excretion increased 54% and 40%, respectively. Soluble fiber analysis indicated that the respective diets contained approximately 14 g of soluble fiber, which was assumed

to be ß-glucan. It therefore appears that ß-glucan, present in oat cereals, is effective in increasing fecal acidic sterol and lipid excretion.

Isolated ß-glucan has been shown to be equally effective in increasing the viscosity of the intestinal fluid of poultry as ß-glucan found naturally in barley (White et al., 1981). Although several studies in poultry indicate that ß-glucan increases the fat content of feces leading to depressed weight gains (Burnett, 1966; Gohl et al., 1978 and Classen et al., 1985), there may not be a similar increase in viscosity in the human intestine as has been reported in chickens. Clinical studies incorporating purified ß-glucan in human diets have not been reported.

The present study indicates that the amount of ß-glucan incorported into the mixed diet may have been too low for detecting measureable increases in bile acid and fat excretion. However, there are limits to the amounts of soluble fiber and fiber containing foods which can be consumed. The incorporation of approximately 9 g/day of barley ß-glucan into the diet was contained in 236.5 g of barley flour, an amount that would be difficult to include in a normal diet on a daily basis. Pectin fed in amounts of 9-12 g/day has been associated with abdominal cramps and discomfort, flatulence (Kay and Truswell, 1977) and diarrhea (Durrington et al., 1976). As well, significant losses of body weight were noted in a study by Anderson and coworkers (1984) when 14.5 g/day of soluble fiber was fed. The amount of ß-glucan utilized in the present study did not cause body weight changes in subjects. However, when the subjects were consuming the BD, some subjects complained of

increased looseness and frequency of stools and abdominal cramps. These symptoms appeared to be less severe as the study period continued.

Results from studies which investigated oat cereals suggests that the amount of ß-glucan fed in the present study may have been insufficient to cause increases in fat and bile acid excretion. In the studies of Kirby and coworkers (1981) and Anderson and associates (1984), 14 and 14.5 g/day of ß-glucan from oat bran were fed with concommitant increases in bile acid excretion. However, the populations investigated were hyperlipidemic subjects and therefore, a direct comparison to the present study cannot be made. Judd and Truswell (1981) studied the effects of rolled oats in normolipidemics and observed increases in bile acid and fecal fat excretion. Again, a comparison with the results reported here is difficult to make as the soluble fiber fractions of the rolled oat supplement were not quantitated.

Stasse-Wolthuis and associates (1980) incorporated natural pectin into the diets of normolipidemics in an amount, 7.5 g/day, similar to the present study. They observed a similar lack of change in bile acid excretion and fecal fat. Purified pectins, fed at levels of 9-15 g/day increased bile acid and fat excretion (Kay and Truswell, 1977; Stasse-Wolthius et al., 1980).

In the study reported here, fiber containing foods, except for the barley flour, were excluded or kept to a minimum as calculated from food composition tables. As well, food sources of soluble fiber, especially pectins, were not included. In a number of feeding trials, undefined fiber sources have been present in the basal and test diets which may have enhanced fecal bile acid and fat excretion. In a study by Kirby

and associates (1981), the test diet contained 7 g of unidentified soluble fiber sources while 6 g was included in the experimental diets fed by Anderson and coworkers (1984). In other studies, the crude fiber method (Kay and Truswell, 1977) or the neutral detergent fiber analysis (Ross and Leklem, 1981) was used to quantify fiber. Neither method measures water soluble fractions (Lanza and Butrum, 1986) and therefore the true soluble fiber content of these diets may be greatly underestimated.

In the present study the normolipidemic subjects ingested the ß-glucan for 15 days. Studies with normolipidemics indicate that a 15 day time period may be inadequate to observe changes in bile acid and fecal fat excretion. In the studies of Ross and Leklem (1981); Kay and Truswell (1977) and Stasse-Wolthuis and coworkers (1980), purified pectin was fed for 18, 21, and 35 days, respectively. These researchers noted increased bile acid and fat excretion of 6% and 0; 33% and 44%; and 51% and 75%, respectively. Similar data have also been reported by Judd and Truswell (1981). They fed rolled oats to normal subjects for 21 days and noted increases in bile acid and fat excretion. In contrast, hyperlipidemic subjects who ingested soluble fiber for 10 days showed increases in bile acid and fat excretion (Kirby et al., 1981).

The quantitation of bile acids and fecal fat is affected by individual variations in fecal outputs. Human studies have reported extreme variation in fecal output of individuals irrespective of diet. In the present study, fecal wet weights ranged from 45 to 170 g/day. Judd and Truswell (1981) reported similar variability in the fecal output of

their subjects which ranged from 83 to 219 g/day during both a control and test diet. Ross and Leklem (1981) and Stasse-Wolthuis and coworkers (1980) also reported large individual differences between fecal weights when subjects consumed commercial pectin, fruits and vegetables, and control diets. Cummings and associates (1978) noted that large individual variations in fecal wet weights can occur which are independent of fiber source and level of intake. The fecal weights for several series of five-day collection periods of twenty healthy subjects consuming their normal diets were measured by Wyman and coworkers (1978). They noted a wide variation in fecal wet weights between series of collections within the same subject as well as between subjects for the same collection period. Their study indicated the importance of a three to five day fecal collection period. In the present study, fecal collections were made for a five day period at the conclusion of each dietary period.

