THE EFFECTS OF OAT FIBER AND CORN BRAN ON BLOOD SERUM
CHOLESTEROL AND TRIGLYCERIDE LEVELS

THESIS

Presented to the Graduate Council of the
North Texas State University in Partial
Fulfillment of the Requirements

For the Degree of

MASTER OF SCIENCE

By

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Denton, Texas
August, 1985
Broeder, Craig E., *The Effects of Oat Fiber and Corn Bran on Blood Serum Cholesterol and Triglyceride Levels.*
Master of Science (Home Economics), August, 1985, 46 pp.,
8 tables, bibliography, 37 titles.

Forty Sprague Dawley rats were randomly placed in five
groups with eight rats per group. Each group varied in
dietary composition for fiber type and carbohydrate source.
Groups one and two received oat fiber and either sucrose or
corn starch as the carbohydrate source. Groups three and
four received corn bran as the fiber source and either
sucrose or corn starch as the carbohydrate source. Group
five (considered the control group), received Purina
standard rat chow.

Analysis of variance showed only significant differ-
ences for food intake, and the control group had a
significantly higher food intake. Weight gain, serum
cholesterol and triglyceride levels showed no significant
differences.
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>LIST OF TABLES</td>
<td>iv</td>
</tr>
<tr>
<td>LIST OF ILLUSTRATIONS</td>
<td>v</td>
</tr>
<tr>
<td>Chapter</td>
<td></td>
</tr>
<tr>
<td>I. INTRODUCTION</td>
<td>1</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td></td>
</tr>
<tr>
<td>Sucrose</td>
<td></td>
</tr>
<tr>
<td>D-Fructose</td>
<td></td>
</tr>
<tr>
<td>Carbohydrate Digestion</td>
<td></td>
</tr>
<tr>
<td>Carbohydrate Absorption</td>
<td></td>
</tr>
<tr>
<td>Dietary Fiber</td>
<td></td>
</tr>
<tr>
<td>Pectins</td>
<td></td>
</tr>
<tr>
<td>Cellulose</td>
<td></td>
</tr>
<tr>
<td>Hemicellulose</td>
<td></td>
</tr>
<tr>
<td>Plant Gums</td>
<td></td>
</tr>
<tr>
<td>Lignins</td>
<td></td>
</tr>
<tr>
<td>Dietary Fiber's Effect On Serum Lipids</td>
<td></td>
</tr>
<tr>
<td>Chylomicrons</td>
<td></td>
</tr>
<tr>
<td>Very Low Density Lipoproteins</td>
<td></td>
</tr>
<tr>
<td>Low Density Lipoproteins</td>
<td></td>
</tr>
<tr>
<td>High Density Lipoproteins</td>
<td></td>
</tr>
<tr>
<td>Apoproteins</td>
<td></td>
</tr>
<tr>
<td>II. METHODS</td>
<td>23</td>
</tr>
<tr>
<td>III. RESULTS</td>
<td>29</td>
</tr>
<tr>
<td>IV. DISCUSSION</td>
<td>34</td>
</tr>
<tr>
<td>Proposed Experimental Design</td>
<td></td>
</tr>
<tr>
<td>Conclusion</td>
<td></td>
</tr>
<tr>
<td>BIBLIOGRAPHY</td>
<td>43</td>
</tr>
</tbody>
</table>
LIST OF TABLES

<table>
<thead>
<tr>
<th>Tables</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. Physiochemical Properties of Various Dietary Fiber Types</td>
<td>11</td>
</tr>
<tr>
<td>II. Composition of Diets For Groups One to Four</td>
<td>23</td>
</tr>
<tr>
<td>III. Food Intake Results</td>
<td>30</td>
</tr>
<tr>
<td>IV. Total Weight Gain Or Loss</td>
<td>31</td>
</tr>
<tr>
<td>V. Total Serum Cholesterol Levels</td>
<td>32</td>
</tr>
<tr>
<td>VI. Total Serum Triglyceride Levels</td>
<td>33</td>
</tr>
<tr>
<td>VII. Ranking of Variance</td>
<td>36</td>
</tr>
<tr>
<td>VIII. Proposed Experimental Group Divisions</td>
<td>39</td>
</tr>
</tbody>
</table>
LIST OF ILLUSTRATIONS

<table>
<thead>
<tr>
<th>Figures</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Leading Causes of Death United States</td>
<td>1</td>
</tr>
<tr>
<td>2. Changes in U.S. Carbohydrate Intakes</td>
<td>4</td>
</tr>
<tr>
<td>3. Structural Requirements of Active Sugar Transport</td>
<td>9</td>
</tr>
<tr>
<td>4. Comparison of Lipoprotein's Floatation &amp; Migration Rates</td>
<td>16</td>
</tr>
<tr>
<td>5. Food Intake Percentages Based on Gram Weight</td>
<td>24</td>
</tr>
<tr>
<td>6. Separation Method Used for Cholesterol/Triglyceride Analysis</td>
<td>25</td>
</tr>
</tbody>
</table>
CHAPTER I

INTRODUCTION

Cardiovascular related heart diseases (CHD) are among the leading causes of death in the United States today (Figure 1.) (26). CHD risk factors are numerous and not well understood at this time. These factors are based primarily on epidemiological studies such as the Framingham Studies instead of controlled studies like the more recent Lipid Research Clinics Coronary Primary Prevention Trial. Hence, these factors are primarily based on an association with CHD and may or may not be a direct cause of CHD (16).

