EFFECT OF DIETARY FIBER AND CARBOHYDRATE SOURCE ON GLUCOSE TOLERANCE, INSULIN RESPONSE AND LIPOGENIC ENZYME ACTIVITY

by

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INTRODUCTION

Researchers look to discover the etiology of disease and the relationship of nutrition to disease. Fiber in the diet has been ignored until recent progress in the knowledge of fiber composition, coupled with epidemiological observations has indicated that dietary fiber plays a significant role in health maintenance (1). Advances in fiber research have been limited by the inconsistent definition of fiber and its components.

Fiber can be divided into two categories: crude fiber (CF) and dietary fiber (DF). A test for crude fiber was developed 150 years ago to estimate the indigestible components in animal feed (2, 3). Crude fiber is defined by the association of Official Analytical Chemists as "the residue of a feeding material after treatment with boiling sulfuric acid, sodium hydroxide, water, alcohol, and ether" (1). This method accounts for approximately 20 per cent of hemicelluloses, 30 to 50 per cent of cellulose and 10 to 50 per cent of lignins present in plant cell walls (2, 4). Crude fiber estimates do not include many of the cell wall polysaccharides that are undigested by man (5) and are composed primarily of lignin and cellulose (3).

Dietary fiber includes components of plant cell walls remaining after hydrolysis by enzymes in the human digestive tract (3, 5-6). Constituents of dietary fiber include hemicelluloses, pectic substances, gums, and musilages in addition to the components of crude fiber (2, 5-10). Components of dietary fiber are classified as follows.

TABLE 1

Components of dietary fiber (3)

Principal sources in the diet	Description	Classical nomenclature
Structural materials of plant cell wall	Structural polysaccharides	Pectic substances
		Hemicelluloses
		Cellulose
	Noncarbohydrate constituents	Lignin
		Minor components
Nonstructural materials either found naturally or used as food additives	Polysaccharides from a variety of sources	Pectic substances
		Gums
		Musilages
		Algal polysaccharides
		Chemically modified polysaccharides

Delineation between crude and dietary fiber is necessary because dietary fiber levels are 3-5 times greater than estimated crude fiber levels (2). Underestimation of fiber in food occurs because during crude fiber determination the water and alkali-soluble hemicelluloses are removed and therefore not measured (1).

Fiber content in food composition tables is reported on a crude fiber basis providing only a rough estimate of fiber in the diet. Lack of agreement on methods of analysis for dietary fiber make development of more

accurate tables difficult. A model by Saunders (11) may establish the relationship between dietary fiber and crude fiber. A straight-line correlation was substantiated where DF (y) and CF (x) equal:

$$y = 4.17\ x - 0.27$$

Bran is formed in the milling process of flour (12) and is composed of the external layers of the grain. These layers include the pericarp which consists of the epidermis, cuticle, and cross-cell and tube-cell layers; the seed coat; the hyaline layer; and the aleurone layer. Separation of the endosperm and outer layers is not complete; part of the endosperm is removed with the bran layer. The endosperm may be responsible for altering the physical properties of bran.

Epidemiological observations stimulated research by Burkitt (13, 14), Walker (15) and Trowell (2, 4, 16) into the incidence of non-infective diseases such as diverticular disease, carcinoma, hemorrhoids, appendicitus, irritable colon, ulcerative colitis, coronary heart disease, diabetes mellitus, gall stones, and obesity in rural African populations. The high-fiber, high carbohydrate diet of the Africans was postulated to have a protective effect.

The incidence of "fiber-related" diseases such as diabetes mellitus parallels the consumption of fiber and refined food (4, 7, 17-20). In historical perspective, in 1770 in western societies over 500 g of bread were consumed daily compared with 180 g daily in 1970 which is a drop in crude fiber from cereals from 1.1 to 2.8 g per day to 0.4 g per day in 1970 (21). The average crude fiber intake in African men is 10 to 25 g daily whereas British adults consume only 4 g of crude fiber daily (6). Decreased fiber consumption resulted from decreases in wheat flour intake, invention of the

roller mill to increase the refinement of flour, reduction in potato intake, and substitution of fiber-depleted cereals for oatmeal porridge (21). In England, during wartime food restriction when high-fiber National flour was produced, diabetic death rates fell by over 50 per cent (4, 18). The rarity of diabetes mellitus in rural Africans and the increased incidence in urban Africans suggests fiber may be a protective factor in diabetes mellitus.

The effects of fiber are attributed to its physico-chemical properties (22). Current claims concerning the effects of fiber and the status of the claims are listed in Table 2 (20, 23-36, 42). Fiber is not a panacea however, and may decrease absorption of minerals such as calcium, iron, copper, zinc, selenium, and magnesium (24, 37-41, 42).

The purpose of the following study was to determine effects of type and amount of dietary fiber and carbohydrate on glucose tolerance, insulin secretion during a glucose load, and lipogenic enzyme activity in liver tissue. Throughout the rest of the paper the term fiber shall be used to represent dietary fiber, unless otherwise specified. Wheat bran which is designed for research was used as the fiber source.

TABLE 2

Claims of dietary fiber and status of the claims (42)

Definite Value:
Constipation problems - increasing water in feces.
Reducing calorie value of foods.
Probable Value:
Treating diverticular disease.
Reducing serum cholesterol (certain types).
Improving glucose tolerance.
Fiber May Be Related to Prevention Of:
Colon-rectal cancer.
Ischemic heart disease.
Gallstones.
Dental Caries.
Appendicitis.
Hiatus hernia.
Varicose veins.
Obesity.
Phlebitis.
Hemorrhoids.
Irritable bowel, ulcerative colitis.
Harmful effects of massive doses of some food additives.

REVIEW OF LITERATURE

Glucose Tolerance

Claims of dietary fiber are listed in Table 2; however, the credibility of these claims is not clear when the results from all studies are compiled. Glucose tolerance is a "measure of the ability of the body to utilize a known amount of glucose" (43). This test provides an index of the metabolic effect of changes in dietary carbohydrate.

Fiber in animal diets. Trowell and Woodgreen (6) in testing the dietary fiber hypothesis fed rats high fiber mixed-vegetable diets with 2.6 g CF per 100 kcal. Control animals received Purina® laboratory rat chow containing 1.2 g CF per 100 kcal. "Fiber-deficient" controls developed a decreased glucose tolerance. This study suggests that the fiber level is the important criterion in carbohydrate tolerance. However, Lin and Anderson (44) found that a high carbohydrate starch-bran diet was associated with slightly higher plasma glucose levels, but glucose tolerance tests were unaltered. Harland and co-workers (45) also suggested that adding various fiber sources such as soya flock, alfacel, wheat bran, sugar cane pulp, and sugar beet pulp act to increase fasting blood glucose levels. The data from studies by Lin and Anderson (44) and Harland and co-workers (45) suggest that source rather than level of dietary fiber is the critical factor in glucose metabolism.

Human fiber studies. Wide variations of fiber source have been used in human studies. Guar gum and pectin resulted in the flattening of glucose tolerance curves in all studies reviewed (17, 46-47). Both normal and diabetic subjects were used in these studies. Diabetic subjects provide a good

test model because of their sensitivity in carbohydrate metabolism to dietary change and the application of enhanced glucose tolerance in diabetes. Improved glucose tolerance was noted when 26 g of crude fiber as corn bran, soy hulls, freeze dried apple powder, or freeze dried carrot powder were incorporated in the diet (48). When two types of wheat bran (hard red spring wheat and soft white wheat) were used by Munoz and co-workers (48) no effect on glucose tolerance occurred. Results in human studies support the concept that the source of fiber may mediate its effect on glucose tolerance. One study not supporting the effectiveness of fruit and vegetable fiber found that high fiber diets (high fiber diets contained five times the amount of fiber as low fiber diets) composed of fruits and vegetables did not significantly alter fasting blood glucose (49). The study did not measure the effects of a glucose load however.

Fiber is hypothesized to alter glucose tolerance by changing glucose absorption. Alterations in glucose absorption rates could be influenced by the gel forming properties of the water soluble fractions of fiber (50). This property would decrease the rate of glucose diffusion, thereby increasing the area in the intestine over which absorption could occur. Any change in the gastric emptying time or transit time would decrease the rate of glucose absorption and produce a slower rise in blood glucose. In addition, fiber may modify the digestive process by altering the release of gastrointestinal hormones or pancreatic secretions. This theory by Cummings (50) proposes that, in general, fiber improves glucose tolerance by decreasing the rate of glucose absorption through modification of the digestive process.

Effects of carbohydrate source in rat studies. A second major factor

to consider when evaluating glucose tolerance curves is the source of carbohydrate in the diet; specifically, sucrose or simple carbohydrates versus complex carbohydrates such as starch. In rat studies, significant impairment of glucose metabolism was detected when sucrose represented the main carbohydrate source (51-53). Impairment, measured by response to a glucose tolerance test and the corresponding insulin response, could be induced by as little as 33 per cent sucrose (52). Carbohydrate composed 70 per cent of the calories in the study by Vrana et al. (53). Development of glucose impairment was related to the amount of sucrose in the diet (52).

Carbohydrate source in human studies. In a human study by Crapo and colleagues (54), simple carbohydrates elicited a higher glucose response curve than starch. Results of Cohen and co-workers (55) support this trend for improved glucose tolerance with complex carbohydrates versus sucrose. Results of animal and human studies appear to concur that simple carbohydrates impair glucose metabolism.

Carbohydrate level in rat studies. Not only is the source of carbohydrate important, but the percentage of calories from carbohydrate significant. Eaton (56) found that a long-term glucose supplementation produces normal serum glucose levels and a normal-to-improved glucose tolerance in rats. Diabetes therapy has traditionally recommended restricted carbohydrate intake to prevent abnormal glucose curves. However studies with diabetic patients demonstrated lower fasting glucose levels and improved glucose tolerance when a high carbohydrate diet was consumed (57-61). To test the effect of a high carbohydrate diet, patients were initially fed a basal diet containing from 40 (60) to 45 (57-59, 61) per cent of calories from carbohydrate.

Subjects were then placed on a high carbohydrate diet containing 75 (59-60) to 85 (57-58, 61) per cent of calories from carbohydrate. Glucose tolerance and insulin secretion were measured in all, but one study to demonstrate the effect of dietary treatment on carbohydrate metabolism (57-59, 61). The one study (60) measured glucose tolerance only. Similar results were reported in normal men (62). Recent research suggests that high carbohydrate diets improve glucose tolerance and need not be rigidly restricted in the diabetic diet.

Non-specific Factors Influencing Glucose Tolerance. The data presented thus far concerning glucose tolerance appears to be fairly consistent; however, the relationship of results in the studies seems questionable after comparing the experimental methods. Differences between studies which could influence results include:

1. Size of the glucose load and method of glucose administration. Glucose dosage ranged from 250 mg per 100 g body weight to 350 mg per 100 g body weight. Glucose was given either orally or intravenously.
2. Level of carbohydrate in the diet. By percentage of calories, carbohydrate content ranged from 54 per cent to 85 per cent.
3. Length of dietary regime. Length of studies varied from 48 hours to 13 weeks. Classification of these studies as short-term or long-term varied between investigators.
4. Age of the animal. Glucose tolerance is known to decrease markedly with age (63). Age of the animal is of particular importance in long-term studies.

5. Body weight of the subject. Evidence suggests that glucose tolerance decreases with obesity in humans (63).
6. Diurnal variation. A decrease in glucose tolerance is observed when the test is performed in the afternoon (63).

The influence of these factors should be considered before conclusions on the results of these studies are made.

Insulin

Insulin is an endocrine hormone produced by the B-cells of the islets of Langerhans in the pancreas (64). The pancreas produces many substances both endocrine and exocrine. Insulin-containing cells aggregate in the center of islet tissue, disturbances of this normal cell distribution may be functionally related to pathological disorders such as diabetes mellitus (65).

Many of the same factors which stimulate glucose tolerance, stimulate insulin secretion. Hormone secretion may be directly regulated by stimulation of the B-cells of the pancreas or indirectly by influencing the serum glucose level. The pancreas is extremely sensitive to blood glucose levels; hyperglycemia is a potent stimulus for insulin release (66). Therefore any factor altering blood glucose will indirectly stimulate insulin secretion.

Fiber and insulin secretion in humans. Supplementation of the diet with guar gum and pectin resulted in lower insulin responses which corresponded with lower glucose levels (17, 47). Munoz and co-workers (48) who tested the effects of various fruit and vegetable powders and cereal fibers found only the fruit and vegetable fibers effective in improving insulin release and/or peripheral activity. The cereal fibers, including wheat bran had no significant

effect on insulin release. Since wheat bran did not affect the glucose tolerance curve, no effect on insulin would be expected. When wheat bread was combined with baked beans and rice to form a high fiber diet, insulin response was less than half that caused by a liquid, fiber depleted meal (67). The liquid meal was composed mainly of glucose and dextrose. This study may have been actually comparing the effects of high carbohydrate diets with a complex and a simple source of carbohydrate. The physical form of fiber was demonstrated by Haber et al. (20) to influence insulin response. Disruption of fiber by mechanical processing such as blending produced greater insulin release than did the intact food. The results of Behall and co-workers (49) dispute the effects of dietary fiber on carbohydrate metabolism. No change in fasting glucose levels or insulin was detected following treatment with a high fiber diet. The literature for the most part, however, supports lower insulin levels with high fiber diets composed of fruit or vegetable fiber. When wheat bran is the fiber source no effects have been noted.

Fiber in animal studies. In the literature reviewed no study paired insulin release and dietary fiber although glucose tolerance was measured. A possible cause for lack of data in this area is the procedural difficulty of obtaining adequate blood samples to run both insulin and glucose determinations. The ineffectiveness of wheat bran in improving glucose tolerance in the experiments suggests, however, that wheat bran would have no effect on insulin secretion (44-45).

Carbohydrate source in human studies. When starch and sucrose were paired as the carbohydrate source (carbohydrate composed 30% of calories), fasting serum insulin levels and response to a glucose load were significantly

higher with the sucrose than with the starch diet (68). This result paralleled the glucose tolerance response. When sucrose and glucose were paired as the carbohydrate source, both diets significantly lowered plasma in insulin levels (62). In this study (62), carbohydrate accounted for 80 per cent of calories. This level of carbohydrate would be categorized as a high carbohydrate diet. The reduced plasma insulin may be influenced more by the level of carbohydrate rather than source in this study.

Carbohydrate sources in animal studies. Results support that simple carbohydrate induces a greater and faster insulin response than does complex carbohydrate (51, 54, 69-70). Changes in insulin secretion were noted in both fasting serum insulin levels and postprandial response. Hallfrisch and associates (70) reported the most marked difference between sucrose and starch meal-fed rats was four hours after the glucose load. At four hours insulin levels in sucrose meal fed rats were 52 μU/ml as opposed to 29 μU/ml for starch meal fed rats. With ad libitum diets insulin response was 77 μU/ml for sucrose fed rats and 35 uU/ml for starch fed rats. The insulin levels in sucrose fed rats were nearly twice as high as starch fed rats. The elevation of serum insulin levels correlates with the increased blood glucose found with sucrose diets; the higher levels of blood insulin in sucrose fed rats may in part be in response to repeated high levels of blood glucose. A study by Vrana and coworkers (53), however, suggests sucrose fed rats do not develop hyper- or hypoinsulinism. A deteriorated glucose tolerance was noted, but was attributed to factors other than sucrose feeding.

Studies where sucrose is the main source of dietary carbohydrate represent fiber-depleted diets. When the effect of carbohydrate source is viewed

from this perspective, then diets with high sucrose feeding would be expected to produce results similar to studies with low fiber. In most of the literature this relationship is supported.

Carbohydrate levels in animal studies. Lowering of insulin response when glucose tolerance is enhanced by high carbohydrate feeding would be expected. Eaton (56) found that basal insulin levels and insulin response to oral glucose were reduced even when serum glucose levels were normal and glucose tolerance was normal to slightly improved. This finding suggests that high carbohydrate diets may act through more than one pathway (control of blood glucose) to alter insulin response.

Carbohydrate levels in human studies. Initial examination of research supports the premise that improved glucose tolerance produced by high carbohydrate feeding results in lowered insulin response (71). However, insulin response to high carbohydrate diets in other research suggests secretion of insulin does not follow this highly predictable pattern. In contrast, Kolterman and co-workers (72) found that both short-term and long-term feeding of a high carbohydrate diet led to hyperinsulinemia. Intermediate results have also been found by other workers. The reports on the effect of high carbohydrate feeding on insulin response range from a lowering of fasting insulin levels with no effect on insulin response to oral glucose (58), to slight lowering of insulin response (59), to no significant effects on insulin response in the fasting state or during a glucose tolerance test. Data from these studies on high carbohydrate diets appear to be very contradictory; however, factors other than carbohydrate level in the diet must be taken into consideration when examining the results.