In the present study, fecal wet weights were not significantly increased for subjects fed the BD diet. These observations are similar to the study of Judd and Truswell (1981) who noted that individual stool weights were unaffected by rolled oat supplementation. Ross and Leklem (1981) noted a mean increase in stool output of 26% in six of eight subjects consuming purified pectin for 18 days. However, Kay and Truswell (1977) found no changes in fecal weights when nine normolipidemics ingested isolated pectin for 21 days.

Some investigators have suggested that subjects with very low fecal output may need more dietary fiber than their counterparts to increase fecal weight (Cummings and Stephen, 1980; Schweizer et al., 1987). This

appears to be true for subjects HP and BK, whose fecal outputs were, on average, 55% and 32% of those of the other subjects. Individual differences in bacterial flora and in gastrointestinal motility could contribute to the variability in fecal output observed in various studies on soluble fibers.

The sequence in which the barley and wheat diets were consumed by the subjects significantly ($p<0.05$) affected fecal output, which suggests that there may be a 'carry-over' effect of dietary fiber. This hypothesis has been supported by Eastwood and associates (1973). In the present study, it was noted that the subjects who ingested the BD followed by the WD had a higher mean fecal output on both diets than the subjects fed the reverse diet order. As well, bile acid excretion was higher for two of the five subjects when the WD treatment followed the BD treatment. The research of Kay and Truswell (1977); Ross and Leklem (1981) and Judd and Truswell (1981) appear to support these results. In these studies, control diets both preceded and followed the soluble fiber diet periods. Bile acid excretion was 20 to 27% higher during the control periods following pectin or rolled oat supplementation, than during the preceding control period. If ß-glucan produces viscous gels in the human gut, as is indicated in animal studies, carry-over effects could be present. This suggests that a 'washout' period in which subjects resume their normal diets for a short time period may be beneficial to ensure that there are no residual dietary affects. Jenkins and coworkers (1979) implemented a three week 'free' feeding period when investigating the effects of several types of fiber on fecal output. It may also be useful to precede the test diet with a control period. Kay

and Truswell (1977); Stasse-Wolthuis and coworkers (1980) and Ross and Leklem (1981) preceded their fiber diets with periods of dietary control ranging from four to seventeen days. Pre-experimental periods are useful in stabilizing the gut function of subjects and ensuring that no residual effects of previous diets will affect the data of interest. Although fecal weights were significantly affected by the sequence of diets, the data for bile acid and fecal fat excretion were not. Fecal lipid output was less affected by inherent differences in individual stool production than was the data for bile acids. Subjects HP and BK, who had the lowest fecal weights also had the lowest uncorrected values for bile acids and fecal fat excretion. PEG corrected data for these subjects resulted in values which differed greatly from the other subjects.

None of the published research referred to here utilized internal markers for the correction of fecal output. It is therefore debatable whether the fecal data reported did, in fact, truely quantitate bile acid and fecal fat excretion. If data had been corrected for intestinal transit time, perhaps there would be less effect of fiber. For example, if the uncorrected data in the present study had been used in the analysis, fecal bile acid excretion would have been increased as a result of the BD. The mean bile acid output, uncorrected by PEG values, for all subjects consuming the BD was 781.0 μmoles/day, which was 10% higher than for the WD (699.0 μmoles/day). Similarly, uncorrected fecal fat was higher for the BD, 3.20 g/day versus 3.00 g/ day for the WD. When the results were analyzed using the uncorrected data for HP and BK plus the corrected data for the other subjects, bile acid excretion was 832.0

umoles/day for the BD, compared to 768.0 umoles/day with the WD, an increase of 8%. This result is similar to that of Ross and Leklem (1981) who found a non-significant increase in bile acid excretion of 6% with the incorporation of pectin into mixed diets. Fecal fat was also higher for the BD than the WD, 3.40 g/day versus 3.10 g/day, when data uncorrected for HP and BK was used in the analysis. These examples indicate the differences in the interpretation of results that may occur when fecal data is corrected by internal markers. The use of the internal marker, PEG, in the present study produced statistically insignificant results that may not have been observed had uncorrected fecal data been analyzed. This suggests that data of previous researchers, who did not correct fecal data, must be interpretated with caution.

In the present study, dietary constituents known to affect bile acid metabolism, primarily dietary cholesterol and the amount and type of fat, were controlled for in both diets. The BD and WD fed here incorporated 36% of total energy as fat. This is similar to the diets utilized in the research of Stasse-Wolthuis and coworkers (1980), Judd and Truswell (1981), Kirby and associates (1981) and Anderson and coworkers (1984), who incorporated 37%, 36%, 38%, and 36% of total energy as fat, respectively. These investigators noted increases in bile acid excretion of 51%, 35%, 54%, and 40%, respectively. In the present study, the fatty acid composition of both diets was similar. PUFA represented 9% of total energy, while SFA and MUFA made up 10% and 17% of total energy, respectively. The diets fed by Stasse-Wolthius et al., (1980); Kirby et al., (1981) and Anderson et al., (1984) contained similar fatty acid compositions as described in this study. Therefore, differences between

the results of fecal measurements noted in these reports and those observed in the present study, would not appear to be due to the total dietary fat or the fatty acid composition of the diets.

