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Barbara Baker Campbell

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Ronda Greenwalt
Major professor

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EFFECT OF DIETARY FIBER ON GLUCOSE TOLERANCE IN RATS

By

Barbara Baker Campbell

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ABSTRACT

EFFECT OF DIETARY FIBER ON GLUCOSE TOLERANCE IN RATS

By

Barbara Baker Campbell

The effects of pectin (P), methylcellulose (MC) and wheat bran (WB) and two levels of dietary carbohydrate on glucose tolerance were investigated in male Sprague-Dawley rats. Two fiber-free diets containing high (70% kcal) or low (40%) carbohydrate were formulated using a cornstarch: sucrose (3:1) mix. P and MC were added to these control diets at a level of 1.9 g/100 kcal. WB was added to provide 3.6 g NDF/100 kcal. After a four week feeding period, rats were given oral glucose tolerance tests (OGTT) using 1) glucose alone and 2) glucose + fiber. Irrespective of previous diet, glucose + fiber resulted in a lower glucose response at 30 to 60 minutes and an elevation at 120 to 180 minutes when compared to tests using glucose alone. Following an OGTT using glucose alone, no difference was found in the total area under the glucose response curve (GRC) for rats fed high or low carbohydrate, fiber-free diets. Addition of P to these diets lowered the GRC; WB produced no change. MC added to the high carbohydrate diet lowered the GRC but did not alter the response when added to the low carbohydrate diet. These results suggest that alterations in the GRC occur not only when fiber is added to the glucose test load but also after chronic ingestion

of fiber. These chronic effects appear to depend on the type of dietary fiber. In a second experiment to evaluate the mechanism(s) whereby chronic ingestion of fiber affects glucose tolerance, rats were fed the high carbohydrate, fiber-free diet with or without added P or WB. Consumption of P or WB did not affect the rate of serum glucose disappearance or immunoreactive insulin (IRI) and pancreatic glucagon (IRG_p) concentrations following an intravenous glucose tolerance test when compared to controls. Serum glucose was decreased following an OGTT in rats fed P, but IRI and IRG_p were not changed. The enteroglucagon response, however, was lower at 30 minutes. P feeding increased the gastric emptying rate of the glucose load and this corresponded to an earlier peak response of serum glucose. Since the traditional OGTT misses this early response, increased gastric transit may partially explain the decreased serum glucose at later sampling times. The lower fasting blood glucose observed in rats after chronic consumption of WB may be explained by their decreased basal concentration of IRG_p.

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CHAPTER 1 - ACUTE AND CHRONIC EFFECTS OF DIETARY FIBER AND CARBOHYDRATE LEVEL ON GLUCOSE TOLERANCE IN RATS

INTRODUCTION

Dietary fiber is not a single substance nor is it an inert, indigestible material that simply passes unchanged through the gastrointestinal tract. Dietary fiber possesses specific physico-chemical properties. These properties are dependent on the structure and composition of the fiber source and determine the physiological effects of dietary fiber.

Interest in the physiological role of dietary fiber originated with a series of epidemiological observations of inverse relationships between dietary fiber intake and disease incidence. Dietary fiber is now proposed to be beneficial for ischaemic heart disease, diabetes, colon cancer, gallstones, constipation, irritable bowel syndrome, obesity management and dental caries. General recommendations for an increased consumption of dietary fiber are promoted for the population in general (Food and Nutrition Board, National Research Council, 1980; US Senate Select Committee on Nutrition and Human Needs, 1977; US Department of Health and Human Services, 1980). The rationale is that high fiber intakes are associated with improved health and well being. High fiber diets are specifically advocated for diabetic patients.

Epidemiological and clinical studies suggest that both acute and long-term consumption of dietary fiber may influence carbohydrate metabolism and diabetic control.

In acute tests, addition of dietary fiber to a glucose load or test meal results in a flattening of the serum glucose response curve. This postprandial decrease in serum glucose peak height and nadir is observed predominantly after ingestion of water soluble, gel-forming fiber sources. It is proposed that the acute, intraluminal effect of dietary fiber may be due to a delay in the absorption of associated digestible carbohydrate. However, the mechanism for the long-term chronic improvements in carbohydrate metabolism that remain despite lack of the concomitant presence of fiber in the gut has not been clearly identified. Therefore, it was the general aim of this study to characterize and compare the effects of acute and chronic ingestion of dietary fiber on glucose metabolism in normal rats and to elucidate the physiological mechanism(s) producing the sustained alterations induced by fiber.

REVIEW OF LITERATURE

Dietary Fiber - Physical and Chemical Characteristics

Dietary fiber is defined as the sum of lignin and the polysaccharides that are not digested by the endogenous secretions of the human digestive tract (Trowell et al., 1976). Crude fiber, the time honored concept of fiber, is not dietary fiber. Crude fiber is the residue that remains after a food has been treated with both acid and alkali (Association of Official Analytical Chemists, 1980). This remaining material is composed of cellulose and lignin. Dietary fiber includes not only cellulose and lignin but also hemicellulose, pectic substances, gums, mucilages and algal polysaccharides. These unavailable carbohydrates are structural materials of the plant cell wall (cellulose, hemicellulose, pectic substances and the non-carbohydrate, lignin) or non-structural materials found naturally or used as food additives (pectic substances, gums, mucilages, algal polysaccharides or chemically modified polysaccharides (Southgate, 1976).

The major structural polysaccharide in the plant is cellulose. It is a high molecular weight compound ($6x10^5$)

composed of β-1,4-linked glucose units. These units form straight chain polymers with strong hydrogen bonding between adjacent chains resulting in a fibrillar structure with crystalline properties. Cellulose is characterized by high mechanical strength, resistance to acid and alkali digestion and insolubility in water. Amorphous regions on the molecule will absorb water and swell so that every gram of cellulose can hold approximately 3 g of water (Schaller, 1978). This water holding property of cellulose may result in physiological alterations in gastrointestinal transit time, in intraluminal pressure or in fecal weight or electrolyte content (Eastwood and Kay, 1979). Cellulose passes through the small intestine of non-ruminants with little or no hydrolysis. However, about 50% is digested by microflora residing in the large intestine (Cummings, 1981).

Modified celluloses, either microcrystalline or carboxymethylcellulose, are used as food additives. Microcrystalline cellulose is produced by acid hydrolysis to depolymerize the fibrillar structure. This modified cellulose will form a stable suspension and can be used as an emulsifier (Royal College of Physicians of London, 1980). Carboxymethylcellulose is produced by the etherification of the free hydroxyl groups of the glucose sub-units. Methylcelluloses are viscous and have gelling properties (Reiser, 1984).

Hemicellulose forms the matrix of the plant cell wall in which the cellulose fibrils are enmeshed. The hemicellulose component of dietary fiber consists of a wide variety of polysaccharide polymers which contain either pentose or hexose "backbone" sugars with various degrees of branching. The pentosans are composed chiefly of β-1,4-linked anhydro-D-xylose units with a variety of different side chains, 4-O-methyl-α-D-glurono and L-arabino being the most common (Southgate, 1976). Acidic hemicelluloses contain a high proportion of uronic acids attached to the xylose polymer. The hexose hemicelluloses or mannans are either galacto- or glucomannans. Small amounts are found in the cell walls but most is contained in seeds where they are storage forms. Soybeans contain appreciable amounts of galactomannans (Royal College of Physicians of London, 1980).

The parent xylan chain of hemicellulose is insoluble, but a high degree of substitution favors water solubility. Wheat flour soluble pentosans will form viscous solutions in water (Hoseney, 1984). Hemicellulose, like cellulose, has water holding capacity and can bind cations depending on the presence of uronic acids (Cummings, 1976). Approximately 50-80% of hemicellulose is metabolized in the human large intestine (Southgate and Durnin, 1970; Williams and Olmstead, 1936).

In addition to hemicellulose, pectic substances form the matrix of the plant cell wall. The major constituent of this group of non-cellulosic polysaccharides is α-1,4,-linked D-galacturonic acid. Rhamnose residues occur at frequent intervals and side chains of galactose and arabinose may be present. The carboxyl groups of the uronic acids are esterified with methyl groups. Technically, highly methylated pectic substances are pectins, whereas pectinic acids are only partly methylated and pectic acids are devoid of methyl groups (Southgate, 1976). However, the term pectin or pectic substances is used collectively to describe these water soluble galacturonans.

Citrus fruits, apples and sugar beet pulp are rich sources of pectin (Theander and Aman, 1979). Two properties of pectin that may be important in nutrition are its ability to form gels and its divalent ion binding capacity. Gel formation may modify gastric emptying, mouth to cecum transit time or small intestine absorption (Eastwood and Kay, 1979). Pectin appears to be almost completely metabolized in the large intestine with less than 5% recovered in the feces (Holloway et al., 1983).

Plant gums are not part of the cell wall structure but are a sticky exudate formed at the site of injury to plants. They are a complex group of highly branched polymers containing mainly glucuronic or galacturonic acids with xylose, arabinose and mannose. Plant gums can be

dispersed in water to give a thickening or gelling effect (Selvendran, 1984). Karaya gum is used as a bulk forming laxative. Gum arabic or gum tragacanth are used commercially as stabilizers, emulsifiers or thickeners in foods.

In contrast to the acidic nature of plant gums, mucilages are predominantly neutral polysaccharides. Mucilages are found in the seed coat of many seeds. They retain water and protect the seed against dessication (Aspinall, 1970). Locust bean gum from the endosperm of carob seeds and guar gum from the legume Cyanopsis tetragonolobus are seed galactomannans. Guar gum is used in small amounts as a thickener and stabilizer in foods. Both gums and mucilages have biochemical and physical properties that resemble the pectic substances and certain hemicelluloses. They absorb water to yield viscous gels.

Algal polysaccharides are complex cell wall polysaccharides extracted from algae. There are two main types, alginates and sulfated galactans. Alginic acid is a linear polymer of D-mannuronic and L-guluronic acids. Similar to cellulose, it has a fibrillar structure. The calcium alginates are water insoluble whereas sodium and potassium alginates are soluble (Cummings, 1976). There are two major kinds of sulfated galactans, agar and carageenan. Agar is insoluble in cold water but soluble in boiling water producing a clear solution which on cooling sets to give a rigid gel (Southgate, 1976). Agars will gel at

a low concentration and are stable over a range of pH and ionic strengths. Carageenan forms gels with potassium ions to give a brittle gel or with calcium to give an elastic gel.

Lignin is a highly insoluble, non-carbohydrate component of plant cell walls. It is a series of aromatic polymers formed by the condensation of phenolic alcohols. Lignin infiltrates around the carbohydrate compounds of the plant cell wall producing an expansion of the cell wall. Most plant tissues are only lightly lignified. Lignin is extremely resistant to chemical and microbial degradation. Therefore, it is not digested to any significant extent in the human gut, and it can inhibit the breakdown of associated cell wall carbohydrate (Cummings, 1981). Lignin possesses a range of functional organic groups that could be important in the adsorption of nutrients or bile salts in the digestive tract (Barnard and Heaton, 1973).

Wheat bran is not a component of dietary fiber, rather it is a rich natural fiber source. It has an approximate fiber content of 40-50 g/100 g and consists primarily of hemicellulose (29%) with smaller amounts of cellulose (9%), lignin (3%) and pectin (2%) (Anderson and Clydesdale, 1980). Approximately 15% of the cellulose and 60% of the non-cellulosic polysaccharides of wheat bran are digested by the human gastrointestinal tract (Cummings,

1981).

As has been discussed, dietary fiber is not a single chemical entity but a heterogenous mixture of several types of polysaccharides and lignin. Physiological actions attributable to one compound may not be attributable to another. The composition and physical properties of dietary fiber will vary with the foods present in the diet. With the exception of refined sucrose and vegetable oils, all plant foods contain some dietary fiber. Most of the intake of dietary fiber comes from cell walls in fruits, vegetables, nuts, cereals and food additives (Selvendran, 1984). It is estimated that the average intake of dietary fiber in Great Britain and the United States is 20-27 g/person/day with 10-15 g derived from vegetables, 8 g from cereals and 2-4 g from fruits and nuts (Bingham and Cummings, 1978). Cereal products and legumes have a high hemicellulose content, fruits are rich in pectin, and vegetables are a good source of both hemicellulose and cellulose (Southgate et al., 1976; Southgate, 1977). The typical diet contains a mixture of the components of dietary fiber and rarely includes just any one polysaccharide in a purified form. Experiments performed to define the physiological effects of dietary fiber commonly substitute purified fibers for natural sources of fiber. However, isolation procedures to obtain a purified fiber may radically alter the physical properties

of the cell wall components. Purified fiber sources may then behave quite differently physiologically than natural sources (VanSoest, 1978).

Dietary Fiber and Carbohydrate Metabolism

Studies on the effects of plant fibers on carbohydrate metabolism are diverse and difficult to consolidate. There are differences in studies with respect to 1) types of fiber used - various purified fibers, natural fiber sources and high fiber foods, 2) the population studied- normal humans, patients with diabetes mellitus, both insulin dependent diabetes mellitus (IDDM) and non-insulin insulin dependent diabetes mellitus (NIDDM) and rats, 3) the type of test administered - glucose tolerance after ingestion of glucose and fiber combined, glucose alone or a high fiber test meal with serum glucose sampled at various times throughout the day, 4) the length of time of fiber ingestion - a response measured after a single high fiber supplement or a long-term high fiber diet modification, 5) the composition of the diet into which the fiber source is incorporated - either "high" carbohydrate (60-70% of the energy) or "low" carbohydrate (40-50% of the energy). Despite these many variables it appears the basic consensus of opinion is that a variety of plant fibers will improve carbohydrate tolerance in both normal men and diabetics. To delineate the possible mechanism for this improvement, the organization of this

review of the literature is based on the comparison of those studies in which dietary fiber is present or absent in the gastrointestinal tract at the time of the test load. Acute effects are defined as those observed after a single high fiber test meal or a glucose load with added fiber. Chronic or sustained effects are those occurring after a fiber-free glucose test load following an extended period of fiber feeding. These chronic effects may then be related to adaptive changes.

Acute Effects

Numerous studies have shown that the serum glucose and insulin response to an oral glucose load or a test meal can be attenuated by the presence of various dietary fibers in the test meal in both normal and diabetic subjects. Jenkins and coworkers (1978a) fed normal volunteers 14.5 g of various fibers together with 50 g of glucose. The fibers used were guar, tragacanth, citrus pectin, methylcellulose and wheat bran. All fibers tended to reduce the peak rise in serum glucose concentration and elevate the two hour concentration in response to the test load. A viscous fiber, guar gum produced the greatest flattening. The plasma insulin response to glucose loads or test meals supplemented with viscous polysaccharides are also lower than fiber-free test loads or meals (Jenkins et al., 1977; Jenkins, 1980a). The insulin response appears

to be proportionately flattened in relation to the blood glucose concentration. This decrease may be mediated directly by glucose or by the glucose stimulated gastrointestinal hormones GIP and GLI (Morgan et al., 1979) known to stimulate insulin release (Dupre et al., 1973; Moody et al., 1980).

In patients with chemical diabetes characterized by postprandial hyperglycemia, Monnier and collaborators (1978) supplemented oral glucose tolerance tests with pectin. A decrease in blood glucose values in response to this glucose-pectin load was observed. Addition of guar or pectin or a combination of guar and pectin to test meals has also been shown to improve the glucose and/or insulin response to the test in normal men (Gold et al., 1980; Smith and Holm, 1982; Goulder et al., 1978; Levitt et al., 1980; Jenkins et al., 1977) and subjects with IDDM (Smith and Holm, 1982; Poynard et al., 1982; Morgan et al., 1979) or NIDDM (Jenkins et al., 1976a; Levitt et al., 1980). In addition, other water-soluble gel forming fibers such as konjac mannan (Ebihara et al., 1981a) and locust bean gum (Tsai and Peng, 1981) added to a glucose test load have been shown to cause a decrease in the post-load serum glucose response.

These acute effects appear to depend on the viscous gel-forming fiber source being intimately mixed with the food or drink and are not found if the fiber is taken

separately before the tolerance test (Jenkins et al., 1979a) or merely sprinkled on the food (Williams et al., 1980). In rat studies, Blackburn and Johnson (1981) have established that ingestion of guar gum as a dry component mixed with the diet will give rise to an increase in the apparent viscosity of the stomach and small intestine contents.

While addition of barley bran (Vaaler et al., 1980) or cellulose (Monnier et al., 1978) are ineffective, other fiber sources such as wheat bran or high fiber foods which are rich in insoluble fibers and do not tend to be gel formers also improve glucose tolerance. Psyllium hydrophilic mucilloid composed primarily of hemicellulose has a high water holding capacity and will decrease the serum glucose response when added to a glucose test load (Welsh et al., 1982). In normal subjects addition of wheat bran to a glucose load (Jeffreys, 1974) or consumption of a high fiber coarse bran cereal (Connell, 1980) will improve the serum glucose response. Addition of 50 g wheat bran to a 50 g glucose tolerance test improved the serum glucose response in a group of hyperglycemic non-insulin dependent diabetics treated with diet and oral agents (Hall et al., 1980). Kay and coworkers (1981) demonstrated a positive effect on carbohydrate tolerance when the fiber source was supplied from high fiber foods. Five maturity onset diabetics were maintained on a 30 g dietary fiber/day

natural fiber diet for fourteen days. These subjects responded to a 13 g high fiber test meal with a reduced rise of plasma glucose, insulin and GIP. However, the extent of the modulation in the postprandial responses attributable to the previous high fiber diet cannot be excluded.

These acute effects of dietary fibers require the presence of fiber in the test meal, yet heterogeneous types of dietary fibers with dissimilar physical and chemical properties appear to lower the response of serum glucose and associated hormones to a test load. While the flat response curve suggests a direct intraluminal effect on the rate or completeness of glucose absorption, the mechanism of action attributable to one indigestible polysaccharide may not necessarily be the mode of action of another. Possible factors that could affect the availability of digestible food components may be changes in the rate of gastric emptying, small intestinal transit time, intraluminal diffusion and the thickness of the unstirred water layer.

The gastric emptying rate is a major determinant of the rate at which orally administered substances are absorbed (Heading, 1980). Alterations in gastric emptying by the presence of dietary fiber in a test load may then make a major contribution to the slow rate of absorption of digestible carbohydrate. The viscous polysaccharides

such as guar gum and pectin appear to prolong gastric emptying. In eight healthy humans, Holt et al. (1979) studied the effect of 10 g of pectin and 16 g of guar consumed in 400 ml of ^{113}Indium labelled orange juice. The gastric emptying of indium was significantly reduced by the presence of the fibers. There was a decrease from 54% to 34% in the amount emptied in 30 minutes and a corresponding increase from 23 to 50 minutes in the mean half-time of indium in the stomach. In addition, in further tests, an intravenous injection of propantheline bromide, a pharmacological inhibitor of gastric emptying, produced a flattening effect similar to pectin on the serum glucose response to an oral glucose load. Using a ^{113}Indium labelled porridge breakfast meal with 10 g of added guar gum, Leatherdale et al. (1982) showed an increase in the gastric emptying half-time of indium from 30 to 88 minutes in normal subjects. However, in a group of non-insulin diabetic patients with existing slow emptying rates, an effect was not observed. In a study using twelve NIDDM subjects, Ray and coworkers (1983) showed that addition of 6 g of guar gum and 3 g of wheat bran to a breakfast meal improved the postprandial serum glucose rise and decreased the mean rates of gastric emptying. At 150 minutes following ingestion of the meal with added fiber, 45% of the solids and 24% of the liquids remained in the stomach compared with 9% of the solids and

3% of the liquids in the fiber-free test meal.

Mean gastric transit was increased from 69 to 112 minutes in seven obese subjects after ingestion of 200 g of a milky drink containing 2 g of added guar (Wilmhurst and Crawley, 1980). In five patients with the dumping syndrome addition of 10.5 g high methoxy pectin to a hypertonic glucose drink prolonged the gastric emptying and decreased the rise in serum glucose and insulin responses (Leeds et al., 1981).

In animal studies where more direct measurements of gastric emptying rates are possible, the results are in accord with those seen in man. Rats given a glucose test load containing locust bean gum had three and one-half times the amount of glucose remaining in their stomachs at 75 minutes when compared to control rats (Tsai and Peng, 1981). Addition of konjac mannan to a glucose load administered to rats was also shown to decrease the gastric emptying rate. At sixty minutes after a glucose-konjac mannan test load, 30% of the glucose remained in the stomach compared to an almost complete emptying with glucose alone (Ebihara et al., 1981b).

Although it is implicitly suggested that the increased viscosity of the meal due to the added fiber slows the gastric emptying rate, Leeds et al. (1979) verified this by using high, medium or low viscosity meals of glucose and guar gum. There was a direct correlation between the

increasing viscosity and the stomach emptying half-time. Erlein and Prove (1982) also showed that the mean rates of gastric emptying were lower and the half-times of gastric emptying were extended as meal viscosity increased from a viscosity of 1 to 10^6 centipoise. Viscous fiber converts a liquid meal into a semi-solid and this may cause the stomach to handle it as a solid. Solid meals have been shown to empty more slowly than liquid meals (Dozois et al., 1971). A fiber-produced increase in viscosity appears to be necessary for the acute effects of some dietary fibers on glucose metabolism. Jenkins et al. (1980a) showed that when four gel forming fibers, guar, tragacanth, pectin or methylcellulose were used there was a correlation between the flattening of the serum glucose and the viscosity of the test load obtained with these four fibers. Use of wax-coated guar gum (O'Connor et al., 1981) or hydrolyzed guar (Jenkins et al., 1976b), neither of which were able to increase the viscosity of an aqueous solution, eliminated the ability of these fibers to flatten the serum glucose response.

While carbohydrate gelling agents may delay gastric emptying, impairment of absorption within the small intestine by impeding nutrient diffusion to or across the absorptive mucosal surface may be possible and contribute to improved glucose tolerance. Results of studies carried out in experimental animals using everted jejunal sacs or

rings in vitro (Johnson and Gee, 1981; Elsenhans et al., 1980) or perfused loops of intestine in vivo (Low et al., 1982) suggest that solutions of guar and other viscous polysaccharides may specifically inhibit intestinal glucose absorption. Low and coworkers (1982) isolated a 1.5 meter jejunal loop from pigs. This loop was perfused for six hours with a glucose solution with or without guar gum (5 g/liter). The presence of guar gum in the perfusate caused reductions of 41% to 74% in the amount of glucose absorbed. These authors concluded that a possible mode of action of guar gum in decreasing postprandial hyperglycemia is to decrease the rate of glucose uptake per unit of jejunum. However, even though the rates of absorption were drastically reduced, the total length of the small intestine is far longer than necessary for complete absorption of nutrients (Crane, 1977) and thus there may not be effects on total absorption.