The primary biological effects associated with insulin are initiated by hormone-receptor interaction. According to Cuatrecasas (73), a receptor is defined by its ability to recognize and interact with a high degree of specificity, to produce metabolically significant occurrences. The insulin receptor is a glycoprotein with surface peptides which probably act in transmitting the hormone signal (73). Each receptor molecule appears to be composed of 4 subunits which disassociate with binding of insulin to the receptor (74). Failure of a normal concentration of insulin to produce a normal biological response is termed insulin resistance (75). Insulin resistance is a state usually associated with glucose metabolism. Resistant states can therefore include states of decreased responsiveness where the maximum response is reduced, but the dose-response relationship remains unchanged or states of decreased sensitivity where the maximum response is unaltered, but the dose required to produce a normal response is increased (75). Resistant states can be reduced to three instances: 1) prior to insulin-receptor interaction, 2) at the receptor, and 3) at steps following insulin-receptor interaction. Theoretically factors creating resistance at the pre-receptor site decrease the concentration of free insulin. Receptor resistance to insulin could result through changes in the receptor affinity or number. The final point in modification of insulin action can be altered by any changes in the "coupling function" or steps in the pathway to biological activity. Insulin resistance could account for hyperinsulinism producing a normal glucose curve.

The reverse of insulin resistance is an increased sensitivity whereby hormone-receptor interactions are enhanced to produce a biological effect

with lower insulin levels. An increased sensitivity to insulin would account for the normal to slightly improved glucose tolerance noted with lower insulin levels. The factors outlined in non-specific factors modifying glucose tolerance (p. 9) also influence insulin sensitivity and resistance.

Lipogenic Enzyme Activity

One pathway of glucose metabolism is synthesis of fatty acids in liver cells and adipose tissue (76). Activities of several enzymes are an indicator of lipogenesis; lipogenic enzymes include fatty acid synthetase, citrate cleavage enzyme, acetyl CoA carboxylase, malic enzyme, 6-phosphogluconate dehydrogenase and glucose-6-phosphate dehydrogenase (77). Lipid synthesis requires NADPH (78). Two enzyme systems that produce NADPH reducing equivalents are: glucose-6-phosphate dehydrogenase (G-6-P-D) and malate dehydrogenase (malic enzyme).

Benyen and co-workers (79) postulate the rate of lipogenic enzyme activity is controlled by two types of mechanisms: 1) long-term mechanisms and 2) short-term control. The long-term mechanism, requiring six hours to come into effect, changes absolute amounts of the enzyme and is influenced by nutritional and hormonal factors. The short-term control, taking 15 to 30 minutes to become active, changes the activity of the existing enzyme through hormonal controls.

G-6-P-D. Insulin is considered the stimulatory hormone of lipogenesis (79). However, not all research supports the claim that lipogenic enzymes are under the direct hormonal regulation of insulin. Rudack and associates (80) found that insulin had no direct effect on the regulation of G-6-P-D

levels in rat liver. The synthesis of G-6-P-D increased in proportion to the caloric consumption of carbohydrate, regardless of the ability of the carbohydrate source to stimulate insulin secretion from the pancreas. Kukulansky and Yagil (81) placed mice with streptozotocin-induced diabetes on a fat-free diet. The results demonstrated that lipogenic enzymes could be induced in the absence of insulin. When exogenous insulin was paired with an otherwise noninducing diet, such as high fat or laboratory chow, insulin did not increase the enzyme activity. The diet regime of no fat can also be considered a high carbohydrate diet since 62 per cent of calories were from carbohydrate. The two studies (80-81) yield similar results, i.e., control of enzyme activity by carbohydrate intake with no direct effect of insulin, if the fat free diet of Kukulansky and Yagil (81) is considered a high carbohydrate diet and the high fat diet is considered low carbohydrate.

In contrast, Weber and Convery (82) found that hypoinsulinism decreases G-6-P-D activity and addition of insulin increases enzyme activity. Insulin acts to increase G-6-P-D activity by increasing enzyme synthesis (77, 83-84). Differences in the studies may be accounted for by distinguishing the direct and indirect influences of carbohydrate and insulin. Possibly the actions of carbohydrate are indirect by stimulating insulin secretion or vice versa.

One factor influencing enzyme activity which has received little attention is dietary fiber. Lin and Anderson (44) fed both normal and diabetic rats a starch-bran diet or a high sucrose diet. Normal sucrose-fed animals had significant increases in the level of G-6-P-D activity. Enzyme activity was significantly lower on the starch-bran diet. However, diabetic rats irrespective of diet had similar levels of G-6-P-D. Because fiber was not paired

with sucrose also, the differences noted may stem from differences in dietary carbohydrate source. Cohen and co-workers (85) fed rats high carbohydrate diets with either starch or sucrose as the carbohydrate source. Results indicated that increasing the carbohydrate level produced increased lipogenic activity. However, G-6-P-D activity was elevated significantly more when sucrose was the carbohydrate source.

Malic enzyme. The trends noted for G-6-P-D are followed by malic enzyme. The study cited by Lin and Anderson also examined the activity of malic enzyme. As expected, the enzyme activity was significantly lower in starch-bran diets of normal rats. The possibility that differences in malic enzyme activity are a result of differences in carbohydrate source rather than addition of dietary fiber is supported by Cohen et al. (85). As previously described rats on a high sucrose diet had higher levels of malic enzyme activity than rats fed starch diets. Fitch and Chaikoff (86) also found levels of malic enzyme elevated when rats were fed a diet high in simple carbohydrate. Only two sources of simple carbohydrate, glucose or fructose, were tested in this study.

Activity of malic enzyme was reported by McCormick and co-workers (87) to be regulated by insulin. Rats with chronic hyperinsulinism displayed elevated enzyme activity that was directly proportional to their plasma insulin levels. Hyperinsulinemia in fetal monkeys enhanced lipogenic and NADPH-producing enzyme activities (88). Malic enzyme is absent in fetal development and therefore was not measured (88-89).

In sum, both G-6-P-D and malic enzyme respond to experimental treatments with similar results. Although evidence is not clear, lipogenic enzymes appear to be under the control of insulin and dietary carbohydrate.

MATERIAL AND METHODS

Animals and Their Care

Sixty male weanling rats[1] weighing from 50 - 75 g were housed in (23) individual stainless steel cages in a temperature controlled laboratory. Room lighting was controlled with a 12-hour light-dark cycle. Animals were maintained on a commercial stock diet[2] for five days and then randomly assigned to six experimental diets for ten weeks. Feed intake was recorded weekly and weight gain was measured every other day.

Diet

Experimental diets (Table 3, 4) contained three levels of dietary fiber (0, 6, and 18 per cent) with either sucrose or cornstarch as the carbohydrate source. Standardized wheat bran[3] was used as the source of fiber (Appendix 1GA) Adjustments for lipid, protein, and digestible carbohydrate in wheat bran were made in the diet formulation. Diets were not isocaloric as a result of varying the fiber level. Animals were allowed feed and water ad libitum.

Diets were mixed in a Hobart Automatic Mixer. All ingredients except fat were mixed for five minutes to allow distribution of the dry components. Fat was added and diets were mixed for an additional 30 minutes with frequent scraping of the bowl. Mixed diets were refrigerated and stored in cartons

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[1] Sprague Dawley rats, Gibco Animal Resource Laboratory, Madison, Wisconsin.

[2] Purina® Laboratory Rat Chow 5012; St. Louis, Missouri.

[3] A.A.C.C. certified food grade wheat bran; American Association of Cereal Chemists, St. Paul, Minnesota.

TABLE 3

Diet composition

Ingredient[1]	g/100 g					
	0% Fiber		6% Fiber		18% Fiber	
	Sucrose	CS[2]	Sucrose	CS	Sucrose	CS
Casein, vitamin free	22.50	22.50	20.33	20.33	15.87	15.87
dl Methionine	0.20	0.20	0.20	0.20	0.20	0.20
Corn oil	5.00	5.00	4.77	4.77	4.31	4.31
Beef tallow	5.00	—	4.77	4.77	4.31	4.31
Sucrose	62.30	—	49.82	—	—	—
Cornstarch	—	62.30	—	49.82	—	25.00
Wheat bran[3]	—	—	15.10	15.10	45.30	45.30
Mineral mix[4]	4.00	4.00	4.00	4.00	4.00	4.00
Vitamin mix[5]	1.00	1.00	1.00	1.00	1.00	1.00

[1] Diet components were obtained from Teklad Test Diets, Madison, Wisconsin 53711.

[2] Cornstarch.

[3] A.A.C.C. certified food grade wheat bran supplied by American Association of Cereal Chemists, St. Paul, Minnesota 55121.

[4] William Briggs Modified Mineral Mix 71709.

[5] Vitamin Mix 40060.

TABLE 4

Ingredients contributing to specific nutrient categories

	g/100 g					
	0% Fiber		6% Fiber		18% Fiber	
	Sucrose	CS[1]	Sucrose	CS	Sucrose	CS
Protein						
-- Casein	21.94	21.94	19.83	19.83	15.48	15.48
-- Wheat Bran	—	—	2.16	2.16	6.65	6.65
Fat						
-- Wheat Bran	—	—	0.79	0.79	2.36	2.36
-- Corn Oil and Beef Tallow	10.00	10.00	9.55	9.55	8.60	8.60
Carbohydrate, digestible						
-- Sucrose or Cornstarch	62.30	62.30	49.80	49.80	25.00	25.00
-- Wheat Bran	—	—	6.08	6.08	18.25	18.25
Carbohydrate, nondigestible						
-- Wheat Bran	—	—	6.07	6.07	18.21	18.21
	Per cent of Calories					
Protein	20.55	20.55	21.74	21.74	24.58	24.58
Fat	21.08	21.08	23.00	23.00	27.39	27.39
Carbohydrate	58.37	58.37	55.25	55.25	48.03	48.03

[1] Cornstarch.

lined with polyethylene bags. Individual animal feed was stored in pint jars.

Tissue Preparation

Blood Collection. Animals were tested at six and ten weeks. Prior to testing, animals were fasted for 14 hours. Blood was collected for glucose tolerance and insulin secretion determinations at 0, 30, 60, and 90 minutes. An oral glucose load of 350 mg per 100 g body weight was administered to unanesthetized rats with a three inch curved intubation needle.[1] The initial blood sample was collected before the glucose load was given.

Blood samples were collected from the tail vein. To enhance bleeding, tails were dipped in warm water (43^{o}) and then rinsed with alcohol. Bleeding was stopped by applying pressure.

Collection tubes were heparinized. Blood was centrifuged for 10 to 15 minutes in an International Centrifuge (Model SBV No. 1419H) with a free-swinging head at 2000 x g to obtain plasma.

Plasma was immediately assayed for glucose content within 30 minutes of blood collection to prevent cellular metabolism of the glucose. Plasma for insulin determinations was frozen (16^{o}) after collection and stored for later testing. Six week plasma insulin samples were pooled according to group and time period. Terminal plasma samples (10 weeks) were stored individually, but before the insulin assay each experimental group was divided in half and plasma samples were pooled producing two samples per experimental group per time period.

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[1] Popper and Sons, Inc., New Hyde Park, New York.

Liver and Epididymal Tissue. Animals were sacrificed using a cervical dislocator.[1] Liver and epididymal fat pads were removed and placed immediately in an ice bath. Tissues were blotted dry and organ weights were obtained. Approximately one gram of liver tissue was homogenized[2] in cold .25 M sucrose buffer. The homogenate was made to a final volume of 10 ml and centrifuged at 4^{o} for 30 minutes at 26,5000 x g.[3] The clear supernatant cytosol fraction was extracted and stored at -16^{o} until assayed. Young and co-workers reported that enzyme activity was stable for several weeks if stored at -15^{o} (90).

ANALYTICAL TECHNIQUES

Enzyme Activity

Liver tissue cytosol fractions were assayed for the combined activity of the hexose monophosphate shunt dehydrogenases (D-glucose-6-phosphate, E.C.1.1.49 and 6-phospho-D-gluconate, E.C.1.1.1.44) and "malic enzyme" (NADP-malate dehydrogenase, E.C.1.1.1.40).

Hexose monophosphate shunt dehydrogenases. The combined activity of the hexose monophosphate shunt dehydrogenase was determined by the method of Glock and McLean (91). Shunt activity was based on the appearance of an absorption band at 340 mu produced by the reduction of NADP. The reaction was initiated by the addition of the tissue cytosol and reacted for 4 minutes at 25^{o}. Blanks without NADP were made for each sample. Concentration and volume of reagents are listed on Table 5. The reaction sequence is as follows:

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[1] Cervical disolocators, Inc., Wausau, Wisconsin.

[2] Brinkman Homogenizer (PT 10-35) with stainless steel probe.

[3] Sorvall Automatic Refrigerated Centrifuge (Model RC2-B).

TABLE 5

HMPD (Glucose-6-phosphate dehydrogenase and
6-phosphogluconate dehydrogenase)
reagent mixture for combined enzyme activity

Reagent[1]	Concentration	Volume/Cuvette	
		Sample	Blank
Glycylglycine Buffer	0.25 M pH 7.6	0.5 ml	0.5 ml
$MgCl_2$	0.10 M	0.5	0.5
NADP	0.0015 M	0.2	——
Glucose-6-Phosphate	0.05 M	0.1	0.1
Enzyme Homogenate	1 - 10 Dilution	0.1	0.1
Distilled Water		1.1	1.3
	Total Volume	2.5	2.5

[1] Sigma Chemical Company, St. Louis, Missouri.

$$\text{Glucose-6-phosphate} + NADP^{+} \longrightarrow \text{6-phosphogluconate} + NADPH + H^{+}$$

$$\text{6-phosphogluconate} + NADP^{+} \longrightarrow \text{Ribulose-5-phosphate} + CO_2 + NADPH + H^{+}$$

The combined activity of the hexose monosphosphate shunt enzymes can be assayed because in each reaction of the sequence, one mole of NADPH is produced. With excess substrate, the amount of enzyme present is proportional to the rate of NADPH production.

NADP-malate dehydrogenase. Malic enzyme activity was determined by the method of Ochoa (92). The concentration and volume of reagents are shown in Table 6. Enzyme activity was based on the generation of NADPH and measured with a spectrophotometer[1] at 340 mu for 4 minutes at 25°. Nonsubstrate controls were run for all assays. The NADPH production is a result of the following reaction:

$$\text{L-Malate}^{-} + NADP^{+} \xrightarrow{Mn^{++}} \text{Pyruvate}^{-} + CO_2 + NADPH$$

Protein determination. Total protein in the tissue samples was determined by the method of Lowry et al. (93). This is a colorimetric assay that is based on the tyrosine and tryptophan content of the proteins. In the procedure, protein complexed with copper sulfate in alkaline solution.[2] In the second reaction, phosphomolybdic - phosphotungstic reagent[3] was reduced by the

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[1] A Gilford recording spectrophotometer (Model 2430) was used and results recorded on a Beckman chart recorded (Model GC-5).

[2] A 0.5% $CuSO_4 \cdot 5\ H_2O$ in 1% Na - K Tartrate (Reagent B) was diluted by 50 ml of 2% $NaCO_3$ in 0.1 N NaOH (Reagent A) to form the alkaline copper solution (Reagent C).

[3] "Folin-Ciocalteau" reagent.

TABLE 6

NADP - malic dehydrogenase ("malic enzyme") reaction mixture

Reagent[1]	Concentration	Volume/Cuvette	
		Sample	Blank
Glycylglycine Buffer	0.25 M pH 7.4	0.30 ml	0.30 ml
$MnCl_2$	0.05 M	0.06	0.06
NADP	0.000675 M	0.24	0.24
Malate	0.030 M pH 7.4	0.10	—
	1 - 10 Dilution	0.10	0.10
Distilled Water		2.20	2.20
	Total Volume	3.00	3.00

[1] Sigma Chemical Company, St. Louis, Missouri.

protein-copper complex to produce the final color development. The Coleman double beam spectrophotometer (Model 1240) at 660 mu was used to measure color development. Standards[1] and a blank of distilled water were prepared for each trial of unknowns. Protein content of unknown samples was derived from a standard curve.

The Lowry method is the method of choice because of its sensitivity (a range from 10 ug/ml to 240 ug/ml). And sucrose buffer interferes with the biuret method at low protein concentrations (94).

Glucose Tolerance Tests

Plasma glucose was determined by the glucose oxidase method according to the procedure of Fales (95) as modified by Cooper and McDaniel (96). The assay is based upon the oxidation of glucose in a colorimetric reaction. The plasma is first reacted with a barium hydroxide - zinc sulfate reagent[2] to precipitate the protein. The protein-free filtrate is reacted with an enzyme solution[3] containing glucose oxidase and peroxidase to produce a rose-pink color. The reaction sequence occurs as follows:

$$\text{Glucose} + H_2O + O_2 \xrightarrow[\text{Oxidase}]{\text{Glucose}} \text{d-gluconic acid} + H_2O_2 \text{ Hydrogen Peroxide}$$

$$H_2O_2 + \text{ortho-dianisidine (indicator)} \xrightarrow{\text{peroxidase}} \text{oxidized chromogen} + H_2O$$

---

[1] The standard stock solution contained 100 mg Bovine Albumin Fraction V in 100 ml of 0.25 M sucrose buffer with 1000 ug protein per ml.

[2] Carbon-dioxide free solutions of 2.2% zinc sulfate ($ZnSO_4 \cdot 7\ H_2O$) and 0.12 N barium hydroxide ($Ba(OH)_2 \cdot 8\ H_2O$) were dispensed from separate burettes into the plasma.