In human studies reported, bile acid excretion appears to be related to the intake of dietary cholesterol. Judd and Truswell (1981) fed an average of 390 mg/day of cholesterol in their diets and noted a 35% increase in bile acid excretion, while Kay and Truswell (1977) observed an increase of 33% when 425 mg of cholesterol was fed per day. Similarily, the diets of Kirby and coworkers (1981) and Anderson and associates (1984) incorporated approximately 440 mg of cholesterol daily and noted increases of 54% and 40% in bile acid excretion, respectively, in hyperlipidemics. In contrast, Ross and Leklem (1981) fed 260 mg of cholesterol daily and noted a non-significant increase of 6% in fecal bile acids. In the present study, 210 mg and 225 mg of cholesterol were incorporated into the BD and WD, respectively, with no changes in bile acid or fat excretion. These amounts are much lower than the average dietary cholesterol intake of the general population which is estimated to be approximately 400-500 mg/day (Grundy, 1983). The ingestion of a low cholesterol diet may have had more important effects on bile acid excretion than did the barley ß-glucan.

This study indicates that a natural source of ß-glucan incorporated into a diet containing moderate fat and low cholesterol content, does not appear to affect bile acid and fat excretion. The amount of fiber and the length of time that the fiber was fed as well as the sequence of feeding are variables which were shown to affect the results of the present study. As in other reported studies, there were individual

variations in fecal output. Caution must be employed when attempting to draw conclusions from studies where fecal data has not been corrected by the use of internal markers. It is therefore difficult to conclusively ascertain whether soluble fibers have an affect on fecal acidic steriod and fat excretion in humans.

4.2 SERUM LIPIDS

4.2.1 Serum Total Cholesterol

In the present study, serum cholesterol levels were significantly ($p<0.05$) lower than baseline values when subjects consumed the BD, but not the WD. Mean decreases of serum cholesterol levels of 11% and 9% occurred during the BD and WD, respectively. Several reports are in agreement with these results. Kay and Truswell (1977) observed a reduction of 13% in serum cholesterol levels from baseline values while in a study by Stasse-Wolthius and coworkers (1980), serum cholesterol levels decreased 10% from baseline. In both studies, purified pectins were fed. Stasse-Wolthius and associates (1980) also reported reductions of serum cholesterol levels of 4% from baseline values when fruits and vegetables were included in diets fed to normolipidemic subjects.

When the effects on serum cholesterol levels of the BD were compared to the WD, a significantly ($p<0.05$) different response were noted between the two treatments. However, analysis of variance revealed that this effect was due, not to significant differences between the two diets, but to inter-subject variability. In other words, the subjects were not acting alike in their responses to the respective dietary treatments and this contributed variability to the results. Hegsted

(1986) has also observed individual variability of serum cholesterol values in response to changes in the diet. An examination of the individual data for serum cholesterol (Figure 4) indicates this variability.

In six of the eight subjects, serum cholesterol levels decreased during the initial two week dietary period, irregardless of the diet treatment. The mean serum cholesterol of the subjects according to their respective dietary sequence are indicated in Figure 3. It can be noted that both the BD and the WD treatments reduced mean serum cholesterol concentrations of the subjects similarily after the initial two week period. These data suggest that both diets were approximately equally hypocholesterolemic for the subjects.

Several constituents in the diets fed in the present study may have contributed to the overall reduction of serum cholesterol levels noted on both dietary treatments during weeks one and two. The hypocholesterolemic effects of moderate fat, low cholesterol diets are well established. In the present study, approximately 36% of total energy was contributed by fats. This amount is lower than the average fat intake of Canadian males, ages 19 to 35, which was estimated by the Nutrition Canada Survey (1977) to be approximately 40% of total energy. As well, approximately 22% of the fat in the test and control diets was of vegetable origin. PUFA comprised 9% of total energy while SFA represented 10% of total energy. In the present study, dietary cholesterol levels were 69 mg/1000 kilocalories (210 mg/day) in the BD and 77 mg/1000 kilocalories (225 mg/day) in the WD. These amounts are less than 300 mg/day which is the amount of cholesterol recommended for low cholesterol intakes. Other researchers have shown significant reductions in plasma

cholesterol levels with diets comprised of similar fatty acid compositions and amounts of dietary fats and cholesterol as those incorporated into the diets of the research reported here. Brussaard and coworkers (1980) noted that mean serum cholesterol levels decreased significantly ($p<0.05$) approximately 9% from baseline values when normolipidemic subjects followed a moderate fat, high PUFA diet for a 2.5 week period. This diet consisted of approximately 30% of total energy as fat and 11% as PUFA. The cholesterol content of the diet was 300 mg/day.

In subjects consuming a basal diet consisting of 40% of total energy as fat and 300 mg/day of cholesterol, serum cholesterol levels decreased approximately 9% from baseline values (Schonfeld et al., 1982). This diet was fed to six healthy men for three to four weeks. The percentage of total energy represented by PUFA, MUFA and SFA were not reported.

Since both diets in the present study had apparently similar hypocholesterolemic effects, the unequal distribution of the subjects during the treatment periods may have favoured a greater hypocholesterolemic effect of the BD. A pre-experimental period may have been beneficial in order to stabilize the blood lipids of subjects and to ensure that there were no carry-over effects of previous diets. In the present study, a low-fiber diet composed of the same amounts of fat and cholesterol as during the experimental WD and BD periods may have been useful.

The data of Figure 3 shows that, whereas the WD produced a rise in serum cholesterol levels to pre-experimental values during the second dietary period, the BD treatment maintained serum cholesterol concentrations at reduced levels. This observation suggests that barley ß-glucan

appears to have hypocholesterolemic properties which may not have been measureable with the experimental conditions of the present study. Keys and coworkers (1965) have observed that dietary periods of two weeks may not be long enough to realize the ultimate effects of dietary alterations on serum cholesterol levels. Other researchers have indicated that three to four week dietary periods may be necessary to fully monitor serum cholesterol responses in some individuals (Mattson et al., 1972).