Johnson and Gee (1981) incubated everted sacs of rat jejunum in 28 mM glucose solutions with either 0, 0.1, 0.25, or 0.5 g/dl guar gum. They found an inverse relationship between the viscosity of the solution and the glucose uptake. By use of everted rings of rat small intestine, Elsenhans and colleagues (1980) also showed that the inhibition of uptake of 3 mM methyl-D-glucoside, D-galactose or leucine was inversely correlated to the viscosity of the incubation media. This inhibition was

reversible by washing the tissue and re-incubating in a guar-free solution or by increasing the agitation of the incubated tissue. Detailed studies of the transport kinetics revealed that this was consistent with an increased resistance of the unstirred water layer. There was no difference in the Vmax of transport, but the Km was increased by the presence of guar gum. Thomson and Dietschy (1980) have shown that a high unstirred water layer resistance may alter the difference between the apparent Km and the true Km by a hundredfold. However at concentrations far above the established Km for glucose (0.8 mM - 1.5 mM) (Crane, 1960; Thomson and Dietschy, 1980) the unstirred layers may not affect glucose transport. In oral glucose tolerance tests the administration of concentrated glucose solutions (500 - 1850 mM) produces saturating conditions within the small intestine especially early in the test period. Only as the intraluminal concentration goes down may gelling agents gain increasing influence and affect events in absorption by altering the unstirred water layer (Elsenhans et al., 1980).

In rats, 4.9 mg of glucose was present in the upper small intestine as late as 75 minutes following gastric incubation of a 10% solution containing 500 mg glucose (556 mM) (Tsai and Peng, 1981). Although the volume present was not reported, this amount of glucose would need to be in approximately 18 - 28 ml to approach the

Km for glucose. This volume would appear to exceed the capacity of the upper small intestine of rats. Forster and Hoos (1977) infused a 278 mM (5%) glucose solution containing 1% of various gums (sodium alginate, guar, carageenan, methylcellulose or carboxymethylcellulose) into the small intestine of rats. Blood glucose concentrations during a sixty minute glucose absorption period were not significantly influenced by the presence of the viscous gums.

While the unstirred layer may impose a barrier to solutions containing low glucose concentrations, Blackburn et al. (1984) have proposed that viscous fibers improve glucose tolerance by inhibiting convective solute movement in the gut lumen. This convective movement is responsible for bringing nutrients from the bulk phase to the absorptive mucosal surface. In vitro dialysis experiments showed that as the concentration of guar was increased, the half-time to reach equilibrium with a continuously stirred external isotonic medium was also increased. The increased guar gum mediated viscosity thus appeared to restrict the rate at which glucose could reach the dialysis membrane.

This concept of bulk density interference may be one possible mechanism whereby the water insoluble fibers may affect glucose tolerance. Whereas soluble polysaccharides may produce a viscous intraluminal gel or coat the absorptive lining of the gut, the ability of an insoluble

fiber to affect absorption is probably limited to adsorption of matter to its own surface, or bulk volume and water holding capacities. Water insoluble fiber components in natural foods such as those contained in wheat bran and fiber rich cereals, legumes or lentils reduce post-prandial glycemia but not as effectively as water soluble viscous fibers. Wheat bran with its relatively large particle size may provide a bulk density in the gut lumen similar to ground foodstuff. Jenkins and colleagues (1980a) put 2 g carbohydrate portions of cooked and ground soya beans or lentils plus 3 mM glucose in closed dialysis bags. The glucose transfer to the outside after a three hour incubation in a stirred phosphate water bath was 40% for soya beans and 65% for lentils compared to a food-free glucose solution. It appeared that food trapped the glucose and made it unavailable for transfer to the outside compartment.

In addition to an inhibition of nutrient diffusion to the absorptive mucosal surface, the bulk provided by insoluble fiber could adsorb the associated digestible carbohydrate. Psyllium which contains primarily hemi-cellulose but also cellulose and lignin has a fiber composition similar to bran. In in vitro studies, the centrifuged precipitate of a glucose-water-psyllium slurry was resuspended in 50 ml of water. Samples of the water were removed after brief intermittent mixing at multiple

sequential time points over a three hour period. The psyllium-glucose precipitate continued to release glucose over a three hour period with most of the glucose released during the first thirty minutes. In corresponding in vivo tests, psyllium added to a glucose solution lowered the peak serum glucose response and prolonged its rate of fall without altering total glucose absorption (Welsh et al., 1982).

Bran fiber is also an efficient fecal bulking agent (Cummings et al., 1978). Since intestinal transit time is inversely related to fecal weight (Burkitt et al., 1972; Stasse-Wolthuis et al., 1979) it is not surprising that addition of wheat bran to the diet has been shown to speed intestinal transit (Paylor et al., 1975). Very rapid mouth to cecum transit may result in decreased time of exposure of nutrients at the site of absorption with possible malabsorption. Malabsorption after a test load containing glucose with added fiber could cause an attenuated glycemic response.

Mouth to cecum transit is measured by the production of hydrogen gas by cecal flora from the unabsorbable disaccharide lactulose and measurement of the expiration of this hydrogen in the breath. Malabsorption is assessed by the recovery of xylose in the blood or urine after an oral dose. In a series of experiments using these techniques, Jenkins (1980a) added various fibers to a

glucose/xylose/lactulose drink. A direct relationship ($r=0.885$, $p \leq 0.02$) was found between increased mouth to cecum transit time and the viscosity attributable to the added fiber. However, addition of wheat bran caused a forty-five minute decrease in transit time. Whereas the delayed transit time for the viscous fiber, guar reduced xylose absorption over a two hour period, the recovery of xylose was complete by eight hours. Wheat bran with a decreased transit time also had a depressed recovery of xylose at two hours. While the total amount of xylose recovered after an eight hour period was not given, this decreased rate of absorption coupled with an increased transit rather than a decreased transit rate may suggest malabsorption.

Chronic Effects

Not all the actions of dietary fiber on carbohydrate tolerance can be ascribed to a direct interaction of dietary fiber within the gastrointestinal tract. There is evidence of sustained effects on glucose tolerance after discontinuation of fiber feeding. Thirty-seven patients with diverticular disease consumed 24 g of wheat bran daily for at least six months. After an overnight fast, plasma glucose concentrations were reduced in response to an oral glucose load (Brodribb and Humphreys, 1976). Thirty-eight subjects with impaired glucose

tolerance added 20 g raw bran to their usual diets. After one month, the serum glucose and insulin responses to an oral glucose tolerance test (OGTT) were reduced. Subjects who continued this regime for an additional month maintained the improved metabolic situation while those who discontinued bran returned to pre-treatment conditions (Bosello et al., 1980). Oral glucose tolerance improved in healthy young men when they added 26 g of either corn bran, soy hulls or apple and carrot powder to a low fiber (1 g crude fiber/1000 kcal) standardized diet for one month. However, no effects on glucose tolerance were observed after consumption of bran from either soft white or red hard spring wheat (Munoz et al., 1977). An improvement, although slight, in glucose tolerance was observed following a four week daily consumption of 52 g soy hull fiber in persons with type II diabetes (Mahalko et al., 1984).

In animal studies, Cannon et al. (1980) have reported significantly lower postprandial glucose and insulin levels in rats after feeding guar or carboxymethylcellulose (8%) for eight weeks. Feeding wheat bran did not improve glucose tolerance. Chronic ingestion of either guar (8%), pectin (5%), cellulose (10%) or a multifiber source containing pectin, bran, cellulose and lignin decreased the serum glucose response to a glucose load in rats (Track et al., 1981; Schwartz and Levine, 1980). A diet

providing 14 g of fiber from soy beans fed for 45 days reduced the serum glucose response to a glucose load in both normal and streptozotocin diabetic rats (Madar, 1983). Throughout a forty day feeding period, streptozotocin diabetic rats consuming a diet containing 5% bagasse (sugar cane fiber) had significantly lower fasting blood glucose concentrations (Yamashita et al., 1980). Non-fasting serum glucose concentrations were also lowered in both normal and alloxan diabetic rats after consuming a diet containing 14% raw bran (Nygren and Hallmans, 1982).

Long term ingestion of fiber supplemented diets has also been shown to improve diabetic control. Miranda and Horowitz (1978) compared the effects of a diet containing cellulose enriched bread (20 g crude fiber/day) to a low fiber diet (3 g crude fiber/day). Eight insulin-dependent men were maintained on each diet for ten days each. The insulin dose was kept at a constant level during both dietary periods. Plasma glucose concentrations measured throughout the day averaged 121 mg/dl on the high fiber diet and 169 mg/dl on the low fiber diet. Addition of approximately 25 g/day of guar gum in the form of a crispbread for five days reduced urinary glucose excretion and fasting plasma glucose concentrations in nine diabetic patients. Six subjects continued eating guar over an eight week period. There was a progressive fall in their

insulin doses from 46 U to 36 U/day (Jenkins et al., 1978b) and this remained fairly stable for up to 20 weeks (Jenkins et al., 1979b). In a study of patients with both stable and labile IDDM, Monnier and coworkers (1981) fed diets supplemented with wheat bran (1 g fiber/15 g available carbohydrate) for 10-15 days. A decrease in glycosuria, glycosylated hemoglobin and post-lunch glucose concentrations in the stable diabetics coupled with post-dinner improvements in serum glucose in the labile diabetics was observed. No change in either fasting blood sugar or insulin requirements was noted in either group of patients. Metabolic control following a two month period of diet supplementation with 20 g of guar and 10 g of wheat bran was studied in twelve obese, poorly controlled non-insulin dependent diabetic subjects. The urinary excretion of glucose was reduced and fasting plasma glucose concentration dropped from 301 mg/dl to 184 mg/dl (Ray et al., 1983).

In addition to diets supplemented with purified fibers, diets rich in high fiber foods have also been shown to produce long term improvements in carbohydrate metabolism. These diets typically provided 60-70% of the total daily energy from carbohydrate with three-fourths of this in the complex form providing 50-80 g of dietary fiber. The remaining additional energy was derived from approximately 10-20% fat and 20% protein. Insulin therapy was

discontinued in NIDDM patients receiving 15-38 U/day of insulin and cut by 35-46% in IDDM patients receiving 22-25 U/day after following this dietary program (Anderson, 1982). With continued adherence to this diet, improvements have been maintained over a fifteen month period (Anderson and Ward, 1978). A decrease in fasting blood sugar coupled with a discontinuation of insulin and oral hypoglycemic agents in NIDDM patients was maintained over a two to three year period with continued compliance to a high carbohydrate, high fiber diet (Barnard et al., 1983). In a series of studies, Simpson and colleagues (1979a, 1979b, 1981) have confirmed the positive results of high dietary fiber in diabetic patients. The basal, mean pre-prandial and two hour postprandial blood glucose and urinary glucose were decreased in both NIDDM and IDDM patients consuming a high carbohydrate, very high fiber (97 g/day) diet for six weeks.

Some studies (Anderson, 1979; Jenkins et al., 1980b) have suggested that the favorable impact of high carbohydrate, high fiber diets may be due to the high carbohydrate content of the diet. Merely increasing the carbohydrate content of the antecedent diet has been shown to improve the plasma glucose response to a glucose challenge (Himsworth, 1979; Brunzell et al., 1971). However, the study of Riccardi and colleagues (1984) indicates that the high fiber content of the diet,

independent of the carbohydrate content, appears to be accompanied by significant improvements in glucose metabolism. Diabetic patients were fed three consecutive diets in a random order for ten day periods each. Two diets provided 53% of the energy from carbohydrate, 17% from protein and 30% from fat. One of these diets was low in fiber (16 g/day) while the other was high in fiber (54 g/day). The third diet provided 42% carbohydrate and 20 g/day dietary fiber. Daily blood glucose profiles and two hour postprandial glucose concentrations were significantly lower during the high fiber dietary period. When these patients were maintained on either the 42% or 53% carbohydrate, low fiber diet, the improvements disappeared. In addition, in studies (Munoz et al., 1977; Miranda and Horowitz, 1978; Monnier et al., 1981) employing addition of fiber supplements to the diet, the carbohydrate content remained unchanged and improvements were produced by the increasing fiber content. However, concomitantly increasing the carbohydrate content may synergistically increase the magnitude of the improvement in carbohydrate metabolism.

From the literature presently available, it appears that some purified fibers, natural fiber sources and commonly available high fiber foods in the diet can promote alterations in the physiological response to carbohydrate ingestion. The mechanisms for these long

term effects that are progressive with time and remain despite lack of fiber in the gut at the time of glucose load have not been clearly identified. Possible factors that may explain the cumulative metabolic effect of chronic dietary fiber consumption include changes in the hormonal responses to a carbohydrate load, volatile fatty acid production or alterations in the structure or function of the stomach or small intestine.

Diets rich in fiber may alter hormonal responses which in turn could modify the uptake and subsequent metabolism of nutrients in hepatic or extra-hepatic tissues. Serum glucagon concentrations throughout the day were significantly lower during high fiber diets (Miranda and Horowitz, 1978). Fasting serum glucagon (Yamashita et al., 1980; Madar, 1983) and the glucagon responses to an oral glucose tolerance test after a fiber rich antecedent diet (Munoz et al., 1977) were also decreased. Since glucagon stimulates the release of glucose from the liver, a decreased secretion of pancreatic glucagon in response to fiber ingestion may lead to better utilization and clearance of a glucose load.

An increase in insulin sensitivity or secretion in response to a carbohydrate load may also improve glucose metabolism. In the study of Munoz et al. (1977) a decrease in the serum glucose response coupled with no apparent difference in the insulin response curve following an oral

glucose load was observed. Evaluation of the insulin response at the glucose peak indicated that an increase in insulin secretion had occurred. The decrease in insulin requirements in diabetic subjects after high fiber diets (Anderson, 1982; Jenkins et al., 1978b; Simpson et al., 1981) may suggest an enhanced sensitivity to insulin. In thirty-three IDDM patients maintained on a 70% carbohydrate, high fiber diet (70 g/day), decreased fasting blood glucose concentrations and lower insulin doses were coupled with an increase in insulin binding to isolated monocytes (Anderson, 1979). Pedersen et al. (1982) fed diets with the carbohydrate content increased from 33% to 48% of the calories and dietary fiber increased from 33 to 63 g/day. Decreased insulin requirements were coupled with increased insulin binding to isolated monocytes in IDDM patients. In a study of seven NIDDM subjects, Ward and coworkers (1982) fed 60% carbohydrate diets with 100 g/day of dietary fiber. A lowered fasting blood sugar without a change in fasting insulin was associated with increased insulin binding to isolated monocytes. However, since the ambient insulin level may reciprocally regulate its own receptor (Gavin et al., 1974) it might be suggested that the increase in insulin binding may be a consequence rather than a cause for the reduced insulin requirement. In normal subjects an increase in the carbohydrate content of the diet, which is

thought to increase insulin sensitivity (Brunzell et al., 1971), did not appear to be mediated by increased binding to insulin receptors (Wigand et al., 1979; Beck-Nielsen et al., 1978; Kolterman et al., 1979).

The mechanism by which dietary fiber may alter hormonal responses has not been determined. Jenkins et al. (1980c) have proposed that the slow absorption of glucose attributable to the presence of fiber in the gastrointestinal tract may affect the handling of subsequent fiber-free carbohydrate loads. These investigators have shown that addition of fiber to one glucose load resulted in lower levels of 3-hydroxybutyrate and free fatty acids after four hours and improved the tolerance in response to a second guar-free glucose load. By prolonging the time course over which carbohydrate is absorbed, dietary fiber may minimize the endocrine response and prevent rebound hypoglycemia, mobilization of free fatty acids and ketone body synthesis. The subsequent meal regardless of whether or not it contains fiber may then be taken up more readily by peripheral tissues. One meal may influence the next and metabolic alterations may carry over to each successive day.

Physiological use of volatile fatty acids as an energy source may also affect carbohydrate metabolism. The microflora of the large intestine is able to ferment dietary fiber and produce volatile fatty acids; however

there is great variability in the extent of breakdown of different types of fiber. Lignin is resistant to degradation whereas cellulose and to an even greater extent the non-cellulosic polysaccharides pectin, guar and hemicellulose are more easily fermented (Cummings, 1981; Holloway et al., 1983; Nyman and Asp, 1982). Substantial concentrations of the volatile fatty acids: acetate, propionate and butyrate in a molar ratio 60:25:15 may be produced by anaerobic degradation of dietary fiber (Cummings, 1984). Studies in both animals and man have shown that these short chain fatty acids are rapidly absorbed from the cecum or colon (McNeil et al., 1978) and may provide part of the daily energy requirements. In non-ruminant herbivores such as the rabbit or pony, volatile fatty acids provide 30-40% of the basal energy requirement. In monogastric omnivores, either man or pigs, anywhere from 2.5-10% of the energy requirement may be provided depending on the intake of dietary fiber (McNeil, 1984). The contribution of volatile fatty acids to the energy intake of rats has been determined to be approximately 4.7% (Yang et al., 1970).

Local oxidation of the volatile fatty acids with butyrate as the predominant substrate has been shown to provide an energy supply for colonic epithelial cells (Roediger, 1970). However, the volatile fatty acids are also transported in the portal vein, and the liver appears

to be the main organ utilizing these absorbed organic acids. Some acetate is available for extra-hepatic metabolism (Marty and Vernay, 1984; Snoswell et al., 1982; Buckley and Williamson, 1977). Depending on the overall metabolic state, the volatile fatty acid carbon may be channelled into gluconeogenesis (propionate), lipogenesis or ketogenesis (acetate, butyrate) (Remesy and Demigne, 1983; Williams and Spray, 1971). In addition, the energy supply derived from fermented dietary fiber may be available later in the post absorptive period compared with immediate energy supplied from digestible carbohydrate. The portal venous blood concentration of volatile fatty acids was sustained for one, six and eleven hours after food removal in rats fed diets containing 10% pectin, oat bran or standard cereal-based laboratory chow (Illman et al., 1982; Storer et al., 1983). Compared to fiber-free control diets, diets containing 10% oat bran resulted in higher hepatic portal vein concentrations of propionate and butyrate but not acetate. This increased concentration of volatile fatty acids peaked at two hours postprandially and remained elevated for the following four hour period (Chen and Anderson, 1984). In rats fed uncooked potato starch, an available substrate for fermentation similar to dietary fiber, there was an increased and constant absorption of volatile fatty acids at three and seven hours after feeding.

Maximal concentrations were present in the portal blood at twenty-three hours after feeding. At this late post absorptive period, volatile fatty acids were also increased in the arterial blood (Demigne and Remesy, 1982).

The specific effects of a constant, delayed supply of large amounts of metabolites from the fermentation of plant fiber on the various metabolic pathways and subsequent effects on carbohydrate or lipid metabolism or hormonal responses have not been determined. However, the possibility that volatile fatty acids may affect glucose metabolism does exist. In studies using isolated hepatocytes from fasted rats, Anderson and Bridges (1980) showed that whereas addition of acetate or butyrate to the incubation media stimulated gluconeogenesis and inhibited glycolysis, addition of propionate had the opposite effect and appeared to enhance glucose utilization and decrease glucose production. While the overall physiological significance cannot be assessed from this isolated in vitro system, the potential may exist and some effects of plant fiber on glucose metabolism may be mediated through these short chain fatty acids.

The long term effects of dietary fiber on glucose metabolism may also be associated with biochemical and morphological modifications of the small intestine. Dietary fiber may interact directly with the intestinal mucosa to produce adaptive changes in the functional

capacity of the small intestine. Using scanning electron microscopy, Cassidy et al. (1981) have shown that long term ingestion of pectin may produce an abnormal mucosal ultrastructure. Rats that were fed pectin or alfalfa (15 g/100 g diet) for six weeks showed apical cell swelling and loss and disarray of microvilli in the jejunum. Jacobs (1983) demonstrated crypt cell hyperplasia, increased cell loss from villi and decreased villus height in rats fed diets containing 10% pectin for four weeks. These observations are consistent with an observed decrease in the brush border enzymes, alkaline phosphatase and leucyl-β-naphthylamidase with pectin feeding (Brown et al., 1979). The lower enzyme content may thus imply an increase in cell sloughing with the cells of the villi achieving a less mature state. However, addition of wheat bran to the diet does not appear to affect the jejunal wet weight, cell size or DNA synthesis (Jacobs and White, 1983) or cause distortion and disruption of the jejunal microvilli (Cassidy et al., 1981).

Several investigators (Brown et al., 1979; Forman and Schneeman, 1982; Farness and Schneeman, 1982) have shown that feeding pectin (5-18% of the diet) will also increase the total length and weight of the small intestine. The increase in weight has been attributed to an increase in both the muscle layer and mucosal thickness (Brown et al.,

1979). An increase in length of the small intestine has also been observed when several other soluble polysaccharides were fed to rats for seven to eight weeks (Elsenhans et al., 1982). These authors proposed that the size difference may be caused by the increased work of propelling a viscous gel along the small intestine or an increased amount of nutrients reaching the distal small intestine and signalling a longitudinal growth response.

In contrast to these possible deleterious effects, Tasman-Jones et al. (1982) have shown that fiber is necessary for the normal developmental changes in the rat small intestine. At birth rats have regular finger-shaped villi. Removal of fiber from the post-weaning diet slows the rate of development of leaf shaped broad ridged villi characteristic of the mature small bowel. Addition of 10% pectin but not 10% cellulose to a fiber-free diet will produce the developmental alterations similar to a chow diet. Ecknauer et al. (1981) fed rats chow, a liquid fiber-free diet or a liquid diet with 24% cellulose. Addition of bulk in the diet changed the villus shape and increased the villus surface area and gut circumference. The development of the adult villus was dependent on the fiber content of the diet.

Reports on the functional correlates of the structural changes in the small intestine with fiber feeding are equally conflicting. Sigleo and coworkers (1984) fed

diets containing either 10% cellulose or 10% pectin to rats for four weeks. Sections of the jejunum were then examined for changes in villus structure and for in vitro transport characteristics by the estimation of the unidirectional flux of analogs of a hexose, 3-O-methyl-glucose, an amino acid, α-aminoisobutyric acid and sodium. Fiber feeding resulted in an increase in intestinal villus length and width and in the transport of all nutrients. On the other hand, Schwartz and Levine (1979) reported that incorporation of 10% cellulose or 5% pectin into the diets of rats for a four week period decreased glucose absorption during a subsequent in vivo perfusion of isolated jejunal segments. The in vitro mucosal to serosal transport of sodium and chloride ions in segments of jejunum isolated from rats previously fed similar diets was also depressed, thus suggesting possible impaired transport by villus enterocytes (Schwartz et al., 1982a). However, in these experiments, impaired intestinal absorption of either glucose or sodium and chloride was not associated with any gross morphological alterations in the small intestine. This decreased absorption with no histological changes as well as the observations of improved absorption with enlargement of the villus may suggest that chronic improvements in glucose tolerance may be associated with other alterations rather than structural changes affecting enterocyte transport function.