Fig. 1--Leading causes of death in United States
In America, CHD affects 640,000 people per year in which 25 per cent are less than the age of sixty five (16). "CHD risk factors include: age, sex, familial history, serum cholesterol and triglyceride levels, the level of systolic and diastolic blood pressure, cigarette smoking, impaired vital capacity, obesity, abnormal glucose tolerance and diabetes, gout and hyperuricemia, hypothyroidism, socio-psychological stress, certain personal behavioral patterns, and physical inactivity" (3).

Several risk factors have shown a strong positive correlation to CHD. Among the strongest correlations are those of serum cholesterol and triglyceride levels. Epidemiological studies have shown that CHD incidents reflect serum cholesterol values of populations around the world (16). In countries where serum cholesterol levels were 185 to 335 mg/dl, there has been found a five-fold increased risk for CHD compared with countries where serum cholesterol is less than 185 mg/dl (16). Research has also shown that increased CHD incidents have been correlated with high circulating levels of serum triglycerides.

Understanding how man can control his circulating cholesterol and triglyceride serum levels may be helpful in the "primary prevention" of CHD (14). "Primary prevention implies control of atherogenic risk factors before the
appearance of clinical evidence of atherosclerotic coronary heart disease” (14).

A significant number of research studies have investigated dietary manipulations of serum cholesterol and triglyceride levels. Areas studied for comprehension of possible primary prevention factors include simple versus complex carbohydrate metabolism, dietary fiber's metabolic effects on serum triglyceride/cholesterol excretion, and saturated versus unsaturated lipids effects on serum triglyceride/cholesterol levels.

Carbohydrates

Dietary carbohydrates appear to have variable effects in their CHD relationship, depending on the carbohydrate class consumed. There are two general classifications of dietary carbohydrates. The first group is called the available dietary carbohydrates which contain monosaccharides, disaccharides, and the polysaccharides. The second is called the unavailable dietary carbohydrates which are also referred to as the various dietary fiber types (29). The simple sugars such as the monosaccharide fructose and the fructose-containing disaccharide, sucrose, have been strongly related to increased serum triglyceride/cholesterol levels. The unavailable carbohydrates such as the dietary fiber types have been shown to decrease serum triglyceride/cholesterol levels. Yudkin and Cohen
were the first researchers to show an etiological correlation with carbohydrates and the development of adult onset diabetes and heart disease (32).

The dietary food supply proportion of simple carbohydrates has increased from 31.9 to 52.8 per cent while complex carbohydrates have decreased from 68.1 to 47.2 per cent over the last seventy-five years. This change can be seen in Figure 2. (32). Since the publication of Yudkin and Cohen's research, researchers have found significant correlations relating type and amount of carbohydrates to altered serum triglyceride/cholesterol levels. Increased levels of cholesterol and circulating triglyceride levels are among the major positively correlated causative factors associated with CHD. Kritchevsky et al. (18) fed rabbits diets containing 40 per cent glucose, fructose, sucrose, lactose, or starch as part of a cholesterol free semipurified diet for four to ten months. Aortic atheroma at ten months was the most severe in the sucrose and fructose fed rabbits (29).

Research correlating CHD with increased dietary intake levels of the simple carbohydrates has led several groups to
make specific recommendations. Recommendations include decreasing dietary simple carbohydrates and increasing complex carbohydrates. The list of recommending groups include 1. The American Heart Association, 2. The Senate Select Committee on Nutrition and Human Needs of the U.S., 3. The Departments of Agriculture and Health, Education, and Welfare, 4. The Surgeon General, 5. The Food and Nutrition Board of the National Research Council (32).

Although a great amount of research has been conducted regarding carbohydrate metabolism and CHD, the exact mechanisms involved remains unclear. It appears that both the mono- and disaccharides, especially sucrose and fructose, are involved in inducing CHD causative lipogenic effects in the body. Yudkin and Cohen's research identified the disaccharide sucrose as the possible etiological agent in heart disease (24).

**Sucrose**

Sucrose is a water soluble nonreducing disaccharide. This disaccharide is hydrolyzed by sucrase in the small intestines. Increased sucrase activity occurs and the rate of intestinal sucrose transport decreases with increased amounts of dietary sucrose intake (24). In research by Michaelis IV (25), varying carbohydrate diets containing 10 to 50 per cent glucose, fructose, or sucrose showed that the highest lipogenic effects involved the disaccharide sucrose.
D-Fructose

D-Fructose is classified among the monosaccharide carbohydrates which are water soluble, colorless, and are crystalline solids (20). Fructose is part of the ketone family and is distinguished by having a carbonyl group attached to any carbon other than the terminal carbon. Fructose usually occurs in a ring structure with beta-D-fructofuranose being the most common form found. Research has implicated that the monosaccharide fructose as the major effector compound increasing lipogenic activities (24).

Sucrose and fructose have been both shown to increase a number of different rat liver lipogenic enzymes:

a. Glucose-6-phosphate dehydrogenase;
b. 6-phosphogluconate dehydrogenase;
c. Malic Enzyme;
d. Pyruvate kinase;
e. Citrate cleavage enzyme synthetase;
f. Acetyl CoA carboxylase;
g. Fatty acid synthetase.

Although both fructose and sucrose have been shown to have effects on lipogenic enzyme systems in the body, their mechanisms are different. The disaccharide effect appears to occur in the small intestines when sucrose cleaves sucrose into two monosaccharides (fructose and glucose), while the fructose effect is mediated in the liver. Presently,
there are two major hypotheses about the disaccharide effect that takes place in the body (24).