The results of the present study suggest that the observed responses of individual serum cholesterol levels appear to be due more to a general hypocholesterolemic effect of both dietary treatments than to the inclusion of the ß-glucan in the BD.

4.2.2 Serum Low Density Lipoprotein Cholesterol

Serum LDL-C levels responded in a similar manner as serum cholesterol. A significant ($p<0.05$) difference was noted between the BD and WD in the present study. However, as was indicated for serum cholesterol responses, this effect was shown to be due to significant ($p<0.05$) inter-subject variability. As is indicated by the data in Figure 5, mean serum LDL-C levels were decreased during the initial two week dietary period, for both diets. A possible stabilization effect on this lipoprotein fraction was also indicated during weeks three and four of the dietary study. These responses may be due more to the total diet than by the ingestion of the soluble fiber. Diets containing moderate amounts of total fats and cholesterol, which characterize the two experimental diets in the present study, have been shown by other researchers

to reduce serum LDL-C levels. Schonfeld and coworkers (1982) noted a reduction of 15% in serum LDL-C as a result of a test diet comprised of 40% of total energy as fats and 300 mg/day of cholesterol. These researchers also observed variable individual responses of serum cholesterol and LDL-C levels to dietary alterations. The data shown in Figure 6 indicates the variable responses of individual serum LDL-C concentrations to either dietary sequence in the present study. It is therefore difficult to isolate the response of serum LDL-C to the ß-glucan due to the large individual variability inherent in this data.

4.2.3 Serum High Density Lipoprotein Cholesterol

In the present study serum HDL-C concentrations were unaffected by the inclusion of ß-glucan in the BD. This is consistent with the reports of Stasse-Wolthuis and coworkers (1980) and Judd and Truswell (1981) who fed diets containing soluble fibers as fruits and vegetables and rolled oats, respectively, to normolipidemics. Generally, soluble fiber has not been shown to affect serum HDL-C levels (Table 2).

The hypocholesterolemic effects of the amounts of dietary fat and cholesterol observed with both diets did not appear to affect individual serum HDL-C levels consistently (Figure 8). Brussaard and associates (1980) noted that a controlled diet consisting of 30% of energy as fat and 300 mg/day of cholesterol had no effect on serum HDL-C levels in normolipidemics. It therefore appears that neither the total diet nor the ß-glucan affected serum HDL-C levels in the research reported here.

4.2.4 Serum Triglycerides

In the present study, no significant ($p<0.05$) differences occurred between the BD and the WD in the responses of serum triglycerides. This result is in agreement with the research of Kay and Truswell (1977). As well, the investigations of Jenkins and coworkers (1979), Khan and associates (1981), Simons and coworkers (1982) and Aro and associates (1984) indicated that serum triglycerides were unaffected by the supplementation of guar gum in both normolipidemic and hyperlipidemic subjects.

Individual serum triglyceride responses to both diets were highly variable and inconsistent (Figure 10). However, during the initial two week dietary period, the BD and the WD resulted in similar mean reductions in serum triglycerides of the subjects (Figure 9). It cannot be ascertained whether the larger decreases in serum triglycerides observed in subjects initially consuming the BD are due to the effects of the soluble fiber, or to the fact that the three subjects with highest initial triglyceride levels and the most dramatic reductions were randomly assigned to begin the study with the BD. In support of the latter possiblity, it can be noted that the subjects consuming the BD during weeks three and four did not experience a further reduction in serum triglycerides, but instead a gradual increase was observed. It is not possible to conclusively determine the effects of the diet or the ß-glucan on serum triglycerides due to the large inter-subject variability in responses that occurred for both diets.

4.2.5 Serum Very Low Density Lipoprotein Cholesterol

Serum VLDL-C concentrations were not significantly ($p<0.05$) different between the two diets in the present study. Few investigations have examined the response of serum VLDL-C concentrations to soluble fiber ingestion. Khan and associates (1981) observed no reductions in this lipoprotein fraction when twenty-four normolipidemics consumed guar gum for one month. The inclusion of oat bran and oat meal in a fat-modified diet also produced no significant changes in serum VLDL-C levels (Van Horn et al., 1986). The effects of the two dietary periods on individual serum VLDL-C concentrations were similar to individual serum triglycerides (Figures 11 and 12). Initial mean serum VLDL-C levels were higher for subjects following the BD to WD sequence than for those subjects following the opposite sequence. This may be due to the data of subject SM, whose initial and final serum VLDL-C values were approximately two to three times higher than those of the other subjects. The results of the present study suggest that the ingestion of soluble fiber supplements do not effect serum VLDL-C concentrations in normolipidemics.

The results of the present research suggest that the two dietary treatments, the BD and the WD, were equally hypocholesterolemic. This was primarily due to the moderate fat and low cholesterol contents of the diets. Even though the BD was found to significantly lower serum cholesterol levels, the unequal distribution of the subjects consuming the two diets was probably responsible, in the most part, for this result. The actual effects of the ß-glucan on serum lipids may there-

fore have been negated by the properties of the diets, as well as the unequal distribution of the subjects in the experimental design. Individual variability in the responses of serum lipids also makes it difficult to conclusively determine the effects of the ß-glucan in this study.