A sustained increase in the unstirred water layer resulting in a depression of the transport rate to the mucosal surface may be produced by prolonged consumption of dietary fiber. Wheat bran (20%) added to the diet of rats appears to selectively increase the number of goblet cells per villus (Schneeman et al., 1982). The production of mucus by these cells could then increase the unstirred water layer and affect nutrient absorption in the small intestine. The viscous polysaccharides may interact with the mucopolysaccharides of the mucosal surface and produce a sustained superficial film thus increasing the unstirred water layer. In in vitro studies using everted sacs of rat jejunum, Johnson and Gee (1981) showed that glucose transport fell in sacs pre-incubated with guar gum and then exposed to a subsequent guar-free incubation. Blackburn and Johnson (1981) also demonstrated that guar gum need not be present in the bulk mucosal solution to exert a rate limiting effect on absorption. Pre-perfusion of isolated rat intestinal loops with guar gum caused a decrease in the rate of subsequent glucose absorption.

Chronic consumption of dietary fiber may also produce a sustained alteration in the rate of gastric emptying and improve carbohydrate tolerance similar to acute ingestion of dietary fiber. Schwartz et al. (1982b) studied the effects of fiber ingestion on gastric

emptying in healthy volunteers. The subjects consumed 20 g/day of either apple pectin or α-cellulose added to their diets. Gastric emptying time, glucose tolerance and hormonal responses were measured after an overnight fast followed by ingestion of a low fiber, carbohydrate breakfast meal surface labelled with ^{99m}technetium. Gastric emptying half-time doubled after previous consumption of pectin but not cellulose. Plasma glucose, insulin, glucagon, motilin, gastrin or human pancreatic polypeptide responses were unchanged. Glucose tolerance was not enhanced in the presence of the sustained delayed gastric emptying. The significance of this adaptive delay in gastric emptying as a causative agent in the improvement of carbohydrate metabolism is therefore uncertain. However, chronic delayed absorption due to alterations in the small intestine may represent a possible mechanism of action for the prolonged effects of dietary fiber.

CHAPTER 1

ACUTE AND CHRONIC EFFECTS OF DIETARY FIBER AND CARBOHYDRATE LEVEL ON GLUCOSE TOLERANCE IN RATS

Introduction

Some purified fibers (Jenkins, 1980a; Ebihara and Kiriyama, 1982; Schwartz and Levine, 1980; Cannon et al., 1980), natural fiber sources (Munoz et al., 1977; Brodribb and Humphreys, 1976; Mahalko et al., 1984; Bosello et al., 1980) and high fiber foods (Kay et al., 1981) promote alterations in the physiological response to oral glucose tolerance tests (OGTT). The improvement is seen when fiber is incorporated into the glucose test load (acute effects) (Jenkins, 1980a; Ebihara and Kiriyama, 1982; Schwartz and Levine, 1980; Kay et al., 1981) or when the glucose load is given without added fiber after an extended period of feeding fiber supplemented diets (chronic effects) (Madar, 1983; Schwartz and Levine, 1980; Cannon et al., 1980; Munoz et al., 1977; Brodribb and Humphreys, 1976; Mahalko et al., 1984; Bosello et al., 1980).

The acute effects of fiber have been attributed to a slower rate of gastric emptying (Holt et al., 1979; Leeds

et al., 1981) and/or intestinal absorption (Caspary, 1980) of associated digestible carbohydrate, thus indicating a gut mediated mechanism. However, the chronic effects of dietary fiber have not been clearly defined nor their mechanism of action elucidated.

In acute tests incorporation of wheat bran (Jenkins, 1980a) but not barley bran (Vaaler et al., 1980) or cellulose (Monnier et al., 1978) into a carbohydrate test load will improve glucose tolerance; however the viscous gel-forming types of dietary fiber have been found to be more effective (Jenkins et al., 1978a; Ebihara and Kiriyama, 1982). In contrast, both soluble and insoluble types of dietary fiber have produced, with apparent equal effectiveness, chronic effects on glucose tolerance.

It has been suggested that the favorable impact of the long term feeding of high fiber diets may be due to the concomitant increase in the carbohydrate content of the diet (Anderson, 1979; Simpson et al., 1981; Heaton et al., 1982). Indeed it has been shown that the plasma glucose response of normal subjects to an oral glucose challenge is improved when the preceeding diet has a higher percentage of the total energy derived from carbohydrate (Brunzell et al., 1971; Himsworth, 1939). However, several studies have shown improvements in carbohydrate metabolism by increasing the fiber content of the diet alone (Munoz et al., 1977; Miranda and Horowitz, 1978;

Monnier et al., 1981). Whether the effects on glucose metabolism obtained by altering either the dietary carbohydrate or the dietary fiber level are independent or synergistically dependent has not been determined. An increase in insulin sensitivity also has been proposed to explain the sustained effects of a diet rich in fiber (Pedersen et al., 1982; Ward et al., 1982). This would suggest a systemic improvement in glucose metabolism as opposed to an effect mediated through the gastrointestinal tract.

In order to characterize the effects of chronic feeding of dietary fiber on glucose metabolism, the objectives of this study were 1) to compare the acute and chronic effects of various fiber sources, pectin (P), methylcellulose (MC) and wheat bran (WB) on oral glucose tolerance in rats; 2) to distinguish if the level of carbohydrate influenced the effect of various types of fiber on glucose tolerance; 3) to determine if the alteration in OGTT after chronic fiber feeding is a gut-mediated phenomena.

Materials and Methods

Animals and Diets

Male Sprague-Dawley rats (Spartan Research Animals, Inc., Haslett, MI) weighing 190-250 g were individually housed in wire-bottom stainless steel cages in a

temperature controlled room (22°C) lighted from 0700 to 1900 hours. Rats were randomly divided into groups of six to eight each and fed one of eight purified diets: high carbohydrate, fiber-free (HC-FF), low carbohydrate, fiber-free (LC-FF) or these same diets with the addition of pectin (HC-P or LC-P), methylcellulose (HC-MC or LC-MC) or wheat bran (HC-WB or LC-WB) (Table 1). The distribution of energy in the high and low carbohydrate diets was made to approximate the composition of diets commonly used in human studies (Anderson and Ward, 1979). It is recognized, however, that the total amount of carbohydrate, despite a higher complex to simple ratio in these high carbohydrate diets, is similar to the recommended level in standard AIN-76 purified diets for rats (American Institute of Nutrition, 1977). In formulating the low carbohydrate diets, corn oil was substituted on an equal metabolizable energy basis for corn-starch and sucrose.

Pectin and methylcellulose are water soluble, viscous gel forming purified fiber sources. They were used to simulate the conditions of the fiber supplemented diets of Jenkins and coworkers (1980b). Wheat bran contains both water soluble and insoluble fiber components: cellulose, hemicellulose, pectin and lignin. It was used in these studies because it is a natural fiber source adding fiber to the diet while also providing part of the protein, available carbohydrate and fat. This means of

Table 1. Composition of diets

	High carbohydrate[1]				Low carbohydrate[2]			
	HC-FF	HC-P	HC-MC	HC-WB	LC-FF	LC-P	LC-MC	LC-WB
	g/397 kcal							
Basal[3]	5	5	5	5	5	5	5	5
Casein[4]	21	21	21	16	21	21	21	16
Cornstarch	53	53	53	45	31	31	31	23
Sucrose	16	16	16	16	9	9	9	9
Corn oil	5	5	5	3	18	18	18	16
Pectin[5]	-	7.5	-	-	-	7.5	-	-
Methylcellulose[6]	-	-	7.5	-	-	-	7.5	-
Wheat bran[7]	-	-	-	36	-	-	-	36
Energy (kcal/g)[8]	4.0	3.7	3.7	3.3	4.8	4.4	4.4	3.8

[1]High carbohydrate (HC) % kcal: protein 19; fat 11; carbohydrate 70.

[2]Low carbohydrate (LC) % kcal: protein 19; fat 41; carbohydrate 40.

[3]Contained 0.3 g DL-methionine, 0.2 g choline chloride, 1 g AIN vitamin mix and 3.5 g AIN mineral mix (American Institute of Nutrition, 1977).

[4]Sodium caseinate, United States Biochemical Corp., Cleveland, OH; 90% protein.

[5]USP 60, Hercules, Inc., Wilmington, DE.

[6]Methocel A4C Premium, Dow Chemical, Midland, MI.

[7]AACC Certified Food Grade Wheat Bran (#R07-3691, containing (%): neutral detergent fiber, 40.2; crude fiber, 8.9; protein, 14.3; fat, 5.2; available carbohydrate, 21.8.

[8]Assumed 4 kcal/g for casein, cornstarch and sucrose, 9 kcal/g for corn oil.

adding fiber to the diet is similar to the substitution of high fiber foods used in the high fiber diets employed by Anderson and colleagues (1980).

It was assumed that pectin and methylcellulose did not supply significant energy and these fiber sources were added to the fiber-free diets to maintain a constant protein to calorie ratio. The amounts of casein, corn oil and cornstarch in HC-WB and LC-WB were adjusted to account for the protein, carbohydrate and fat in wheat bran. Pectin and methylcellulose were added to the diets at a level of 1.9 g fiber source/100 calories. Wheat bran was added to the diets at a level of 3.6 g neutral detergent fiber/100 calories. This amount of fiber provided approximately 3% crude fiber which approaches the 4.5% maximum crude fiber in ground cereal-based stock diet (Wayne Lab-Blox, Allied Mills, Chicago, IL). This chow diet was fed to a ninth group of rats. Water and food were provided ad libitum for four weeks. Food intake was recorded every three days and body weights were determined weekly.

Chronic Glucose Tolerance Tests

At the end of the four week feeding trial oral glucose tolerance tests were initiated. Tests were conducted over a two week interval while rats continued on their respective diets. To randomize testing, only one rat from

any diet group was tested per day.

Food cups were removed from the cages at 1000 hours on the day preceding the test. At approximately 0900 hours on the day of the test, the rats were weighed and a fasting blood sample was taken by clipping the tail and collecting a 300 µl sample directly into a 6x50 mm test tube. D-glucose, in an aqueous solution containing 300 mg/ml, at a dose of 150 mg/100 g body weight was then delivered into the stomach of each rat by means of a stainless steel feeding tube. This dosage is within the range recommended by the National Diabetes Data Group for children and adults (National Diabetes Data Group, 1979). Additional blood samples from the tail were obtained at 30, 60, 120 and 180 minutes post glucose loading. The rats were placed in a plastic restrainer only during sample collections. At all other times, the animals were returned to their cages and had free access to water.

Acute Glucose Tolerance Tests

All acute glucose tolerance tests were administered three days after the completion of the chronic glucose tolerance tests. The acute tests followed the same general protocol as the chronic tests but with differences in the composition of the test load. Rats fed diets HC-FF, HC-P, LC-FF or LC-P were given a test load containing 150 mg/100 g body weight D-glucose in a 300 mg/ml

aqueous solution containing 4% (w/v) pectin. This pectin-glucose solution had a viscosity of 220 poise (Brookfield viscometer, model RVT). Rats maintained on HC-FF, HC-MC, LC-FF or LC-MC were given a tolerance test with the test load containing the standard dose of D-glucose with 4% (w/v) added methylcellulose. This solution was prepared according to the manufacturer's specifications and had a viscosity of 550 poise. Because neither a solution or suspension could be made with wheat bran, fasted rats previously fed HC-FF, HC-WB, LC-FF or LC-WB were offered 3 g of wheat bran and given a 20 minute consumption period immediately before the administration of the standard glucose solution. The rats ate an average of 1.13 ± 0.11 g with no significant differences between groups. Rats which were given multiple tests (HC-FF, LC-FF) were allowed a minimum of three days between testing.

Intravenous Glucose Tolerance Tests

Because previous ingestion of HC-P and HC-WB diets appeared to alter the response to an OGTT, a second set of twenty-one rats weighing approximately 190-220 g were randomly assigned to HC-FF, HC-P or HC-WB diets as previously described for a four week period. At the end of this time period rats were fasted overnight and then anesthetized with an intraperitoneal injection of 35 mg/kg sodium pentabarbital (Sigma Chemical Co., St. Louis, MO).

The right jugular vein was then exposed and cannulated. The catheter was flushed with a solution of heparin in saline (50 U/ml). A 1.0 ml fasting blood sample was collected and a 0.5 g/kg body weight dose of D-glucose was then infused into the vein. Additional 1.0 ml blood samples were collected at 5, 15, 30 and 60 minutes post glucose loading. After each collection, the blood volume was replaced with saline. Throughout the test period, body temperature was maintained using a low wattage light as a heat source. Blood samples were collected into tubes containing 0.1 ml proteolytic enzyme inhibitor (Trasylol, FBS Pharmaceuticals, New York, NY), gently inverted, refrigerated, allowed to clot and then centrifuged. The sera were frozen for later determinations of glucose, insulin and pancreatic glucagon.

Chemical Analyses

Serum samples from chronic, acute and intravenous glucose tolerance tests were analyzed for glucose content by the glucose oxidase method (BMC Reagent Set - Glucose, Boehringer Mannheim, Dallas, TX). Samples from the intravenous glucose tolerance test were also analyzed for insulin and pancreatic glucagon. Insulin was determined according to radioimmunoassay principles of Yalow and Berson, 1960 (MicroMedic Test Delivery Systems, Micromedic, Inc., Horsham, PA). Serum glucagon was determined using

anti-glucagon serum (AGS) 18, antibodies which bind specifically to pancreatic glucagon, and the method of Foa et al., 1977.

Data Analyses

The total incremental areas under the glucose response curves were calculated by integration, utilizing trapezoidal rules, of the serum glucose concentrations from 0-180 minutes using fasting serum glucose concentrations as the baseline. The fractional clearance rate of glucose after an intravenous glucose infusion (K_G value) was calculated according to Soeldner, 1971.

All data are expressed as means ± SEM. Analysis of variance and Bonferoni t tests (Gill, 1978) were used to detect mean differences between groups. Paired t tests were used in comparing the results of acute and chronic oral glucose tolerance tests. The criterion of significance was $P \leq 0.05$.

Results

Body Weight and Food Intake

The mean final body weight of rats fed HC-WB was significantly lower ($P \leq 0.05$) than rats fed LC-WB (Table 2). Rats fed HC-WB also had a lower initial body weight (211 ± 5 g) when compared to LC-WB fed rats (236 ± 4 g) so that the total body weight gain for the two groups

Table 2. Body weight and estimated metabolizable energy intake of rats fed high or low carbohydrate diets with or without various fiber sources for a four week period[1].

Diet	Body Weight		Energy Intake[2]
	Final	Gain	
	g	g	kcal
HC-FF	370±9	139±7	2381±106
HC-P	374±2	144±7	2414±74
HC-MC	366±3	141±5	2244±76
HC-WB	361±5	151±6	2319±30
LC-FF	383±11	158±4	2474±50
LC-P	374±8	138±5[4]	2403±119
LC-MC	380±4	179±5[3,4]	2446±107
LC-WB	383±6[3]	147±8	2142±50[4]

[1]Means±SEM, each mean represents values for six rats in fiber fed groups, seven rats in HC-FF group and eight rats in LC-FF group.

[2]Based on the calculated energy value for each diet.

[3]Significantly different ($P \leq 0.05$) from rats fed high carbohydrate diets with the same fiber source.

[4]Significantly different ($P \leq 0.05$) from rats fed fiber-free diets within a carbohydrate level.

was similar. There was no significant differences in body weight gain or energy intake between rats fed high carbohydrate fiber-free diets or this same diet with added fiber. Whereas rats fed LC-P had a decreased ($P \leq 0.05$) body weight gain when compared to rats fed LC-FF, rats fed LC-MC had an increased ($P \leq 0.05$) body weight gain when compared to either LC-FF or HC-MC fed rats. There were no significant differences in total energy intake to account for these differences. Conversely, rats fed LC-WB had a decreased energy intake when compared to fiber-free low carbohydrate fed controls yet no significant differences in body weight gain.

Oral Glucose Tolerance Tests

At the end of the four week feeding trial, mean fasting serum glucose was significantly lower ($P \leq 0.05$) in rats fed wheat bran diets than in rats fed fiber-free control diets (Table 3). Standard laboratory stock diets providing a type and amount of dietary fiber similar to that of diets with added wheat bran also significantly lowered ($P \leq 0.05$) fasting serum glucose concentrations when compared to fiber-free high or low carbohydrate diets.

Figure 1 and Appendix Table A1 shows the results of the chronic glucose tolerance tests in rats previously fed high or low carbohydrate diets with or without added

Table 3. Fasting blood glucose concentrations (FBS)[1].

Diet	FBS, mg/dl
HC-FF	104±4
HC-P	100±5
HC-MC	106±5
HC-WB	89±5[2]
LC-FF	103±4
LC-P	118±6
LC-MC	104±7
LC-WB	87±4[2]
Chow	80±2[2]

[1]Means±SEM, each mean represents values for six rats in fiber-fed groups, seven rats in HC-FF and eight fats in the LC-FF group.

[2]Significantly different ($P \leq 0.05$) from rats fed fiber-free diets within a carbohydrate level.

fiber for a four week period. Because of variations in fasting glucose concentrations among groups, the results are presented in Figure 1 as the change in serum glucose above basal levels for a three hour period. Rats fed high carbohydrate diets with added wheat bran or stock diets showed a significant increase both at 30 and 180 minutes after the glucose load when compared to the fiber-free fed group (Figure 1A). Although the increase in serum glucose in rats fed pectin and methylcellulose was similar to control at 30 minutes, blood glucose concentration then decreased and was significantly less ($P \leq 0.05$) than controls at 60 minutes. This decrease was still apparent at 120 minutes in pectin fed rats. Rats fed low carbohydrate diets with added fiber did not have significantly different glucose tolerance than rats fed fiber-free low carbohydrate diets (Figure 1B). However, the serum glucose response of rats fed wheat bran or pectin added to low carbohydrate diets followed similar trends as these same fiber sources added to high carbohydrate diets. Serum glucose concentrations of rats fed LC-P were lower at 60, 120 and 180 minutes than the glucose concentrations of rats fed LC-FF. Rats fed LC-WB had elevated serum glucose at 60 and 180 minutes after glucose loading when compared to LC-FF fed rats. Despite the lowered serum glucose response observed when methylcellulose was added

Figure 1. The increase in mean serum glucose concentration following an oral dose of glucose (chronic tests) in rats fed standard lab chow or high carbohydrate (Figure 1A) or low carbohydrate (Figure 1B) diets with or without added fiber for four weeks. Asterisks indicate that the value for the fiber fed rats was significantly different than that of rats fed fiber-free diets. Vertical lines indicate standard errors of the mean.

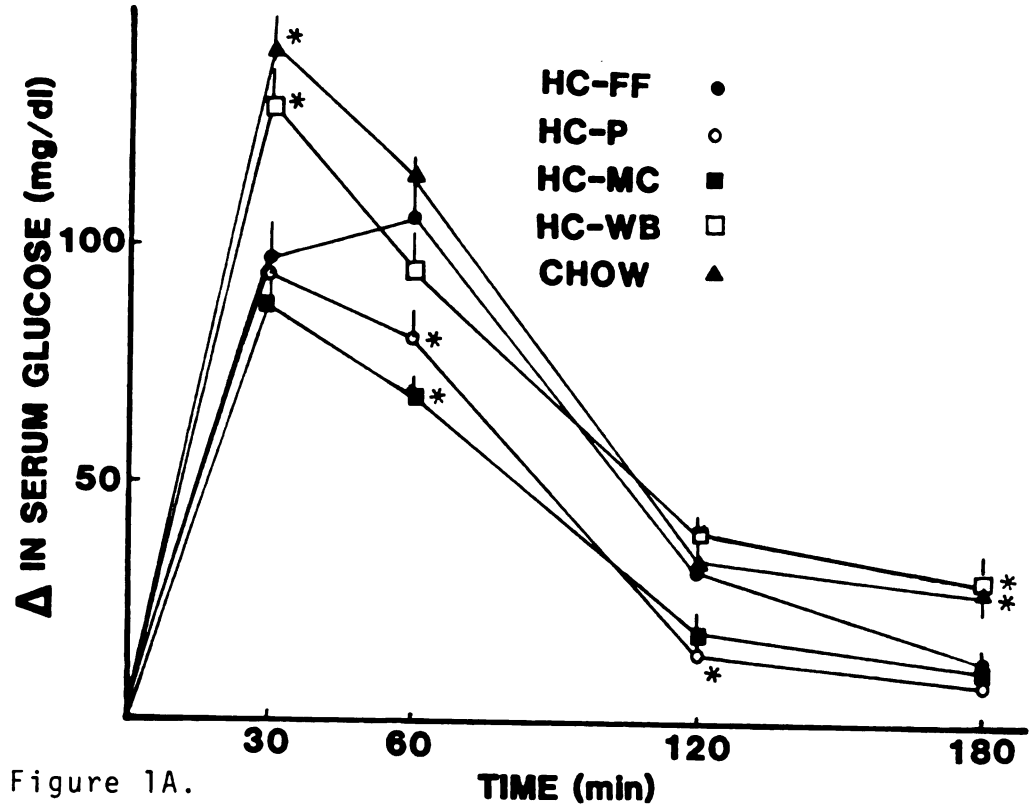

Figure 1A.