1. Feeding of disaccharides results in more rapid influxes of monosaccharides in the portal blood and liver than does feeding of monosaccharide equivalents.

2. Disaccharides are more effective than monosaccharides in causing the release of an insulintrophic gastrointestinal hormone into the blood, which in turn increases the magnitude of the lipogenic response.

Each hypothesis leads to many questions concerning its worth as a viable explanation for the monosaccharide and disaccharide effects.

Studies using various carbohydrate types with concentrations greater than 70 per cent of the total diet demonstrated that serum lipids were affected. The type of effect was based on the kind and amount of carbohydrate ingested. Macdonald and Braithwaite (23) compared in man two diets in which one was 75 per cent sucrose and the other was 75 per cent liquid raw starch. The results varied for each carbohydrate source; the 75 per cent sucrose diet increased serum triglycerides while serum cholesterol levels remained unchanged. The 75 per cent raw starch diet did not demonstrate increases in the triglyceride level and was accompanied by a decrease in cholesterol levels.

In man, moderate (40-60 per cent) carbohydrate diets also showed increased serum triglyceride levels when greater than 40 percent of the total carbohydrate came from sucrose.
When less than 30 percent of the calories are replaced by sucrose, research demonstrated little effect on the serum triglyceride or cholesterol blood levels in man (1).

Carbohydrate Digestion

Carbohydrate digestion begins in the mouth with the polysaccharide substrates being acted on by salivary amylase. However, the polysaccharide substrates seldom remain in contact with amylase for any appreciable amount of time. Therefore, little digestion takes place. In the stomach, amylase action is inhibited once the pH falls below the 6.6 to 6.8 range. The major site of carbohydrate digestion is in the small intestine. The disaccharides are hydrolyzed closer to the villus (8). Alpha-amylase is the major carbohydrate digestive enzyme. This enzyme hydrolyzes the alpha-1,4 glycosidic bonds. In straight chain starches, hydrolysis yields maltose and maltotrioses while branch chain hydrolysis leads to limit dextrin mixtures containing the alpha-1,6 linkages averaging six glucose residues each (6).

Carbohydrate Absorption

Carbohydrate absorption varies depending on the sugar type. Cori (7) made the first suggestion that intestinal membranes are selective for simple sugar. Cori found different absorption rates for various sugar types (galactose > glucose > fructose > manose > xylose > arabinose).
About thirty-five years later, Wilson and Landau (31) explained why some sugars required active transport and others did not. These researchers proposed that active transport required a 6-carbon D-pyranose ring with an hydroxal group at the second carbon position (See Figure 3.).

Fructose, which has been positively correlated with CHD, uses a different form of transport called facilitated carrier mechanism for absorptive transport. Microelectrode techniques have shown actively transported sugars involve both chemical and Na⁺ coupled electrical potential differences (27). Sugar absorption mechanisms have been shown to be so efficient that all digested carbohydrates are transported through the intestinal wall by the time the diet contents reach the lower jejunum (6).

![D-Pyranose Ring with a C-2 OH Group](image)
Dietary Fiber

Dietary fiber's importance has been clearly established in the diet of man, however its mechanism is unclear. It is generally accepted that fiber can interact considerably with various components of the diet (12). Fiber has several physiochemical features that are outlined in Table I (30). Three of the above features that appear to influence lipid metabolism most are water holding capacity, gel formation, and adsorption. One problem in establishing a dietary fiber mechanism is based around difficulties in fiber classification. Some researchers classify only the nondigestable polysaccharides of plant origin and lignin as dietary fibers, while others include waxes, cutin, indigestible protein, minerals and other substances abound in the plant cell wall to the dietary fiber classification (11). This classification ambiguity has made it difficult for researchers to develop a specific mechanism for the dietary action of fiber. The plant origin fibers are considered to be pectins, cellulose, hemicellulose, and gums.

Pectins

Pectins are found in plant cell walls and intercellular layers (11). Pectins have been implicated in the following: hypocholesterolemic effect, increased fecal sterol and lipid secretion, increased binding of bile salts, and binding of polyvalent cations (12). Although these effects have been
TABLE I

PHYSIOCHEMICAL PROPERTIES OF VARIOUS DIETARY FIBER TYPES

<table>
<thead>
<tr>
<th>Property</th>
<th>Type of Dietary Fiber or Component</th>
<th>Predicted Physiological Response</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water-holding Capacity</td>
<td>Polysaccharides enriched with polar groups: Particle size of material</td>
<td>Increased stool bulk, laxation, decreased intraluminal pressure</td>
</tr>
<tr>
<td>Gel Formation</td>
<td>Pectin, mucilaginous polysaccharides</td>
<td>Delayed gastric emptying, delayed nutrient absorption, increased transit time</td>
</tr>
<tr>
<td>Cation Exchange</td>
<td>Free ionic (carboxyl) groups (hemicelluloses)</td>
<td>Trace element imbalance</td>
</tr>
<tr>
<td>Adsorption</td>
<td>Lignin, pectin, mucilaginous fibers</td>
<td>Bile acid imbalance, laxation</td>
</tr>
<tr>
<td>Bacterial Digestibility</td>
<td>Polysaccharides</td>
<td>Altered microbial growth, altered chemical environment</td>
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</table>
known for some time, little is known about their chemistry and mode of action. Pectins have complex structures in which their main constituent is galacturonic acid units (12).

