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University of Alberta

The effect of fermentable dietary fiber on glucose tolerance and immune function in dogs

by

Stefan Massimino 

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements for the degree of Masters of Science

in

Nutrition and Metabolism

Department of Agricultural, Food and Nutritional Science

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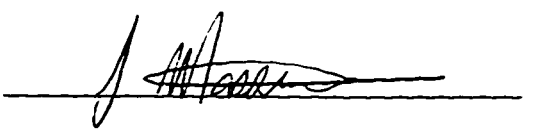

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled *The effect of fermentable dietary fiber on glucose tolerance and immune function in dogs* by *Stefan Patrick Massimino* in partial fulfillment of the requirements for the degree of *Masters of Science* in *Nutrition and Metabolism*.

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Date of Committee Approval: OCT 2/97

Abstract

The purpose of this study was to determine if glucose tolerance or immune function were affected by longterm (14 d) fiber ingestion. Dogs (n=16) received isonitrogenous isoenergetic diets differing in fermentability of dietary fiber using a randomized cross-over design. Proglucagon mRNA, intestinal GLP-1(7-36)NH_2 and incremental area under the curve (AUC) for plasma GLP-1(7-36)NH_2 and insulin were increased ($p < 0.05$) while the incremental AUC for plasma glucose decreased ($p < 0.05$) in dogs fed high fermentable fiber after an oral glucose tolerance test. Jejunal villi height, D-glucose Vmax and GLUT2 and SGLT1 transporter abundance were increased in dogs fed high fermentable fiber ($p < 0.05$). Immune function of isolated lymphocytes were not affected by diet nor were immune phenotypes except for CD4:CD8 ratio and % B-lymphocytes in the periphery ($p < 0.05$). In conclusion, fiber fermentability is important in glucose homeostasis and can affect lymphocyte population distributions in the periphery.