[3] One ml of a 1% ortho-dianisidine-methanol indicator solution was diluted in a 200 ml aliquot of enzyme solution (250 mg glucose oxidase, Type II, and 50 mg peroxidase, RZ 0.3, dissolved in 1:1 glycerol buffer solution).

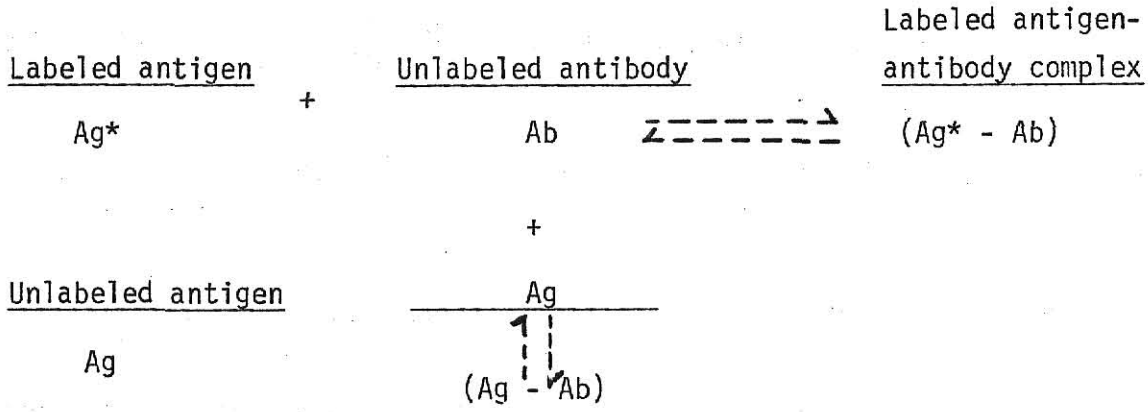
The reaction is stopped by adding 6 N sulfuric acid. A Coleman double beam spectrophotometer (Model 124C) at 540 mu was used for colorimetric determinations. The amount of color produced is proportional to the amount of glucose oxidized.

The glucose oxidase method is the only assay procedure that has absolute specificity for glucose (96). This method is precise within ± 3 mg per 100 ml; thus, the method of choice.

Insulin Assay

Thawed plasma samples for insulin determination were recentrifuged to remove the fibrin or other solids. A double antibody radioimmunoassay procedure[1] according to the method of Hales and Randle (97) was used.

In the radioimmunoassay procedure isotopically labeled and unlabeled insulin compete for binding sites on an anti-insulin antibody. As the amount of unlabeled hormone increases, the binding of labeled hormone to the antibody decreases following a saturation kinetics curve. The competitive binding reactions are as follows:



[1] Amersham insulin radioactive immunoassay kit (IM.78), Amersham Corp., Arlington Heights, Illinois.

The amount of unlabeled insulin can be measured by separating the bound fraction and counting the radioactivity. A second antibody is required to precipitate and separate the antigen - antibody complex. For this assay, the second antibody was reacted with the first antibody prior to use as a reagent. The insulin binding reagent was guinea pig anti-insulin antibody which was reacted with rabbit anti-guinea pig antibody. The binding reagent was added to the human insulin standard[1] and the test plasma and incubated for six hours. In the second reaction, radioactive insulin[2] was added to the previous solution and incubated for 18 hours to allow equilibrium of the labeled and unlabeled insulin - antibody complex. The bound hormone was separated by centrifugation. Radioactivity of the precipitate was measured with a scintillation gamma counter.[3]

Table 5 contains the assay protocol. Incubation times were lengthened to increase precision. The assay was developed to test human insulin levels but other laboratories (98) found a 90 per cent cross-reactivity with rat plasma.

STATISTICAL ANALYSIS

A 3 X 2 factorial design with three levels of dietary fiber and two sources of carbohydrate were used in the study. Data was analyzed by analysis of variance using an F - distribution with n-1 or 5 degrees of freedom (99).

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[1] Freeze-dried human insulin was reconstituted to contain 1000 microunits of insulin per ml.

[2] Insulin ^{125}I prepared from purified bovine insulin, which has a potency of 24 international units/mg.

[3] Tracor Analytic, Scintillation Gamma Counter (Model 1185).

TABLE 7

Insulin assay protocol

Reagents[1]	Total Counts	Standard	Blank	Unknown
Phosphate buffer, µl	—	—	200.0	—
Insulin standard dilution, µl	—	100.0	—	—
Unknown plasma, µl	—	—	—	100.0
Binding Reagent, µl	—	100.0	—	100.0
		Incubate at 2 - 4°C		
Insulin ^{125}I, µl	100.0	100.0	100.0	100.0
		Incubate at 2 - 4°C		
Phosphate buffer, µl	—	700.0	700.0	700.0
		Centrifuge, decant, and count.		

[1] Amersham Insulin radioimmunoassay kit (IM.78) Amersham Corp., Arlington Heights, Illinois.

RESULTS AND DISCUSSION

Body weight gain, feed intake and feed efficiency ratios

Body weight gain. Weight of animals at the beginning and end of the experimental period was not significantly among groups (Table 8). Weight gain was measured every other day and averaged at two-week intervals. Mean daily weight gain was not significantly affect by the experimental treatment (Table 9).

The fiber hypothesis proposed by Trowell (2), suggests that fiber-depleted diets may lead to obesity. Haber and co-workers (20) propose two mechanisms through which low fiber diets could lead to obesity in addition to their faster and easier consumption and their reduced satiety value. First, eating is stimulated by a fall in blood glucose; meals which trigger a rebound fall in plasma glucose (such as a concentrated carbohydrate meal) are more rapidly followed by another meal. Second, stimulation of insulin release promotes synthesis and deposition of fat. Hyperinsulinism, which is associated with obesity, can lead to inappropriate lipid metabolism.

Supplementation of fiber in this study did not demonstrate that fiber affected weight gain. Southgate and co-workers (36) found supplementation of wheat bran in human diets had little effect on energy metabolism. Results of this study agree that fiber has no effect on weight gain.

Feed Intake. Feed intake was measured weekly. Mean daily feed intake in grams and kilocalories is presented in Table 9. Animals on a high fiber diet consumed significantly more (P 0.05) diet (g) than did rats on a low fiber. diet. Caloric density of the diets decreased as the level of fiber increased.

TABLE 8

Initial and final body weight[1] and weight of the epididymal fat pad in rats (n=10)

Diet	Initial Bd Wt[2] (g)	Final Bd Wt[2] (g)	Epididymal Fat Pad Wt/100 g bd wt[1,2]
Low Fiber[4] Sucrose	87.25±3.87[6]	442.50[abc]±10.52	1.69±0.09
Low Fiber[4] Cornstarch	87.85±3.87	455.25[abc]±10.52	1.64±0.09
Medium Fiber Sucrose	86.85±3.87	437.75[abc]±9.41	1.71±0.08
Medium Fiber Cornstarch	87.25±3.87	449.75[abc]±9.41	1.74±0.08
High Fiber[5] Sucrose	87.90±3.87	421.39[a]±9.92	1.58±0.08
High Fiber Cornstarch	81.85±3.67	438.50[abc]±9.41	1.68±0.08

[1] Total duration of the experiment 10 weeks.

[2] No significant differences between groups.

[3] Values in the same column with different superscripts are significantly different at $P < 0.05$.

[4] n=8

[5] n=9

[6] Mean ± S.E.

TABLE 9

Weight gain, food intake, energy intake and feed efficiency ratios (FER). (Time=71 days; n=10)

Diet	Mean Daily Wt Gain	Mean Daily Feed Intake		Caloric Density	FER[4]
	g/day	g/day	kcal/day	kcal	
Low Fiber[2] Sucrose	$4.87^{a}\pm0.20$	$15.94^{ab}\pm0.57$	$68.04^{a}\pm2.44$	4.2696	$7.16^{a}\pm0.16$
Low Fiber[2] Cornstarch	$5.04^{a}\pm0.16$	$15.74^{a}\pm0.57$	$67.20^{a}2.20$	4.2696	$7.53^{a}\pm0.27$
Medium Fiber Sucrose	$4.86^{a}\pm0.13$	$16.44^{ab}\pm0.26$	$66.34^{a}\pm1.05$	4.0454	$7.33^{a}\pm0.15$
Medium Fiber Cornstarch	$5.05^{a}\pm0.16$	$17.05^{ab}\pm0.41$	$68.77^{a}\pm1.65$	4.0454	$7.33^{a}\pm0.10$
High Fiber[3] Sucrose	$4.74^{a}\pm0.13$	$18.27^{c}\pm0.29$	$65.77^{a}\pm1.03$	3.6016	$7.20^{a}\pm0.15$
High Fiber Cornstarch	$4.93^{a}\pm0.11$	18.29 ± 0.23	$67.68^{a}\pm0.82$	3.6016	$7.30^{a}\pm0.17$

[1] Values in the same column with different superscripts are significantly different at the $P<0.05$ level.

[2] n=8

[3] n=9

[4] FER: Feed Efficiency Ratio = $\frac{\text{wt gain}}{\text{kcal}} \times 100$

However, total caloric intake was not significantly different between experimental groups. Rats tend to consume feed for total energy rather than on a weight basis. The analysis of variance in Table 10 illustrated that high levels of fiber were strongly ($P<0.001$) related to the grams of diet consumed.

Southgate (22) found that undigestible carbohydrate does disappear from the digestive tract. However, as the level of non-digestible carbohydrate increases, the apparent digestibility of other caloric nutrients in the diet decreases. This balance in digestibility produces a net input of metabolizable energy of 0.03 to 0.38 per cent from fiber. The low energy yield of fiber accounts for the increased food consumption on a weight basis with a low calorie dense diet.

Feed efficiency ratio. The ability to convert calories to weight gain is termed the feed efficiency ratio (FER). The formula for calculating FER is as follows:

$$FER = \frac{\text{Weight gain}}{\text{Calories}} \quad X \quad 100$$

Since fiber had no effect on weight gain, or energy intake, the FER would not be affected. Mean values for FER's are contained in Table 9. The results support that dietary treatment had no significant effects on FER's.

Glucose Tolerance

Trial 1 (Six weeks). Glucose tolerance tests were conducted at six and ten weeks. The results are plotted in Figure 1. No significant differences were noted between diets in the 0 and 30 minute periods. At 60 and 90 minutes,

TABLE 10

Analysis of variance for feed intake (g/day) of rats

Source of Variance	Degrees Freedom	Least Squares Mean[2] and Significance
Fiber[1]	2	68.20***
High		18.53[a]
Medium		16.70[b]
Low		15.84[b]
Carbohydrate[1]	1	1.59[ns]
Sucrose		16.87[ns]
Cornstarch		17.18[ns]
Fiber x Carbohydrate[1]	2	1.66[ns]

[1] Values represent the sum of squares for the variation in the category.

[2] Values in the same column with different superscripts are significantly different at the $P < 0.05$ level.

*** Significant at the $P < 0.001$ level.

ns Not significant.

THIS BOOK CONTAINS NUMEROUS PAGES WITH DIAGRAMS THAT ARE CROOKED COMPARED TO THE REST OF THE INFORMATION ON THE PAGE.

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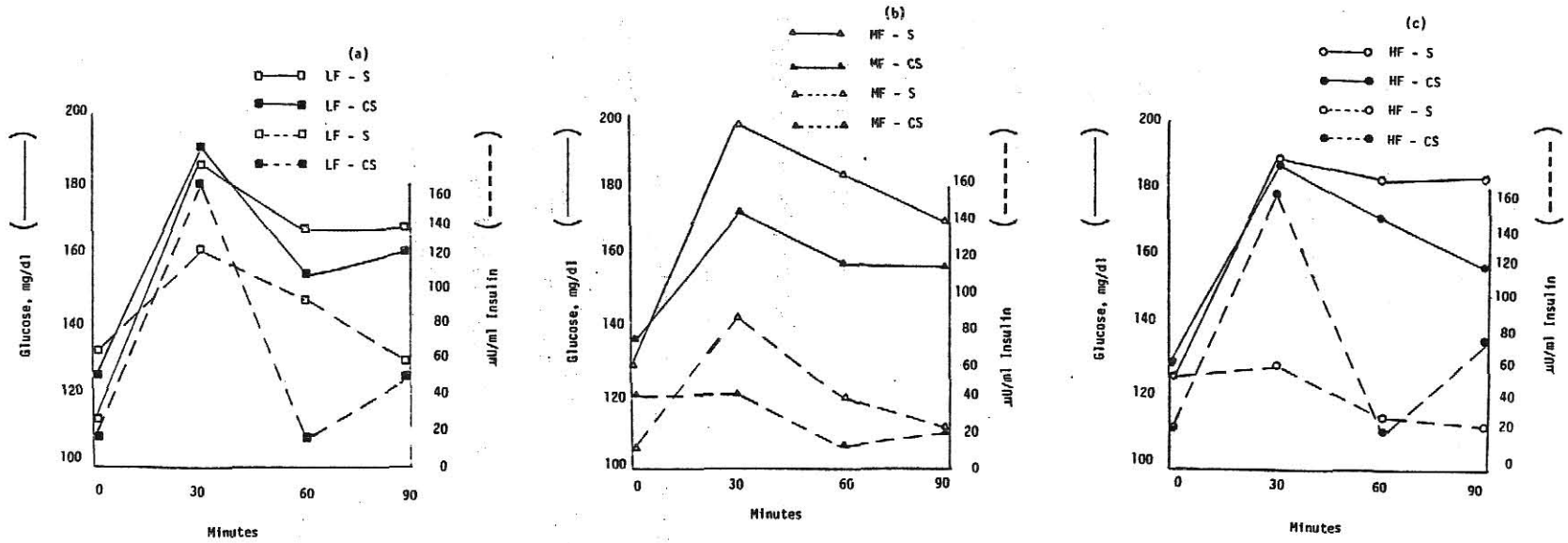


Figs. 1a, 1b, 1c Glucose tolerance and serum insulin levels at six weeks (Trial 1) (a) low fiber treatments, sucrose (LF-S) and cornstarch (LF-CS), (b) medium fiber treatments, sucrose (MF-S) and cornstarch (MF-CS); and (c) high fiber treatments, sucrose (HF-S) and cornstarch (HF-CS). Each glucose tolerance point represents the group mean. Insulin points represent the pooled insulin response for the group.

however, the source of carbohydrate affected the results. Rats fed cornstarch diets with medium or low levels of fiber had significantly lower ($P<0.05$) glucose levels than sucrose fed rats on medium or low fiber levels at the 60 minute interval. High fiber diets negated the effects of carbohydrate source at 0, 30 and 60 minute intervals.

At 90 minutes the only significant difference was between animals on the high fiber sucrose and high fiber cornstarch diet. The glucose level of cornstarch fed rats was significantly ($P<0.05$) lower than for sucrose fed rats. The analysis of variance of factors influencing glucose tolerance (Table 11) found carbohydrate source significant at the $P<0.05$ for the 60 and 90 minute interval.

The source of carbohydrate is an important factor influencing glucose tolerance. Simple carbohydrate causes an impaired glucose tolerance curve while complex carbohydrates such as starch produce a normal to improved glucose tolerance curve (51-52, 54, 68, 70). Hallfrisch et al. (70) found the effect of carbohydrate source on glucose tolerance and insulin secretion to increase with time. Cornstarch produced significantly lower glucose and insulin responses. Results of the present study agree that cornstarch has a more pronounced effect on glucose tolerance at 60 and 90 minutes than at 0 or 30 minutes.

Crapo and co-workers (54) propose that the differences in glucose response to sucrose and starch are related to digestion of the molecules. Starch digestion is a two-step process: luminal digestion of starch to dextrins and maltose and mucosal digestion at the brush border where monosaccharides are formed. Luminal digestion could be altered by prolonged gastric transit time, differences in fiber content, or other factors which decrease

TABLE 11

Analysis of variance for rat glucose tolerance
Trial 1 (6 weeks)

Source of Variance	Degrees Freedom	Least Square Mean and Significance			
		0 min	30 min	60 min	90 min
Fiber[1]	2	1525.10[ns]	79.30[ns]	2336.54[ns]	200.70[ns]
High		127.91	186.91	176.20	170.25
Medium		132.06	184.73	172.05	163.41
Low		118.92	188.41	160.61	164.49
Carbohydrate[1]	1	818.08[ns]	858.04[ns]	3822.14[*]	2568.99[*]
Sucrose		122.23	190.35	178.43	173.63
Cornstarch		130.36	183.02	160.43	158.47
Fiber x Carbohydrate[1]	2	205.29[ns]	2237.05[ns]	746.68[ns]	663.89[ns]

[ns] Not significant.

[*] Significant at the 0.05 level.

[1] Numbers represent the sum of squares.

the availability of starch to the luminal enzymes. These factors which alter luminal digestion limit glucose absorption. The difference in glucose response to starch and sucrose appears to center around the process of luminal digestion.

In summary, starch elicits a lower glucose response than sucrose. Carbohydrate source significantly ($P<0.05$) influenced glucose tolerance at 60 and 90 minutes. This suggests that at different points in time different factors influence glucose tolerance. In the glucose test, as time increased carbohydrate source became a factor. Specifically, starch produced a lower glucose response than sucrose.