**Cellulose**

Celluloses are the most abundant organic compounds in nature. Celluloses are composed of beta-1,4, glucose linkages [up to 10,000 sugar residues long] (17). In man, the cellulase enzymes are missing and prevents the digestion of this biomolecule (20). Cellulose's principal role is of structural nature within the plant walls.

**Hemicelluloses**

Hemicelluloses are cell wall polysaccharides similar to celluloses but are smaller in size [often less than 200 sugar residues] and contain both pentose and hexose sugars (17). The hemicelluloses in combination with pectins form the backbone matrix of the plant cell walls (11). Once the matrix is formed, cellulose fibers are woven into the matrix.

**Plant Gums**

Plant gums are very complex structures. They contain several of the following components: D-glucuronic acid, D-galacturonic acid, as well as xylose, arabinose and mannose (11). The plant gums appear to be produced when a plant becomes injured and are not considered part of plant cell walls (11).
Lignins

Lignins are non-polysaccharide components that make-up the cell matrix of several plants. They offer mechanical support and help conduct the plant solutes (11). They are complex polymers formed by about forty oxygenated phenyl-propane units including coniferyl, sinaply, and p-coumaryl alcohols that have undergone complex dehydration (17). Of all naturally occurring polymers, lignins are the most resistant to digestive enzymes.

Dietary Fibers Effect On Serum Lipids

Dietary influences on lipids and cholesterol absorption, metabolism, and excretion have been major areas of research for answers to the causes of CHD. Evidence supporting the beneficial effects of dietary fiber in preventing hypercholesterolemia is numerous. Demonstrated either through epidemiological studies or experimental design some dietary fiber types have been shown beneficial as hypocholesterolemic effectors while others are not. It has been recently reported by the Lipid Research Clinics Program that a reduction of total plasma cholesterol by 13.4 per cent and reduction of low-density lipoprotein cholesterol by 20.3 per cent reflected a 24 per cent reduction in definite CHD death and a 19 per cent reduction in non-fatal myocardial infarction (21, 22). In this program, hypocholesterol type diets were used in one group, while the other
group used the serum cholesterol reducing drug cholestyramine.

Eastwood and Mitchell (9) stated that vegetable type fibers adsorb conjugated bile acids in the small intestines while unconjugated bile acids are adsorbed in the colon. This may be one way dietary fiber is helpful in lowering serum cholesterol levels since the conversion of cholesterol to bile acids is the major excretory cholesterol pathway.

Fiber binding mechanisms will vary from one fiber type to another. Dietary fiber binding mechanics for bile acids are influenced by both the chemical and physical forms of the fibrous material, the type of bile acid, its micellar form, pH, and osmolality (30). These various influences have been demonstrated in several studies and are believed to occur in the upper and lower intestines (17).

The following dietary fiber types appear to decrease atherogenic development: straw chow, oats, soy bran, rice bran, some fruits and vegetables, alfalfa, legumes, and mucilaginous or gel-forming fibers seem to have the greatest effect in lowering serum lipids (17). One way that gelling and mucilaginous fibers affect serum lipoproteins is believed to occur by decreasing lipid absorption (30). These fiber types have the ability to isolate micellar components on a non-selective basis. Both cholesterol and triglyceride absorption has been shown to decrease with diets greater then 15 per cent in fiber content (30).
Dietary fiber has been shown to decrease serum triglycerides and cholesterol content by increasing bile acid excretion with rats fed 5 percent pectin and 1 percent cholesterol diets. In man, vegetarian diets have shown higher sterol and bile acid excretion levels when calculated on the basis of milligrams per gram of feces (19). Oat bran has been shown to significantly decrease serum cholesterol and increase fecal bile acid loss while corn bran has not. Oat bran has a high content of beta-glucans which makes it mucilaginous in nature. Judd (15) has shown that oats were four times as effective compared to wheat in binding bile acids. Oat bran has been shown as a rich source of water soluble fiber [14.8 per cent]. Several animal experiments lend support for the hypothesis that dietary fiber may have a protective role against CHD.

It appears that these dietary fibers have their greatest effect by decreasing low-density lipoproteins [LDL] (30). Oat bran has been demonstrated to selectively decrease LDL concentrations with either no effect on high-density lipoproteins [HDL] or slight increases in HDL concentrations. In either respect, oat bran favorably alters the HDL/LDL ratios.

LDL are just one class of lipid carrying molecules known to exist in man. Lipoproteins are responsible for lipid transportation through the body's blood system. They help balance the influx/efflux of plasma cholesterol and
other lipid molecules. Lipoproteins play important roles in dietary absorption, synthesis, transportation, turnover, removal, metabolism, and excretion of dietary fats and cholesterol (10).

Lipoproteins are classified according to electrophoresis migration and ultracentrifuge floatation rates (13). A lipoprotein's density decreases as lipid content increases (Figure 4.) thus affecting its migration and floatation rates. These lipoproteins result from the many catabolic and anabolic reactions that occur in the body.

**Fig. 4--Comparison of lipoprotein's floatation & migration rates**

**Chylomicrons.**--They are formed in the body's intestinal mucosal cells and predominately contain triglyceride molecules along with six to ten percent cholesterol (4, 13, 5). After their formation, they leave the intestinal mucosal
cells and enter the lymphatic system until they are deposited in the blood via the subclavian lymphatic vein. In the lymph, chylomicrons will contain esters of cholesterol that will vary with the amount of cholesterol present in the diet. After entry into the blood, chylomicrons have both hepatic and non-hepatic metabolic phases (13). The chylomicron metabolic by-products are called chylomicron remnants. They are believed to be the major feedback inhibitor of cholesterol synthesis occurring in the liver (13). The main physiological function if chylomicrons is the transport of triglycerides, mainly of dietary origin, to tissues for immediate sources of energy or for energy storage materials (5).