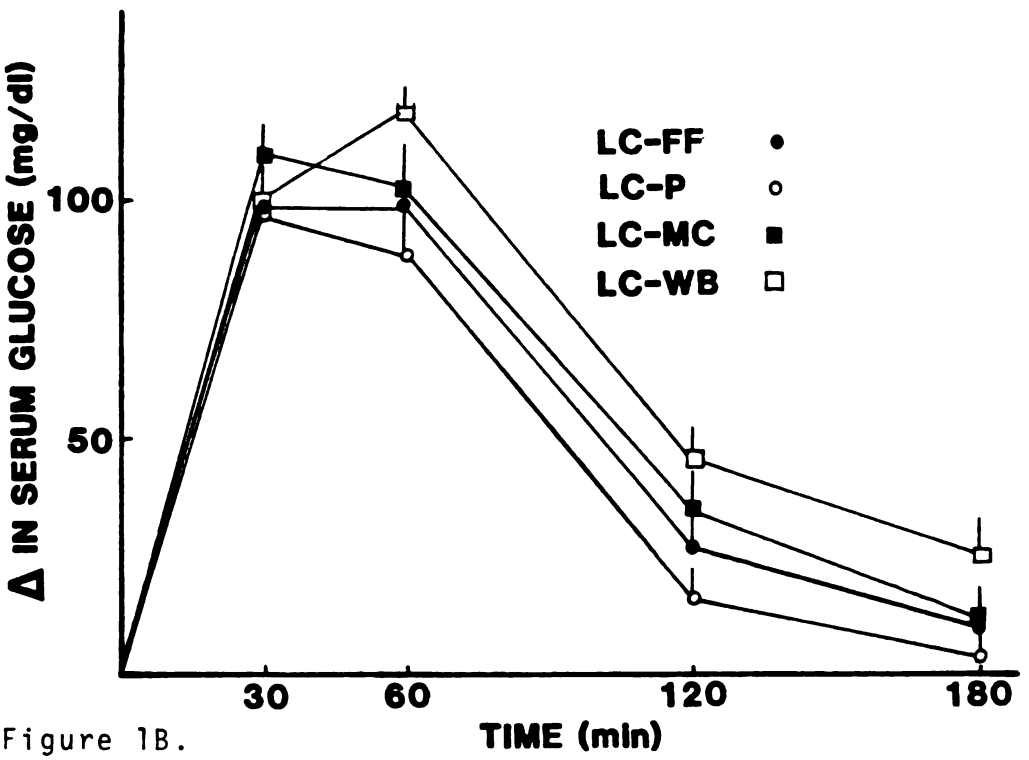

Figure 1B.

to high carbohydrate diets, a similar effect was not seen with low carbohydrate diets.

When a dietary fiber source was added to the glucose test load (acute tests), irrespective of the previous diet, the serum glucose was lower at 30-60 minutes and elevated at 120-180 minutes when compared to serum glucose concentrations after a test load of glucose alone (Figures 2 and 3; Appendix Table A2).

The integrated areas of the serum glucose concentrations following an oral load were calculated to give an index of the total 180 minute response for both chronic and acute tolerance tests (Table 4). In chronic tests, there was no difference in area under the glucose response curve for rats previously fed HC-FF or LC-FF diets. Inclusion of pectin in these diets lowered ($P \leq 0.05$) the total incremental area and addition of wheat bran slightly increased ($P \leq 0.10$) the total area when compared to fiber-free control diets.

While the serum glucose response to an acute OGTT was flattened due to a lower peak rise and decreased nadir, there were no significant differences in the total areas for acute and chronic OGTTs for rats fed a particular diet. In the acute tests, addition of pectin to the glucose test load did not significantly affect the total area for rats previously fed HC-FF, HC-P, LC-FF or LC-P diets. However the order of magnitude of the total areas

Figure 2. Comparison of the increase in mean serum glucose following an oral dose of glucose alone (chronic tests) or glucose with added fiber (acute tests) for rats fed HC-FF (Figure 2A) or LC-FF (Figure 2B) diets. Asterisks indicate that the value for glucose load containing fiber was significantly different than that of rats administered a fiber-free glucose test load. Vertical lines indicate standard errors of the mean.

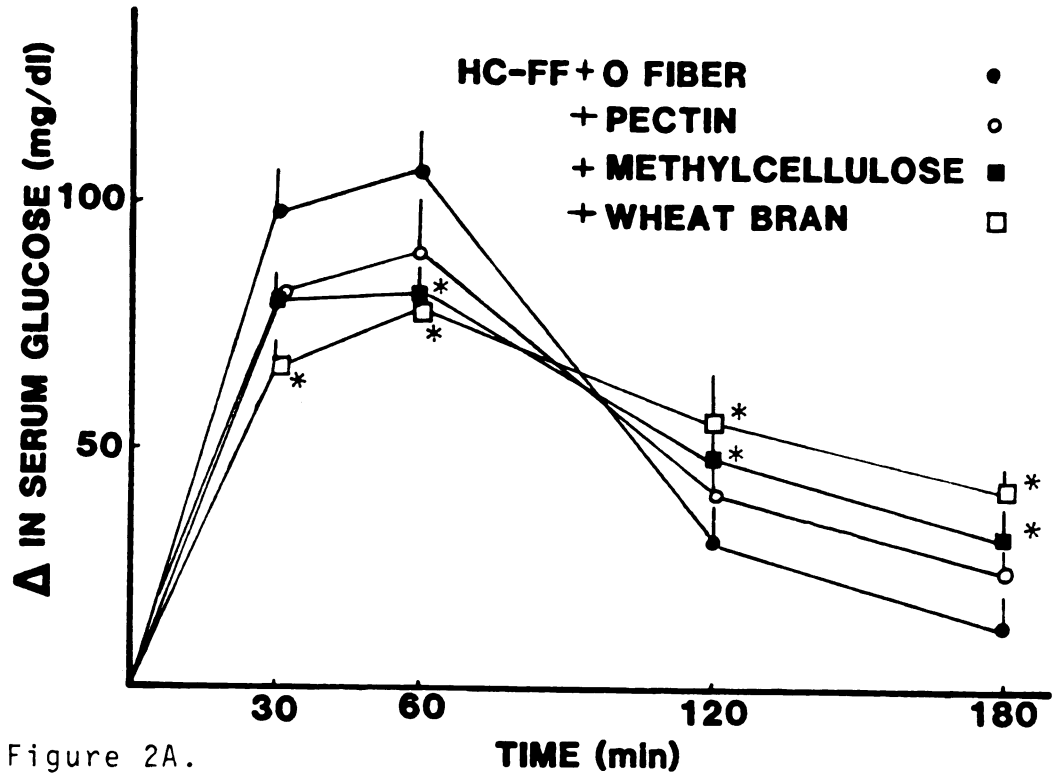

Figure 2A.

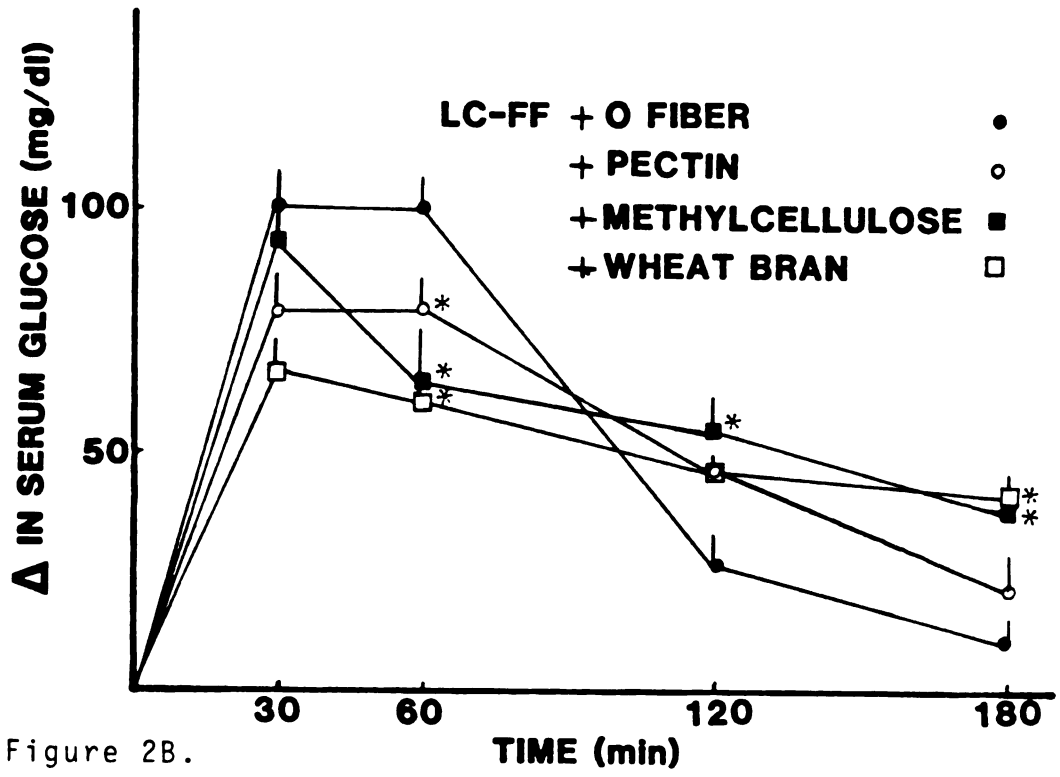

Figure 2B.

Figure 3. Comparison of the increase in mean serum glucose concentration following an oral dose of glucose alone (chronic test) or glucose plus added fiber (acute test) for rats fed high or low carbohydrate diets with added pectin (Figure 3A), methylcellulose (Figure 3B) or wheat bran (Figure 3C). Asterisks indicate that the value for the glucose load containing fiber was significantly different than that of rats administered a fiber-free glucose test load. Vertical lines indicate standard errors of the mean.

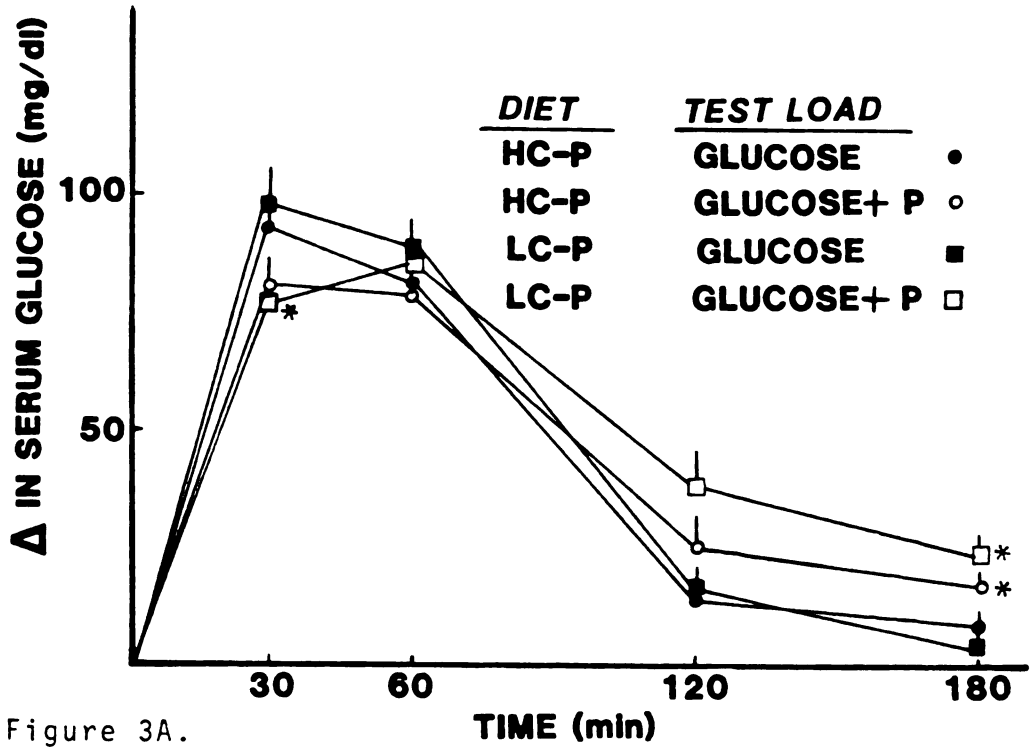

Figure 3A.

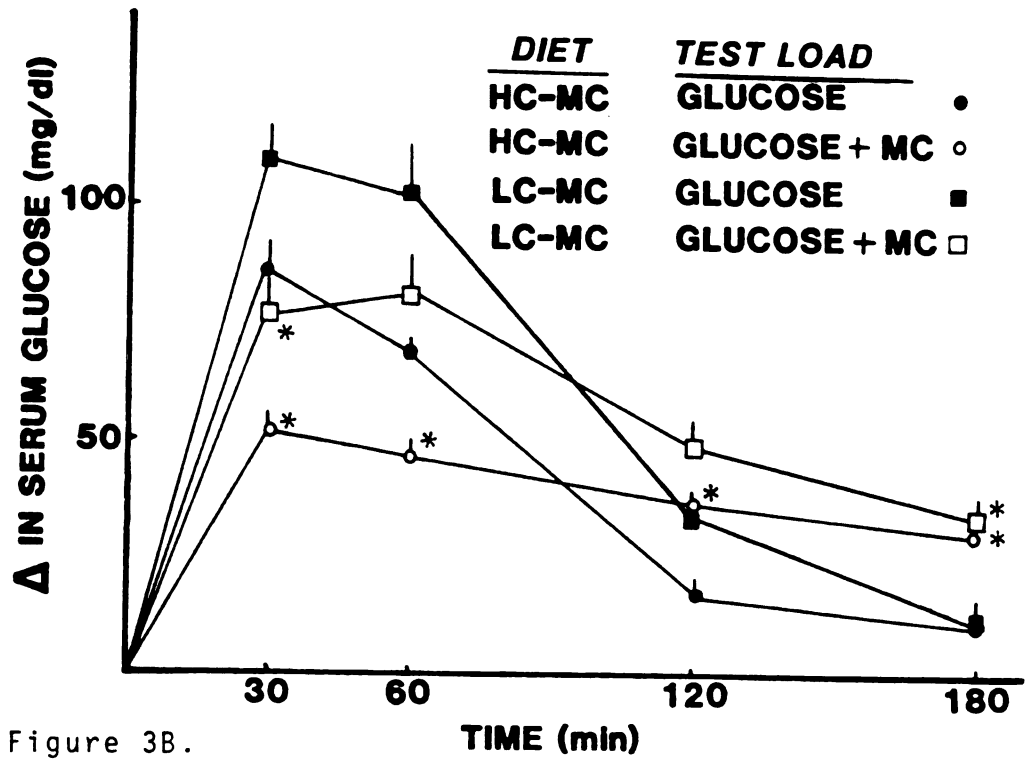

Figure 3B.

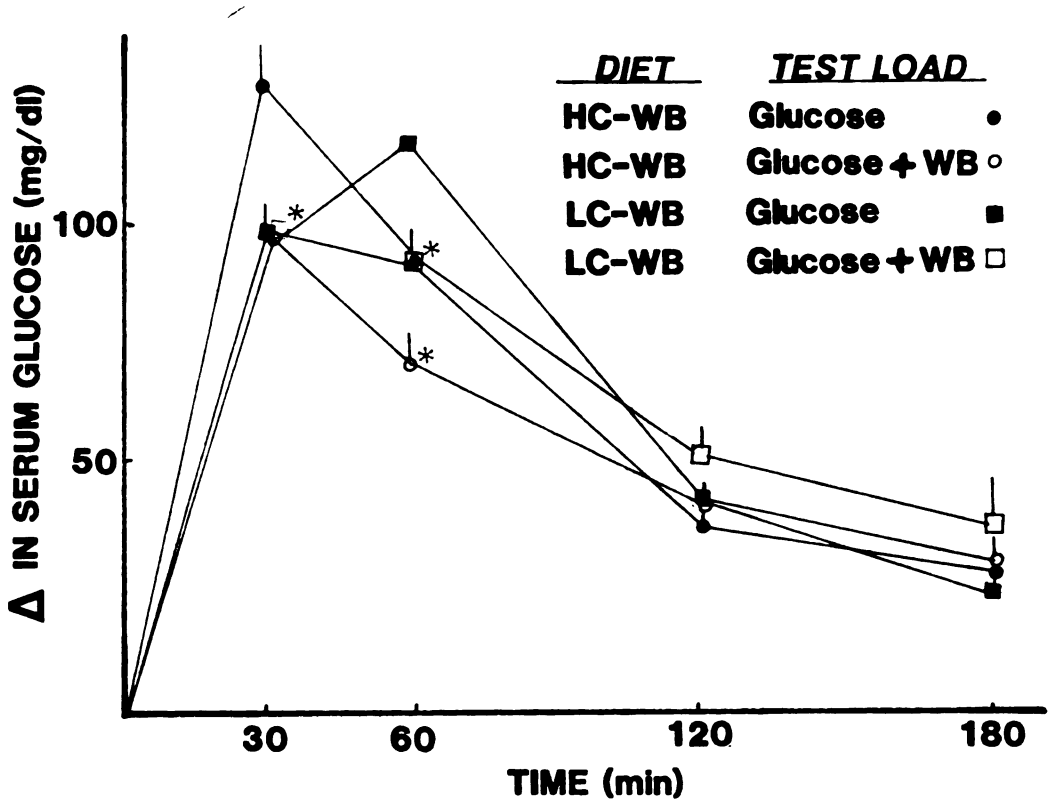
DIET
TEST LOAD
HC-WB
Glucose
HC-WB
Glucose + WB
LC-WB
Glucose
LC-WB
Glucose + WB
Δ IN SERUM GLUCOSE (mg/dl)
100
50
30
60
120
180
TIME (min)

Figure 3C.

Table 4. Incremental areas under the serum glucose response curve for chronic and acute OGTTs in rats fed high or low carbohydrate diets with or without added fiber sources[1].

Diet	Chronic OGTT[2]	Acute OGTT[3]		
		+Pectin	+Methyl-cellulose	+Wheat bran
			mg dl^{-1} min	
HC-FF	10006±987	9763±941	9940±547	9856±556
HC-P	7843±692[4]	8100±767		
HC-MC	7173±816[5]		6973±688[5]	
HC-WB	11544±709			9770±663
LC-FF	9490±1068	9213±963	8573±875	10070±1037
LC-P	8013±340[4]	9097±957		
LC-MC	10130±1045		9880±896	
LC-WB	11300±718			11740±964

[1] Mean±SEM

[2] Load dose was 1.5 g glucose/kg body weight.

[3] Load dose was 1.5 g glucose/kg body weight with added pectin (4% w/v), methylcellulose (4% w/v), or wheat bran (1.1±0.1 g).

[4] Values are different from values from rats fed the fiber-free diets ($P \leq 0.05$). Values from HC and LC fed rats were combined for analysis because there were no significant interactions between fiber and carbohydrate level.

[5] Values are different from values from rats fed the fiber-free diet ($P \leq 0.05$) values from HC and LC were analyzed separately because there was an interaction of carbohydrate level with methylcellulose.

after the acute tests with added pectin followed the same pattern as the total area following chronic tests (Spearman's rank correlation (r_s)=1.0, $P \leq 0.05$). When methylcellulose was added to the glucose load, rats previously fed HC-MC, but not LC-MC, had a decrease in the integrated response area when compared to those fed HC-FF or LC-FF diets. This response pattern is similar to the inconsistency observed in chronic tests for HC-MC and LC-MC fed rats. However, the rank correlation of the total incremental areas after the acute and chronic OGTTs for rats previously fed HC-FF, HC-MC, LC-FF or LC-MC was r_s=0.8. This value was not significant. Rats given wheat bran with a glucose load showed no significant differences in the total area under the glucose response curve dependent on previous diet. There was an insignificant correlation (r_s=0.4) between the areas after acute and chronic tests for rats fed HC-FF, HC-WB, LC-FF or LC-WB diets.

Intravenous Glucose Tolerance Tests

The final body weights of HC-FF, HC-P, HC-WB rats given an intravenous glucose tolerance test were 322±7 g, 315±7 g and 330±3 g, respectively. Since the glucose load was calculated on the basis of body weight there was no difference in the administered load dose. Table 5 shows the rate of glucose disappearance (K_G) in response

Table 5. The rate of serum glucose disappearance following an intravenous infusion of glucose in rats fed high carbohydrate diets with or without various fiber sources[1,2]

Diet	K_G (%/min)[3]
HC-FF (7)	1.35±0.30
HC-P (6)	1.63±0.47
HC-WB (7)	1.51±0.31

[1]Glucose load dose was 0.5 g/kg body weight.

[2]Mean±SEM; number of rats per treatment is indicated in parentheses.

[3]Calculated as the least squares slope of the natural log of the glucose concentration vs. time relationship between 5 and 30 minutes after the glucose infusion.

to the intravenous glucose infusion. No significant difference was found in the rate of glucose disappearance in rats fed HC-FF, HC-P or HC-WB diets. Figure 4 (Appendix Table A3) shows the serum glucose, insulin and pancreatic glucagon concentrations in response to the glucose load. At 15 minutes post glucose infusion, the serum glucose concentrations for rats fed HC-WB were significantly lower ($P \leq 0.05$) than values for rats fed HC-FF diets. Since the concentration of serum glucose at 5 minutes post infusion was also lower than control values, the glucose disappearance rate was not affected. Serum insulin concentrations were elevated at 5 minutes post glucose infusion for rats fed HC-WB diets and at 30 minutes for rats fed HC-P diets. Neither insulin value was significantly different from control fiber-free diet fed rats. The dietary treatments did not affect pancreatic glucagon concentrations during the intravenous glucose tolerance tests.

Discussion

The flattened serum glucose response to an oral glucose tolerance test observed in this study with pectin or methylcellulose added to the glucose load is consistent with the previous reports for acute oral tolerance tests in men (Jenkins et al., 1978) and rats (Schwartz and Levine, 1980). This reduced glycemic response has been

Figure 4. Serum glucose (4A), insulin (4B) and pancreatic glucagon (4C) following an intravenous glucose load (0.5 g/kg BW) in rats fed high carbohydrate diets with or without added fiber. Asterisks indicate the value is significantly different from that of rats fed fiber-free diets. Vertical lines indicate standard errors of the mean.

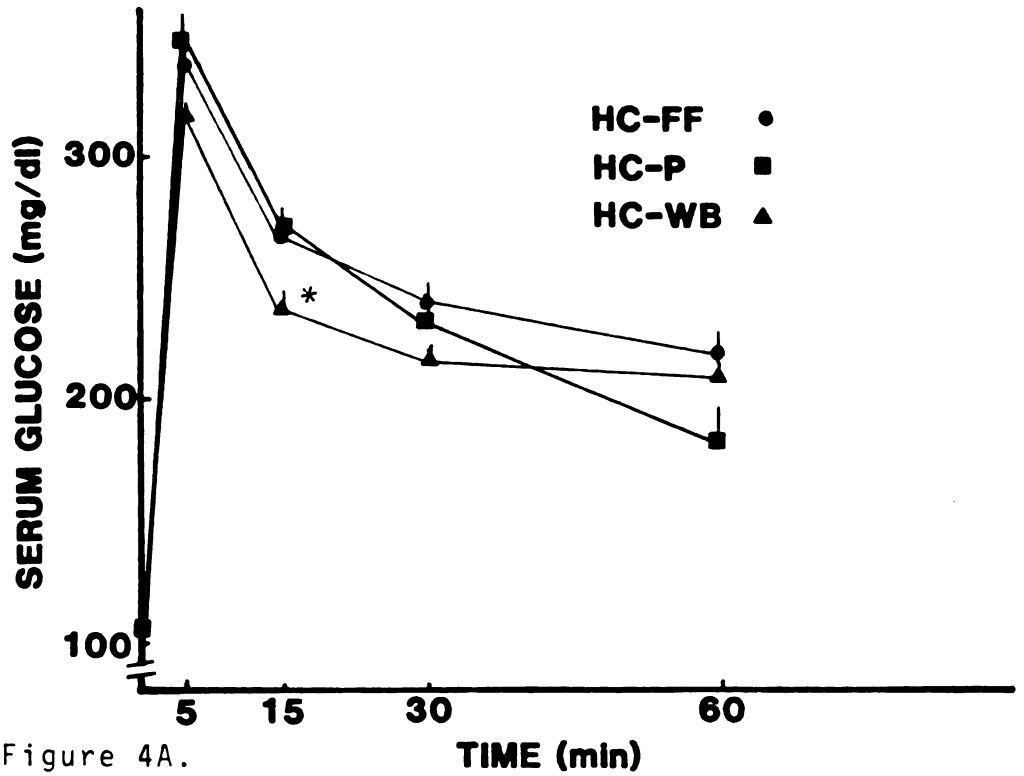

Figure 4A.