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List of Abbreviations

ADF = acid detergent fiber

ANOVA = analysis of variance

AOAC = Association of Official Analytical Chemists

AUC = area under the curve

BBM = brush border membrane

BLM = basolateral membrane

BW = body weight

cAMP = cyclic adenosine monophosphate

DM = diabetes mellitus

DNA = deoxyribonucleic acid

DPIV = dipeptidyl peptidase IV

FITC = fluorescein isothiocyanate

FOS = fructooligosaccharide

GALT = gut associated lymphoid tissue

GIP = glucose dependent insulinotropic polypeptide

GLP-1 = glucagon-like peptide-1

GLP-2 = glucagon-like peptide-2

GLUT2 = sodium independent D-glucose transporter

GLUT5 = sodium independent fructose transporter

HFF = high fermentable fiber

IFN-γ = interferon gamma

Ig = immunoglobulin

IP-2 = intervening peptide-2

Kd = dissociation constant

Km = Michaelis constant

LFF = low fermentable fiber

mAb = monoclonal antibody

MHC = major histocompatability complex

MPF = major proglucagon fragment

mRNA = messenger ribonucleic acid

NDF = neutral detergent fiber

NIDDM = non-insulin dependent diabetes mellitus

NK cell = natural killer cell

NSP = non-starch polysaccharide

OGTT = oral glucose tolerance test

OM = organic matter

PC = prohormone convertase

PE = phycoerythrin

Pre-Exp = pre-experimental

RIA = radioimmunoassay

SAS = statistical analysis system

SCFA = short chain fatty acid

SEM = standard error of the mean

SGLT-1 = sodium dependent D-glucose cotransporter

TCR = T-cell receptor

TNF-α = tumor necrosis factor alpha

TPN = total parenteral nutrition

Vmax = maximal transport rate

I. Literature Review

1.NIDDM

Diabetes mellitus (DM) is a chronic metabolic disease characterized by hyperglycemia and disturbances in carbohydrate, fat and protein metabolism. DM is associated with absolute or relative deficiencies in the secretion and/or action of the hormone insulin (WHO, 1994; DeFronzo et al., 1992). Epidemiological studies have shown that DM affects almost all populations and all age groups. Approximately 60 million people worldwide have diabetes (Global Health Situation, 1993) and that number is constantly rising (King and Rewers, 1991). Non-insulin dependent diabetes mellitus (NIDDM) is the most common form of diabetes comprising approximately 85% of people with the disease. The world health organization (WHO) has estimated that the number of individuals worldwide with NIDDM may exceed 100 million by the year 2000 (WHO, 1994). Defective insulin response to glucose and decreased insulin sensitivity are the major metabolic impairments related to NIDDM (Efendic et al., 1986; Zimmet, 1992). It is well established that dietary fiber is effective in the dietary treatment of NIDDM. Studies have demonstrated that consumption of a diet rich in dietary fiber results in elevated plasma insulin concentrations and improved glucose tolerance during oral glucose tolerance tests in NIDDM subjects (Miranda et al., 1978; O'Dea et al., 1989; Pastors et al., 1991), lower serum cholesterol and blood pressure (Anderson and Chen 1979; Anderson 1983), whereas consumption of dietary fiber with a meal results in decreased postprandial hyperglycemia and lower insulin requirements (Anderson et al., 1995; Jenkins et al., 1980a; Jenkins et al., 1980b; Wolever et al., 1979).

2.Dietary Fiber

2.1. Structural and Physical Properties

The definition of dietary fiber is problematic and measurements are dependent upon methodology used. The broadest definition of dietary fiber is that component of the diet which is resistant to hydrolysis by mammalian digestive enzymes and is anaerobically fermented to some extent by the microflora in the large intestine. Different dietary fibers have differing physiochemical properties with regards to solubility, cation-exchange capacity and fermentative properties (McBurney 1991; McBurney and Sauer, 1993; Bourquin et al., 1993).

2.2. Dietary Fiber & Health

The medical and public sector began to increase their attention towards dietary fiber in the 1970s when possible links between fiber intake and certain diseases were made, including coronary heart disease (Morris et al., 1977), hypertension (Sacks et al., 1975), several western-diet associated gastrointestinal disorders (Burkitt and Trowell 1975) and cancer (Report from the international agency for research on cancer intestinal microecology group, 1977). Since the suggestion by Trowell (1975) that diabetes may be a fiber-deficiency disorder, fiber has become an important component of diabetic diets. Traditionally, the beneficial effects of dietary fiber were attributed to its mechanical effects in the gut which essentially result in less energy consumption and/or slower absorption (Leeds, 1987; Wolever et al., 1991; Jenkins et al., 1980c). Although these effects are beneficial, recent studies have also demonstrated improvements in glucose control with long term ingestion of soluble, fermentable dietary fiber (Pastors et al., 1991; Reimer 1997). Fiber supplements taken at a meal have been shown to produce beneficial effects on glucose tolerance to subsequent meals (Trinick et al, 1986; Jenkins et al., 1980a) and long-term consumption of fiber results in improved glucose tolerance (Aro et al., 1981; Hagander et al., 1984; Lovejoy and DiGirolama 1992; Groop et al., 1993). In healthy humans, NIDDM and in adipocytes and monocytes isolated from individuals with

NIDDM, the ingestion of high fiber diets is associated with significant improvements in peripheral insulin sensitivity (Fukagawa et al., 1990; Hallfrisch et al., 1995; Anderson et al., 1986; Lovejoy and DiGirolamo, 1992; Pederson 1982; Hjollund 1983; Ward 1982). Long term ingestion of fiber supplemented diets has been reported to significantly flatten the glucose curve and decrease fasting plasma insulin and glucose concentrations (Fukagawa et al., 1990; Hallfrisch et al.,1995; Pastors et al., 1991). All these effects cannot be attributed simply to the presence and/or physical properties of dietary fiber, thus suggesting an alternate mechanism(s). Reimer and McBurney (1996) reported that supplemental fiber (30%) significantly increased ileal, cecal and total colon weight and total colon length. As well, Tappenden et al (1996) reported that SCFA increased mucosal, submucosal and total intestinal weight, including ileal protein, DNA and RNA concentrations. Many studies have established a correlation between cell proliferation and significantly greater plasma levels of proglucagon-derived peptides (Sagor et al., 1982; Dowling 1982; Bloom and Polak 1982; Sagor et al., 1983). As well, plasma enteroglucagon levels are significantly elevated with consumption of fermentable dietary fiber (Southon et al., 1987; Gee et al., 1996), suggesting a role for short chain fatty acids (SCFA).