Very Low Density Lipoproteins (VLDL).-- Very low density lipoproteins are rich in endogenous triglycerides synthesized in the liver and contain cholesterol of hepatic origin. In VLDL, the triglyceride ratio to cholesterol is approximately five to one (13). As VLDL enter the circulation, they are enriched with cholesterol esters by lecithin-cholesterol-acyl-transferase (LCAT) enzyme action (5). Very low density lipoproteins' main physiological action is the transportation of hepatic synthesized triglycerides to the tissues. Lipoprotein lipases act upon the VLDL molecules making them available for either cellular
energy or energy storage materials as seen in the chylomicrons (5).

Low Density Lipoproteins (LDL).--Low density lipoproteins are basically conversion by-products of VLDL metabolism. After VLDL delivers triglycerides to the body tissues, free and esterfied cholesterol, phospholipids, and apolipoproteins accumulated in the VLDL molecule (5). Eventually, the continued loss of triglycerides and the continued accumulation of cholesterol esters transforms VLDL to Intermediate density lipoproteins (IDL) and then finally into LDL. Low density lipoproteins' principal role is the transportation of cholesterol to body cells.

High Density Lipoproteins (HDL).--High density lipoproteins appear to originate in the liver where they arrive in the blood rich in APO E and are disc shaped in structure. However, HDL biogenesis is not clearly defined as of present (5). It is believed that HDL acquire their principal constituents in the blood. High density lipoproteins are considered to be cholesterol carriers to the liver for excretion preparation as bile acids in our feces.

Apoproteins.--They perform important metabolic functions related to specific domains on the three-dimensional structure of individual proteins (28). Originally named by their respective discoverers, ABC nomenclature proposed by
Alaupavic (2) has been adopted by most as a universal standard.

All known apoproteins bind to lipids and have amphipathic designations. The hydrophobic amino acids interact with the fatty acid portion of phospholipids and the hydrophilic portion interacts with the polar region of phospholipids (28). In addition, apoproteins have been shown to have catalytic functions with various enzymes. An example of these functions has been shown between APO CII to lipoprotein lipase (LPL). Lipoprotein lipase emulsion rates are increased in the presence of APO CII due to a decrease in random LPL substrate activity (28).

The following experiment investigated two contrasting fiber types: Corn bran versus oat fiber in relationship to metabolic changes occurring in blood lipids. The hypothesis was that oat fiber would have the most significant effect in lowering total serum cholesterol and triglyceride levels. In order to investigate this hypothesis two different carbohydrate sources were used, sucrose and corn starch. Sucrose being considered a hypercholesterolemic effector while corn starch was considered the hypocholesterolemic effector.


CHAPTER II

METHODS

Forty Sprague-Dawley male rats were used in this experiment. Each rat was housed in a separate feeding chamber. The rats were given four weeks to become accustomed to the new surroundings. During this time, food and water were given without restriction. After the four week adjustment period, the rats were randomly chosen and divided into five groups with eight rats per group. The experiment lasted a total of 16 weeks. Groups one to four varied in either the dietary fiber and/or dietary sugar intakes as seen in Table II. Group 5 (considered controls) continued eating the Purina standard rat chow.

TABLE II

COMPOSITION OF DIETS FOR GROUPS 1 TO 4

<table>
<thead>
<tr>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
<th>Group 4</th>
</tr>
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<tbody>
<tr>
<td>Sucrose</td>
<td>Corn Starch</td>
<td>Sucrose</td>
<td>Corn Starch</td>
</tr>
<tr>
<td>Oat Fiber</td>
<td>Oat Fiber</td>
<td>Corn Bran</td>
<td>Corn Bran</td>
</tr>
<tr>
<td>Vitamin Mix</td>
<td>Vitamin Mix</td>
<td>Vitamin Mix</td>
<td>Vitamin Mix</td>
</tr>
<tr>
<td>Mineral Mix</td>
<td>Mineral Mix</td>
<td>Mineral Mix</td>
<td>Mineral Mix</td>
</tr>
<tr>
<td>Casein</td>
<td>Casein</td>
<td>Casein</td>
<td>Casein</td>
</tr>
<tr>
<td>Corn Oil</td>
<td>Corn Oil</td>
<td>Corn Oil</td>
<td>Corn Oil</td>
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The total nutrient intake percentages based on gram weight can be seen in Figure 5.

**Food Intake Percentages**

![Food Intake Percentages](image)

Fig. 5--Food Intake Percentages Based On Gram Weight

All rats were fed 50 grams of their respective diets every other day and water was available 24 hours per day. The feedings occurred approximately the same time each day between 6:30 am. and 9:00 am. Food intakes were measured on each feeding day by subtracting the remaining food amounts from the initial 50 grams given. Water intakes were not measured. Once a week each rat was weighed at the start of that experimental week (Sunday). Any gains or losses in weight were noted and recorded.

At the end of the sixteenth week, all forty rats were fasted twelve hours before their final weighing. After
weighing, each rat was anesthetized with CO₂ and a blood sample was removed via the inferior vena cava after a abdominal cavity incision was performed. The amount of blood removed from each rat varied from eight to fifteen ml of blood. Blood samples were centrifuged in order to separate out blood serum samples. The blood serum samples were then placed in freezer storage until gas liquid chromatography (GLC) preparation took place. The methods used for GLC preparation can be seen in Figure 6. Two-tenths of a millileter of serum plasma were used in lipid separations for total serum cholesterol/triglyceride analysis.