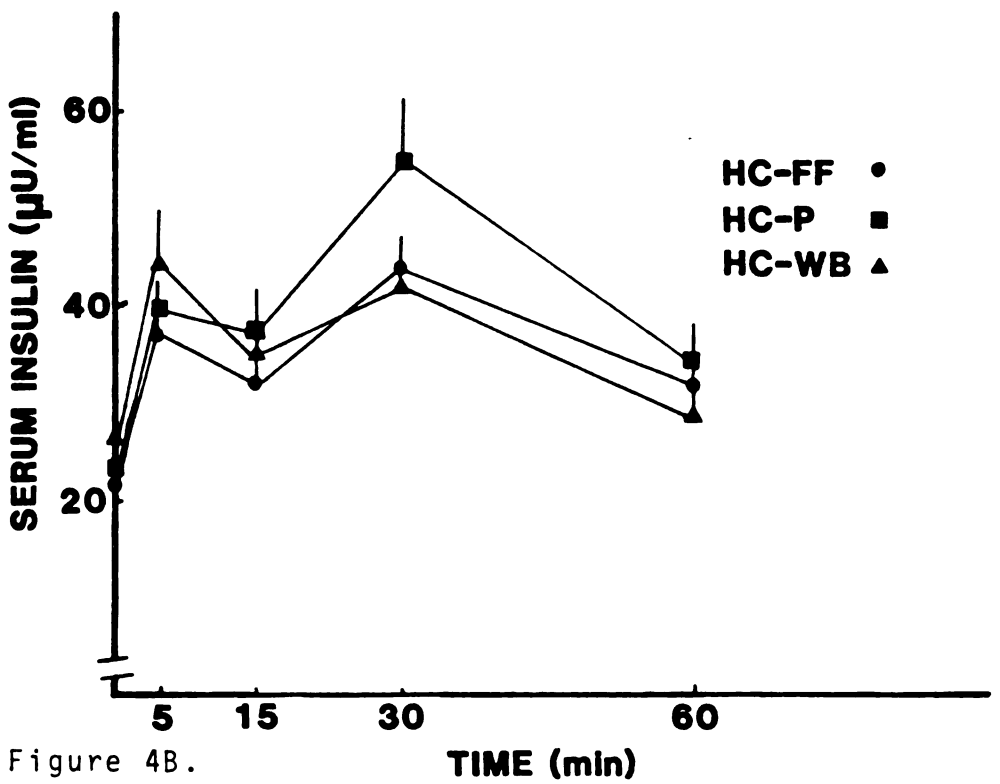

Figure 4B.

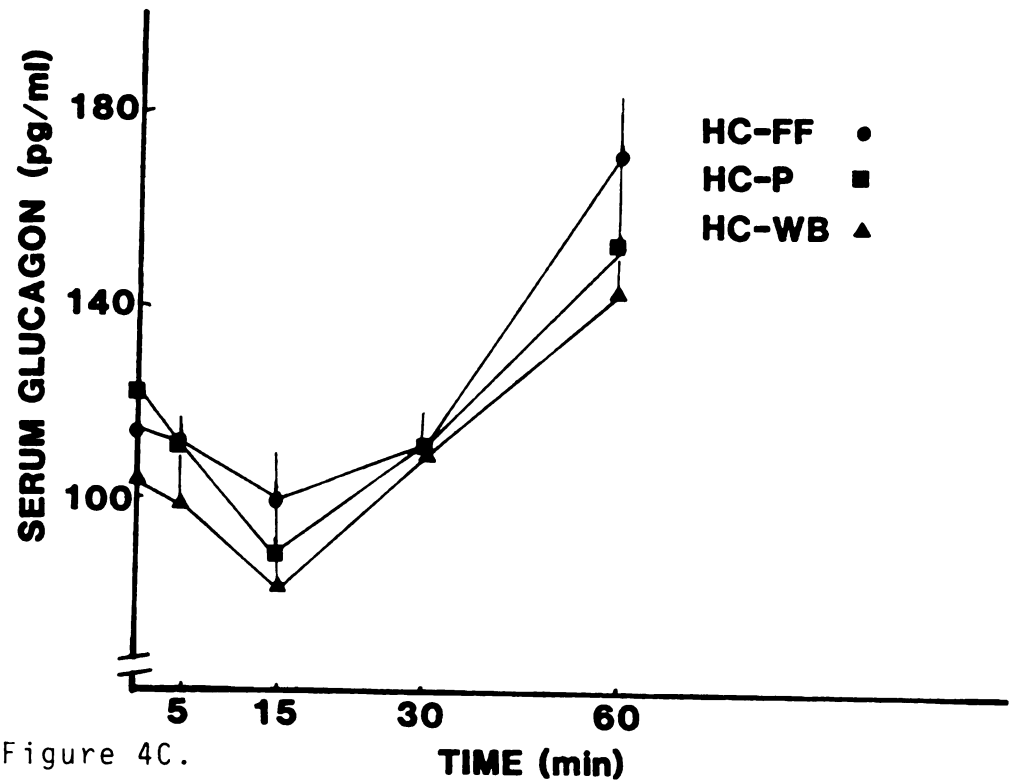
SERUM GLUCAGON (pg/ml)
180
140
100
5
15
30
60
TIME (min)
HC-FF
HC-P
HC-WB

Figure 4C.

correlated with the increased viscosity of the glucose-fiber test solutions (Ebihara and Kiriyama, 1982). Gel forming dietary fibers such as pectin or methylcellulose convert the liquid glucose into a semi-solid mass that may reduce the rate of gastric emptying (Holt et al., 1979) and possibly the rate of diffusion of glucose in the gut (Caspary et al., 1980). Jenkins and coworkers (1980a) used xylose load tests and breath hydrogen release after lactulose, fiber supplemented meals to correlate intestinal absorption and transit time. They determined that absorption is merely delayed and not reduced when certain fibers are included in the test meal. In the present study, the incremental areas under the serum glucose response curves were similar for both chronic and acute oral glucose tolerance tests for any diet group. The similarity of these areas as well as elevated serum glucose concentrations at later times during acute tests also suggests delayed absorption rather than malabsorption.

Consumption of wheat bran immediately prior to the glucose load likewise appeared to flatten the serum glucose response. While this result agrees with studies by Jenkins et al. (1978a) it is difficult to explain on the basis of slowed gastric emptying and/or absorption because of gel formation. On the contrary, wheat bran has been shown to decrease the mean transit time of lactulose in the gut (Jenkins, 1980a) possibly causing

malabsorption due to rapid gut transit. However, from the present study it would appear unlikely that a lack of complete absorption of glucose occurred. The total area under the serum glucose response curve was of a similar magnitude after wheat bran consumption in both acute and chronic tests in the same rats. Although not a water soluble fiber, perhaps wheat bran with its water holding capacity and physical mass acts as an intraluminal bulking agent and reduces the rate of glucose diffusion to the absorptive mucosal surface. In studies reported by Jefferys (1974) and Hall et al. (1980) addition of wheat bran to a glucose load also reduced the serum glucose response.

In addition to an acute effect of fiber, these experiments show that a chronic ingestion of dietary fiber can cause an alteration in the serum glucose response to an oral glucose tolerance test. The effects of chronic fiber ingestion appear to be independent of the level of carbohydrate in the diet. The integrated areas of the serum glucose response curves for rats previously fed 70% or 40% carbohydrate diets were not significantly different. It has been proposed that the higher the carbohydrate content of the preceding diet, the more efficiently an oral glucose load is cleared from the circulation due to an increase in insulin sensitivity (Himsworth, 1939; Brunzell et al., 1971). However, other investigators

have shown no change in the glucose response in normal men (Coulston et al., 1983), non-insulin dependent diabetics (Weinsier et al., 1974) or persons with hypertriglyceridemia (Liu et al., 1983) when the energy derived from carbohydrate was increased from 40 to 60%. Track et al. (1982) showed that diets with a much greater difference in carbohydrate content (50% vs. 14%) fed to rats did not alter the subsequent glucose response to an oral glucose tolerance test. Insulin responses during the OGTT were not determined in their study or in the present experiment. However, because the serum glucose values were similar after both the high and low carbohydrate diets, the insulin concentration would have to be decreased in the groups fed the high carbohydrate diets to indirectly demonstrate improved tissue sensitivity to insulin. Both Coulston et al. (1983) and Liu et al. (1983) observed an increase in insulin concentration with increased carbohydrate in the diet while serum glucose remained unchanged. This result would suggest a possible deterioration rather than an improvement in insulin sensitivity.

In a more recent study by Riccardi and coworkers (1984) in patients with type I and type II diabetes, an increase in carbohydrate from 42% to 53% of the energy without changing the low fiber content of the diets did not affect fasting or pre- or postprandial serum glucose

concentrations. A decrease in these parameters was obtained when the fiber content of the diet was increased from 16 g to 54 g by substitution with fiber-rich foods. Addition of dietary fiber thus appeared to mediate the positive effects on serum glucose. This result employing a relatively small change in carbohydrate content is consistent with findings in our study using a larger difference in carbohydrate content.

The effects of chronic fiber ingestion on glucose tolerance appear to be dependent not only on the presence of fiber in the diet but also on the type of fiber when compared to control fiber-free diets. Addition of pectin to the diet lowered the total area under the serum glucose response curve. This improvement in serum glucose response agrees with that of Schwartz and Levine (1980) in rats fed a 62% (w/w) sucrose diet with 5% added pectin for a five week period. These workers attributed the decrease in serum glucose response to impaired intestinal absorption due to adaptive, morphological changes in the small intestine. In intestinal perfusion studies using isolated jejunal loops from rats previously fed dietary pectin, they demonstrated a significant decrease in the absorption of a 10 mM glucose solution. However, dietary pectin did not alter mucosal thickness, villus height or crypt depth measured as structural correlates of absorptive function. In contrast, Sigleo and coworkers (1984) showed an

increase in villus length and width in rats previously fed diets with 10% pectin. These morphological alterations were coupled with an increase in the in vitro absorption of 3-O-methylglucose. Furthermore, Murray and colleagues (1980) demonstrated no changes in the specific activity of rat jejunal Na-K-ATPase after a 28 week period of high fiber diet. These studies suggest that impaired absorption may be associated with alterations other than structural changes affecting enterocyte transport.

Another possible mechanism for impaired glucose absorption following long term fiber ingestion may be a sustained increase in the unstirred water layer. Most studies (Blackburn et al., 1984; Elsenhans et al., 1980; Ebihara et al., 1981) have shown an increase in the unstirred water layer and a decrease in intestinal absorption when a gel-forming fiber is added to the glucose perfusate irrespective of the previous diet. However, more applicable to a chronic situation, Johnson and Gee (1981) have shown that merely pre-incubating everted intestinal sacs with guar gum followed by a guar-free glucose incubation is sufficient to inhibit glucose transport.

Increasing the unstirred water layer resistance has been shown to have a profound effect on the apparent Michaelis constant (Km) with little or no effect on the maximal transport capacity (Vmax) of glucose (Elsenhans

et al., 1980; Thomson and Dietschy, 1980). Because an oral glucose tolerance test load would likely produce substrate concentrations in the small intestine far above the Km, an effect on small intestinal absorption would not be expected. In addition, at high concentrations of glucose there is a larger contribution of passive permeability to glucose absorption (Thomson and Dietschy, 1980). Only as the intraluminal concentration of glucose is decreased, such as later in the oral glucose tolerance test period or at the relatively low concentrations of glucose (10 mM) used in the perfusion studies, would an effect of dietary fiber on small intestinal absorption be likely. The results of our chronic oral glucose tolerance tests and those of Schwartz and Levine (1980) show a decrease in serum glucose beginning at 30 minutes after the commencement of the oral load, a time point when the concentration of glucose in the gut might be expected to be the greatest.

While decreased small intestinal absorption of glucose due to increased unstirred water layer resistance or other adaptive structural alterations may be questionable, changes in gastric emptying may in part contribute to the improved glucose response associated with chronic fiber ingestion. As discussed previously, the acute effect of dietary fiber on glucose tolerance has been attributed in part to delayed gastric emptying (Holt

et al., 1979). The extent of an alteration in gastric emptying on glucose tolerance in previously fiber-fed rats has not been determined. In the present experiment, while the total area under the serum glucose response curve is similar for both acute and chronic tests, the shape of the response curve differs. In chronic tests the decrease in serum glucose at early post-load times is not as extensive as that seen in acute tests and there is a continued depression of serum glucose throughout the three hour test period as opposed to an elevation of serum glucose at 120-180 minutes seen in acute tests. Unless early delayed gastric emptying was coupled with a decrease in small intestinal absorption at later time points, the present studies would suggest that delayed gastric emptying is not solely responsible for the effects of chronic pectin ingestion on oral glucose tolerance.

The alternative possibility is that enhanced serum glucose clearance occurred in pectin fed rats. Net tissue concentration is dependent on the coordination of two processes, stimulation of glucose uptake and inhibition of endogenous glucose production. The metabolic clearance rate is largely insulin dependent (Cherrington et al., 1978) and the tolerance to a glucose load depends on two factors: the responsiveness of the pancreatic β-cells to glucose with consequent secretion of insulin and the sensitivity of the peripheral glucose utilizing tissues

to secreted insulin. Studies in patients with either non-insulin dependent diabetes or insulin dependent diabetes (Ward et al., 1982; Anderson, 1979; Pedersen et al., 1982) have attributed the beneficial effects of high carbohydrate, high fiber diets to increased insulin sensitivity. This increase in tissue sensitivity to insulin was indirectly demonstrated by decreased fasting glucose, lower insulin requirements and increased hypoglycemic episodes. These events were then associated with an increase in insulin binding to isolated monocytes. In the present experiment, however, it is unlikely that the decrease in serum glucose in pectin fed rats was due to an increased tissue sensitivity to insulin. Results of the intravenous glucose tolerance tests for these rats showed there was no difference in the disappearance rates of glucose or the corresponding insulin levels from those of rats fed fiber-free diets. Beck-Nielson and Pedersen (1978) have shown a positive correlation between the disappearance rate of glucose after an IVGTT and insulin binding to monocytes as a measure of insulin sensitivity in normal subjects. The K_G values for this population ($1.0\text{-}3.0 \times 10^{-2}$ min^{-1}) were in the range observed in our rats. In addition, if an increase in sensitivity to insulin had occurred in pectin fed rats, a lowered concentration of insulin in response to similar concentrations of glucose or conversely, a lowered glucose in response to

an equal insulin concentration would have been expected.

This lack of alteration in the intravenous glucose tolerance test compared to a possible increased uptake after the chronic OGTT in pectin-fed rats could be due to an increased β-cell responsiveness and secretion of insulin that is gut mediated through either a neural or hormonal mechanism. The gut hormones gastric inhibitory polypeptide (GIP) and enteroglucagon (GLI) released by enteral nutrients, have been shown to stimulate insulin secretion (Dupre et al., 1973; Moody et al., 1980).

Alternatively, the better clearance of an oral glucose load may be due to an inhibition of endogenous glucose production mediated through a reduction in pancreatic glucagon secretion. Both Munoz and collaborators (1979) and Miranda and Horowitz (1978) observed a decrease in pancreatic glucagon in conjunction with lowered serum glucose concentrations after chronic fiber feeding. However, in the present study, no difference was observed in serum glucagon concentration following an intravenous glucose load. The degree of hyperglycemia per se would also affect the extent to which hepatic glucose production is reduced (Liljenquist et al., 1979). In pectin fed rats there was a lower level of glycemia following an oral glucose load compared to control rats. A decrease in the inhibition of hepatic glucose production would then be anticipated and this would cause an apparent elevation as

opposed to a reduction in serum glucose concentration.

Addition of methylcellulose to the high carbohydrate diets resulted in a similar yet more extensive alteration in serum glucose response to that mediated by pectin feeding. Methylcellulose is a water soluble, viscous gel-forming methoxy derivative of the insoluble dietary fiber cellulose. It has similar physical properties to pectin and accordingly, similar physiological effects would be expected. In a study by Cannon et al. (1980) in rats that were fed 8% carboxymethylcellulose added to a diet containing 50% of the total energy supplied by corn-starch an attenuation of the serum glucose response was seen following a carbohydrate load. However, in the present experiment, when rats were fed low carbohydrate diets with added methylcellulose no difference was seen in the handling of a glucose load when compared with control fiber-free fed rats. A similar pattern after acute oral glucose tolerance tests was observed in these rats. The shape of the response curve in these acute tests was similar but the overall area was not decreased to the extent seen in HC-MC fed rats. The reason for this discrepancy in results for HC-MC and LC-MC fed rats is not apparent.

The major effect of adding wheat bran to the high and low carbohydrate diets appeared to be a decrease in fasting serum glucose concentrations. If absolute

serum glucose values are considered instead of the relative elevation from basal levels, the glucose response becomes more similar to that of rats fed fiber-free diets. In other studies, long-term feeding of wheat bran (Bosello et al., 1980) or natural foods with a large proportion of cereal based fiber sources (Kiehm et al., 1976; Barnard et al., 1983) have been shown to lower fasting glycemia. This decrease appears to be specific because chronic consumption of pectin does not produce a lowered basal glycemia (Gold et al., 1980).

In contrast to blood glucose concentrations prior to the OGTT, fasting blood glucose was not decreased in HC-WB fed rats used in the IVGTT. The higher values may have been a consequence of the anesthesia (Furner et al., 1972) and thus can not be compared to values from unanesthetized rats. In the early time points after the infusion of glucose in these intravenous tests, the lower glucose levels for the wheat bran fed rats correspond to a slight yet statistically non-significant elevation of insulin and reduction of glucagon. However when the incremental concentrations, as opposed to the absolute concentrations, are considered the alterations become non-existent. This similarity in relative changes between wheat bran and control rats may suggest an alteration in basal set point rather than an increased secretory responsiveness of the pancreas in rats fed

wheat bran.

In summary, it appears that sustained ingestion of dietary fiber as well as including fiber in the test meal can affect oral glucose tolerance. The chronic effects of dietary fiber ingestion are dependent on the fiber source, gut mediated and independent of the dietary carbohydrate content within the range of 40-70% of the total energy. Further experiments are necessary to elucidate the mechanism for the observed chronic effects of dietary fiber.

CHAPTER 2

LONG TERM EFFECTS OF DIETARY PECTIN OR WHEAT BRAN ON GLUCOSE TOLERANCE AND GASTRIC EMPTYING IN RATS

Introduction

It is well established that addition of fiber to a glucose load or a meal enhances glucose tolerance in healthy volunteers (Jenkins et al., 1978a), insulin dependent (Smith and Holm, 1982; Morgan et al., 1979) and non-insulin dependent diabetics (Levitt et al., 1980) and animal models (Ebihara et al., 1981; Tsai and Peng, 1981). In addition, several studies have reported chronic effects apparent after extended periods of dietary fiber supplementation when fiber is not included in the test load. Oral glucose tolerance was improved after consumption of 24 g of bran a day for a six month period by patients with diverticular disease (Brodribb and Humphreys, 1976) or in normal men by daily consumption of 26 g of either corn bran, soy hulls or apple and carrot powders for a thirty day period (Munoz et al., 1977). In type II diabetics, 52 g/day of soy hull fiber (Mahalko et al., 1984) or 20 g/day of raw bran added to the diets of individuals with chemical diabetes (Bosello et al., 1980)

resulted in an improvement in oral glucose tolerance. In rat studies, chronic ingestion of 8% guar (Cannon et al., 1980), 5% pectin or 10% cellulose (Schwartz and Levine, 1980) or a multifiber source containing pectin, bran, cellulose and lignin (Track et al., 1981) decreased the serum glucose response to a glucose load. In both normal and streptozotocin diabetic rats 14 g of soy bean fiber/day for a forty-five day period reduced the serum glucose response to a glucose load (Madar, 1983).

Our previous study in rats demonstrated and further defined the sustained effect of chronic dietary fiber consumption. Rats that were fed diets with added pectin showed a decreased serum glucose response to an oral glucose load whereas rats fed wheat bran showed no improved response to a glucose load but rather a decreased fasting glycemia. In pectin fed rats, the improved glucose response was observed after an oral but not an intravenous glucose tolerance test. Therefore, it was suggested that the chronic effect of dietary fiber may be gut mediated. However, the exact mechanisms for these long term effects that seemingly are dependent on the fiber type and remain despite the lack of the concurrent presence of the fiber in the gastrointestinal tract have not been clearly identified. Possible factors that may explain the metabolic effect of chronic dietary fiber consumption include gut mediated changes in the hormonal response to a

carbohydrate load and/or alterations in the structure or function of the stomach or small intestine affecting nutrient absorption.

Both gastric inhibitory polypeptide (GIP) and glucagon-like immunoreactivity (GLI; enteroglucagon) originate in the gastrointestinal tract (Sjolund et al., 1983) and are insulin secretagogues (Dupre et al., 1973; Moody et al., 1980; Gutman et al., 1973). It is therefore possible that either of these hormones may mediate an increased insulin response to glucose thus producing an improved oral glucose tolerance test. However, in addition to the proposed ability of GLI to stimulate insulin secretion, a strong association has been demonstrated between the serum concentration of this hormone and conditions with stimulated intestinal growth (Bloom, 1972; Bloom and Polak, 1982; Sagor et al., 1983; Gornacz et al., 1984; Besterman et al., 1978; Uttenthal et al., 1982) and gastric emptying rate (Ralphs et al., 1975; Jenkins et al., 1980d; Lauritsen et al., 1982). Coincidently, prolonged ingestion of certain dietary fibers has been associated with changes in the length, weight and morphology of the small intestine (Elsenhans et al., 1982; Brown et al., 1979; Forman and Schneeman, 1982; Farness and Schneeman, 1982; Cassidy et al., 1981; Jacobs, 1983) and rate of gastric emptying (Schwartz et al., 1982).