Addition of wheat bran to the diet had no significant effect on glucose tolerance. The analysis of variance on the effects of wheat bran is contained in Table 11. Similarly, rats fed a starch-bran diet or a sucrose diet in the study by Lin and Anderson (44) had no change in glucose tolerance. Harland and co-workers (45) even found that addition of various fiber sources such as wheat bran, soya flock, alfacel, sugar cane and sugar beet pulp act to increase fasting blood glucose levels. A relationship between fiber and glucose tolerance has been found using other sources of fiber. Glucose tolerance is improved with supplementation of fruit and vegetable fiber (6). In human studies, glucose tolerance is unaltered by supplementation of wheat bran (49), but improved by addition of fruit and vegetable fibers (46-49). These data suggest that chemical composition of fiber rather than level of fiber is the critical factor in glucose metabolism. The chemical composition of wheat bran is markedly different from other sources of fiber (48). Wheat bran is categorized as the most true lignin (10). This chemical composition could account

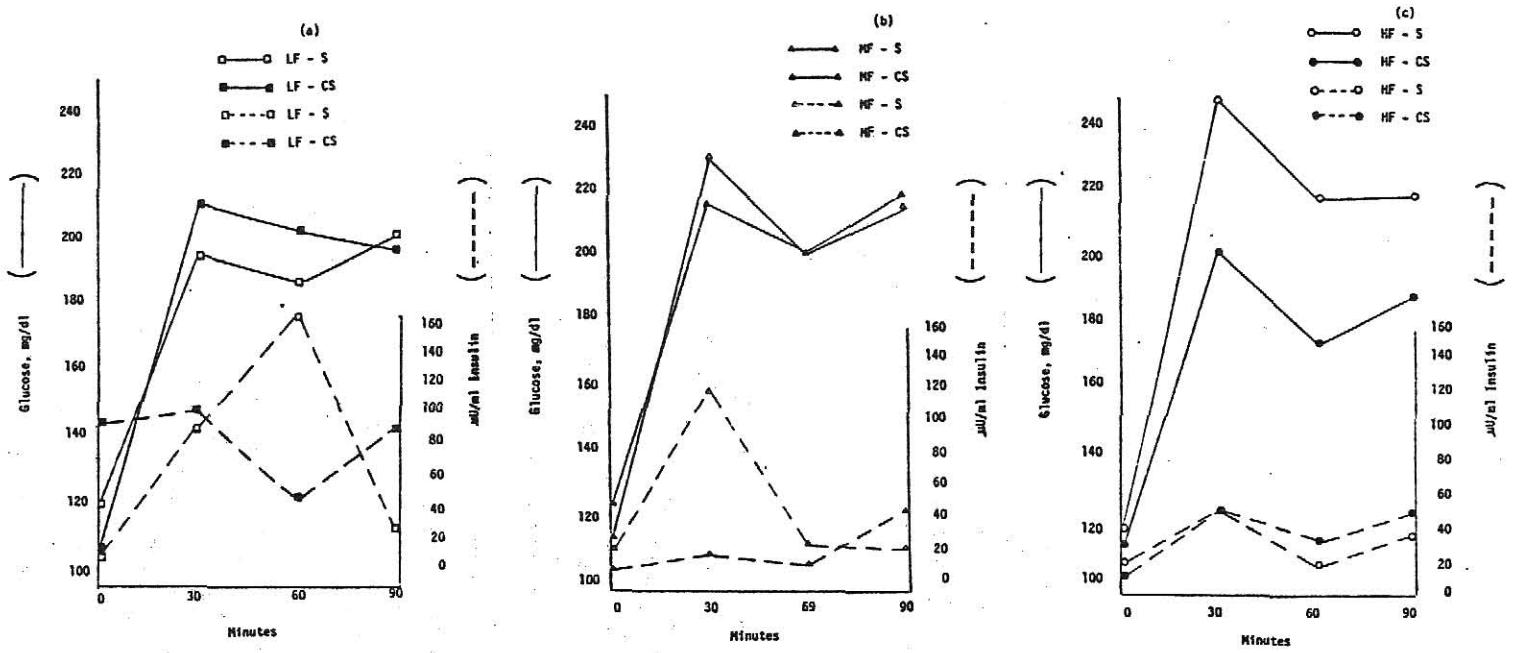
for the inability of wheat bran to alter glucose tolerance.

Glucose values at each time interval during the tolerance test may be slightly elevated in all groups. A normal human fasting blood glucose level is approximately 90 mg% glucose (this value varies according to the reference) with a peak of 140 mg % glucose and a return to normal within 90 to 120 minutes (100-101). Experimental results are consistently at the upper end of the scale or slightly outside the limit. This does not necessarily constitute abnormal carbohydrate metabolism. Two factors should be taken into consideration: size of the glucose load and diurnal variation.

The glucose load was 350 mg glucose per 100 g body weight based on the procedures of other research (53). The glucose load is not standardized; other researchers have given 250 mg glucose per 100 g body weight (102, 44). The higher level of glucose may be too great for the system to respond to appropriately causing a slightly abnormal or high normal tolerance curve.

In addition, diurnal variation can affect the results. Diurnal variation is the fluctuation caused by the time of the day. A decreased glucose tolerance is noted when the test is administered in the afternoon (103). This fluctuation is the result of normal variation in insulin secretion. Tests in this experiment were performed in the afternoon possibly accounting for slightly high glucose tolerance results.

Trial 2 (Ten weeks). Results of terminal glucose tolerance tests are contained in Figure 2. Dietary treatment had no significant effects on glucose tolerance in trial 2 with two exceptions. At 0 minutes the low fiber cornstarch fed rats had significantly lower ($P < 0.05$) levels of blood glucose than did low fiber sucrose fed rats. At 60 minutes, the high fiber diet with



Figs. 2a, 2b, 2c Glucose tolerance and serum insulin levels at ten weeks (Trial 2) (a) low fiber treatments, sucrose (LF-S) and cornstarch (LF-CS), (b) medium fiber treatments, sucrose (MF-S) and cornstarch (MF-CS); and (c) high fiber treatments, sucrose (HF-S) and cornstarch (HF-CS). Each glucose tolerance point represents the group mean. Insulin points represent the pooled insulin response for the group.

cornstarch produced a significantly lower glucose response ($P<0.05$) than did the sucrose diet. Analysis of variance (Table 12) indicated carbohydrate source did not significantly influence the glucose tolerance curve. The two isolated points of significance are probably due to chance. Again as with trial 1 (six weeks) the level of fiber had no effect on glucose tolerance.

No significant differences due to dietary treatment were found, but the glucose curve was elevated in each group. Comparison of the results in trial 2 to a typical glucose curve (Table 13) indicates that the experimental glucose curves exceed values for a typical curve. Results of glucose tolerance tests at the six week interval were also slightly high. When the results of each trial were compared, the glucose tolerance curve in trial 2 was found to be significantly higher ($P<0.05$) than in trial 1.

Several factors or a combination of these factors may be responsible for the decreased glucose tolerance noted in trial 2. Included among these are increased age of the animal, "obesity", or chromium deficiency. Siperstein (63) reported that glucose tolerance decreased with aging. Romero-Bracho and Reaven (104) observed deterioration of glucose response begins at four weeks. Weanling Sprague-Dawley rats were used in the study; glucose tolerance tests were performed at four and twelve weeks. Their testing period closely corresponds to the interval used in this study. Romero-Bracho and Reaven (104) attributed the change in glucose tolerance to an age-related factor plus the heavier weight accompanying aging.

At a cellular level, changes in glucose tolerance stem from a decreased rate of glucose transport into the cell. Glucose transport is facilitated by insulin binding to target cell receptors. Reiser and Hallfrisch (69) noted

TABLE 12

Analysis of variance for rat glucose tolerance
Trial (10 weeks)

Source of Variance	Degrees Freedom	Least Square Mean and Significance			
		0 min	30 min	60 min	90 min
Fiber[1]	2	23.44[ns]	4061.33[ns]	227.31[ns]	2738.80[ns]
High		117.78	226.78	198.16	203.02
Medium		118.63	223.92	202.46	218.12
Low		117.02	206.46	198.26	202.93
Carbohydrate[1]	1	130.01[ns]	3267.44[ns]	1340.42[ns]	2020.08[ns]
Sucrose		119.80	226.27	204.33	214.19
Cornstarch		115.82	211.83	194.92	201.84
Fiber x Carbohydrate[1]	2	1239.50	8036.65[ns]	8213.65[ns]	3338.27[ns]

[ns] Not significant.

[1] Numbers represent the sum of squares.

TABLE 13

Typical insulin and glucose levels after an oral glucose tolerance test in humans (102)

Variable		Time			
		0 min	30 min	60 min	90 min
Glucose (mg,dl)	Mean	90	142	119	121
	Range	(73-104)	(87-183)	(77-160)	(58-116)
Insulin (μU/ml)	Mean	19	101	102	64
	Range	(7-34)	(26-160)	(34-216)	(21-121)

an age-related decrease in insulin sensitivity in rats. Insulin insensitivity is defined by a greater than normal concentration of insulin to produce a normal response (75). The decreased glucose tolerance is then a secondary effect of decreased insulin responsiveness.

The research by Romero-Bracho and Reaven (104) stated that animal weight along with age affected glucose tolerance. Rats were categorized as obese if weighing more than 300 g (105-106). This weight was arbitrarily chosen because at 300 g alterations in insulin binding were noted. The final weight of rats in this study at 70 days of age averaged 440.86 (Table 8). The standard weight of laboratory rats as defined by the National Academy of Sciences is 290.57 g at 70 days (107). The final body weight of all groups was greater than one standard deviation above the norm (290.57 g). This suggests that the animals in this study were not only obese by the standards set by other researchers, but also by the National Academy of Science's standards.

Weight of the epididymal fat pad was obtained (Table 8). Dietary treatment had no effect on deposition of epididymal fat. Differences in adiposity affect glucose tolerance with an increase in adiposity decreasing glucose tolerance (69). Reiser and Hallfrisch (69) determined adiposity by measuring the weight of the epididymal and perirenal fat pad and the soluble protein content of the adipose tissues. Results in this study suggest that fat deposition and glucose tolerance were not significantly affected by dietary treatment; however, only epididymal fat was measured in this study.

Obesity decreases glucose tolerance by altering insulin binding characteristics. An actual decrease in the number of insulin receptors was noted by Olefsky and Reaven (106). Decreased receptor number is in part caused by the hyperinsulinism which accompanies obesity. A full discussion of insulin

resistance will be presented with the insulin results.

In summary, the experimental animals were considered overweight by both the standards of other researchers and the National Academy of Sciences standards (107). Obesity impairs glucose tolerance by decreasing insulin binding and therefore glucose transport.

The final factor potentially modifying glucose tolerance is chromium which has been designated the glucose tolerance factor (108). The first known sign of chromium deficiency in rats is an impaired glucose tolerance. This alteration of glucose tolerance can occur when rats are maintained under ordinary conditions with various purified diets and laboratory chows (109). In a strictly controlled study eliminating environmental chromium, other deficiency symptoms such as impaired growth, fasting hyperglycemia, glucosuria and elevated serum cholesterol appeared. These symptoms did not develop under ordinary conditions. An earlier study in this laboratory using the same experimental diets showed no change in serum cholesterol after six weeks of feeding (110). This result suggests that a severe deficiency was not present.

The National Academy of Sciences recommends supplementation of 2-5 ppm of chromium in the water to prevent deficiency. Water used in the experiment contained 0.000 ppm chromium (111). Chromium can be airborne with the concentration varying with the location. In Washington, D.C., 0.08 ug chromium/ m^3 was detected. On the other hand, no chromium was detected in a Vermont laboratory after a six month collection of electrostatically precipitated dust (109).

The dietary ration supplied no added chromium. William-Briggs Modified Mineral Mix (112) which contains no chromium was used in the diet. Analysis

of standardized wheat bran did not indicate the presence of chromium (113). Any chromium present in the diet would come from exogenous contamination.

The presence of a chromium deficiency is difficult to ascertain. If a chromium deficiency exists it is probably very marginal which accounts for the fact that glucose tolerance was not impaired immediately, but developed over time as the body stores were reduced.

The mechanism of chromium action in glucose tolerance is thought to be by potentiating the effect of insulin (180, 114). In chromium deficiency, peripheral tissues may become less sensitive to insulin causing abnormal glucose metabolism (115, 108). The potentiating effect of chromium is hypothesized to occur by initiation of disulfide exchange between insulin and membrane receptors (108). This step is the initial reaction of insulin with the membrane site which allows transport of glucose across the cell membrane.

In review, two general factors are involved in glucose intolerance: diurnal variation and size of the glucose load. Glucose tolerance curves are normally higher in the afternoon than in the morning because of the low insulin release at this phase of the diurnal cycle. A high glucose load can deplete insulin stores and produce elevated glucose curves. The glucose load given in this study (350 mg per 100 g body weight) may not by itself be sufficient to deplete glucose stores, but when given in addition to a high carbohydrate diet may produce an abnormal glucose curve.

The glucose tolerance was significantly higher in trial 2 (ten weeks) than at trial 1 (six weeks). The time-dependent change suggests an age-related factor or development of a nutrient deficiency is involved. Aging itself decreases glucose tolerance by decreasing receptor sensitivity to

insulin. Insensitivity to insulin reduces glucose transport and therefore increases the level of circulating insulin creating a glucose intolerance. Chromium deficiency produces an impaired glucose tolerance by altering the binding of insulin to the receptor. Signs of a chromium deficiency would not be noted until internal stores were reduced. The diet was not supplemented with chromium so that any dietary chromium was from external contamination. Because of the possibility of exogenous chromium entering the diet, any chromium deficiency which existed was probably marginal.

All factors that affect glucose tolerance act either directly or indirectly through insulin response. The ability of such a diverse group of factors to exert a common effect glucose tolerance suggests that one factor alone is not responsible for the altered glucose tolerance, rather a combination of factors is acting to change the glucose tolerance.

Diet was able to exert some effect on glucose tolerance with cornstarch producing a lower glucose response than sucrose in the 60 and 90 minute test interval at six weeks. In trial 2 (ten weeks) no significant differences in dietary treatment were demonstrated. As wheat bran was used as the source of fiber no changes were expected. Overall, dietary treatment had minimal effects on glucose tolerance.

Insulin Response

Serum sample pooling made statistical analysis of insulin response between groups impossible. The insulin value plotted with the glucose tolerance curves for each group aid in interpreting glucose tolerance data (Figures 1 and 2). A typical insulin response to glucose challenge is contained in

Table 13. The experimental insulin values at 60 and 90 minutes appear low. The trend of normal insulin release which peaks at 30 minutes is noted in all but two groups in both the six week and ten week test. The pancreas appears to initially respond to the stimulus for glucose secretion; however, at 60 and 90 minutes insulin release is decreased. Low insulin response to glucose challenge is typical of diabetes mellitus (100). The ability to secrete insulin initially suggests that the pancreas is overstimulated and insulin stores exhausted rather than a symptom of diabetes. Eaton and Kipnis (56) were able to exhaust pancreatic insulin stores by feeding a high carbohydrate diet (40 per cent dextrose) and then administering an oral glucose tolerance test which resulted in decreased insulin secretion.

Insulin release is a multiphasic with two separate pools. The first pool of insulin is available for immediate release; whereas,release from the second pool is gradual and coupled with synthesis of new insulin (117). The first storage pool responds to acute stimuli and is a small finite reservoir (116). Insulin response from this pool is an important determinant of glucose tolerance. Chronically high levels of carbohydrate may cause hyperinsulinism. When the additional stress of a glucose load is paired with chronic hyperinsulinism,the system may not be able to respond normally to the overload. Exhaustion of insulin stores may also be caused by an excessive glucose load. The 350 mg glucose per 100 g body weight used for the glucose tolerance test in this study may have been too large of a test dose for the animals to metabolize normally. This decrease in insulin response may account for the elevated glucose levels at 60 and 90 minutes.

The pooled group insulin values were used in calculations to provide

an index of insulin activity. The following formulas were used:

$$(117) \quad CIRp = \frac{Ip \ (100)}{Gp \ (Gp - 70)}$$

$$(118) \quad GTp = \frac{10^6}{Gp^2 \ (Gp - 70)}$$

CIRp = Corrected Insulin Response at glucose peak

GTp = Glucose Tolerance Parameter

Gp = Glucose Peak

Ip = Insulin Peak

The first formula is an index of pancreatic function (insulin release) which is independent of the glucose level reached. This formula is a ratio of the amount of insulin released in response to the glucose stimuli (48). The second formula is an index of peripheral insulin sensitivity or responsiveness of target cell to insulin.

CIRp. Mean values of insulin release are contained in Table 14. In trial 1 (six weeks), the low fiber sucrose group was significantly different ($P<0.03$) from other groups except for animals fed the high fiber cornstarch diet. Results for rats fed the low fiber cornstarch diet were significantly different ($P<0.03$) from all other groups excluding the high fiber cornstarch group. The value for the high fiber cornstarch treatment was higher than expected. The high value may be a result of experimental error. The probability of error occurring is increased for CIRp values because pooled group insulin values were used in the calculations. The medium fiber and high fiber sucrose groups were not significantly different.