Chapter V

CONCLUSION

It is concluded that ß-glucan, found naturally in barley flour, did not appear to have an effect on fecal acidic steroid and fat excretion in humans with the experimental conditions of the present study. Barley ß-glucan did not appear to have an effect on serum cholesterol, LDL-C, HDL-C, triglycerides, or VLDL-C levels, with the present research design. The results of the present study suggest that a 'carry-over' effect of dietary fiber exists in the gut. The sequence of feeding had an effect on the sterol excretion and may be explained, in part, by a 'carry-over' effect of the fiber. The amount of ß-glucan and the length of fiber ingestion may have been inadequate to produce measureable changes in fecal bile acids, fat excretion and serum lipid levels. In the present study, individual variability in fecal output and serum lipid responses to dietary alterations limited the conclusions that could be drawn from the data.

Although soluble dietary fibers have been shown to have hypocholesterolemic properties in many reported investigations, the results of the present research suggests that the metabolic basis for this response may not be an increase in fecal bile acid and fat excretion. Most of the data upon which this theory is based was not corrected for fecal output by internal markers. The results of the present study indicate that this hypothesis may not be reflective of the role of soluble fiber in lipid metabolism.

The hypocholesterolemic effects of ß-glucan may have been negated in the present study by the nature of the diets. The present research supports the finding that moderately low fat, low cholesterol diets are effective at decreasing serum cholesterol and LDL-C concentrations. In view of the published investigations which have shown increases in fecal bile acid and fat excretion as well as hypocholesterolemic effects of ß-glucan, it is necessary that subsequent experimental examination of these relationships should occur. The total composition of the experimental diet must be taken into account as a possible additive factor. The results of this study suggest that additional research is needed in order to define more accurately the metabolic effects of soluble fibers in the human gastrointestinal tract.

The present research does not support a hypocholesterolemic effect of the soluble fiber, ß-glucan, through increased fecal acidic steroid and lipid excretion in humans.

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APPENDIX A

TYPICAL DAILY MENUS

Barley Diet	g	Wheat Diet	g
Breakfast			
Orange Juice Crystals	15	Orange Juice Crystals	15
Corn Flakes Cereal[1]	28	Corn Flakes Cereal	28
Milk, 2% B.F.	125	Milk, 2% B.F.	125
Barley Biscuit[2]	100	White Bread	62
		Wheat Bran	3.8
Peanut Butter	17	Peanut Butter	17
Morning Snack			
Barley Biscuit	50	Wheat Biscuit[3]	50
Diet Jam	15	Diet Jam	15
Lunch			
Beef pattie, lean	100	Beef pattie, lean	100
Lettuce	20	Lettuce	20
Tomatoes, fresh	75	Tomatoes, fresh	75
Barley Biscuit	50	White Bread	62
Pears, canned	125	Pears, canned	125
Milk, 2% B.F.	125	Milk, 2% B.F.	125
Afternoon Snack			
Barley biscuit	50	Wheat biscuit	50
Diet Jam	15	Diet Jam	15
Dinner			
Beef pattie, lean	125	Beef pattie, lean	125
Cream-style corn	100	Cream-style corn	100
Instant mashed potatoes	30	Instant mashed potatoes	30
Barley biscuit	100	Wheat biscuit	100
Fruit cocktail, canned	125	Fruit cocktail, canned	125
Milk, skim	250	Milk, skim	250
Evening Snack			
Barley biscuit	50	Wheat biscuit	62
Diet jam	15	Diet jam	15
Peanut butter	34	Peanut butter	34
Fat			
Vegetable oil margarine[4]	30	Vegetable oil margarine	37.5
		Shortening, Crisco[5]	10

[1]Kellogg Salada Canada Inc., Rexdale, Ontario

[2]Recipe for a daily serving (400 g): (in grams) barley flour, 236.5; Crisco shortening, 44; skim milk powder, 29; salt, 3; and baking powder, 4.

[3]Recipe for a daily serving (200 g): (in grams) wheat flour, 120; Crisco shortening, 21; skim milk powder, 13.5; salt, 2; and baking powder, 2.

[4]Fleishmann's Corn Oil margarine, Nabisco Brands, Ltd., Toronto, Ontario

[5]Proctor and Gamble Inc., Toronto, Ontario.

APPENDIX B

MEAN DAILY ENERGY, PROTEIN, CARBOHYDRATE, AND FAT INTAKES OF SUBJECTS[1]

SUBJECT	BARLEY				WHEAT			
	ENERGY KJ (KCal)	PROTEIN g (%)[2]	CARBOHY-DRATES g (%)	FAT g (%)	ENERGY KJ (KCal)	PROTEIN g (%)	CARBOHY-DRATES g (%)	FAT g (%)[2]
BI	12770 (3052)	108.5 (14.2)	388.7 (50.9)	123.5 (36.4)	12602 (3012)	116.1 (15.4)	387.6 (51.5)	123.6 (36.9)
SM	12770 (3052)	108.5 (14.2)	388.7 (50.9)	123.5 (36.4)	13457 (3216)	123.8 (15.4)	423.3 (52.6)	129.2 (36.2)
MM	12770 (3052)	108.5 (14.2)	388.7 (50.9)	123.5 (36.4)	12602 (3012)	116.1 (15.4)	387.6 (51.5)	123.6 (36.9)
GW	12770 (3052)	108.5 (14.2)	388.7 (50.9)	123.5 (36.4)	12564 (3003)	116.1 (15.4)	383.7 (51.1)	123.4 (37.0)
AL	12426 (2970)	107.2 (14.4)	378.6 (51.0)	119.6 (36.2)	11847 (2831)	113.1 (16.0)	357.7 (50.5)	117.8 (37.4)
HP	12770 (3052)	108.5 (14.2)	388.7 (50.9)	123.5 (36.4)	12602 (3012)	116.1 (15.4)	387.6 (51.5)	123.6 (36.9)
RW	12770 (3052)	108.5 (14.2)	388.7 (50.9)	123.5 (36.4)	12577 (3006)	115.6 (15.4)	387.6 (51.5)	123.5 (37.0)
BK	12770 (3052)	108.5 (14.2)	388.7 (50.9)	123.5 (36.4)	12602 (3012)	116.1 (15.4)	387.6 (51.5)	123.6 (36.9)
Mean	12727	108.3	387.4	123.0	12607	116.6	387.7	123.5
±SD[3]	121.6	0.46	3.6	1.4	431	3.1	17.7	3.0