Although these fiber induced alterations have not been related to an improved oral glucose tolerance, it may be possible that enteroglucagon through its ability to stimulate insulin secretion and/or control gastrointestinal growth and motor function may be involved in the fiber induced improvements in oral glucose tolerance. Therefore, in addition to evaluating the pancreatic hormonal responses and gastric emptying rate as a mechanism(s) to explain the chronic effects of wheat bran and pectin on glucose metabolism, the present study also included an examination of enteroglucagon as a possible gut mediator of these fiber-induced events.

Materials and Methods

Animals and Diets

Male Sprague-Dawley rats (Harlan Industries, Inc., Indianapolis, IN) were individually housed in hanging wire-bottom stainless steel cages in a temperature controlled room (22^{o}C) with a 12-hour light/dark cycle. A fiber-free diet with 70% of the total energy derived from carbohydrate was formulated (HC-FF). Either 7% pectin (HC-P) or 30% wheat bran (HC-WB) was added (w/w) to the basal HC-FF as previously described. Water and food were available ad libitum. Food intake was recorded every three days and body weights were determined weekly.

Experiment 1

Two sets of thirty rats each, weighing 190-225 g, were randomly assigned to HC-FF or HC-P diet groups for a four week feeding period. At the end of this period, following a 22-24 hour fast, rats were given an OGTT. Forty-two glucose tolerance tests were conducted over a one week period with three rats from each group tested per day. Serum samples were collected in a fasted state and at 30, 60, 120 and 180 minutes after a glucose load. Rats were returned to their respective diets at the conclusion of the test. During the following week, the quantity of glucose retained in the stomach at 30, 60, 120 and 180 minutes after an oral glucose load was determined. An equal number of rats from each diet group were tested on any given day.

Experiment 2

Rats weighing 185-210 g were distributed into diet groups as follows: twenty-eight rats each, HC-FF and HC-P and fifty rats, HC-WB. After a four week feeding period and an overnight fast, rats previously fed HC-FF or HC-P were given an OGTT. In order to assess the early response to an OGTT, blood samples were collected before and at 10, 20, 30 and 60 minutes after a glucose load. In eighteen of the tolerance tests, samples from three rats were pooled for glucose, insulin and glucagon analyses. In

six of the tests there was additional sampling at 120 and 180 minutes; in these tests, samples were not pooled and only glucose and insulin were determined. Twenty-nine OGTTs were performed on rats fed HC-WB. Samples were collected before and at 10, 20, 30, 60, 120 and 180 minutes after a glucose load. In twenty-one of the tolerance tests, samples from three rats were pooled for glucose, insulin and glucagon analyses. In eight OGTTs samples were not pooled, and glucose and insulin were determined. Gastric emptying at 5, 10, 20, 30, 60, 120 and 180 minutes after a glucose load was determined in rats previously fed HC-WB diets in the week following the completion of all oral glucose tolerance tests. Additionally, seven rats from each group (HC-FF, HC-P and HC-WB) were fasted for 22-24 hours prior to removal of the upper gastrointestinal tract for length and weight measurements.

Glucose Tolerance Tests

Oral glucose tolerance tests (OGTT) were administered following the same procedure as previously described for chronic glucose tolerance tests. However, the collected sample volume was increased from 300 µl to 600 µl at each time point. Blood samples were collected into chilled 6x50 mm test tubes containing proteolytic enzyme inhibitor (Trasylol; FBA Pharmaceuticals, Inc., New York, NY), allowed to clot and then centrifuged. The separated serum

samples from three rats were then pooled and frozen for later determinations of glucose, insulin and glucagon.

Gastric Emptying

After a 22-24 hour fasting period, rats were administered glucose solutions in the same manner and amount as in the glucose tolerance tests. Rats were then returned to their cages. At five minutes prior to the specified sampling times rats were anesthetized by an intra-peritoneal injection of 0.5 mg/kg body weight sodium pentabarbital. When eye reflexes had disappeared, the abdominal cavity was opened by means of a mid-line incision. The stomach was ligated at the gastroesophageal junction and at the pylorus. The stomach, with contents intact, was excised, removed and placed in a small beaker. Ligatures were removed and glucose retained in the stomach and released and collected. The stomach was then split longitudinally and subjected to repeated rinses with distilled water. This combination of stomach contents and rinses was refrigerated and saved for glucose analysis later the same day. Gastric emptying was expressed as a percentage of the administered glucose dose retained in the stomach at a specified time post intubation. The recovery of glucose immediately after delivery into the stomach was 94-98%.

Gastrointestinal Length and Weight Measurements

Fasted rats were anesthetized with sodium pentabarbital. The stomach and the entire small intestine from the gastroduodenal junction to the ileocecal junction was removed and stripped of mesentry. The stomach was separated from the small intestine and split longitudinally and rinsed. The length of the entire small intestine was measured against a horizontal scale. The intestine was then flushed with distilled water and drained. The mucosal layer was separated from the muscle and serosal layer by running the intestine through a closed forceps. Both stomachs and small intestines were then placed in a 50^{o}C drying oven until a constant weight was obtained.

Chemical Analyses

Serum samples and appropriately diluted gastric contents were analyzed for glucose content by the glucose oxidase method. Serum samples were also analyzed for insulin and glucagon content. Insulin was determined by a radioimmunoassay procedure employing pre-coated assay tubes (Micromedic Test Delivery Systems, Micromedic, Inc., Horsham, PA). Serum immunoreactive glucagon (IRG) concentration was determined using the method of Foa et al. (1977) except that a dextran coated charcoal slurry was used to separate free from bound radioactivity. Two antiglucagon sera (AGS) were used in this assay: 1) AGS 10

which cross-reacts with extracts of intestinal mucosa and measures "total" IRG and 2) AGS 18 which is specific for the carboxyl terminus of the molecule and is believed to measure A-cell ("pancreatic") glucagon (IRG_p) (Matsuyama et al., 1977). The difference between "total" IRG and IRG_p was used to estimate "gut" glucagon or enteroglucagon (IRG_g). This is the IRG fraction of other than A-cell origin believed to be secreted mostly by the mucosa of the small intestine.

Data Analyses

All calculations were performed on a Hewlett-Packard Model 97 programmable calculator or a Texas Instruments Model 99/4A microcomputer. The incremental area under the glucose or insulin response curves was calculated by integration, utilizing trapezoidal rules, of the increase above fasting serum concentration over a designated time period. Treatment means were compared by Student's t test (two means) or by analysis of variance and Dunnett's t test (three means). Slopes (b) of linear regression describing the gastric emptying of glucose were used to calculate the fractional disappearance rate (k) of glucose ($k=b/0.434$). The fractional disappearance rates were compared statistically by Bonferoni t tests using the variance estimated from the difference between slopes of individual regression equations (Gill, 1978). The

criterion of significance was $P \leq 0.05$.

Results

Glucose Tolerance Tests

Experiment 1. The final body weights for rats maintained on HC-FF or HC-P diets were 322±2 g and 321±2 g, respectively. Since the glucose load was calculated on the basis of body weight there was no difference in the administered load dose between groups. Table 1 shows the serum concentrations of glucose, insulin and pancreatic and gut glucagon prior to the glucose load. There was no difference between diet groups in any of these measurements. Changes in serum glucose in response to an oral glucose load are shown in Figure 1; Appendix Table B1. The increase in serum glucose concentration (Figure 1A) above basal levels was significantly diminished ($P<0.05$) at all sampling time points in rats previously fed diets with added pectin. This sustained attenuation resulted in a significant decrease ($P<0.05$) in the total three hour incremental serum glucose response (Table 1). Serum insulin was also decreased at all time points in HC-P fed rats (Figure 1B) resulting in a decrease in the total three hour response (Table 1). This decrease, however, did not reach a level of statistical significance. Previous feeding of pectin did not alter the concentration of pancreatic glucagon in response to a glucose load (Figure 1C);

Table 1. Serum glucose, insulin and glucagon concentrations after a 22-24 hour fast and the incremental areas of the serum glucose and insulin response, following an oral glucose load, Experiment 1.

Parameter[1]	Diet Group	
	HC-FF	HC-P
Fasting Serum Concentration		
Glucose, mg/dl	111±4	110±3
Insulin, μU/ml	16±1	16±1
Pancreatic glucagon, pg/ml	116±7	111±4
Gut glucagon, pg/ml	596±42	577±55
Incremental Serum Response[2]		
Glucose, mg/dl^{-1} min	8607±516	6143±520[3]
Insulin, μU ml^{-1} min	1674±197	1281±196

[1]Values are mean±SEM for seven pooled samples in each group; each sample represents three rats.

[2]0-180 minutes time period.

[3]Significantly different ($P \leq 0.05$) from rats fed HC-FF.

Figure 1. The increase in mean serum glucose (1A), insulin (1B), pancreatic glucagon (1C) and gut glucagon (1D) from fasting serum concentrations following an oral dose of glucose in rats fed a high carbohydrate fiber-free (HC-FF) diet or a high carbohydrate diet with added pectin (HC-P) for four weeks. Asterisks indicate that the value for HC-P fed rats was significantly different than that of rats fed HC-FF diets. Vertical lines indicate standard errors of the mean.

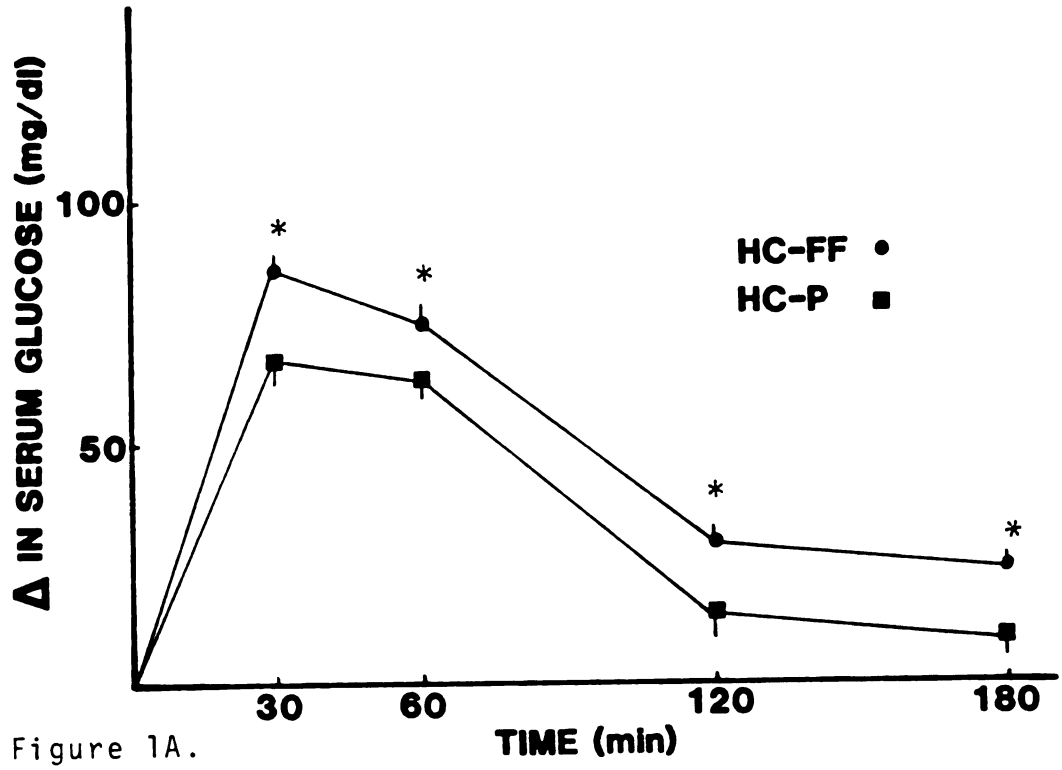

Figure 1A.

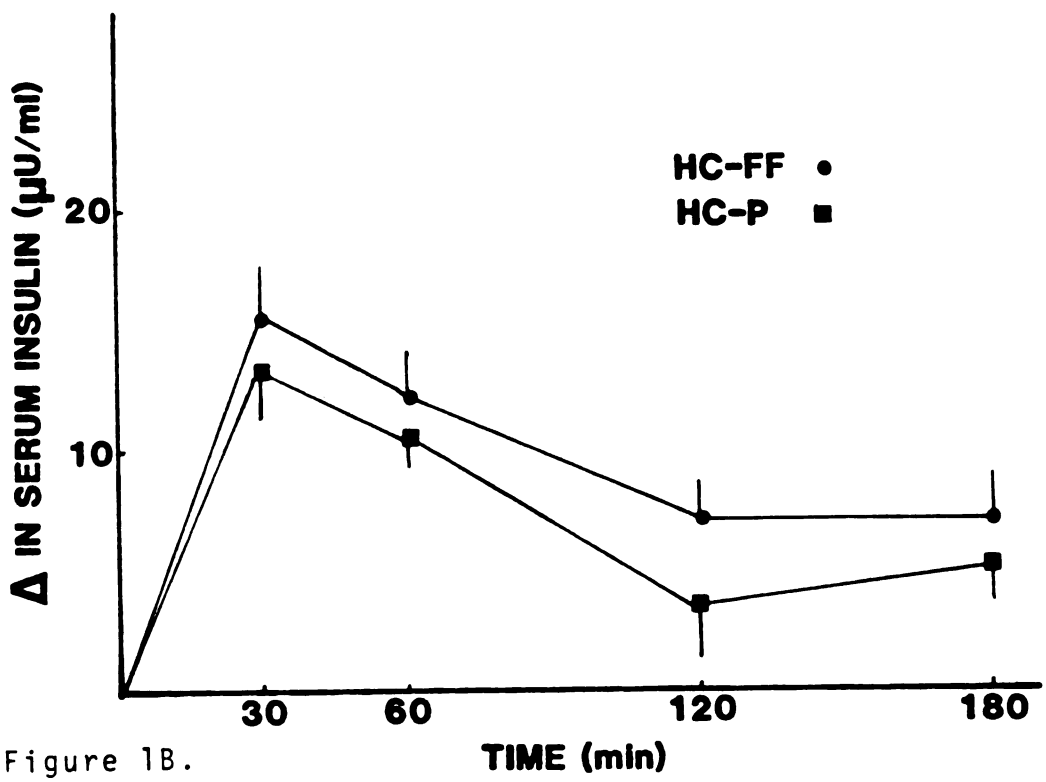

Figure 1B.

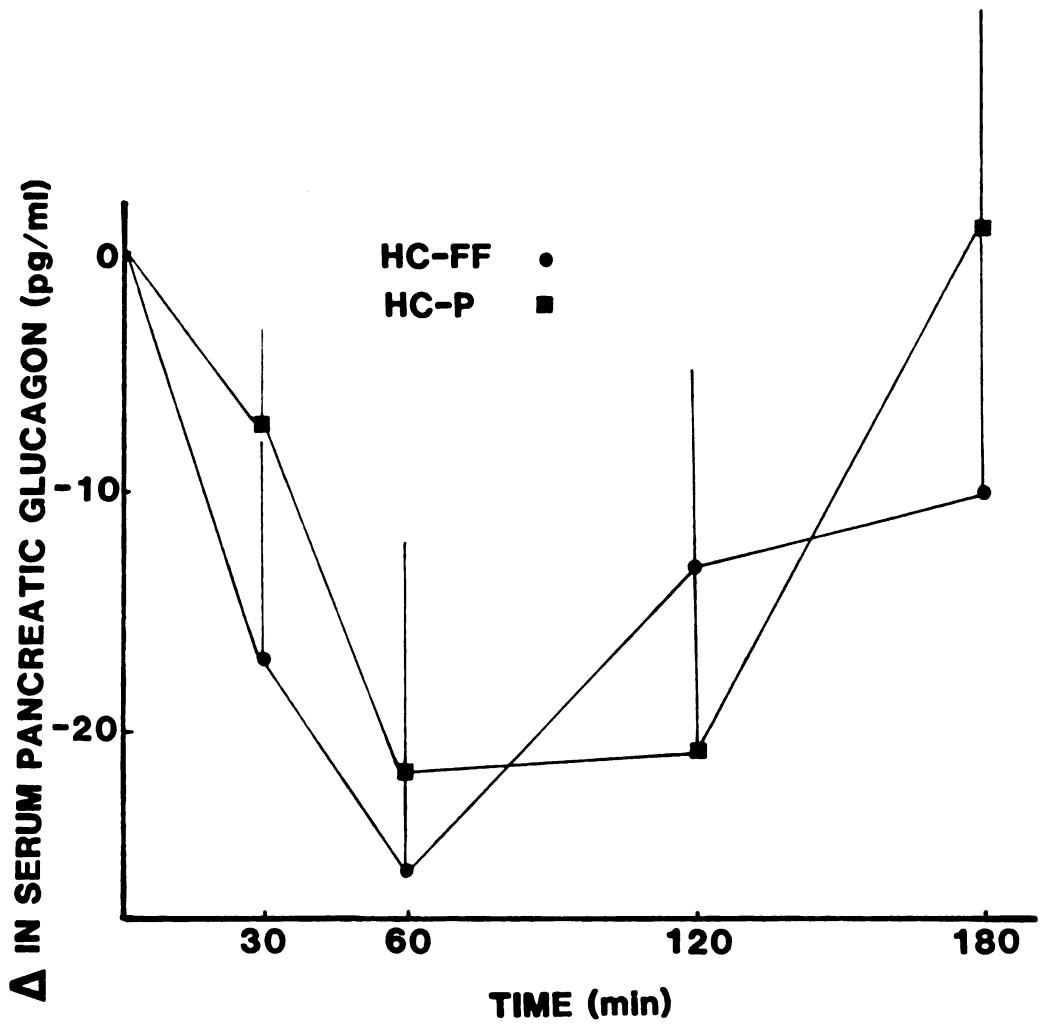
HC-FF
HC-P
0
-10
-20
Δ IN SERUM PANCREATIC GLUCAGON (pg/ml)
30
60
120
180
TIME (min)

Figure 1C.

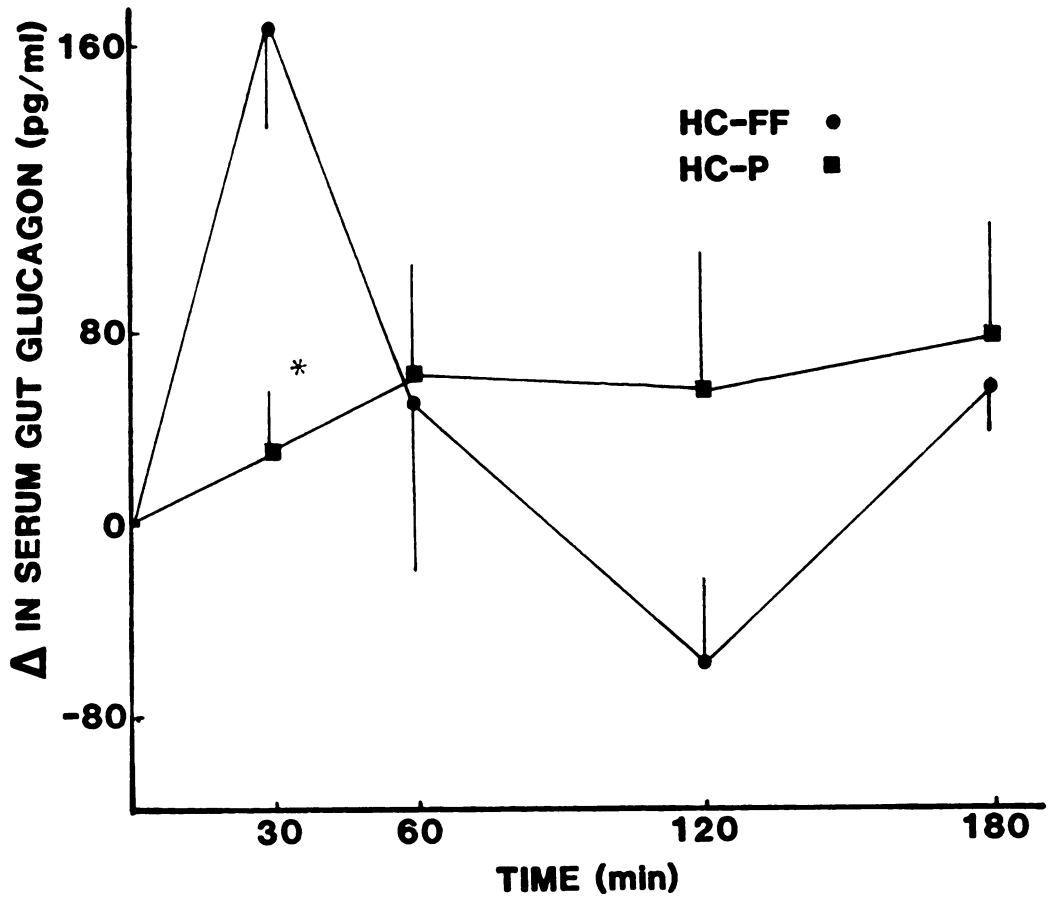
160
80
0
-80
Δ IN SERUM GUT GLUCAGON (pg/ml)
HC-FF
HC-P
*
30
60
120
180
TIME (min)

Figure 1D.

however, the serum enteroglucagon response at 30 minutes following a glucose load was significantly lowered ($P<0.05$) in pectin fed rats (Figure 1D).

Experiment 2. The final body weights for rats fed HC-FF, HC-P or HC-WB were 309±4 g, 306±2 g and 318±3 g, respectively. Neither fiber fed group differed from the control fiber-free fed group. Prior to the glucose load, HC-P and HC-FF fed rats had similar basal concentrations of serum glucose, insulin and glucagon, whereas HC-WB fed rats had significantly lower ($P\leq0.05$) glucose and pancreatic glucagon concentrations (Table 2). Figure 2 shows the increase in serum glucose, insulin, pancreatic glucagon and gut glucagon for a sixty minute period following an oral dose of glucose in rats previously fed HC-FF, HC-P or HC-WB diets. The incremental areas of these serum glucose and insulin response curves are given in Table 3. Rats fed HC-P showed an earlier rise in serum glucose concentrations at 10 and 20 minutes post glucose loading followed by a decrease ($P\leq0.05$) at 30 minutes when compared to fiber-free fed rats. At 180 minutes the serum glucose was also significantly decreased ($P\leq0.05$) in HC-P rats (Appendix Table B2). Whereas the peak glucose response was reached at 30 minutes after the load dose in HC-FF rats, it occurred at 20 minutes in HC-P fed rats. Therefore, if the 10 and 20 minute sampling time points are included, the 0-60 minute incremental serum glucose

Table 2. Serum glucose, insulin and glucagon concentrations after a 22-24 hour fast, Experiment 2.