1. .2 ml of Plasma
2. 50 ul of Tridecanoin (1ug/ul)
3. Added 2 ml isopropanol
4. Mixed on "Vortex" mixer
5. Added 1 ml of deionized water
6. Added 3 ml of Hexane
7. Mixed on "Vortex" mixer
8. Let settle and siphoned out lipid fractions

Fig. 6--Separation Method Used For Cholesterol and Triglyceride Analysis

The quantitative lipid profiles of the plasma were determined by means of GLC essentially as described by
Kuksis and his associates (1,2). The separations were performed on a 18" X 2 mm I.D. glass column packed with 1% Dexsil-300 (Carborane Silicone) on 100-120 mesh Supelcoport (Supelco chromatography supplies, Supelco-Park, Bellefonte, Pa.) using helium as a carrier gas in the temperature range of 185-350°C. The injection port was maintained at 325°C and detector at 350°C. After one minute isothermal period at 185°C the oven was temperature programmed at 6°C/min. up to 310°C where it was held for two minutes and then reprogrammed at 5°C/minute up to 350°C where it was held for five minutes.

The retention times and peak areas were recorded by means of an electronic integrator (Sigma-15 Perkin-Elmer). The peak identification and calculation of areas was performed in relation to the tridecanoylglycerol internal standard and were expressed as mg per 100 ml of plasma.

The data were expressed in means including the standard deviations for total food intake, total weight gain or loss, total serum cholesterol, and total serum triglyceride levels. One-way ANOVA statistical procedures were also used to test the variance about the means at a .05 alpha level along with the Duncan multiple range test. Since some raw data points seemed abnormally high or low, non-parametric statistics might have been appropriate to use. However as shown in the results section of the next chapter, one-way analysis of variance indicated non-parametric statistics
would not show any significantly different results from the parametric tests already performed and thus were not used.
CHAPTER BIBLIOGRAPHY


CHAPTER III

RESULTS

The experimental results have been listed as follows: food intake, total weight gain or loss, total serum cholesterol levels, and total serum triglyceride levels. The only area in which any significant difference between respective group means was seen occurred for food intake. All other areas indicated there were no statistical differences between means.

Intake values between the five groups ranged from a minimum value of 893.91 grams for group two (Oats and Corn Starch) to a maximum value of 1290.7 for group five (Standard Rat Chow). Analysis of variance indicated that there was a significant difference (at .05 alpha level) between group five's intake and the remaining four groups during this experiment (F value =13.81). Duncan's multiple range test verified that group five appeared to cause the significant F value of the one-way ANOVA (P = .0001). Table III shows these results.

Analysis of total weight gains for all rats indicated that there were no significant differences between group means using one-way ANOVA. The F value at a .05 alpha level was 0.28 with P equal to 0.8904. One interesting observation about the group weight gain data concerns group five.
### TABLE III

**FOOD INTAKE RESULTS**

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean*</th>
<th>Duncan Grouping Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 5 (Standard Rat Chow)</td>
<td>1290.7 ±101.82</td>
<td>A **</td>
</tr>
<tr>
<td>Group 4 (Corn Starch/Corn Bran)</td>
<td>986.7 ±101.38</td>
<td>B</td>
</tr>
<tr>
<td>Group 3 (Sucrose/Corn Bran)</td>
<td>947.4 ±121.43</td>
<td>B</td>
</tr>
<tr>
<td>Group 1 (Sucrose/Oat Fiber)</td>
<td>903.9 ±130.39</td>
<td>B</td>
</tr>
<tr>
<td>Group 2 (Corn Starch/Oat Fiber)</td>
<td>893.9 ±160.30</td>
<td>B</td>
</tr>
</tbody>
</table>

* Intakes listed in grams
** Alpha = .05, F Value = 13.81, P = 0.0001

Although group five had a significantly larger food intake than the other groups; they had the second lowest group weight gain mean (29.13 ±26.47). Group two (Oats and Corn Starch) which had the lowest food intake also had the lowest group weight gain mean (22.5 ±49.93) as would be expected. Standard deviations for this data set are considered highly variable leading to a large overlap among all groups. However, group five did show the lowest standard deviation among all test groups (+26.47) which was considered a factor.
in the data analysis conclusions in the next section. Table IV shows these results.

TABLE IV

TOTAL WEIGHT GAIN OR LOSS

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 3.</td>
<td>42.1 ±34.15</td>
</tr>
<tr>
<td>(Sucrose/Corn Bran)</td>
<td></td>
</tr>
<tr>
<td>Group 4.</td>
<td>37.3 ±50.13</td>
</tr>
<tr>
<td>(Corn Starch, Corn Bran)</td>
<td></td>
</tr>
<tr>
<td>Group 1.</td>
<td>35.5 ±38.69</td>
</tr>
<tr>
<td>(Sucrose/Oat Fiber)</td>
<td></td>
</tr>
<tr>
<td>Group 5.</td>
<td>29.1 ±26.48</td>
</tr>
<tr>
<td>(Standard Rat Chow)</td>
<td></td>
</tr>
<tr>
<td>Group 2.</td>
<td>22.5 ±49.93</td>
</tr>
<tr>
<td>(Corn Starch/Oat Fiber)</td>
<td></td>
</tr>
</tbody>
</table>