[1]Calculated values using U.S.D.A. Handbook #8, *Composition of Foods* (Watts and Merrill, 1963).

[2]% of total energy

[3]Standard Deviation

Appendix C

ANALYSIS OF VARIANCE: FECAL EXCRETION

Table 1: Total Bile Acids: PEG corrected for all subjects

DEPENDENT VARIABLE: BILECORR

SOURCE	DF	SUM OF SQUARES	MEAN SQUARE	F VALUE
MODEL	9	3280138.04504167	364459.78278241	3.25
ERROR	6	671972.16933333	111995.36155555	PR > F
CORRECTED TOTAL	15	3952110.21437500		0.0823

R-SQUARE	C.V.	ROOT MSE	BILECORR MEAN
0.829971	31.2247	334.65708054	1071.76875000

SOURCE	DF	TYPE I SS	F VALUE	PR > F
SEQU	1	713808.61537500	6.37	0.0450
PERSON(SEQU)	6	2159712.80400000	3.21	0.0906
DIET	1	111572.70062500	1.00	0.3568
SEQU*DIET	1	295043.92504167	2.63	0.1557

SOURCE	DF	TYPE III SS	F VALUE	PR > F
SEQU	1	713808.61537500	6.37	0.0450
PERSON(SEQU)	6	2159712.80400000	3.21	0.0906
DIET	1	210876.74504167	1.88	0.2191
SEQU*DIET	1	295043.92504167	2.63	0.1557

TESTS OF HYPOTHESES USING THE TYPE III MS FOR PERSON(SEQU) AS AN ERROR TERM

SOURCE	DF	TYPE III SS	F VALUE	PR > F
SEQU	1	713808.61537500	1.98	0.2087

Table 2: Total Bile Acids: PEG corrected for six subjects plus uncorrected for two subjects

DEPENDENT VARIABLE: BILECOR2

SOURCE	DF	SUM OF SQUARES	MEAN SQUARE	F VALUE
MODEL	9	2230700.81004167	247855.64556019	12.16
ERROR	6	122309.90933334	20384.98488889	PR > F
CORRECTED TOTAL	15	2353010.71937501		0.0033

R-SQUARE	C.V.	ROOT MSE	BILECOR2 MEAN
0.948020	17.8483	142.77598549	799.94375000

SOURCE	DF	TYPE I SS	F VALUE	PR > F
SEQU	1	312286.84704167	15.32	0.0079
PERSON(SEQU)	6	1898482.85733333	15.52	0.0020
DIET	1	16841.55062500	0.83	0.3984
SEQU*DIET	1	3089.55504167	0.15	0.7105

SOURCE	DF	TYPE III SS	F VALUE	PR > F
SEQU	1	312286.84704167	15.32	0.0079
PERSON(SEQU)	6	1898482.85733333	15.52	0.0020
DIET	1	19474.21504167	0.96	0.3661
SEQU*DIET	1	3089.55504167	0.15	0.7105

TESTS OF HYPOTHESES USING THE TYPE III MS FOR PERSON(SEQU) AS AN ERROR TERM

SOURCE	DF	TYPE III SS	F VALUE	PR > F
SEQU	1	312286.84704167	0.99	0.3588

Table 3: Total Fecal Lipid: PEG corrected for all subjects

DEPENDENT VARIABLE: FATCORR

SOURCE	DF	SUM OF SQUARES	MEAN SQUARE	F VALUE
MODEL	9	54.69262042	6.07695782	1.52
ERROR	6	23.93897333	3.98982889	PR > F
CORRECTED TOTAL	15	78.63159375		0.3135

R-SQUARE	C.V.	ROOT MSE	FATCORR MEAN
0.695555	44.4311	1.99745560	4.49562500

SOURCE	DF	TYPE I SS	F VALUE	PR > F
SEQU	1	11.99301042	3.01	0.1337
PERSON(SEQU)	6	34.98453333	1.46	0.3283
DIET	1	2.64875625	0.66	0.4463
SEQU*DIET	1	5.06632042	1.27	0.3028

SOURCE	DF	TYPE III SS	F VALUE	PR > F
SEQU	1	11.99301042	3.01	0.1337
PERSON(SEQU)	6	34.98453333	1.46	0.3283
DIET	1	4.57332042	1.15	0.3255
SEQU*DIET	1	5.06632042	1.27	0.3028

TESTS OF HYPOTHESES USING THE TYPE III MS FOR PERSON(SEQU) AS AN ERROR TERM

SOURCE	DF	TYPE III SS	F VALUE	PR > F
SEQU	1	11.99301042	2.06	0.2015

Table 4: Total Fecal Lipid: PEG corrected for six subjects plus uncorrected for two subjects