Dietary treatment influenced results (Figure 3a). In general, as the level of dietary fiber increased the pancreatic response decreased. The medium fiber value was lower than the high fiber response; however, the high value for the high fiber cornstarch diet probably skewed the results. The effect

TABLE 14

Index of insulin response: pancreatic response (CIRp) [1] and peripheral sensitivity (GTp) [2]

Diet	GTp 1 [3,4]	GTp 2 [5]	CIPp 1 [3]	CIRp 2 [5]
Low Fiber Sucrose	0.26±0.08	0.15±0.04	0.572^{a}±0.06	0.5014^{a}±0.05
Low Fiber Cornstarch	0.27±0.08	0.19±0.04	0.7777^{b}±0.06	0.3816^{a}±0.05
Medium Fiber Sucrose	0.19±0.07	0.15±0.03	0.3161^{c}±0.08	0.3713^{a}±0.05
Medium Fiber Cornstarch	0.42±0.07	0.17±0.03	0.2670^{c}±0.06	0.0781^{bc}±0.05
High Fiber Sucrose	0.23±0.08	0.13±0.04	0.2498^{c}±0.06	0.1371^{bc}±0.05
High Fiber Cornstarch	0.25±0.07	0.22±0.03	0.7270^{ab}±0.58	0.2163^{c}±0.05

[1] $CIRp = \frac{Ip\ (100)}{Gp\ (Gp - 70)}$

CIRp = Corrected Insulin Response at glucose peak.

[2] $GTp = \frac{10^{6}}{Gp^{2}\ (Gp - 70)}$

GTp = Glucose Tolerance Parameter.

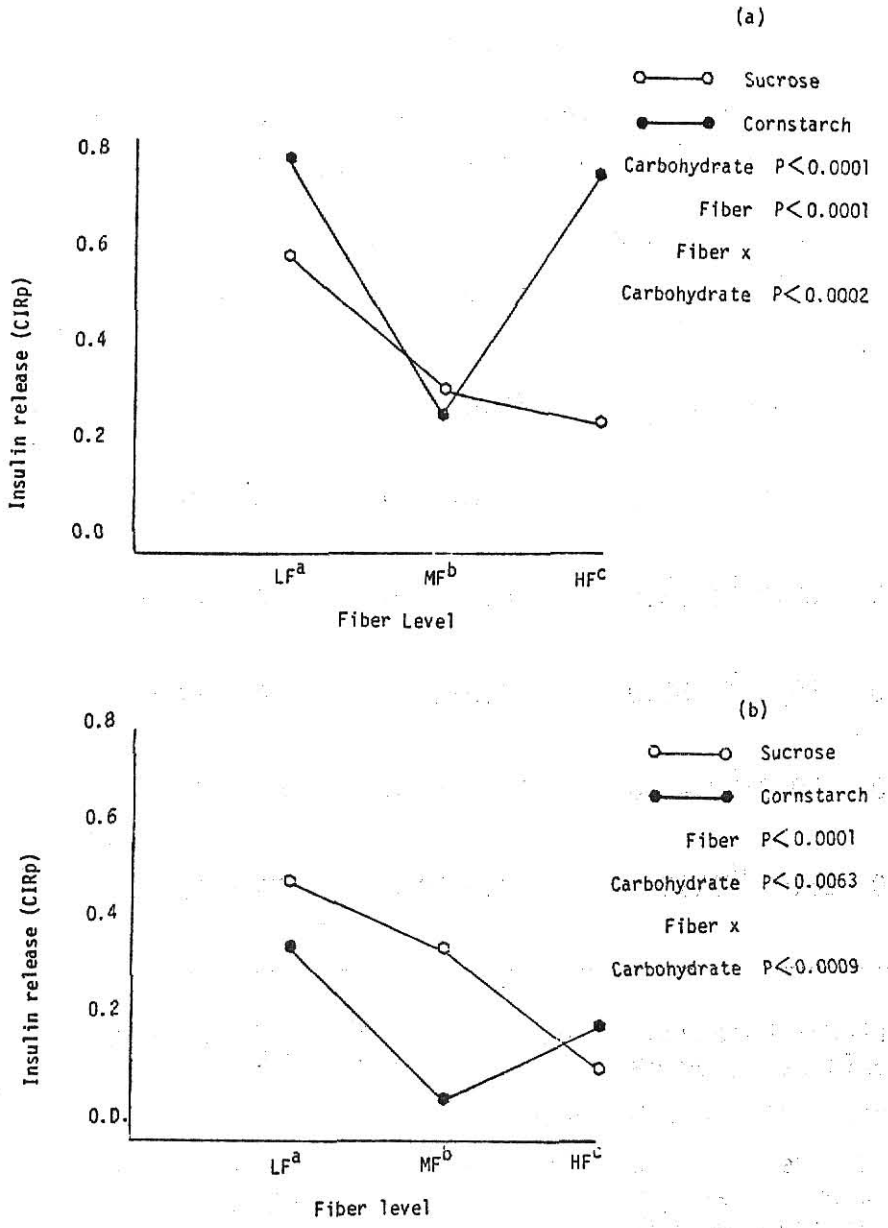
Gp = Glucose peak (Calculations are performed using the Gp for each rat and the pooled insulin peak for the group)

Ip = Insulin peak (group mean)

[3] Values are calculated using data from the six week test interval, mean ± S.E.

[4] Values in the same columns with different superscripts are significantly different at the $P<0.01$.

[5] Values are calculated by using data from the ten week interval, mean ± S.E.



Figs. 3a, 3b Effects of dietary fiber and carbohydrate interaction in pancreatic insulin response at a) six weeks and b) ten weeks. Levels of fibers with different superscripts are significantly different at the P<0.0001 level. LF - low fiber, MF- medium fiber, HF - high fiber.

of fiber was significant at the $P<0.0001$ level. Carbohydrate source altered insulin response ($P<0.0001$) with cornstarch producing a greater insulin release than sucrose. The interaction between fiber and carbohydrate was highly significant ($P<0.0002$).

Results of the ten week test are similar to those at six weeks (Table 14). No significant differences were noted between the low fiber or the medium fiber sucrose treatments. The medium fiber cornstarch group result was significantly different ($P<0.05$) from all other groups except the high fiber sucrose group. No significant differences were noted between the high fiber treatments. Differences due to the level of dietary fiber were significant at the 0.0001 level (Figure 3b); as in trial 1, insulin response decreased as the level of fiber increased. Source of carbohydrate produced differences significant at the $P<0.01$ level. Contrary to results in trial 1, starch decreased the insulin response. The interaction of fiber and carbohydrate was highly significant ($P<0.0001$).

In a study by Munoz and co-workers (48), human subjects were fed a basal low fiber diet for 30 days. At the end of this period, glucose tolerance and insulin response were measured. Subjects were then placed on a high fiber diet (26 g) with fiber sources of wheat bran, corn bran, soy hulls, freeze-dried apple powder, or freeze-dried carrot powder. The insulin response at the glucose peak (CIRp) was not altered by addition of wheat bran; however, other sources of fiber significantly increased insulin response. The differences in the effects of fiber were attributed to the different chemical composition of the fiber source.

In the present research, wheat bran had the opposite effect expected:

insulin response was decreased. Reduced insulin response could be caused by a number of factors. One, if insulin release is normally stimulated to a greater extent by a high fiber diet, then insulin reserves may be lower and insulin response to acute stimuli would be diminished. Alternatively, the fiber may negatively interact with gastrointestinal hormones which stimulate insulin release or increase activity of hormones antagonizing insulin. In the experiment by Munoz and co-workers (48) wheat bran increased the fasting glucagon level and glucagon response to an oral glucose tolerance test. A final factor is diurnal variation. Jarrett and co-workers (103) found the release of insulin is lower in the afternoon than in the morning. Afternoon testing may have produced falsely low results. In addition, insulin values are reduced when samples are hemolyzed (101). Samples used did show slight hemolysis. However, conclusive evidence which assigns cause in unavailable; the decreased insulin response is probably caused by a combination of these factors.

The different effects of source of carbohydrate in trial 1 and 2 was unexpected. Since starch produces a slower rise in blood glucose than sucrose, a lower insulin response would be expected. Research with both rats (51, 52, 53) and humans (54, 55) has consistently found starch produces an improved glucose tolerance curve in comparison to sucrose. The insulin release parallels glucose response. Reiser et al. (68) found fasting serum insulin and insulin response to a glucose load to be significantly higher for the sucrose diet than a starch diet in humans. Studies using rats have found similar results. Simple carbohydrates are reported to stimulate a more rapid and greater insulin response than does complex carbohydrate (69, 70, 54, 52).

In this experiment sucrose produced a lower response than starch in trial 2 (ten weeks), but not in trial 1 (six weeks). The effect of carbohydrate source is highly significant in both trials, decreasing the probability the differences are due to chance. However, as noted previously, results calculated from pooled insulin data are more subject to experimental error. In trial one, the high fiber cornstarch results were higher than all other results except one. This high value may have caused differences to appear which really did not exist.

Another important difference between trials is that CIRp values are lower in trial 2 than trial 1 with one exception. This suggests that the factor decreasing insulin release is strengthened with time. A deficiency of calcium would exhibit this pattern. Calcium is required for release of insulin (66). Bran feeding produced a negative calcium balance (38) and decreased plasma calcium (24) by decreasing intestinal absorption of calcium. The reduction of available calcium would limit the ability of the pancreas to release insulin. On the other hand, diminished insulin release may be the result of further depletion of insulin stores due to chronic overstimulation.

Overall the insulin release in the present study is considerably lower than results obtained by Munoz and co-workers (48). Insulin response of human subjects on high fiber diets (26 g) in the study by Munoz and co-workers (48) ranged from 1.00 to 1.75; in the present study values ranged from 0.08 to 0.73 (CIRp values at six and ten weeks). The low insulin release (CIRp) may indicate that the glucose dose is too large and depletes stores; a nutrient deficiency is present; or that rat and human subjects are not comparable. If insulin release is lower in all dietary treatments, then the

elevated glucose curve could be attributed in part to insufficient levels of circulating insulin. The further decrease of insulin release in trial 2 (ten weeks) corresponds to an increase in the glucose tolerance curve in trial 2. The relationship between these two variables may be coincidental; however, the results suggest that the insulin release was actually lower in all groups.

In general, the data demonstrate dietary treatment influences insulin release. Carbohydrate and fiber interact to enhance effects to the individual components. As the level of fiber increased the insulin response decreased; starch lowered pancreatic response in trial 2. The pattern of response was similar in both trials with insulin release consistently lower in the second trial than in the first. If insulin response is reduced in all dietary treatments, this could account in part for the elevated glucose curves; especially, the exaggerated increase in glucose tolerance which accompanied the further reduction in insulin release in trial two.

GTp. Mean values of peripheral sensitivity are contained in Table 14. Sensitivity was not significantly affected by dietary treatment in either trial 1 or trial 2. The results in trial 1 were significantly different from trial 2 ($P<0.05$). Insulin sensitivity decreased in all groups at the second test period.

The study by Munoz and co-workers (48) using wheat bran found wheat bran had no effect on peripheral insulin sensitivity. This result agrees with the findings in the present study. The insulin activity (GTp) of subjects on high fiber diets (26 g) ranged from 1.17 to 2.50 in the study by Munoz et al. (48). In the present study, GTp ranged from 0.13 to 0.42 (trial 1 and 2

inclusive). The large differences between results of the two studies suggest that insulin sensitivity of rats is below values reported for human subjects studied by Munoz et al. (48).

In the literature carbohydrate source was found to influence insulin sensitivity. Hallfrisch and Reiser have coordinated a number of studies with other researchers (69, 70, 102, 119). The procedure in each study involves feeding a 54 per cent sucrose or cornstarch diet; insulin secretion and other variables relating to carbohydrate metabolism were measured. The results in each study concurred that sucrose decreased insulin sensitivity. A relationship between source of carbohydrate and insulin activity was not demonstrated. The lack of effect of carbohydrate source may be related to the different composition of the diets, i.e., inclusion of fiber; or the low insulin sensitivity values, may reflect some factor which is antagonizing insulin activity and masks the effects of carbohydrate.

Insulin sensitivity is subject to many of the same factors influencing glucose tolerance. Factors which could decrease insulin sensitivity include aging, "obesity," and hyperinsulinism. Elevated fasting serum insulin levels are an indicator of hyperinsulinism. Typical insulin values are contained in Table 13. One-third of the experimental groups had values more than twice the typical insulin value. Hyperinsulinism acts as a negative feedback mechanism on the target cell and reduces the receptor number (116). Some researchers believe responsiveness to insulin is decreased by a decrease in receptor affinity (121, 72, 122). The problem of hyperinsulinism is frequently self-perpetuating because as sensitivity to insulin decreases the insulin released increases to produce a normal response.

Insulin resistance is not only limited to changes in the receptor affinity or concentration. Resistance states can be reduced in three instances: 1) prior to insulin-receptor interaction, 2) at the receptor, and 3) at steps following insulin receptor interaction (75). Changes in receptor affinity or concentration involve resistance at the receptor site. Theoretically factors creating resistance at the pre-receptor site decrease the concentration of free insulin. Factors which could be involved at this point include increased insulin degradation or insulin binding to non-receptor proteins. The final point in modification of insulin action can be altered by any change in the "coupling function" or steps in the pathway to biological activity.

Specific examples of other conditions which decrease insulin sensitivity are aging and obesity. Reiser and Hallfrisch (69) found as adiposity of rats increased insulin sensitivity decreased. Romero-Bracho and Reaven (104) support that heavy rats (body weight>405 g) had a decreased insulin sensitivity. Insulin insensitivity in rats categorized as obese by Olefsky and Reaven (106) (body weight>300 g) was attributed to a decrease in insulin binding as a result of reduced receptor number. The consensus of the literature reviewed is that obesity does decrease sensitivity; however, this factor does not entirely explain the alterations in insulin sensitivity (104).

Romero-Bracho and Reavan (104) postulate the alterations in carbohydrate metabolism are in part influenced by age or an age-related factor. Changes in insulin response are noted as early as four weeks (104). Olefsky and Reaven (106) found insulin sensitivity progressively decreased until 70 days (10 weeks). After this point no further changes were noticed. A study with

human subjects by DeFronzo (123) found insulin sensitivity decreased as age increased due to resistance of peripheral tissues. The most marked decline in humans occurs between 20 and 45 years and levels off after that. In summary, aging decreases insulin sensitivity. This decline begins at an early age and reaches a steady state by approximately 70 days for rats and 45 years for humans. Sensitivity is reduced by decreased insulin binding.

An age-related factor which may influence insulin sensitivity is chromium status. The level of chromium in the body declines with age (124). The reduction in chromium levels may represent a decreased chromium requirement as the organism ceases to grow. On the other hand, low chromium levels may be a sign of deficiency (125). Chromium status of elderly persons in the United States is lower and more unstable than chromium status in aging populations in other parts of the world (114). As chromium facilitates binding of insulin, the decreased insulin response and glucose tolerance may be a result of the lower chromium levels in aging.

To summarize factors affecting insulin sensitivity, dietary treatment had little effect. No significant differences were noted between groups. Insulin sensitivity was significantly reduced at ten weeks compared to six weeks. Hyperinsulism does reduce insulin sensitivity. The data in this study do not clearly implicate hyperinsulinism as a factor in insulin insensitivity. Hyperinsulinism may be involved, but not as a primary factor. Aging and increased body weight also decreased insulin sensitivity. Aging or an age-related factor reduce the insulin binding. This age-related factor may be chromium which is known to decline with aging and potentiates insulin binding. Obesity acts at the level of the receptor to decrease receptor concentration

and/or affinity; the exact mechanism of action is not yet clear. Obesity is not sufficient by itself to produce insulin insensitivity. In addition, potential interference with insulin activity can occur before insulin binds to the receptor or following the "coupling function". A combination of the above factors probably regulates sensitivity.

Lipogenic enzyme activity

Liver Hexose Monophosphate Shunt Enzymes (HMPD). Enzyme activity is expressed in three ways: change in O.D. (optical density) per minute per gram of tissue, change in O.D. per minute per 100 mg cytosol protein, and nMoles NADPH per minute per mg of cytosol protein (Table 15). Dietary treatment caused significant effects ($P<0.02$) on enzyme activity. The low fiber sucrose diet was significantly higher ($P<0.01$) than all other dietary treatments (Figure 4a). Results of groups consuming cornstarch and/or high fiber diets did not differ significantly. The interaction between carbohydrate and fiber was significant at the $P<0.02$ level; this interrelationship is demonstrated in Figure 5a. The medium fiber sucrose diet group was significantly higher ($P<0.02$) than both high fiber groups.

Carbohydrate source produced changes significant at the $P<0.001$ level, with starch diets producing significantly lower enzyme activity than sucrose diets. As the level of fiber increased, the enzyme activity decreased significantly($P<0.001$). Enzyme activity was maximal with the low fiber sucrose treatment. Differences between source of carbohydrate decreased as the level of dietary fiber increased.