Diet Group	Fasting Serum Concentration[1]			
	Glucose mg/dl	Insulin μU/ml	Pancreatic Glucagon pg/ml	Gut Glucagon pg/ml
HC-FF	97±3 (12)	14±1 (12)	119±6 (6)	662±21 (6)
HC-P	93±2 (12)	14±1 (12)	116±6 (6)	559±49 (6)
HC-WB	87±3 (15)[2]	16±1 (15)	90±6 (7)[2]	538±56 (7)

[1]Mean±SEM; where the mean represents the number of samples indicated in the parentheses. Six samples in HC-FF and HC-P and seven samples in HC-WB groups represent three rats each. The additional samples in all groups represent a single rat.

[2]Significantly lower ($P \leq 0.05$) than values for HC-FF.

Figure 2. The increase in mean serum glucose (2A), insulin (2B), pancreatic glucagon (2C) and gut glucagon (2D) from fasting serum concentrations following an oral dose of glucose in rats fed a high carbohydrate fiber-free (HC-FF) diet or a high carbohydrate diet with added pectin (HC-P) or wheat bran (HC-WB) for a four week period. Asterisks indicate that the values for either HC-P or HC-WB fed rats are significantly different than those of HC-FF fed rats. Vertical lines indicate standard errors of the mean.

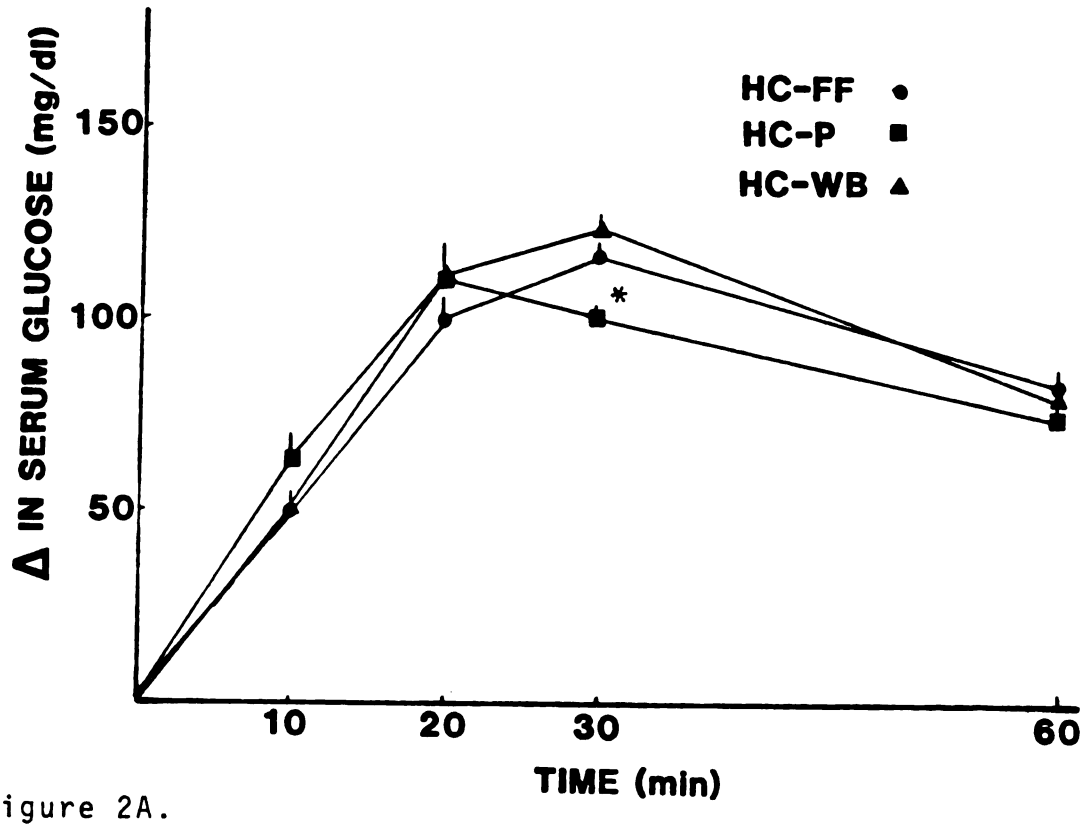

Figure 2A.

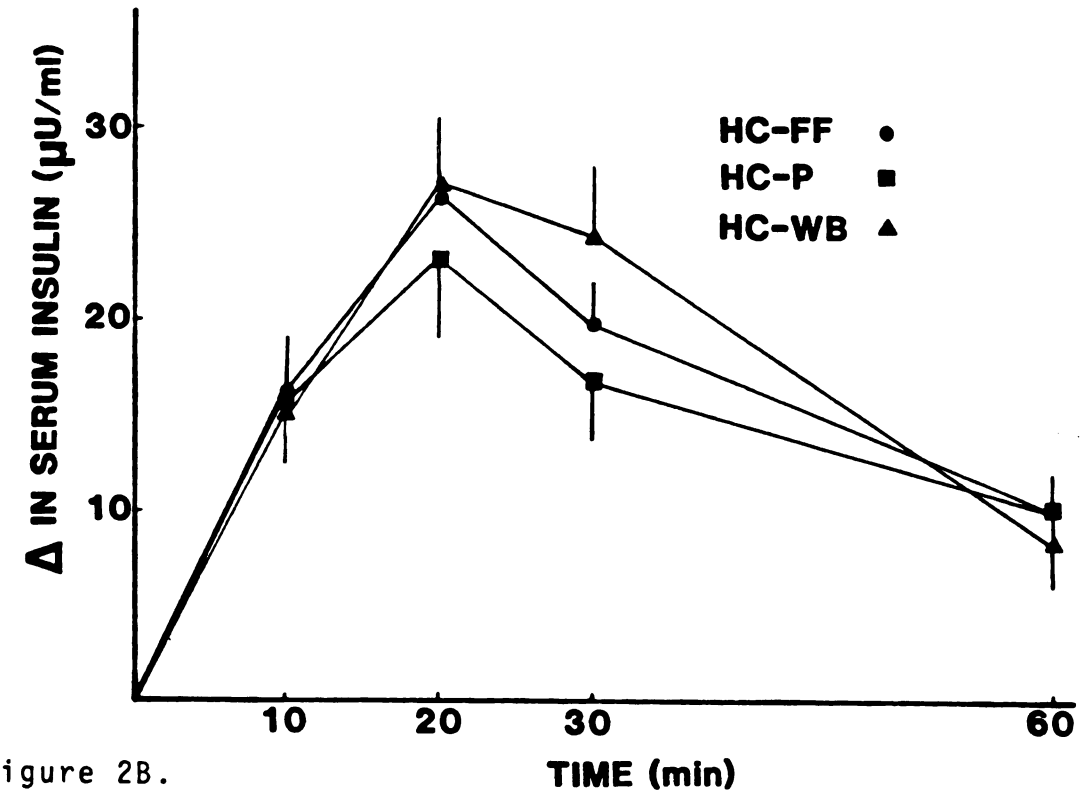

Figure 2B.

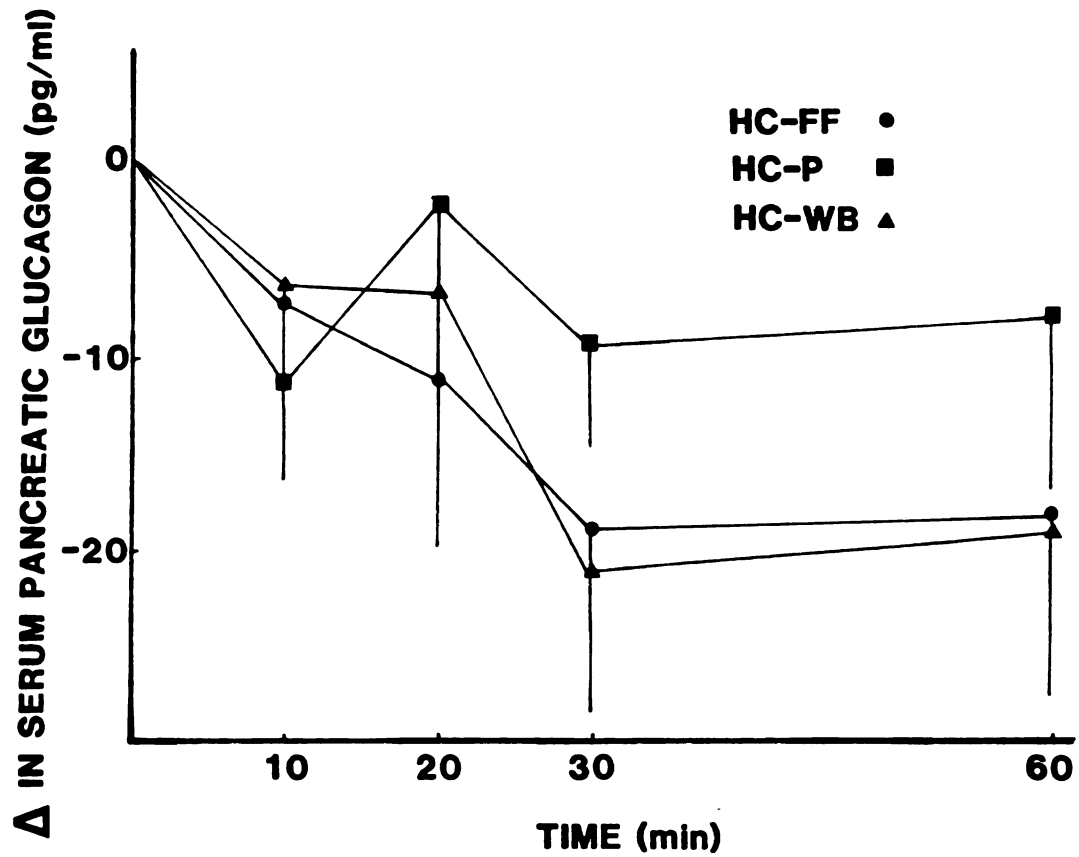
Δ IN SERUM PANCREATIC GLUCAGON (pg/ml)
0
-10
-20
10
20
30
60
TIME (min)
HC-FF
HC-P
HC-WB

Figure 2C.

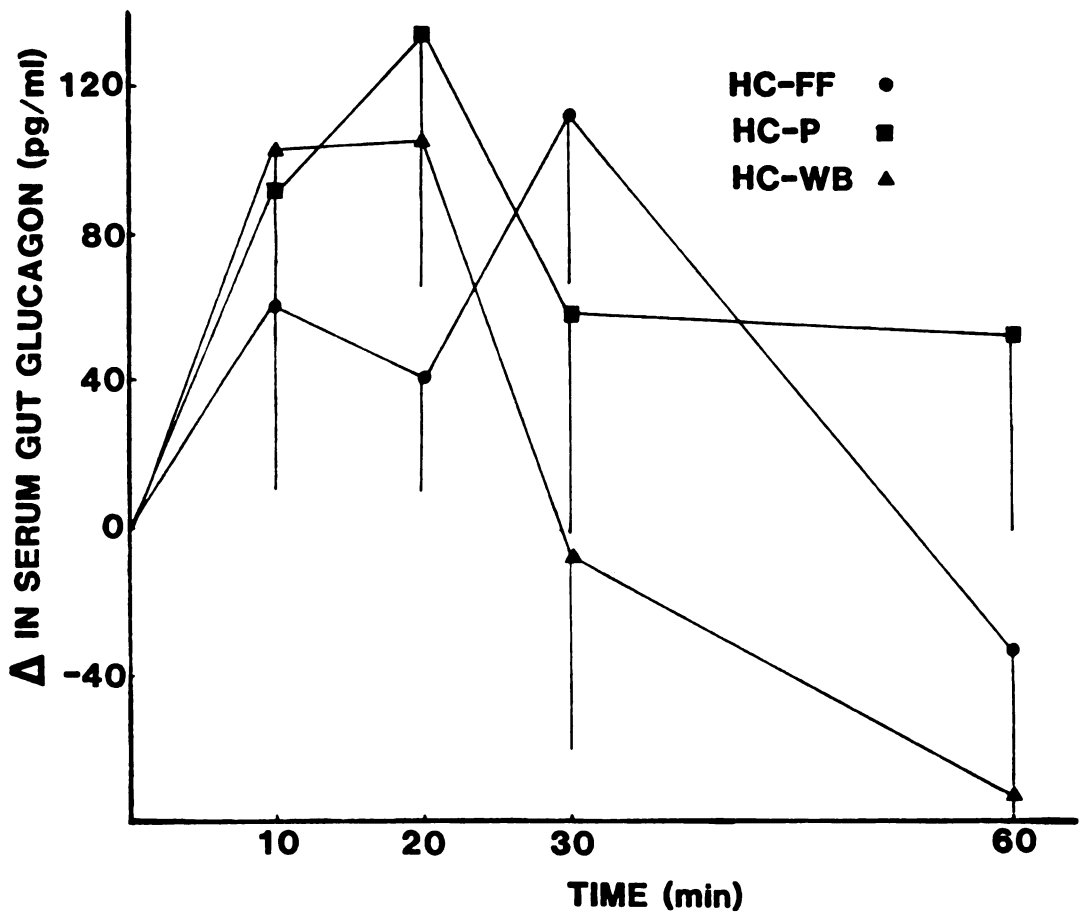
Δ IN SERUM GUT GLUCAGON (pg/ml)
120
80
40
0
-40
10
20
30
60
TIME (min)
HC-FF
HC-P
HC-WB

Figure 2D.

response was similar for HC-P and HC-FF rats (Table 3). However, if these time points are disregarded as in previous OGTTs (Figure 1; Table 1) the total serum glucose response area is lower in HC-P rats (Table 3). Although HC-WB fed rats showed a trend towards a greater increase in the serum glucose response at 20 and 30 minutes following the glucose load, these differences did not reach a level of statistical significance. The peak glucose response occurred at 30 minutes following the load dose in HC-WB fed rats similar to rats fed HC-FF diets (Figure 2). However, at 120 and 180 minutes post-load, serum glucose was significantly decreased in HC-WB fed rats (Appendix Table B2).

Serum insulin (Figure 2B; Table 3; Appendix Table B2), although not different for any diet group, was slightly decreased in HC-P fed rats and increased in HC-WB fed rats when compared to HC-FF fed rats. At 180 minutes, both fiber fed groups had significantly lower serum insulin concentrations (Appendix Table B2). Previous feeding of pectin or wheat bran did not alter the magnitude of the serum glucagon, either pancreatic or gut, response to an oral glucose load. The nadir of pancreatic glucagon occurred at 10 minutes following the glucose load in pectin fed rats compared to 30 minutes in HC-FF or HC-WB fed rats (Figure 1C). The peak enteroglucagon response was at 20 minutes after the glucose load in fiber-fed rats,

Table 3. The incremental areas of the serum glucose and insulin response following an oral glucose load.[1]

Diet Group	Glucose, mg dl^{-1} min Time increment				Insulin, μU ml^{-1} min Time increment			
	0-20	0-30	0-60	0-60[2]	0-20	0-30	0-60	0-60[2]
HC-FF (12)[3]	972 ±72	2054 ±123	4991 ±217	4708 ±212	298 ±41	531 ±63	973 ±78	738 ±47
HC-P (12)	1168 ±117	2219 ±162	4835 ±237	4107[4] ±160	280 ±44	494 ±67	918 ±126	696 ±112
HC-WB (15)	1032 ±87	2196 ±144	5243 ±221	4891 ±168	264 ±30	540 ±47	1078 ±89	925 ±108

[1]Values are the mean±SEM areas for the various incremental time periods.

[2]0-60 incremental period calculated without using values for 10 and 20 minutes post-glucose load.

[3]Number of determinations.

[4]Significantly different ($P \leq 0.05$) than values for rats fed HC-FF.

whereas it was delayed to 30 minutes in HC-FF fed rats (Figure 1D). Because Figure 2 is expressed as the change from fasting concentration, no difference in pancreatic glucagon in HC-WB rats is apparent. However, in addition to a decreased basal concentration of pancreatic glucagon, HC-WB fed rats had lower ($P \leq 0.05$) absolute concentrations of pancreatic glucagon at 10, 20, 30 and 60 minutes following a glucose load (Appendix Table B2).

Gastric Emptying and Gastrointestinal Lengths and Weights

The mean rates of gastric emptying of glucose were accelerated in rats previously fed diets with added fiber (Table 4; Appendix Table B3). However, this effect became statistically significant only in rats previously fed pectin ($P \leq 0.01$).

The dry weights of both the stomach and small intestine from HC-WB fed rats, and to a lesser extent HC-P fed rats, were increased when compared to HC-FF fed rats (Table 5). While the increased ($P \leq 0.05$) length of the small intestine in pectin fed rats may partially explain the increased tissue weight in this group, when expressed on a unit length basis, the small intestines of this group were still significantly ($P \leq 0.05$) heavier than those of HC-FF.

Table 4. Gastric emptying of a glucose solution by rats previously fed fiber-free diets (HC-FF) or diets with added pectin (HC-P) or wheat bran (HC-WB)

Diet Group	Gastric Emptying		
	K, % min[1]	r^2	t1/2[2]
HC-FF (44)[3]	3.20±0.2	0.90	21.7
HC-P (45)	3.95±0.2[4]	0.90	17.6
HC-WB (41)	3.67±0.1	0.94	18.9

[1] Mean±SEM; linear regression of the logarithm of the fraction of the administered glucose remaining vs. time relationship was used to calculate the rate constant (K) of glucose disappearance from the stomach; observations of the fraction of glucose remaining were made at 5, 10, 20, 30, 60, 120 and 180 minutes after a glucose load.

[2] 0.693/K; ("starting index" - time remaining at log 0.5); Hunt and Spurrel, 1951.

[3] Number in parentheses indicate the number of rats tested.

[4] Significantly different ($P \leq 0.01$) than values for rats fed HC-FF diets.

Table 5. Dry weight and length of upper gastrointestinal tract of rats fed fiber-free diets (HC-FF) or diets with added pectin (HC-P) or wheat bran (HC-WB)[1].

Diet Group	Stomach Weight, g/100 g BW	Small Intestine		
		Weight[2], g/100 g BW	Length, cm/100 g BW	Weight/Length, $g/cm \times 10^{-3}$
HC-FF	0.103±0.002	0.143±0.006	30.5±0.4	4.7±0.18
HC-P	0.109±0.002[3]	0.190±0.011[3]	32.7±0.9[3]	5.8±0.30[3]
HC-WB	0.126±0.003[3]	0.267±0.015[3]	31.1±0.5	8.5±0.40[3]

[1]Mean SEM where each mean represents values for twenty-seven rats (stomach weights) or seven rats (intestine weight and length).

[2]Intestine weight does not include mucosa.

[3]Significantly different ($P \leq 0.05$) than the value for rats previously fed HC-FF diets.

Discussion

Results of this study confirm our previous reports and those of others (Munoz et al., 1977; Schwartz and Levine, 1980) demonstrating that antecedent consumption of a diet containing added pectin will decrease the serum glucose response to an oral glucose load. This alteration in the serum response may be indicative of increased clearance or decreased absorption of the administered glucose load. While the primary determinant of glucose disposal is the insulin secretory response (Cherrington et al., 1978; Sherwin and Felig, 1977) it has been suggested that decreased glucagon may be partly responsible for the apparent better utilization and clearance of a glucose load after chronic dietary fiber consumption (Munoz et al., 1977; Miranda and Horowitz, 1978). Increased clearance of a glucose load, therefore, may depend on increased pancreatic hormone responsiveness with increased insulin secretion and decreased glucagon secretion or an altered sensitivity to these hormones at the target tissue level. In the present experiment, the decreased area of the glucose response curve in rats previously fed pectin was coupled with no significant difference in either insulin or glucagon responses when compared to the concentrations in rats fed fiber-free diets. These results would suggest that alterations in the secretion of these hormones was not

responsible for the observed decrease in glucose concentrations.

While this observation does not rule out the possibility of an increase in insulin sensitivity, it is unlikely. The previous determination of the glucose disappearance rate during an intravenous glucose tolerance, thought to correlate with insulin sensitivity (Beck-Nielsen and Pedersen, 1979; Bergman et al., 1979), showed no difference in HC-P fed rats when compared with HC-FF fed rats. Furthermore, to indirectly demonstrate an increase in insulin sensitivity, the observed decrease in glucose concentrations in pectin fed rats would need to be coupled with a close similarity in the absolute levels of insulin for both diet groups. While there were no statistically significant differences in the hormone responses, the concentrations of insulin as well as glucagon were lower in HC-P fed rats. These hormone concentrations appeared to parallel the lowered glucose concentrations. This correspondence of the hormone concentrations to the relative glucose concentrations appears to suggest that the ambient glucose concentration was the primary cause for the hormonal responses as opposed to alterations in the release or activity of hormones mediating an increase in glucose clearance.

An alternate explanation for a decreased area under the serum glucose response curve following sustained pectin

feeding might be a decrease in the rate of absorption of the glucose test load. In acute tests, this is the proposed mechanism. Enhanced glucose tolerance after fiber supplemented oral glucose tolerance tests (acute tests) is thought to be related to delayed glucose absorption mediated by slowed gastric emptying (Holt et al., 1979; Wilmhurst and Crawley, 1980) and/or intestinal absorption (Elsenhans et al., 1980; Blackburn et al., 1984) due to a fiber induced increase in the viscosity of the test substance (Johnson and Gee, 1981; Leeds et al., 1979).

In the present chronic OGTTs without the concomitant presence of fiber, however, two factors suggest the possibility of an increased rate of glucose absorption and an early peak glucose response prior to the customary first sampling at thirty minutes following the test load. First, if delayed absorption had occurred in the pectin fed rats, the shape of the response curve might have varied but the observed decrease in the total three hour incremental area would not necessarily have been expected. Most likely, lower glucose concentrations at early sampling times would be followed by an elevation in serum glucose concentrations at later time points as seen in acute tests (Holt et al., 1979; Jenkins et al., 1978). In addition, in rats previously fed pectin, the increase in gut glucagon concentration was significantly less at thirty minutes

following the glucose load when compared to rats fed fiber-free diets (Figure 1D). Glucagon originating from the gastrointestinal tract is localized in small amounts in the stomach and duodenum with larger amounts in the jejunum and ileum (Matsuyama et al., 1977; Sjolund et al., 1983). Secretion of enteroglucagon is enhanced by the intraluminal presence of carbohydrate or lipids (Holst, 1983). The lowered concentration of enteroglucagon in pectin fed rats at 30 minutes following the oral glucose load and continued flattened response for 180 minutes suggests that the peak stimulus from the ingested glucose may have occurred prior to the thirty minute sampling.