DEPENDENT VARIABLE: FATCORR2

SOURCE	DF	SUM OF SQUARES	MEAN SQUARE	F VALUE
MODEL	9	18.74445375	2.08271708	1.81
ERROR	6	6.89744000	1.14957333	PR > F
CORRECTED TOTAL	15	25.64189375		0.2417

R-SQUARE	C.V.	ROOT MSE	FATCORR2 MEAN
0.731009	32.6947	1.07218158	3.27937500

SOURCE	DF	TYPE I SS	F VALUE	PR > F
SEQU	1	7.93884375	6.91	0.0392
PERSON(SEQU)	6	9.97580000	1.45	0.3327
DIET	1	0.39375625	0.34	0.5797
SEQU*DIET	1	0.43605375	0.38	0.5606

SOURCE	DF	TYPE III SS	F VALUE	PR > F
SEQU	1	7.93884375	6.91	0.0392
PERSON(SEQU)	6	9.97580000	1.45	0.3327
DIET	1	0.59700375	0.52	0.4982
SEQU*DIET	1	0.43605375	0.38	0.5606

TESTS OF HYPOTHESES USING THE TYPE III MS FOR PERSON(SEQU) AS AN ERROR TERM

SOURCE	DF	TYPE III SS	F VALUE	PR > F
SEQU	1	7.93884375	4.77	0.0716

Table 5: Total Fecal Output (Wet Weight)

DEPENDENT VARIABLE: FECOUT

SOURCE	DF	SUM OF SQUARES	MEAN SQUARE	F VALUE
MODEL	9	16035.18004167	1781.68667130	2.69
ERROR	6	3973.01933333	662.16988889	PR > F
CORRECTED TOTAL	15	20008.19937500		0.1205

R-SQUARE	C.V.	ROOT MSE	FECOUT MEAN
0.801430	22.9871	25.73266191	111.94375000

SOURCE	DF	TYPE I SS	F VALUE	PR > F
SEQU	1	8455.00104167	12.77	0.0117
PERSON(SEQU)	6	5731.74333333	1.44	0.3338
DIET	1	1833.98062500	2.77	0.1471
SEQU*DIET	1	14.45504167	0.02	0.8874

SOURCE	DF	TYPE III SS	F VALUE	PR > F
SEQU	1	8455.00104167	12.77	0.0117
PERSON(SEQU)	6	5731.74333333	1.44	0.3338
DIET	1	1788.08504167	2.72	0.1504
SEQU*DIET	1	14.45504167	0.02	0.8874

TESTS OF HYPOTHESES USING THE TYPE III MS FOR PERSON(SEQU) AS AN ERROR TERM

SOURCE	DF	TYPE III SS	F VALUE	PR > F
SEQU	1	8455.00104167	8.85	0.0248

Appendix D

PAIRED T-TEST: SERUM LIPIDS

VARIABLE	N	MEAN	STANDARD DEVIATION	STD ERROR OF MEAN	T	PR>|T|
DIET=B						
TG	8	-13.25000000	30.56024495	10.80467822	-1.23	0.2597
TC	8	-19.37500000	18.50820281	6.54363786	-2.96	0.0211
LDL	8	-13.12500000	16.78806974	5.93547898	-2.21	0.0627
HDL	8	-1.75000000	5.03558764	1.78034808	-0.98	0.3584
VLDL	8	-4.25000000	8.74642784	3.09232922	-1.37	0.2117
DIET=W						
TG	8	-5.25000000	24.95567499	8.82316351	-0.60	0.5706
TC	8	-9.25000000	21.97888597	7.77070966	-1.19	0.2727
LDL	8	-10.37500000	17.50867132	6.19025011	-1.68	0.1376
HDL	8	0.62500000	3.66206421	1.29473522	0.48	0.6440
VLDL	8	-2.12500000	6.79153885	2.40117159	-0.88	0.4055

TG= Triglycerides

TC= Total Cholesterol

LDL= Low Density Lipoprotein Cholesterol

HDL= High Density Lipoprotein Cholesterol

VLDL= Very Low Density Lipoprotein Cholesterol

Appendix E

ANALYSIS OF VARIANCE: SERUM LIPIDS

Table 6: Serum Total Cholesterol

DEPENDENT VARIABLE: TC

SOURCE	DF	SUM OF SQUARES	MEAN SQUARE	F VALUE
MODEL	9	5774.83750000	641.64861111	9.29
ERROR	6	414.60000000	69.10000000	PR > F
CORRECTED TOTAL	15	6189.43750000		0.0067

R-SQUARE	C.V.	ROOT MSE	TC MEAN
0.933015	58.0796	8.31264098	-14.31250000

SOURCE	DF	TYPE I SS	F VALUE	PR > F
SEQU	1	617.60416667	8.94	0.0243
PERSON(SEQU)	6	4394.33333333	10.60	0.0056
DIET	1	410.06250000	5.93	0.0507
SEQU*DIET	1	352.83750000	5.11	0.0646

SOURCE	DF	TYPE III SS	F VALUE	PR > F
SEQU	1	617.60416667	8.94	0.0243
PERSON(SEQU)	6	4394.33333333	10.60	0.0056
DIET	1	222.33750000	3.22	0.1230
SEQU*DIET	1	352.83750000	5.11	0.0646