* Weight gains listed in grams
  Alpha = .05, F Value = 0.28, P = 0.89

One-way ANOVA analysis of serum cholesterol indicated that there were no significant differences between group means (F = 0.52 at .05 alpha level, P = .72). Group five had the lowest mean serum cholesterol levels (65.29 ±13.77). The highest mean serum levels was seen in groups two (Oats and Corn Starch) and three (Corn Bran and Sucrose). Group two had a mean serum cholesterol level of 76.86 ±20.75 while group three had a mean serum cholesterol level of 76.79 ±11.90. Table V shows these results.
### TABLE V

**TOTAL SERUM CHOLESTEROL LEVELS**

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 2 (Corn Starch/Oat Fiber)</td>
<td>76.86 ±20.75</td>
</tr>
<tr>
<td>Group 3 (Sucrose/Corn Bran)</td>
<td>76.79 ±11.90</td>
</tr>
<tr>
<td>Group 1 (Sucrose/Oat Fiber)</td>
<td>69.78 ±33.97</td>
</tr>
<tr>
<td>Group 4 (Corn Starch/Corn Bran)</td>
<td>69.26 ± 8.24</td>
</tr>
<tr>
<td>Group 5 (Standard Rat Chow)</td>
<td>65.29 ±13.77</td>
</tr>
</tbody>
</table>

* Serum Cholesterol Levels listed in mg/100ml.
  Alpha = .05, F Value = 0.52, P = 0.72

One-way ANOVA statistical analysis of total serum triglyceride also showed no significant difference about the group means (F value = .030 at .05 alpha level, P = 0.88). Group three (Corn Bran and Sucrose) had the highest serum triglyceride mean of 69.44 ±38.34 and group four (Corn Bran and Corn Starch) had the lowest serum triglyceride mean of 56.39 ±17.54. These results can be seen in Table VI.
### TABLE VI

#### TOTAL SERUM TRIGLYCERIDE LEVELS

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 3 (Sucrose/Corn Bran)</td>
<td>69.44 ±38.34</td>
</tr>
<tr>
<td>Group 1 (Sucrose/Oat Bran)</td>
<td>69.20 ±45.37</td>
</tr>
<tr>
<td>Group 5 (Standard Rat Chow)</td>
<td>68.38 ±27.28</td>
</tr>
<tr>
<td>Group 2 (Corn Starch/Oat Bran)</td>
<td>59.73 ±20.39</td>
</tr>
<tr>
<td>Group 4 (Corn Starch/Corn Bran)</td>
<td>56.39 ±17.54</td>
</tr>
</tbody>
</table>

* Serum Triglyceride Levels listed in mg/100ml.

Alpha = 0.05, F Value = 0.30, P = 0.88
CHAPTER IV

DISCUSSION

This experimental data showed a significant difference among the means only in the area of food intake. Also, the significant difference related only to the food intake of the animals in group five. According to Sabine (3), dietary fiber effects are considered to be of a protective nature working primarily on exogenous cholesterol. Some dietary fibers bind to cholesterol and bile salts in the small intestine which eventually leads to the loss of cholesterol and bile acids in the feces (3).

The major problem with this study was the experimental design, especially the lack of a true control group or groups. The experiment was designed in order to see the effect of two different dietary fiber types on blood lipid levels (Corn Bran and Oats) using two different forms of carbohydrate sources (Sucrose and Starch). Sucrose was considered the hypercholesterol effector agent and starch was the hypocholesterol effector agent. However, if Sabine (3) is correct in saying that dietary fiber effects exogenous cholesterol, then this experimental design could not have judged the corn bran or oat fiber's serum cholesterol lowering effect. The experimental design should have included groups that did not use cholesterol while other
groups contained one percent cholesterol (2). With this
design change, fiber's effect on exogenous cholesterol would
have been evaluated and the protective mechanisms of oat
fiber and corn bran could have been compared.

Complicating matters even more was the fact that the
Purina standard rat feed group was used as the control group
in this experiment. Purina standard rat feed contains
several different fiber types including oats but not corn
bran. This control group should have only been used as a
means to help determine if these experimental animals were
normal for Sprague Dawley rats used in other experiments.

In an attempt to understand what caused the results
seen in this experiment, a look at variance between dietary
groups was analyzed. This was done by ranking the variance
for each group in each area statistically analyzed and then
a numerical value for place position was assigned as shown
in Table VII.

It is interesting to notice that the groups with the
highest variability were groups one and two which contained
oat fiber. As mentioned above Purina rat chow also contains
oat fiber but not corn bran. One possible reason why there
were no significant differences shown by one-way ANOVA might
be due to the overlap in fiber types between groups one, two
and five.
TABLE VII

RANKING OF VARIANCE

<table>
<thead>
<tr>
<th>Intake</th>
<th>Weight Gain</th>
<th>Total Serum Cholesterol</th>
<th>Total Serum Triglyceride</th>
</tr>
</thead>
<tbody>
<tr>
<td>G2</td>
<td>G4</td>
<td>G1</td>
<td>G1</td>
</tr>
<tr>
<td>G3</td>
<td>G2</td>
<td>G2</td>
<td>G3</td>
</tr>
<tr>
<td>G1</td>
<td>G1</td>
<td>G5</td>
<td>G5</td>
</tr>
<tr>
<td>G5</td>
<td>G3</td>
<td>G3</td>
<td>G2</td>
</tr>
<tr>
<td>G4</td>
<td>G5</td>
<td>G4</td>
<td>G4</td>
</tr>
</tbody>
</table>

G1 = 16 pnts. 
G2 = 15 pnts. 
G3 = 12 pnts. 
G4 = 8 pnts. 
G5 = 9 pnts.