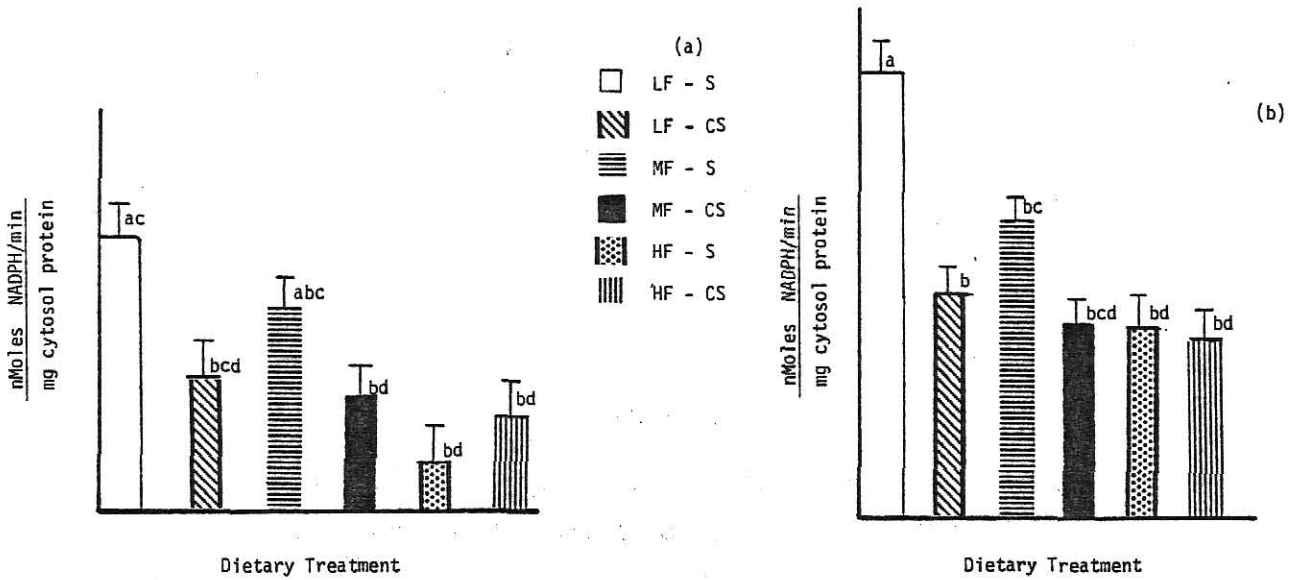
Liver NADP-malate dehydrogenase (malic enzyme). Expression of enzyme

TABLE 15

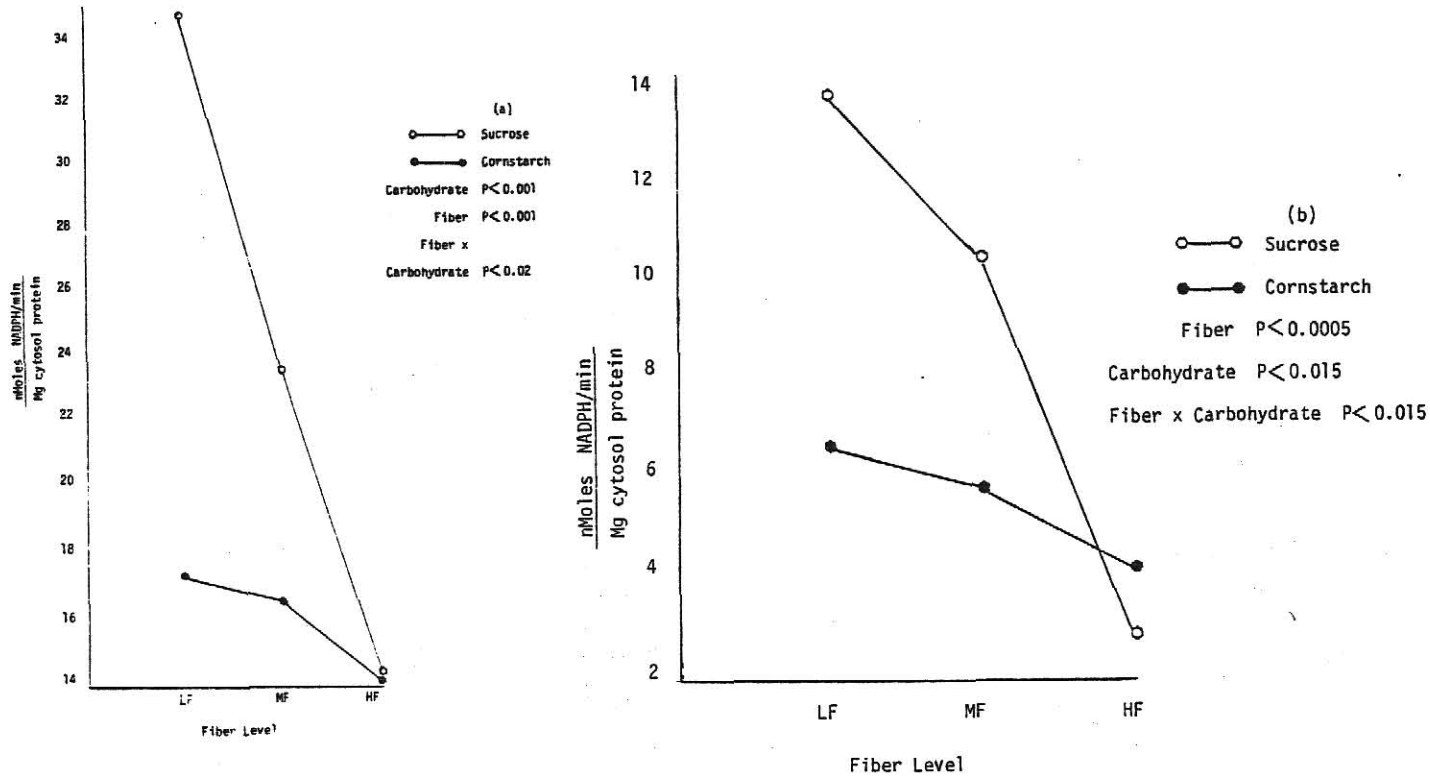
Activity of liver enzymes: hexose monophosphate shunt enzymes and NADP - malate dehydrogenase (Mean±S.E.)

Diet	HMPD			Malate		
	OD/min [1] / g tissue	OD/min / 100 Mg prot	nmoles NADPH/min / Mg prot	OD/min / g tissue	OD/min / 100 Mg prot	nmoles NADPH/min / Mg prot
Low Fiber Sucrose	13.53^{a}±1.13	8.66^{a}±0.70	34.82^{a}±2.83	4.38^{ac}±0.49	2.81^{ac}±0.31	13.56^{ac}±1.52
Low Fiber Cornstarch	6.87^{bcd}±1.13	4.35^{bcd}±0.70	17.50^{b}±2.83	2.19^{bcd}±0.52	1.37^{bcd}±0.34	6.66^{bcd}±1.63
Medium Fiber Sucrose	9.21^{bc} 1.01	5.91^{bc}±0.63	23.79^{bc}±2.53	3.34^{abc}±0.43	2.15^{abc}±0.28	10.39^{abc}±1.36
Medium Fiber Cornstarch	6.77^{bcd}±1.13	4.16^{bcd}±0.66	16.72^{bcd}±2.67	1.95^{bcd}±0.46	1.20^{bd}± 0.28	5.81^{bd}±1.36
High Fiber Sucrose	5.49^{bd}±1.21	3.59^{bde}±0.70	14.45^{bd}±2.83	0.94^{bd}±0.52	0.59^{bd}±0.31	2.84^{bd}±1.52
High Fiber Cornstarch	5.41^{bd}±1.07	3.50^{bde}±0.66	14.07^{bd}±2.67	1.48^{bd}±0.46	1.00^{bd}±0.30	4.82^{bd}±1.43

[1] Values in the same column with different superscripts are significantly different at the P 0.05 level.



Figs. 4a, 4b Liver enzyme activity of a) hexose monophosphate shunt enzymes (glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase) and b) NADP-malate dehydrogenase. 1. Mean $\pm$ S.E., 10 rats per group. Values with the same subscript are significantly different at the $P < 0.025$ level.



Figs. 5a, 5b Interaction of fiber and carbohydrate on lipogenic enzyme activity of a) hexose monophosphate shunt enzymes and b) NADP-malate dehydrogenase. Each point represents the sum of squares.

activity is contained in Table 15. Results were exactly the same in each expression of activity. Differences between the groups are depicted graphically in Figure 4b. The low fiber sucrose diet had significantly ($P<0.01$) higher enzyme activity than all groups except the medium fiber sucrose diet. The medium fiber sucrose diet was substantially higher than the medium fiber cornstarch diet ($P<0.025$) and the high fiber diets ($P<0.001$). These results can be summarized by stating no significant differences were noted when cornstarch or high fiber was included in the diet.

The interrelationship of dietary components is plotted in Figure 5b. Interactions between fiber and carbohydrate were significant at the $P<0.02$ level. Carbohydrate alone was effective in altering enzyme activity ($P<0.02$). The level of fiber was strongly related to enzyme activity ($P<0.0005$) with enzyme activity decreasing as the level of fiber increased. The influence of carbohydrate is negated at high levels of fiber.

Cohen and co-workers (85) studied the effects of high starch and sucrose diets on activity of glucose-6-phosphate dehydrogenase (G-6-P-D) and malic enzyme. The enzyme activity in sucrose fed rats was substantially higher than for starch fed rats even though sucrose fed rats had lower feed intake and body weight than starch fed rats. Fitch and Chaikoff (86) found the level of malic enzyme activity increased when rats were fed a simple carbohydrate diet.

The effect of wheat bran on activity of G-6-P-D and malic enzyme was studied by Lin and Anderson (44). Rats were fed either a starch bran or sucrose diet. Animals on the starch bran ration had significantly lower levels of G-6-P-D and malic enzyme activity than those on a sucrose diet.

The lower enzyme activity with the starch bran diet could result from either presence of a complex carbohydrate or addition of dietary fiber. Because the bran was not paired with sucrose or starch given by itself, the reduced enzyme activity cannot be attributed to one specific diet component. Starch and bran probably both contributed to the diet effects. This experiment re-emphasizes the ability of complex carbohydrates to reduce enzyme activity in contrast with sucrose diets and provides evidence which suggests that wheat bran may also be effective in reducing lipogenic enzyme activity.

In summary, results for HMPD and malic enzyme follow the same pattern. Conclusions in the literature support the experimental results of this study. Activity of liver HMPD and malic enzyme are influenced by both the source of carbohydrate and level of fiber. Sucrose and low levels of fiber increase enzyme activity. Carbohydrate and fiber interact so that the effect of sucrose in the diet is eliminated as the level of fiber increases.

CONCLUSIONS

The following conclusions can be made from the observations in the study:

1. Feed intake (g) increased significantly as the level of fiber increased. On a calorie basis, no difference among group feed intakes was present which supports the concept that rats consume food to meet energy needs.
2. Body weight gain an FER, and index of the ability to convert energy to weight gain, were not significantly affected by dietary treatment.
3. Glucose tolerance at six weeks (trial 1) was improved by starch at 60 and 90 minutes. Carbohydrate source had no effect on glucose tolerance at 0 and 30 minutes. Wheat bran had no significant effects on glucose tolerance.
4. At ten weeks (trial 2), glucose tolerance was unaffected by dietary treatment. Neither carbohydrate source nor level of fiber had an effect on glucose response. Glucose levels were significantly higher at each test interval at ten weeks than at six weeks. The decrease in glucose tolerance with time may indicate involvement of an age-related factor(s).
5. Insulin release (CIRp) was significantly affected by level of fiber, source of carbohydrate and fiber-carbohydrate interaction. As the level of fiber increased the insulin response decreased. Carbohydrate source produced different results at different

points in time. At six weeks (trial 1) sucrose produced a lower insulin response; whereas, at ten weeks (trial 2) starch produced a lower insulin response. At high levels of fiber the effect of carbohydrate source was negated. All values for insulin release (CIRp) appeared low; this deficit was significantly more pronounced at ten weeks (trial 2) than at six weeks (trial 1). Dietary treatment was determined to have a significant effect on CIRp, but factors other than diet appear to influence insulin response.

6. Peripheral insulin sensitivity was not significantly affected by dietary treatment. The GTp values appeared low. Results were significantly lower at ten weeks than at six weeks.
7. Activity of the lipogenic enzymes, hexose monophosphate shunt enzymes and NADP-malate dehydrogenase were significantly influenced by dietary treatment. As the level of dietary fiber increased the enzyme activity decreased. Likewise, cornstarch produced a lower enzyme activity than sucrose. Fiber and carbohydrate interactions were significant. At high levels of fiber the effect of carbohydrate source was eliminated.

In summary, dietary treatment was most effective in altering insulin release and liver lipogenic enzyme activity. Dietary treatment had little effect on glucose tolerance or peripheral insulin sensitivity.

Chemical composition of the fiber source appears to play a significant role in influencing glucose tolerance and insulin sensitivity. Future research might include a second source of fiber such as cellulose or guar gum.

Recommendations to increase clarity of the results include: reduction

of the glucose load to 250 mg per 100 g body weight, morning testing, and chromium supplementation of 2 - 5 ppm. Alteration of these factors would eliminate or reduce the interference of extraneous factors on the results.

SUMMARY

The effects of type and amount of dietary fiber and carbohydrate upon glucose tolerance, insulin response and lipogenic enzyme activity were investigated on weanling (50 - 75 g) male Sprague-Dawley rats. In a 3 x 2 factorial design, each level of fiber (0, 6 and 18%) was paired with sucrose or cornstarch. Rats (10/group) were fed one of the six experimental diets for 70 days (10 weeks). At six and ten weeks, oral glucose tolerance (test dose 350 mg glucose per 100 g body weight) and insulin activity were determined. Insulin activity was determined by calculating the pancreatic insulin release (CIRp) and the peripheral insulin sensitivity (GTp). At ten weeks animals were sacrificed and liver and epididymal tissues were removed. The lipogenic enzymes tested were the hexose monophosphate shunt enzymes (a combined assay for glucose-6-phosphate dehydrogenase and 6-gluconate dehydrogenase) and NADP-malate dehydrogenase.

Dietary treatment had little effect on glucose tolerance or peripheral insulin sensitivity. At six weeks starch reduced glucose levels by 10 per cent at 60 minutes and 9 per cent at 90 minutes; no other significant differences were observed.

Insulin release(CIRp)was reduced by addition of dietary fiber ($P < 0.0001$) and source of carbohydrate ($P < 0.0065$). At ten weeks starch produced a 33 per cent lower insulin response than sucrose, but at six weeks sucrose produced a 36 per cent lower insulin response than starch. As the level of fiber increased the effects of carbohydrate source were eliminated.

Carbohydrate tolerance decreased with time. Glucose tolerance, peripheral insulin sensitivity and pancreatic insulin response were all significantly

lower at ten weeks than at six weeks. This reduction with time may indicate the influence of an age-related factor.

Lipogenic enzyme activity for both enzymes followed the same pattern. High levels of dietary fiber and cornstarch reduced enzyme activity. The influence of carbohydrate source was negated at high levels of dietary fiber.

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APPENDIX

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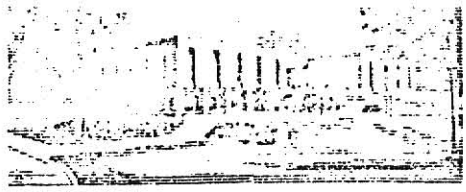

Serving the Cereal Industry

APPENDIX 16A

AACC CERTIFIED FOOD GRADE WHEAT BRAN

Analytical data for the official AACC Certified Food Grade Wheat Bran R07-3691, are summarized below. These values, as is basis, are the average of duplicate analyses.

The various analyses were divided among the following labs, unless otherwise noted: Doty Laboratories, Inc., 1435 Clay Street, North Kansas City, Missouri 64116; Ingman Laboratories, Inc., 324 South Fourth Avenue, Minneapolis, Minnesota 55415; Medallion Laboratories, 9000 Plymouth Avenue North, Minneapolis, Minnesota 55427; and Research 900, 900 Checkerboard Square, St. Louis, Missouri 63188.

The bran was made from a commercial blend of white wheats (87.3% soft white and 12.7% club white), taken after the fifth break roll over a 22 wire to a bran duster with a 1250 micrscreen. The bran was immediately sent through an enzyme deactivation steamer with a residence time of about thirty seconds, exiting at 213-215^{o}F, and 17.2% moisture, and into an insulated screw conveyer which holds the hot bran for about 1.5 minutes. The bran was dried to 10.4% moisture and sacked into "scotchguard" three layer paper, one-layer 2 mil polyethylene bags. The bran is being held at 0^{o}F in Minneapolis.

Requests for samples and information should be made at the letterhead address. Bran is available at $2.00/lb. in 2.5 lb. quantities or at $1.50/lb. in 30 lb. bags, plus shipping costs.