Results from the 0-60 minute response to oral glucose substantiates an earlier rise in serum glucose in pectin fed rats followed by a decrease in serum glucose at later time points (Figure 2). The 0-20 minute incremental glucose response area is increased by 20% in HC-P fed rats. Although this increase is followed by a decrease in glucose concentration at 30 minutes, the total 0-60 minute incremental area for HC-P fed rats is similar to that of HC-FF fed rats. If the incremental serum glucose response area is calculated for the 60 minute period without using the 10 and 20 minute time points, the total area for HC-P fed rats is decreased by 15% when compared with that of HC-FF rats. This apparent decrease in the area of the serum glucose response is consistent with our previous results

and those of Schwartz and Levine (1980) in pectin fed rats.

In rats that had been fed wheat bran, our previous report showed a slight increase ($P \leq 0.10$) in the 0-3 hour incremental area under the serum glucose response curve when compared to that of rats fed fiber-free diets. In the present study, despite a shorter response period (60 minutes), a similar trend for HC-WB fed rats is apparent. Although not statistically significant, the HC-WB fed rats showed an increased peak response and total area when compared with HC-FF rats. Fasting serum glucose was also lower in wheat bran fed rats than in rats fed fiber-free diets. If the absolute serum glucose concentrations are considered instead of the relative elevation from basal levels, the glucose response becomes more similar to that of rats fed HC-FF.

The observed insulin and glucagon concentrations in the 60 minute OGTTs (Figure 2B, 2C) for all rats, irrespective of previous diet, correspond to the glucose concentrations. This parallelism again suggests that the hormone response to an OGTT administered after chronic fiber ingestion is a result of the glucose concentrations.

Other studies in which serum sampling at earlier time points was included in the OGTT agree with the hypothesis of an earlier rise in serum glucose concentrations in fiber fed rats (Cannon et al., 1980; Ebihara and Kiriyama, 1982). To make a more direct comparison of results, data

from the studies of Cannon and coworkers (1980) and Ebihara and Kiriyama (1982) were recalculated as the increase above basal glucose concentrations. Both studies then clearly demonstrate an increased serum glucose at 15 minutes with a shift in the glucose concentration versus time curve to the left in rats previously fed high fiber diets.

Heine and colleagues (1983) demonstrated that results of an OGTT can be influenced by an early rise in serum glucose. These investigators compared fast (1 minute) with slow (10 minute) ingestion of a glucose load dose on the serum glucose response. The fast intake resulted in an earlier (at 10-15 minutes) rise in blood glucose followed by decreased glucose concentrations at later (90-135 minutes) time points. The significant difference at the later time points was attributed to the early increased rise induced by rapid ingestion. These changes in serum glucose following a rapid ingestion of the test load were accompanied by an earlier rise in serum insulin followed by lower concentrations at later time points. The insulin response appeared to correspond with and be dependent on the relative rate of glucose rise.

Changes in the serum concentration of glucose following an oral load are an indirect measure of the rate of glucose absorption. The systemic serum concentration of glucose is the net result of the rate at which glucose enters the circulation by endogenous production or by absorption from

the gut and the rate at which it is eliminated from the circulation by tissue uptake. Gastric emptying rate is a strong determinant of the absorption of orally administered substances (Heading, 1980) and more specifically of oral glucose tolerance (Thompson et al., 1982; Radzuik and Bondy, 1982). Therefore as a more direct assessment of glucose absorption rate following an OGTT, we examined gastric emptying.

Consistent with the early serum glucose response, the half-time of gastric emptying was decreased from 22 to 18 minutes in pectin fed rats. While this may not represent a large physiological difference, it appears sufficient to alter the appearance of glucose in the circulation of rats. In human subjects, a 9 minute time difference in glucose ingestion was related to the altered serum glucose and insulin response to an OGTT (Heine et al., 1983). While the gastric emptying half-time was also decreased in HC-WB fed rats when compared with HC-FF fed rats, the decrease was not as great as that seen in HC-P fed rats. The relative rise in serum glucose in HC-WB rats appeared to correspond to the gastric emptying rate observed in this group.

To correlate the rate of gastric emptying with the rate of glucose absorption, the mean area under the serum glucose concentration time curves at 20, 30, and 60 minutes after an oral glucose load were compared with the percent of

ingested glucose emptied from the stomach. A correlation coefficient of $r=0.99$ ($P \leq 0.001$) implies that the rate of glucose absorption was related to the rate of gastric emptying. The slopes of the individual regression lines: 72±4, 66±11, 78±10, for rats fed HC-FF, HC-P or HC-WB, respectively, were not significantly different. This further implies that gastric emptying is a possible explanation for the observed changes in serum glucose response.

In addition, the rate of gastric emptying of a glucose load has been positively correlated with the rate of increase in serum enteroglucagon concentrations (Ralphs et al., 1975; Lauritsen et al., 1982). The earlier peak rise in serum enteroglucagon in both HC-P and HC-WB fed rats (Figure 2D) is consistent with this observation.

The explanation for the increase in the gastric emptying rate by addition of pectin to the diet is unclear. Differences in the test load volume or composition including acidity and nutrient content which are known to influence gastric emptying rate (Heading, 1980) are unlikely explanations for the observed rate changes. In these experiments, all rats, regardless of previous diet, were administered identical hypertonic glucose loads. Chronic consumption of pectin, but not wheat bran, has been shown to produce morphological alterations in the small intestine (Cassidy et al., 1981; Jacobs, 1983; Jacobs and White, 1983). Duodenal receptors inhibiting gastric emptying in

response to osmotic stimuli may possibly be altered or inactive due to pectin induced structural abberations thus resulting in an increased gastric emptying rate. Analogously, the rapid gastric emptying seen in patients with Zollinger-Ellison syndrome may involve defective duodenal inhibitory receptors due to extensive duodenal and proximal jejunal inflammation (Minami and McCallum, 1984).

An additional explanation for increased gastric emptying rate by chronic pectin ingestion may be adaptive alterations in the motor activity or anatomy of the stomach. The emptying of liquids from the stomach is thought to be primarily a function of the gastro-duodenal pressure gradient (Minami and McCallum, 1984). Post ingestion distention of the stomach signals, either by neural or hormonal means, receptive relaxation and a decreased intragastric pressure. If adaptation to distention is slow, a rise in intragastric pressure with an increased gastric emptying may result. In addition to an increased weight and length of the small intestine observed in this study and those of others (Brown et al., 1979; Forman and Schneeman, 1982; Farness and Schneeman, 1982) addition of pectin or wheat bran to the diet increased the weight of the stomach. This increased weight may represent a larger stomach, a more muscular stomach or a combination of both these factors. Either factor may prevent an equal glucose

test load volume from producing a stomach distention similar to that in HC-FF fed rats. This decreased distention may then cause an elevated pressure resulting in increased gastric emptying. However, if this were the situation, the rats previously fed wheat bran with the greatest increase in stomach weight might be expected to have had the correspondingly fastest gastric emptying rate.

Addition of guar gum, a viscous gel-forming polysaccharide similar to pectin, to meals has been shown to increase gastric motility (Bueno et al., 1981). When compared to the effects of added wheat bran or cellulose, guar gum produced a specific increase in the frequency of postprandial antral contractions. This increased motility was attributed to the greater water holding ability and consequent bulk of the guar fiber source. Whether guar or pectin could result in a sustained alteration or is only apparent when the bulk of the added fiber is present is not known.

In this experiment and our previous report, rats fed wheat bran had a decreased fasting glycemia. Low fasting serum glucose similarly has been found in other studies after prolonged periods of supplemental bran (Bosello et al., 1980; Ray et al., 1981) or high fiber diets derived from unprocessed natural foods (Barnard et al., 1981; Simpson et al., 1981). Because of the comparable fasting insulin concentrations for rats fed either HC-WB

or HC-FF diets in the present study, it is suggested that the lowered basal glycemia may be related to the observed concomitant decrease in pancreatic glucagon. The glucagon response to a glucose load was similar to that of HC-FF fed rats but the fasting glucagon concentrations appeared to be selectively decreased. Although not previously observed in normal subjects, in either diabetic patients (Miranda and Horowitz, 1978) or streptozotocin diabetic rats (Yamashita et al., 1980) maintained on high fiber diets, a decreased glycemia was associated with a decreased concentration of pancreatic glucagon. Glucagon will cause an increase in the serum concentration of glucose (Foa, 1977) and two-thirds of basal hepatic glucose production is glucagon mediated (Cherrington et al., 1981). Low concentrations of glucagon have been found in fasting, reactive and neonatal hypoglycemia (Foa et al., 1975; Foa et al., 1970) and conversely, glucagon secretion is involved in the maintenance and severity of fasting hyperglycemia in human diabetes (Gerich, 1977).

The mechanism(s) by which fiber selectively decreases glucagon in wheat bran fed rats is unclear. The need for endogenous glucose production appears to control the fasting levels of insulin and glucagon. In starvation or after a prolonged period of a low carbohydrate diet, glucagon is elevated and insulin is decreased. When the supply of endogenous glucose is greater, after a glucose infusion or

a high carbohydrate diet, glucagon is decreased and insulin is increased (Muller et al., 1971; Unger, 1972). The present situation with a decreased glucose concentration coupled with decreased glucagon would suggest the possibility of a decreased need for endogenous glucose in wheat bran fed rats. A source of metabolic fuel derived from volatile fatty acids produced by the fermentation of the fiber in the cecum could decrease basal glucose need. These short chain fatty acids are available as an energy source both for the liver and peripheral tissue (Marty and Vernay, 1984; Snoswell et al., 1982; Buckley and Williamson, 1977). Perhaps more importantly, these volatile fatty acids may provide an energy source obtainable as late as 23 hours postprandially (Illman et al., 1982; Storer et al., 1983; Demigne and Remesy, 1982). This delayed supply of metabolites, estimated to contribute 4.7% of the energy needs of rats (Yang et al., 1970), may then spare glucose and lead to a decreased glucagon mediated glucose production. Pectin is a more completely fermented substrate than wheat bran (Cummings, 1981); thus it might be expected that the fasting blood glucose concentrations might likewise be decreased in pectin fed rats if volatile fatty acid production is a reasonable explanation. However in this experiment, the diets contained 7% pectin compared to approximately 30% wheat bran providing 12% dietary fiber. The total amount of fiber together with the type

of fiber will determine the magnitude of volatile fatty acid production. While it seems possible that a high fiber diet may generate volatile fatty acids to provide a late post-absorptive substitute energy source for endogenously produced glucose, this is only speculative and requires further documentation.

In summary, the present study indicates that chronic pectin feeding results in an increased gastric emptying rate as opposed to alterations in the secretion of insulin or glucagon, including that of both pancreatic and gut origin, in response to an OGTT. Since the traditional OGTT misses the early higher serum glucose response, increased gastric transit rate may explain the apparent improvement in chronic glucose tolerance tests following pectin-rich diets. In addition, long term dietary supplementation with wheat bran lowers fasting glycemia and this appears to be related to a decrease in the basal concentration of pancreatic glucagon.

SUMMARY AND CONCLUSIONS

Glucose metabolism was evaluated in male Sprague-Dawley rats after consuming fiber-free high or low carbohydrate diets or these same diets with added pectin, methylcellulose or wheat bran. Following a four week feeding period, rats given an OGTT with either pectin, methylcellulose or wheat bran together with the glucose load (acute OGTT) showed a decrease in serum glucose response at 30 to 60 minutes and an elevation at 120 to 180 minutes when compared to fiber-free glucose test loads. This flattened serum glucose response occurred regardless of previous diet or fiber source added to the test load. In addition to an acute effect of fiber, it was established that previous long term ingestion of dietary fiber can also alter the serum glucose response to an OGTT (chronic OGTT). The total areas under the serum glucose response curves for both acute and chronic tests for any diet group were similar; however, the shape of the response curves varied. These results suggest the chronic, adaptive effect of dietary fiber that is not dependent on the simultaneous presence of dietary fiber in the test load is distinct from the acute effect of fiber on OGTT.

The effects of chronic fiber ingestion appear to be dependent on the type of dietary fiber added to the high and low carbohydrate fiber-free diets. When compared to the control fiber-free diets, dietary pectin lowered the total area under the serum glucose response curve and wheat bran slightly increased the total area under the serum glucose response curve. The effects of chronic fiber feeding in most cases were independent of the level of carbohydrate in the diet within the range of 40-70% of the total energy. However, addition of methylcellulose to high carbohydrate diets but not low carbohydrate diets decreased the area under the serum glucose response curve.

Antecedent consumption of high carbohydrate diets with added pectin or wheat bran did not affect the rate of glucose disappearance or insulin and pancreatic glucagon concentrations following an IVGTT when compared to responses in rats fed fiber-free diets. These results suggest that the chronic effect of dietary fiber observed following an OGTT is gut mediated and may not be related to systemic alterations in the secretion or sensitivity of the counterregulatory hormones of carbohydrate metabolism, insulin and pancreatic glucagon.

The observed chronic effect of dietary pectin on oral glucose tolerance was reproduced in a second experiment and was not associated with changes in the

serum concentrations of insulin or pancreatic glucagon. Serum enteroglucagon, thought to be a likely gut mediator capable of stimulating insulin secretion, was not increased by pectin feeding. These results suggest that an increase in the tissue uptake or utilization of the glucose load was an unlikely mechanism to explain the decreased area under the serum glucose response curve in pectin fed rats.

Delayed absorption as a possible alternate mechanism to explain the decreased serum glucose response following an OGTT in pectin fed rats was investigated. Because the absorption rate of orally administered substances is mainly dependent on gastric emptying rate, this parameter was measured following a glucose load. Chronic consumption of pectin appeared to decrease the half-time required to empty a glucose test load. This observation was supported by including serum sampling at 10 and 20 minutes in addition to the usual 30 and 60 minutes following the ingestion of a glucose test load. Pectin fed rats showed a peak serum glucose response at 20 minutes instead of at 30 minutes as seen in rats fed fiber-free diets. Serum enteroglucagon concentration, which has been associated with gastric emptying rate, also peaked at 20 minutes in rats previously fed pectin. When the total integrated areas for the glucose response curves were calculated with the early sampling times included, the total areas for

pectin and fiber-free fed rats were similar. It is suggested, therefore, that the decreased serum glucose response following an OGTT in rats fed pectin may in part be due to an increased gastric emptying rate of the oral glucose load.

Rats previously fed diets with added wheat bran had a sustained decrease in fasting serum glucose concentration. The lower fasting glucemia resulted in an increase in the relative response above basal concentrations. However, the absolute glucose concentrations in response to an OGTT are similar to those of fiber-free fed rats.

This lower fasting glycemia in wheat bran fed rats was coupled with a lower fasting concentration of pancreatic glucagon. Since pancreatic glucagon is involved in endogenous glucose production, it is suggested that the lowered fasting glucose may be due to the fasting pancreatic glucagon concentrations. It is conceivable that a fiber induced production of volatile fatty acids may mediate a decrease in glucagon. This possibility warrants further investigation.

APPENDICES

Appendix Table A1. Serum glucose concentration following an oral glucose load in rats previously fed high carbohydrate (HC) or low carbohydrate (LC) diets with or without added pectin (P), methylcellulose (MC) or wheat bran (WB)[1,2]

Diet group	Time, minutes[3]				
	0	30	60	120	180
	Glucose, mg/dl[2]				
HC-FF	104±4	202±9	210±10	134±5	118±3
HC-P	100±5	192±3	181±8[4]	115±3[4]	107±3[4]
HC-MC	106±5	192±5	174±3[4]	124±3	117±3
HC-WB	89±3[4]	218±8	183±6[4]	128±4	118±5
LC-FF	103±4	202±6	201±3	128±5	111±4
LC-P	118±6	215±11	206±9	130±7	118±5
LC-MC	104±7	213±8	205±12	138±8	115±4
LC-WB	87±4[4]	187±10	205±5	131±3	112±4
Chow	80±2[4]	221±6	193±6	113±6[4]	108±5[4]

[1] Mean±SEM.

[2] 1.5 g/kg body weight glucose test load.

[3] In relation to administration of test load.

[4] Significantly different ($P \leq 0.05$) from rats fed fiber-free diets within a carbohydrate level.

Appendix Table A2. Serum glucose concentrations following an oral load dose of glucose plus fiber in rats fed high or low carbohydrate diets with added pectin, methylcellulose or wheat bran[1,2].

Test load fiber source	Diet group	Time, min				
		0	30	60	120	180
Pectin, 4% (w/v)	HC-FF	100±7	180±5	190±3	141±6	124±5
	LC-FF	104±6	181±5	183±8	149±9	124±3
	HC-P	96±5	177±5	176±5	122±5	114±3
	LC-P	104±5	182±5	191±2	142±6	128±6
Methylcellulose 4% (w/v)	HC-FF	94±2	161±4	171±3	149±5	135±2
	LC-FF	96±4	161±7	156±8	141±3	135±3
	HC-MC	100±3	152±3	146±6	136±3	130±2
	LC-MC	87±5	165±8	168±8	137±4	121±3
Wheat bran, 1.1±0.1 g	HC-FF	86±4	166±3	166±4	133±6	117±5
	LC-FF	93±3	186±6	157±10	146±5	129±3
	HC-WB	87±4	185±3	159±5	129±2	119±3
	LC-WB	85±7	185±3	177±7	137±3	124±6

[1] Mean±SEM.

[2] 1.5 g/kg body weight glucose test load to which various fibers were added.

Appendix Table A3. Serum glucose, insulin and pancreatic glucagon following an intravenous glucose load in rats previously fed HC-FF, HC-P or HC-WB diets for a four week period.

Diet group	Time, min.	Serum concentration[1]		
		Glucose, mg/dl	Insulin, μU/ml	Glucagon pg/ml
HC-FF	0	108±8	21±4	114±3
	5	340±10	37±5	111±5
	15	265±9	34±3	100±9
	30	240±10	44±3	110±7
	60	218±10	32±3	174±10
HC-P	0	109±4	23±2	123±13
	5	347±12	39±6	112±9
	15	268±8	37±5	89±9
	30	231±14	55±6	111±4
	60	181±12	34±4	153±21
HC-WB	0	109±5	25±4	103±9
	5	318±11	44±6	99±10
	15	236±6[2]	35±4	81±9
	30	214±6	42±4	109±3
	60	212±10	28±4	142±6

[1]Mean±SEM where the mean represents values for seven rats in each diet group.

[2]Significantly lower ($P \leq 0.05$) than values for HC-FF.

Appendix Table B1. Serum glucose, insulin, pancreatic and gut glucagon concentrations following an oral glucose load in rats previously fed HC-FF or HC-P diets for four weeks, Experiment 1.

Diet Group	Time, min	Serum Concentration[1]			
		Glucose, mg/dl	Insulin, μU/ml	Pancreatic Glucagon, pg/ml	Gut Glucagon, pg/ml
HC-FF	0	111±4	16±1	116±7	596±42
	30	195±6	31±2	99±5	763±69
	60	186±6	28±2	90±8	646±39
	120	139±6	23±2	102±10	536±55
	180	134±5	23±2	107±12	649±23
HC-P	0	110±3	16±1	111±4	577±55
	30	177±6[2]	29±2	103±3	608±39
	60	173±3	26±2	89±9	639±41
	120	125±6	19±1	89±12	633±58
	180	119±4[2]	21±1	111±7	655±15

[1] Mean±SEM where the mean represents seven pooled samples; each sample represents three rats.

[2] Significantly lower ($P \leq 0.05$) than values for HC-FF fed rats.

Appendix Table B2. Serum glucose, insulin, pancreatic and gut glucagon concentrations following an oral glucose load in rats previously fed HC-FF, HC-P or HC-WB diets for a four week period, Experiment 2.

Diet Group	Time, min	Serum Concentration[1]			
		Glucose, mg/dl	Insulin, μU/ml	Pancreatic Glucagon, pg/ml	Gut Glucagon pg/ml
HC-FF	0	97±3(12)	14±2(12)	119±6(6)	662±21(6)
	10	146±4	29±3	112±7	723±31
	20	195±5	41±5	108±8	720±24
	30	213±5	34±2	100±8	775±45
	60	179±8	25±2	101±7	634±48
	120	131±3	27±1	-	-
	180	117±2	29±3	-	-
HC-P	0	93±2(12)	14±2(12)	116±6(6)	559±49(6)
	10	155±7	30±3	104±10	659±24
	20	203±6	37±3	114±7	693±27
	30	194±4[2]	30±2	106±7	617±70
	60	169±4	23±2	108±8	611±53
	120	121±2	23±2	-	-
	180	105±1[2]	21±1	-	-
HC-WB	0	87±3[2](15)	16±2(15)	90±5[2](7)	538±56(7)
	10	136±5	32±4	84±11[2]	642±67
	20	198±8	44±2	84±11[2]	643±41
	30	211±5	40±4	69±7[2]	528±63
	60	168±5	24±1	71±9[2]	464±53[2]
	120	116±5[2]	22±1	83±11	505±45
	180	101±3[2]	20±2	83±14	477±51

[1]Mean±SEM, the mean represents the number of samples indicated in parentheses at the 0 time point.

[2]Significantly lower ($P \leq 0.05$) than values for the same parameter measured in HC-FF fed rats.

Appendix Table B3. Percentage of original dose of glucose remaining in the stomach at serial time points after a glucose load in rats previously fed HC-FF, HC-P or HC-WB diets.

Diet Group	Time Post Glucose Load, minutes						
	5	10	20	30	60	120	180
	% of original glucose dose						
HC-FF	92.7 ±2.8(5)	81.1 ±2.7(6)	69.1 ±4.0(7)	50.8 ±3.0(11)	12.3 ±3.2(7)	2.7 ±1.1(5)	0.8 ±0.3(4)
HC-P	93.5 ±2.2(3)	77.2 ±1.8(8)	65.5 ±3.6(6)	40.9[2] ±4.3(9)	9.4 ±1.5(6)	0.7 ±0.4(6)	0.3[2] ±0.1(7)
HC-WB	89.4 ±0.4(3)	78.5 ±2.6(7)	63.8 ±1.6(8)	42.3 ±1.9(8)	9.2 ±3.6(5)	3.0 ±0.9(5)	0.2[2] ±0.1(5)

[1]Values are mean±SEM; number of observations is indicated in parentheses.

[2]Values are significantly lower ($P \leq 0.05$) than values for rats previously fed HC-FF.

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