However, a positive point may be seen through observation of group five. Group five ate a significantly larger amount of feed (1290.7 grams) than the other groups, yet group five had the lowest serum cholesterol levels and second lowest weight gain data. It has been proposed that dietary fiber is helpful in reducing serum cholesterol levels and preventing obesity. This may be the rationale for the data results seen in this experiment concerning the Purina rat chow fed group five. However, Kritchevsky (1) has shown that the beneficial effects of chow meals are not clearly proven in rabbits and this may also be the case for the Sprague Dawley rat.
Proposed Experimental Design

Any future experimental design should include true control groups instead of standard chow fed rats for controls when studying different fiber type effects on serum cholesterol/triglycerides. Standard rat chow has several varieties of fiber types thus making a clear comparison to a single fiber type diet impossible. In the case of this experiment, standard rat chow contained the oat fiber as used in groups one and two but did not contain the corn bran fiber used in groups three and four. The standard rat chow also contained wheat germ, wheat middlings, and alfalfa meal fiber types. Therefore the control diet overlapped with two of the experimental diets possibly causing the lack of significance. This study would be improved by using control groups that did not contain any dietary fiber. This could possibly increase the experiment's chance of significance between groups at the .05 alpha level, and would allow the investigator to measure the effects due to fiber.

Experimental animals should be similar in age and sex. The animals should be housed separately for individual monitoring and to prevent behavior changes associated with group housing effects such as competition for food between dominant and nondominant animals.

Upon arrival, the animals should be initially weighed and placed in their respective cages. An accustomization period should be allowed for approximately a four week
period. Food and water should be adequately supplied with no restrictions placed on consumption during this initial time period. At the end of this period, the rats should be weighed for determining if normal growth rates have been occurring.

After the accustomization period each group should have its members randomly chosen. There should be seven groups containing eight animals per group. Group division should be based on dietary differences. These diets can be seen in Table VIII.

Feeding procedures should be changed from feeding 50 grams every other day as in this past experiment to feeding each rat amounts based on a set percentage of each individual animal's weight and the animal's previous food intake. This could decrease the possibility of a rat being underfed and being placed in an undesired fasting state.

Fasting metabolism differs greatly from normal metabolism depending on the duration of the fast. Initially, there is usually an increased loss of labile proteins and lean body mass. Thus, any decrease in lean body mass of organs such as the liver or pancreas could severely affect their individual functions and lipid metabolism would also be altered. Differences in fasting states may also increase the variation between animals within a group and variation between groups which would affect statistical tests such as ANOVA variance tests as used in this past experiment.
**TABLE VIII**

**PROPOSED EXPERIMENTAL DIET DIVISIONS**

<table>
<thead>
<tr>
<th>DIET 1</th>
<th>DIET 2</th>
<th>DIET 3</th>
<th>DIET 4</th>
<th>DIET 5</th>
<th>DIET 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>_______</td>
<td>_______</td>
<td>Oats</td>
<td>Oats</td>
<td>Corn Bran</td>
<td>Corn Bran</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>Cholesterol</td>
<td>Cholesterol</td>
<td>Cholesterol</td>
<td>Cholesterol</td>
<td>Cholesterol</td>
</tr>
<tr>
<td>Sucrose</td>
<td>Corn Starch</td>
<td>Sucrose</td>
<td>Corn Starch</td>
<td>Sucrose</td>
<td>Corn Starch</td>
</tr>
<tr>
<td>Vitamin Mix</td>
<td>Vitamin Mix</td>
<td>Vitamin Mix</td>
<td>Vitamin Mix</td>
<td>Vitamin Mix</td>
<td>Vitamin Mix</td>
</tr>
<tr>
<td>Mineral Mix</td>
<td>Mineral Mix</td>
<td>Mineral Mix</td>
<td>Mineral Mix</td>
<td>Mineral Mix</td>
<td>Mineral Mix</td>
</tr>
<tr>
<td>Casein</td>
<td>Casein</td>
<td>Casein</td>
<td>Casein</td>
<td>Casein</td>
<td>Casein</td>
</tr>
<tr>
<td>Corn Oil</td>
<td>Corn Oil</td>
<td>Corn Oil</td>
<td>Corn Oil</td>
<td>Corn Oil</td>
<td>Corn Oil</td>
</tr>
</tbody>
</table>

Diet No. 7 will be fed Purina Standard Rat Chow
Alternative methods for blood sample removal should also be used. Blood sampling from the inferior vena cava did not yield sufficient blood amounts for a large variety of tests and prevented repetitive sampling from the same animal. Blood serum amounts should be adequate to include analysis of total cholesterol/triglycerides, lipoproteins (LDL and HDL), and enzyme activities (i.e., LCAT activity).

Fecal bile acid assays are another important investigation area that should be included in an experiment of this type. Several studies have used fecal analysis for attempting to explain the interactions of blood cholesterol levels, enzyme activities, and fecal cholesterol and bile acid excretions.

Statistical analysis should include descriptive statistics such as means and standard deviations. One way ANOVA and Duncan's multiple range test should also be used.

Conclusion

In conclusion, this study cannot claim many statistically supported differences due to fiber type under these experimental conditions. Future experiments need to deal with the problems of excessive variability and the lack of a true control group. Not until these two areas are changed can any significant statements be possibly made regarding the effects of corn bran and oat fiber on serum chole-
sterol/triglyceride levels or any other areas such as possible obesity prevention effects.


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Books


Articles


Public Documents


Unpublished Materials