AACC CERTIFIED FOOD GRADE WHEAT BRAN
R07-3691

Assay	Value[a] (as is basis)	Method[b]
Crude Fiber	8.91%	AOAC-7.050-7.054
Protein	14.3%	AACC-46-10
Moisture	10.4%	AACC-44-40
Fat (Acid Hydrolysis)	5.22%	AOAC-7.047
Ash	5.12%	AACC-08-01
Aerobic Plate Count	16,000/g	AACC-42-11
Acid Detergent Fiber	11.9%	AOAC-7.055-.057
Neutral Detergent Fiber	40.2%	See Below[c]
Lignin	3.2%	AOAC-7.058
Pectin	3.0%	See Below[d]
Water Holding Capacity	9.5 g/g	See Below[e]
Cutin	0.0%	USDA Hndbk #379, pp 9-11
Thiamine (B1)	0.78 mg/100 g	AOAC-43.024-.030
Riboflavin (B2)	0.39 mg/100 g	AACC-86-70
Niacin	20.9 mg/100 g	AOAC-43.044-.046
Pyridoxine(B6)	0.58 mg/100 g	AOAC-43.159-.164
Folic Acid	0.12 mg/100 g	JAOAC,48(6), 1230 (1965)
Pantothenic Acid	2.48 mg/100 g	AOAC-43.130-.138
Vitamin E	2.69 mg/100 g	Gas Chromatorgraphy[f]
Choline	228 mg/100 g	AACC-86-45
Aluminum	5.0 ppm	AOAC-2.096-2.100
Arsenic	$<$0.1 ppm	AOAC-25.012
Barium	45.07 ppm	AACC-40-70
Boron	4.5 ppm	APHA 13th ed, 107B (1971)
Cadmium	2.8 ppm	AACC-40-70
Calcium	0.12 %	AACC-40-21
Cobalt	39.2 ppm	AACC-40-70
Copper	15.6 ppm	AACC-40-70
Iron	122 ppm	AACC-40-70

AACC CERTIFIED FOOD GRADE WHEAT BRAN, R07-3691
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Assay	Value[a] (as is basis)	Method[b]
Lead	2.3 ppm	AACC-40-70
Magnesium	0.43%	AACC-40-70
Manganese	80.0 ppm	AACC-40-70
Mercury	0.002 ppm	AOAC-25.103
Phosphorus	1.04%	AOAC-7.103
Potassium	1.38%	AACC-40-70
Silicon	35.0 ppm	
Selenium	0.1 ppm	See Below[g]
Sodium	0.10%	AACC-40-70
Zinc	54.5 ppm	AACC-40-70
Damaged Starch	3.74%	AACC-76-30A
Total Starch	17.4%	AOAC-14.031
Total Sugar As Invert	7.04%	AOAC-7.066
Pentosan	22.1%	See Below[h]
Phytic Acid	3.36%	See Below[i]
B-Sitosterol	123 mg/100 g	Gas Chromatography[f]
Campesterol	68.8 mg/100 g	Gas Chromatography[f]
Stigmasterol	11.2 mg/100 g	Gas Chromatography[f]
Aflatoxin	<10 ppb	JAOAC 56(4), 803 (1973)
Sanitation[j]	0	AACC-28-60
Pesticides, Phosphorus Containing	< 0.005 ppm	AOAC-29.-
Pesticides, Chlorine Containing	< 0.02 ppm	See Below[k]

Particle Size

ON US #10	1%			
#12	2%		#70	Trace
		Thru	#70	Trace
#14	5%			
#16	11%			
#18	13%			
#20	9%			
#30	33%			
#40	17%			
#50	8%			
#60	1%			

a. Abbreviations: mg - milligrams
ppm - parts per million
ppb - parts per billion

b. AOAC References are 12th edition (1975).

c. Neutral Detergent Giver. Method of Van Soest and Wine, JOAC, 50 (1), 50 (1967), modified by use of hog pancreatic anylase; AACC Fiber Methodology subcommittee.

d. Reported as % galacturonic acid. Medallion Labs modified procedure from: McCready and McComb, Analytical Chemistry, 24 (12), 1986 (1952) and; Bitter and Muir, Analytical Biochemistry, 4, 330 (1962).

e. Water Holding Capacity of the NDF fraction. By Kellogg Co.

f. Method by Medallion Laboratories.

g. Analysis by WARF Institute, Inc., Madison, Wisconsin, according to J. Ag. & Fd. Chem., 9 488 (1961).

h. Method by CPC International, Inc.

i. Modified method by Research 900. Adapted from: Biochem. Z., 64, 422 (1914).

j. Whole insects, insect fragments, whole larvae, larva fragments, rodent hairs, rodent excretion fragments, other contaminants.

k. Modified method by Research 900.

TABLE 17A

Initial body weight, final body weight, mean daily weight gain, mean daily feed intake (g and kcal), feed efficiency ratio (FER) and epididymal fat pad weight of rats

Diet	Initial Weight(g)	Final[1] Weight(g)	Mean Daily Weight Gain (g/day)	Mean Daily Feed Intake g/day	Mean Daily Feed Intake kcal/day	FER = $\frac{\text{wt. gain}}{\text{kcal}} \times 100$	Epididymal Fat Pad Wt/ 100g bd wt
Low Fiber Sucrose							
1	74.5	475.0	5.32	16.0	68.3	7.79	2.13
2	81.0	—	—	—	—	—	—
3	92.5	—	—	—	—	—	—
4	96.0	421.0	4.49	14.6	62.3	7.21	2.00
5	98.0	503.0	5.62	18.7	79.8	7.04	1.46
6	96.0	450.0	4.90	15.6	66.6	7.36	1.35
7	99.0	393.0	4.13	14.1	60.2	6.86	1.60
8	75.5	451.5	5.22	15.9	67.9	7.69	1.54
9	86.0	382.0	4.08	14.7	62.8	6.50	1.67
10	74.0	464.5	5.20	17.9	76.4	6.81	1.75
Mean ± S.E.	87.25±3.87	442.5±10.52	4.87±0.16	15.94±0.41	68.04±1.66	7.16±0.18	1.69±0.09
Low Fiber Cornstarch							
1	80.0	465.0	5.25	15.8	67.5	7.78	1.60
2	86.0	452.5	5.16	13.5	57.6	8.96	1.56
3	89.0	—	—	—	—	—	—
4	93.0	515.0	5.99	17.7	75.6	7.92	1.54
5	100.0	446.0	4.77	16.9	72.2	6.61	1.34
6	85.0	424.0	4.66	14.2	60.6	7.69	1.86
7	66.5	—	—	—	—	—	—
8	85.5	446.0	5.04	15.5	66.2	7.61	1.72
9	110.5	440.5	4.50	15.2	64.9	6.93	1.64

continued, TABLE 17A

Diet	Inital Weight (g)	Final[1] Weight(g)	Mean Daily Weight Gain (g/day)	Mean Daily Feed Intake g/day	Mean Daily Feed Intake kcal/day	FER = $\frac{\text{wt. gain}}{\text{kcal}} \times 100$	Epididymal Fat Pad Wt/ 100g bd wt
9	110.5	440.5	4.50	15.2	64.9	6.93	1.64
10	83.0	453.0	4.92	17.1	73.0	6.74	1.87
Mean ± S.E.	87.85±3.87	455.25±10.52	5.04±0.16	15.74±0.41	67.20±1.66	7.53±0.18	1.64±0.09
Medium Fiber Sucrose							
1	83.5	403.5	4.22	14.8	59.9	7.05	1.81
2	82.0	481.5	5.63	17.1	69.2	8.14	1.72
3	90.0	430.0	5.13	17.4	70.4	7.29	1.83
4	90.0	449.5	4.93	16.3	65.9	7.48	1.63
5	99.0	452.0	4.87	17.4	70.4	6.92	1.58
6	81.5	441.5	5.02	16.5	66.7	7.53	2.17
7	119.5	431.0	4.33	16.8	68.0	6.37	1.52
8	75.0	434.5	4.91	15.8	63.9	7.68	1.78
9	77.0	420.5	4.72	15.8	63.9	7.39	1.31
10	71.0	433.4	4.82	16.1	65.1	7.40	1.71
Mean ± S.E.	86.85±3.87	437.75±9.41	4.86±0.14	16.40±0.36	66.34±1.49	7.33±0.16	1.71±0.08
Medium Fiber Cornstarch							
1	90.0	453.0	4.90	16.2	65.5	7.48	1.69
2	83.5	411.5	4.62	15.9	64.3	7.18	1.96
3	90.0	468.0	5.54	17.2	69.6	7.96	1.91
4	90.0	492.0	5.70	18.6	75.2	7.58	1.60
5	96.0	502.0	5.69	19.0	76.9	7.40	1.67
6	87.0	397.0	4.30	15.3	61.9	6.95	1.24
7	87.0	410.0	4.49	15.4	62.3	7.21	——
8	74.0	450.5	5.20	17.1	69.2	7.51	1.80
9	94.0	466.0	5.07	17.9	72.4	7.00	1.93

continued

continued, TABLE 17A

Diet	Initial Weight (g)	Final[1] Weight (g)	Mean Daily Weight Gain (g/day)	Mean Daily Feed Intake g/day	Mean Daily Feed Intake kcal/day	FER = $\frac{\text{wt. gain}}{\text{kcal}} \times 100$	Epididymal Fat Pad Wt/ 100g bd wt
10	81.0	447.5	4.94	17.4	70.4	7.02	1.82
Mean ± S.E.	87.25±3.87	449.75±9.41	5.05±0.14	17.00±0.39	68.77±1.49	7.33±0.16	1.74±0.08
High Fiber Sucrose							
1	81.5	436.0	4.80	18.1	65.2	7.36	1.84
2	86.0	——	——	——	——	——	——
3	91.0	357.0	3.93	17.3	62.3	6.31	1.13
4	85.0	452.0	5.25	18.7	67.3	7.80	1.26
5	101.0	416.0	4.42	17.2	61.9	7.14	1.91
6	92.0	435.0	4.77	18.0	64.8	7.36	1.61
7	88.0	455.0	5.10	18.5	66.6	7.66	1.62
8	85.5	415.5	4.58	18.5	66.6	6.88	1.90
9	74.0	424.5	4.80	18.0	64.8	7.41	1.24
10	95.0	401.5	4.99	20.1	72.4	6.89	1.75
Mean ± S.E.	87.90±3.87	421.39±9.92	4.74±0.15	18.27±0.38	65.77±1.57	7.20±0.17	1.58±0.08
High Fiber Cornstarch							
1	82.0	411.5	4.51	17.3	62.3	7.24	1.23
2	84.0	426.5	4.82	19.0	68.4	7.05	1.54
3	92.0	435.0	5.41	18.3	65.9	8.21	1.82
4	79.0	433.0	5.06	19.8	71.3	7.10	1.77
5	101.0	428.0	4.56	19.1	68.8	6.63	1.60
6	109.5	453.5	4.81	18.8	67.7	7.10	1.97
7	91.0	422.0	4.68	18.9	68.1	6.87	1.59
8	73.5	447.0	5.06	18.1	65.2	7.76	1.46
9	80.0	492.5	5.63	19.4	69.9	8.05	1.79
10	80.5	436.0	4.80	19.2	69.2	6.94	1.99
Mean ± S.E.	81.85±3.87	438.50±9.41	4.93±0.14	18.79±0.36	67.68±1.49	7.30±0.16	1.68±0.08

[1] Values for rats at 71 days of age.

TABLE 18A

Two-week mean[1] of body weight gain (g) of rats

Diet	Week 1 and 2	Week 3 and 4	Week 5 and 6	Week 7 and 8	Week 9 and 10
Low Fiber Sucrose	113.8±2.63	99.1±3.98	63.9±5.48	32.7[2]±4.08	36.19[2]±4.64
Low Fiber Cornstarch	112.25±2.69	104.9±7.49	66.5±4.75	31.63[2]±4.28	45.5[2]±3.04
Medium Fiber Sucrose	111.5±7.44	89.9±6.41	64.6±4.61	41.22±2.58	42.8±2.99
Medium Fiber Cornstarch	114.0±3.41	104.2±7.44	63.4±4.53	40.2±2.30	41.7±2.98
High Fiber Sucrose	107.5±7.17	96.9±2.98	55.9±3.90	32.9[3]±2.35	41.7[3]±2.70
High Fiber Cornstarch	105.9±1.70	102.8±2.10	60.35±4.05	39.1±3.98	42.3±3.02

[1] Mean ± S.E.

[2] n=8

[3] n=9

TABLE 19A

Two-week mean[1] feed intake (g) of rats (n=10)

Diet	Week 1 and 2	Week 3 and 4	Week 5 and 6	Week 7 and 8	Week 9 and 10
Low Fiber Sucrose	185.8±4.55	232.3±4.60	248.3±8.96	236.8[2]±19.64	223.8[2]±19.75
Low Fiber Cornstarch	181.8±4.15	230.5±8.43	220.2±11.37	221.3[2]±17.04	255.5[2]±7.41
Medium Fiber Sucrose	190.8±4.08	231.9±5.24	239.6±4.75	240.3±7.98	261.1±5.30
Medium Fiber Cornstarch	196.2±4.48	243.8±7.31	251.2±9.15	251.9±6.20	263.6±11.48
High Fiber Sucrose	107.5±7.17	96.9±2.98	55.9±3.90	32.9[3]±2.35	41.7[3]±2.70
High Fiber Cornstarch	105.9±1.70	102.8±2.10	60.3±4.05	39.1±3.98	42.3±3.02

[1] Mean ± S.E.

[2] n=8

[3] n=9

TABLE 20A

Serum glucose levels (mg/dl glucose) and pooled serum insulin response (uU/ml) to an oral glucose tolerance (GTT)

Variable	GTT Six Weeks (mg glc/dl) 0	30	60	90	GTT Ten Weeks 0	30	60	90
Low Fiber Sucrose								
1	78.33	178.06	178.40	163.48	138.02	196.83	185.97	195.48
2	—	—	—	—	—	—	—	—
3	—	—	—	—	—	—	—	—
4	146.12	206.41	164.31	206.03	126.79	193.00	163.66	186.00
5	115.16	200.23	170.63	171.10	134.18	192.23	239.02	195.67
6	63.43	176.94	170.44	176.01	111.95	241.59	170.01	160.61
7	138.68	172.73	167.52	157.82	141.11	171.61	160.94	201.21
8	114.5	170.08	172.29	160.42	119.32	170.62	193.92	—
9	120.91	188.15	139.33	141.59	115.0	133.36	121.42	239.96
10	118.80	196.9	174.80	—	115.30	288.08	290.47	262.48
Mean ± S.E.	111.99± 15.52	186.19± 12.22	167.22± 8.59	168.06± 8.84	125.21± 4.99	198.42± 22.17	190.68± 15.71	205.92± 19.00
Group Insulin Response uU/ml	63.5	122.0	96.0	59.5	17.0	94.75	160.0	28.0
Low Fiber Cornstarch								
1	89.92	169.97	146.57	162.05	131.98	190.50	236.03	133.47
2	161.00	166.10	152.06	—	132.52	129.48	166.84	150.00
3	—	—	—	—	—	—	—	—

continued

continued, TABLE 20A

Variable	GTT Six Weeks (mg glc/dl) 0	30	60	90	GTT Ten Weeks 0	30	60	90
4	149.28	230.62	176.36	179.90	99.44	397.59	190.00	233.12
5	115.91	183.44	163.54	167.48	91.26	170.36	187.99	177.01
6	86.61	168.82	140.20	—	116.37	155.64	170.95	167.66
7	—	—	—	—	—	—	—	—
8	116.89	138.83	159.92	157.63	80.87	146.02	162.04	171.02
9	143.53	186.67	139.01	137.52	106.50	238.41	239.96	251.55
10	143.60	280.67	154.40	—	111.67	288.08	292.95	315.75
Mean ± S.E.	125.84± 15.52	190.64± 12.22	154.01± 8.59	160.92± 10.46	108.83± 5.58	214.51± 22.17	205.85± 15.71	199.95± 17.78
Group Insulin Response uU/ml	16.0	160.0	16.0	53.0	97.0	105.0	52.0	96.0
Medium Fiber Sucrose								
1	91.27	183.12	156.15	181.18	141.07	305.18	161.57	—
2	177.14	239.55	208.67	190.78	101.28	172.16	181.84	161.01
3	232.29	81.05	219.08	—	113.09	168.96	162.04	179.35
4	170.81	229.00	156.27	158.85	109.20	211.30	214.00	—
5	111.39	245.09	192.75	169.84	141.21	298.00	269.91	260.45
6	97.28	276.46	199.61	180.78	133.66	310.27	203.71	193.11
7	97.69	209.92	208.84	155.98	117.67	163.34	171.87	186.83
8	69.55	165.90	171.27	144.32	83.70	196.17	188.10	—
9	132.22	157.76	153.88	155.63	105.18	187.36	189.83	238.78
10	113.53	185.47	196.56	188.15	104.14	296.37	279.79	293.78
Mean ± S.E.	129.32± 13.88	197.33± 10.93	186.31± 7.68	169.50± 7.79	115.02± 4.49	230.91± 19.83	202.27± 14.05	216.19± 19.00