TESTS OF HYPOTHESES USING THE TYPE III MS FOR PERSON(SEQU) AS AN ERROR TERM

SOURCE	DF	TYPE III SS	F VALUE	PR > F
SEQU	1	617.60416667	0.84	0.3939

Table 7: Serum Low Density Lipoprotein Cholesterol

DEPENDENT VARIABLE: LDL

SOURCE	DF	SUM OF SQUARES	MEAN SQUARE	F VALUE
MODEL	9	3840.60000000	426.73333333	8.30
ERROR	6	308.40000000	51.40000000	PR > F
CORRECTED TOTAL	15	4149.00000000		0.0090

R-SQUARE	C.V.	ROOT MSE	LDL MEAN
0.925669	61.0160	7.16937933	11.75000000

SOURCE	DF	TYPE I SS	F VALUE	PR > F
SEQU	1	944.06666667	18.37	0.0052
PERSON(SEQU)	6	2756.93333333	8.94	0.0087
DIET	1	30.25000000	0.59	0.4721
SEQU*DIET	1	108.35000000	2.13	0.1950

SOURCE	DF	TYPE III SS	F VALUE	PR > F
SEQU	1	944.06666667	18.37	0.0052
PERSON(SEQU)	6	2756.93333333	8.94	0.0087
DIET	1	7.35000000	0.14	0.7183
SEQU*DIET	1	108.35000000	2.13	0.1950

TESTS OF HYPOTHESES USING THE TYPE III MS FOR PERSON(SEQU) AS AN ERROR TERM

SOURCE	DF	TYPE III SS	F VALUE	PR > F
SEQU	1	944.06666667	2.05	0.2017

Table 8: Serum High Density Lipoprotein Cholesterol

DEPENDENT VARIABLE: HDL

SOURCE	DF	SUM OF SQUARES	MEAN SQUARE	F VALUE
MODEL	9	211.33750000	23.48194444	1.71
ERROR	6	82.60000000	13.76666667	PR > F
CORRECTED TOTAL	15	293.93750000		0.2654

R-SQUARE	C.V.	ROOT MSE	HDL MEAN
0.718988	659.6170	3.71034590	-0.56250000

SOURCE	DF	TYPE I SS	F VALUE	PR > F
SEQU	1	10.83750000	0.79	0.4091
PERSON(SEQU)	6	150.60000000	1.82	0.2417
DIET	1	22.56250000	1.64	0.2477
SEQU*DIET	1	27.33750000	1.99	0.2084

SOURCE	DF	TYPE III SS	F VALUE	PR > F
SEQU	1	10.83750000	0.79	0.4091
PERSON(SEQU)	6	150.60000000	1.82	0.2417
DIET	1	10.83750000	0.79	0.4091
SEQU*DIET	1	27.33750000	1.99	0.2084

TESTS OF HYPOTHESES USING THE TYPE III MS FOR PERSON(SEQU) AS AN ERROR TERM

SOURCE	DF	TYPE III SS	F VALUE	PR > F
SEQU	1	10.83750000	0.43	0.5355

Table 9: Serum Triglycerides

DEPENDENT VARIABLE: TG

SOURCE	DF	SUM OF SQUARES	MEAN SQUARE	F VALUE
MODEL	9	7668.06666667	852.00740741	1.47
ERROR	6	3484.93333333	580.82222222	PR > F
CORRECTED TOTAL	15	11153.00000000		0.3303

R-SQUARE	C.V.	ROOT MSE	TG MEAN
0.687534	260.5433	24.10025357	-9.25000000

SOURCE	DF	TYPE I SS	F VALUE	PR > F
SEQU	1	264.60000000	0.46	0.5248
PERSON(SEQU)	6	6762.40000000	1.94	0.2200
DIET	1	256.00000000	0.44	0.5314
SEQU*DIET	1	385.06666667	0.66	0.4466

SOURCE	DF	TYPE III SS	F VALUE	PR > F
SEQU	1	264.60000000	0.46	0.5248
PERSON(SEQU)	6	6762.40000000	1.94	0.2200
DIET	1	112.06666667	0.19	0.6759
SEQU*DIET	1	385.06666667	0.66	0.4466

TESTS OF HYPOTHESES USING THE TYPE III MS FOR PERSON(SEQU) AS AN ERROR TERM

SOURCE	DF	TYPE III SS	F VALUE	PR > F
SEQU	1	264.60000000	0.23	0.6452

Table 10: Serum Very Low Density Lipoprotein Cholesterol

DEPENDENT VARIABLE: VLDL

SOURCE	DF	SUM OF SQUARES	MEAN SQUARE	F VALUE
MODEL	9	595.10416667	66.12268519	1.41
ERROR	6	281.33333333	46.88888889	PR > F
CORRECTED TOTAL	15	876.43750000		0.3485

R-SQUARE	C.V.	ROOT MSE	VLDL MEAN
0.679004	214.8250	6.84754619	-3.18750000

SOURCE	DF	TYPE I SS	F VALUE	PR > F
SEQU	1	4.00416667	0.09	0.7800
PERSON(SEQU)	6	472.93333333	1.68	0.2719
DIET	1	18.06250000	0.39	0.5576
SEQU*DIET	1	100.10416667	2.13	0.1943

SOURCE	DF	TYPE III SS	F VALUE	PR > F
SEQU	1	4.00416667	0.09	0.7800
PERSON(SEQU)	6	472.93333333	1.68	0.2719
DIET	1	2.60416667	0.06	0.8215
SEQU*DIET	1	100.10416667	2.13	0.1943

TESTS OF HYPOTHESES USING THE TYPE III MS FOR PERSON(SEQU) AS AN ERROR TERM

SOURCE	DF	TYPE III SS	F VALUE	PR > F
SEQU	1	4.00416667	0.05	0.8292