continued

continued, TABLE 20A

Variable	GTT Six Weeks (mg glc/dl) 0	30	60	90	GTT Ten Weeks 0	30	108	90
Group Insulin Response uU/ml	10.0	85.0	40.0	23.5	22.5	123.5	28.0	27.0
Medium Fiber Cornstarch								
1	90.48	176.97	171.53	—	155.79	247.50	204.07	284.46
2	—	166.37	—	—	110.64	157.12	173.65	165.92
3	318.37	107.68	—	—	119.95	184.77	175.93	167.87
4	134.64	218.85	157.89	165.55	105.60	196.70	194.30	200.30
5	113.88	—	—	—	143.85	299.74	313.07	313.94
6	105.60	185.30	167.89	169.51	136.47	293.73	245.29	243.99
7	86.76	182.23	154.14	138.31	114.44	156.76	155.87	163.34
8	95.62	157.63	138.39	159.07	98.84	237.91	206.16	—
9	130.60	186.15	155.17	143.86	126.55	198.60	159.03	—
10	137.20	168.0	159.52	167.60	110.13	196.38	199.10	241.52
Mean ± S.E.	134.74± 14.63	172.13± 11.52	157.79± 9.18	157.31± 9.54	122.25± 4.99	216.92± 19.83	202.65± 14.05	220.03± 16.76
Group Insulin Response uU/ml	40.25	42.25	14.0	21.5	13.75	23.0	16.0	50.0
High Fiber Sucrose								
1	93.89	163.74	161.54	—	109.98	367.88	282.38	301.04
2	—	—	—	—	—	—	—	—
3	—	178.57	280.36	224.8	119.32	176.45	182.85	—
4	158.56	202.20	190.43	218.85	112.00	244.20	232.59	207.00
5	108.82	200.23	—	—	111.05	271.16	181.82	183.98

continued

continued, TABLE 20A

Variable	GTT Six Weeks (mg glc/dl) 0	30	60	90	GTT Ten Weeks 0	30	60	90
6	136.39	195.98	187.62	198.07	134.31	290.87	177.64	187.63
7	132.24	204.13	161.96	123.15	136.31	175.79	162.71	175.63
8	108.13	163.31	162.71	166.69	128.05	167.04	191.43	150.00
9	124.18	207.63	199.26	186.44	116.01	263.69	254.84	253.38
10	140.80	172.08	110.15	165.20	105.53	288.39	314.30	305.19
Mean ± S.E.	125.38± 15.52	187.54± 11.52	181.75± 8.59	183.31± 8.84	119.17± 5.26	249.50± 20.90	220.06± 14.82	220.48± 17.78
Group Insulin Response uU/ml	53.0	59.0	30.0	10.0	19.5	50.5	18.0	36.5
High Fiber Cornstarch								
1	94.21	179.29	172.82	—	—	—	—	—
2	181.55	190.54	164.85	141.95	107.49	166.56	173.85	138.04
3	206.43	209.18	—	178.27	115.76	205.33	177.78	204.99
4	140.38	180.19	185.36	—	141.07	296.08	167.86	201.3
5	111.09	183.67	176.36	175.59	112.37	183.02	182.62	171.97
6	106.61	172.53	173.15	136.13	106.35	186.21	155.92	163.43
7	134.36	185.95	173.92	129.29	127.42	149.38	177.56	169.21
8	78.88	166.69	149.58	118.49	129.63	175.61	153.06	—
9	130.94	158.86	154.33	195.33	112.68	192.56	185.95	168.78
10	119.92	235.93	185.47	182.41	94.78	281.87	211.76	264.47
Mean ± S.E.	130.44± 13.88	186.28± 10.93	170.65± 8.10	157.18± 8.27	116.39± 5.26	240.06± 20.90	176.26± 14.82	185.55± 16.76
Group Insulin Response uU/ml	21.0	160.0	21.5	77.0	11.0	52.5	33.5	50.0

TABLE 21A

Index of insulin release (CIRp) and peripheral insulin sensitivity (GTp) at six and ten weeks (Trial 1 and 2 respectively)

Diet	GTp 1	GTp 2	CIRp 1	CIRp 2
Low Fiber Sucrose				
1	0.29	0.20	0.63	0.64
2	—	—	—	—
3	—	—	—	—
4	0.17	0.22	0.43	0.67
5	0.19	0.10	0.47	0.40
6	0.30	0.10	0.64	0.39
7	0.33	0.19	0.69	0.61
8	0.33	0.21	0.69	0.67
9	0.24	0.10	0.55	0.39
10	0.20	0.05	0.49	0.25
Mean ± S.E.	0.26±0.08	0.15±0.04	0.57±0.06	0.50±0.05
Low Fiber Cornstarch				
1	0.35	0.11	0.94	0.27
2	0.38	0.37	1.00	0.65
3	—	—	—	—
4	0.12	0.02	0.43	0.08
5	0.26	0.24	0.77	0.47

continued

continued, TABLE 21A

Diet	GTp 1	GTp 2	CIRp 1	CIRp 2
6	0.36	0.34	0.96	0.61
7	—	—	—	—
8	0.43	0.34	1.11	0.61
9	0.25	0.09	0.73	0.23
10	0.06	0.04	0.27	0.14
Mean ± S.E.	0.27±0.08	0.19±0.04	0.78±0.06	0.38±0.05
Medium Fiber Sucrose				
1	0.26	0.05	0.41	0.17
2	0.10	0.27	0.21	0.61
3	0.11	0.28	0.23	0.63
4	0.12	0.15	0.23	0.40
5	0.10	0.05	0.20	0.18
6	0.06	0.04	0.15	0.17
7	0.16	0.25	0.29	0.57
8	0.34	0.21	0.49	0.50
9	0.46	0.10	0.61	0.31
10	0.20	0.05	0.34	0.18
Mean ± S.E.	0.19±0.07	0.15±0.03	0.32±0.08	0.37±0.05
Medium Fiber Cornstarch				
1	0.30	0.06	0.22	0.44

continued

continued, TABLE 21A

Diet	GTp 1	GTp 2	CIRp 1	CIRp 2
2	0.37	0.32	0.26	0.13
3	0.04	0.26	0.05	0.11
4	0.14	0.19	0.13	0.09
5	1.76	0.04	0.85	0.03
6	0.25	0.05	0.20	0.03
7	0.27	0.40	0.21	0.15
8	0.44	0.11	0.30	0.06
9	0.25	0.20	0.20	0.09
10	0.36	0.10	0.26	0.06
Mean ± S.E.	0.42±0.07	0.17±0.03	0.27±0.06	0.08±0.05
High Fiber Sucrose				
1	0.40	0.02	0.38	0.05
2	—	—	—	—
3	0.06	0.27	0.10	0.24
4	0.14	0.10	0.18	0.12
5	0.19	0.07	0.23	0.09
6	0.20	0.05	0.23	0.08
7	0.18	0.31	0.22	0.27
8	0.37	0.22	0.37	0.22
9	0.17	0.07	0.21	0.10
10	0.33	0.04	0.34	0.07
Mean ± S.E.	0.23±0.08	0.13±0.04	0.25±0.06	0.14±0.05

continued

continued, TABLE 21A

Diet	GTp 1	GTp 2	CIRp 1	CIRp 2
High Fiber Cornstarch				
1	0.28	0.24	0.82	0.24
2	0.23	0.32	0.70	0.29
3	0.16	0.18	0.55	0.19
4	0.25	0.05	0.75	0.08
5	0.26	0.26	0.77	0.25
6	0.32	0.25	0.90	0.24
7	0.25	0.29	0.74	0.27
8	0.37	0.31	0.99	0.28
9	0.21	0.22	0.65	0.22
10	0.11	0.06	0.41	0.09
Mean $\pm$ S.E.	0.25$\pm$0.07	0.22$\pm$0.03	0.73$\pm$0.58	0.22$\pm$0.05

TABLE 22A

Activity of liver lipogenic enzymes (hexose monophosphate enzymes and NADP-malate dehydrogenase), liver weight and cytosol liver protein content

Diet	HMPD OD/min g tissue	HMPD OD/min 100mg prot	HMPD nMoles NADPH/min Mg prot	Malate OD/min g tissue	Malate OD/min 100mg prot	Malate nMoles NADPH/min Mg prot	Liver wt (g)	Mg cytosol protein/ml
Low Fiber Sucrose								
1	17.72	11.22	45.09	5.24	3.32	16.02	13.03212	165.815
2	——	——	——	——	——	——	——	——
3	——	——	——	——	——	——	——	——
4	24.33	15.37	61.78	5.73	3.62	17.48	11.38000	157.460
5	14.77	9.93	39.90	5.29	5.29	17.17	15.26308	149.105
6	8.55	5.17	20.78	3.58	2.16	10.45	13.04425	166.370
7	11.66	6.37	25.62	2.69	1.47	7.10	10.26669	183.550
8	7.79	5.63	22.63	2.11	1.52	7.36	11.48141	150.965
9	8.92	6.64	26.69	2.65	1.97	9.51	10.42968	137.075
10	14.46	8.98	36.08	7.79	4.84	23.36	12.58713	186.065
Mean ± S.E.	13.53± 1.13	8.66± 0.70	34.82± 2.83	4.38± 0.49	2.81± 0.31	13.56± 1.52	12.19± 0.58	162.05± 5.98
Low Fiber Cornstarch								
1	5.57	2.77	11.14	2.29	1.14	5.50	11.68987	202.005
2	5.76	3.76	15.10	1.49	0.97	4.69	12.19982	154.420
3	——	——	——	——	——	——	——	——
4	8.53	5.61	22.55	4.57	3.01	14.51	13.37961	149.750
5	4.66	3.34	13.44	——	——	——	11.5408	152.525
6	11.22	6.49	26.08	2.51	1.45	6.99	10.11095	172.655

continued

continued, TABLE 22A

Diet	HMPD OD/min g tissue	HMPD OD/min 100mg prot	HMPD nMoles NADPH/min Mg prot	Malate OD/min g tissue	Malate OD/min 100mg prot	Malate nMoles NADPH/min Mg prot	Liver wt (g)	Mg cytosol protein/ml
7	___	___	___	___	___	___	___	___
8	5.68	4.22	16.96	1.86	1.38	6.66	12.23169	123.255
9	6.12	4.20	16.89	1.72	1.18	5.71	11.50963	152.330
10	7.38	4.45	17.88	0.87	0.53	2.54	10.80427	170.915
Mean ± S.E.	67.87± 1.13	4.35± 0.70	17.50± 2.83	2.19± 0.53	1.37± 0.34	6.66± 1.63	10.43± 1.32	159.73± 8.06
Medium Fiber Sucrose								
1	6.44	4.63	18.61	2.80	2.02	9.74	10.75456	133.920
2	12.28	7.99	32.12	3.12	2.03	9.81	13.56000	157.620
3	6.54	4.04	16.25	2.14	1.32	6.40	11.52539	150.890
4	8.30	4.80	19.30	3.50	2.02	9.78	12.55916	172.910
5	6.96	4.84	19.46	2.11	1.47	7.08	12.4510	156.965
6	12.85	7.74	31.12	4.91	2.96	14.30	12.89151	192.495
7	9.21	5.63	22.62	1.99	1.21	5.87	10.70570	156.395
8	13.22	8.75	35.16	3.87	1.90	9.18	11.60380	157.785
9	8.84	5.86	23.56	2.26	1.50	7.23	13.39789	153.550
10	7.45	4.90	19.71	7.72	5.08	24.53	10.45620	171.285
Mean ± S.E.	9.21± 1.01	5.91± 0.63	23.79± 2.53	3.34± 0.43	2.15± 0.28	10.39± 1.36	11.99± 1.07	160.38± 4.92
Medium Fiber Cornstarch								
1	5.92	3.43	13.78	1.78	1.03	4.97	11.43048	174.980
2	4.02	2.43	9.76	3.18	1.92	9.27	12.29866	177.195

continued

continued, TABLE 22A

Diet	HMPD OD/min g tissue	HMPD OD/min 100mg prot	HMPD nMoles NADPH/min Mg prot	Malate OD/min g tissue	Malate OD/min 100mg prot	Malate nMoles NADPH/min Mg prot	Liver wt (g)	Mg cytosol protein/ml
3	4.76	2.88	11.57	1.53	0.93	4.47	12.13950	183.735
4	4.49	2.85	11.46	1.20	0.76	3.67	15.72936	157.875
5	12.56	7.32	29.44	0.74	0.43	2.09	14.17285	173.390
6	4.21	2.92	11.74	1.52	1.06	5.10	11.04502	160.875
7	——	2.48	9.97	——	0.47	2.28	——	169.420
8	6.61	4.31	17.34	1.41	0.92	4.44	11.54572	173.900
9	——	——	——	2.20	1.50	7.27	13.51694	146.185
10	11.61	8.80	35.39	3.96	3.01	14.52	13.10025	139.730
Mean ± S.E.	6.77± 1.13	4.16± 0.66	16.72± 2.67	1.95± 0.46	1.20± 0.28	5.81± 1.36	11.66± 1.27	165.73± 4.49
High Fiber Sucrose								
1	6.03	3.82	15.60	1.82	1.17	5.65	11.38338	162.395
2	——	——	——	——	——	——	——	——
3	2.31	1.54	6.19	0.30	0.20	6.19	8.88020	149.485
4	——	——	——	——	——		14.60733	——
5	3.06	2.32	9.33	0.10	0.07	0.36	10.65085	133.540
6	6.24	3.98	15.63	1.04	0.65	3.13	13.12466	185.215
7	——	3.84	15.44	——	0.46	2.24	——	150.965
8	5.27	3.30	13.25	0.51	0.31	1.53	11.01718	157.765
9	5.39	3.75	15.08	1.32	0.92	4.45	11.61060	141.250
10	10.11	6.23	25.05	1.49	0.92	4.43	12.74780	163.675
Mean ± S.E.	5.49± 1.21	3.59± 0.70	14.45± 2.83	0.94± 0.52	0.59± 0.31	2.84± 1.52	11.75± 1.24	155.54± 5.58

continued

continued, TABLE 22A

Diet	HMPD OD/min g tissue	HMPD OD/min 100mg prot	HMPD nMoles NADPH/min Mg prot	Malate OD/min g tissue	Malate OD/min 100mg prot	Malate nMoles NADPH/min Mg prot	Liver wt (g)	Mg cytosol protein/ml
High Fiber Cornstarch								
1	4.04	2.81	11.29	1.35	0.94	4.52	11.10150	149.600
2	3.31	2.71	10.90	1.61	1.32	6.36	13.23546	129.100
3	5.21	3.39	13.65	0.40	0.26	1.26	11.89301	153.170
4	——	——	——	——	——	——	13.30346	——
5	6.80	3.85	15.49	1.08	0.61	2.97	12.30245	179.100
6	4.35	2.61	10.49	1.64	0.99	4.76	12.45750	172.395
7	5.85	3.30	13.26	0.39	0.22	1.06	11.86513	181.890
8	6.69	4.32	17.38	0.59	0.38	1.84	12.34282	157.245
9	8.05	5.69	22.89	3.38	2.39	11.54	13.62655	142.250
10	4.37	2.80	11.26	2.92	1.87	9.02	11.05620	150.005
Mean ± S.E.	5.41± 1.07	3.50± 0.66	14.07± 2.67	1.48± 2.67	1.00± 0.46	4.82± 1.43	12.32± 0.28	157.195± 5.84

EFFECT OF DIETARY FIBER AND CARBOHYDRATE SOURCE ON GLUCOSE TOLERANCE, INSULIN RESPONSE AND LIPOGENIC ENZYME ACTIVITY

by

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ABSTRACT

The effects of type and amount of dietary fiber and carbohydrate upon glucose tolerance, insulin response and lipogenic enzyme activity were investigated on weanling (50 - 75 g) male Sprague-Dawley rats. In a 3 x 2 factorial design, each level of fiber (0, 6 and 18%) was paired with sucrose or cornstarch. Rats (10/group) were fed on of the six experimental diets for 70 days (10 weeks). At six and ten weeks, oral glucose tolerance (test dose 350 mg glucose per 100 g body weight) and insulin activity were determined. Insulin activity was determined by calculating the pancreatic insulin release (CIRp) and the peripheral insulin sensitivity (GTp). At ten weeks animals were sacrificed and liver and epididymal tissues were removed. The lipogenic enzymes tested were the hexose monophosphate shunt enzymes (a combined assay for glucose-6-phosphate dehydrogenase and 6-gluconate dehydrogenase) and NADP-malate dehydrogenase.

Dietary treatment had little effect on glucose tolerance or peripheral insulin sensitivity. At six weeks starch reduced glucose levels by 10 per cent at 60 minutes and 9 per cent at 90 minutes; no other significant differences were observed.

Insulin release CIRp was reduced by addition of dietary fiber ($P < 0.0001$) and source of carbohydrate ($P < 0.0065$). At ten weeks starch produced a 33 per cent lower insulin response than sucrose, but at six weeks sucrose produced a 36 per cent lower insulin response than starch. As the level of fiber increased the effects of carbohydrate source were eliminated.

Carbohydrate tolerance decreased with time. Glucose tolerance, peripheral insulin sensitivity and pancreatic insulin response were all significantly

lower at ten weeks than at six weeks. This reduction with time may indicate the influence of an age-related factor.

Lipogenic enzyme activity for both enzymes followed the same pattern. High levels of dietary fiber and cornstarch reduced enzyme activity. The influence of carbohydrate source was negated at high levels of dietary fiber.