2.3. Measurement of Dietary Fiber

It is difficult to measure fiber content of diets and values remain controversial due to the various methods available. The proximate (Weende) system for fiber analysis is the oldest system (Henneberg and Stohmann, 1859) and is a gravametric method where the sample is sequentially refluxed in dilute base and acid. This method measures primarily cellulose (with some noncellulosic components) and this crude fiber underestimates fiber content of foods. Neutral detergent fiber (NDF), developed by Van Soest (1963), uses a combination of chemical extraction (a neutral detergent solution under reflux) and gravametric determination. NDF also underestimates total fiber content because pectin is solubilized (Van Soest 1994). Acid detergent fiber (ADF) also developed by Van Soest (1963) measures cellulose, variable amounts of xylans and other components, however it

solubilizes a significant portion of the lignin. A common practice is to use the NDF as a pretreatment followed by the ADF (Van Soest and Robertson, 1980). A major method for measuring fiber is the Prosky dietary fiber (DF) method (Prosky et al., 1984). This method uses a series of enzymatic and chemical treatments and precipitation in ethanol for fiber isolation. This method is considered to retain all fiber components. The only problem associated with this method is starch and protein removal can be difficult causing overestimation of fiber concentration. Another very important measure of fiber is as non-starch polysaccharides (NSP). The basis of NSP stems from Trowell's definition of fiber as plant polysaccharides and lignin which are resistant to hydrolysis by the digestive enzymes of man (Trowell et al., 1976). Since the only polysaccharide known to be hydrolyzed by digestive enzymes is starch, we can define these undigestable starches as non-starch polysaccharides. NSP can be separated further into cellulose and non-cellulosic polysaccharides (NCP). NSP analysis consists of three main steps; removal of starch and free sugars, hydrolysis of NSP and measurement of the constituent sugars released from NSP (Englyst and Cummings 1986).

3.Short Chain Fatty Acids (SCFA)

When fermentable dietary fiber reaches the colon, it can be fermented by the endogenous anaerobic bacteria which produce short chain fatty acids (SCFA) as end-products (McBurney and Thompson, 1989, McBurney and Sauer, 1993). Although differences do exist between fiber sources in the rate (McBurney et al., 1988) and extent of fermentation (McBurney and Thompson 1987, McBurney and Thompson 1990, McBurney and Thompson 1991; Bourquin et al., 1993), SCFA production generally increases with increasing dietary fiber intake (McBurney and Sauer, 1993). SCFA are absorbed, metabolized, and account for approximately 3-13% metabolizable energy (McBurney et al., 1988). The SCFA found in the highest proportion are acetate, propionate and butyrate (Cummings and Branch, 1986).

3.1. SCFA and Glucose Tolerance

SCFA have been correlated with modulating glucose homeostasis. In ruminants, propionate and butyrate were reported to be insulin secretagogues, independent of glucose concentration (de Jong, 1982), and SCFA infusion have been reported to significantly increase plasma insulin concentrations in sheep after an overnight fast (Husveth et al., 1996). SCFA have been reported to significantly lower hepatic glucose output (Thorburn et al., 1993), fasting plasma glucose (Boillot et al., 1995; Berggren et al., 1996) and urinary glucose excretion (Berggren et al., 1996). Although the mechanism(s) by which SCFA elicit their effects on glucose tolerance are not yet fully elucidated, McBurney et al (1995) reported that splancnic SCFA infusion is not directly responsible.

3.2. SCFA and Proliferative Effects

The ingestion of fermentable dietary fiber or SCFA supplementation of parenteral diets results in a significant increase in distal small and large intestinal mass (Sakata and von Englehardt, 1983; Jacobs and Lupton, 1984; Goodlad et al., 1987a; Goodlad et al., 1987b; Koruda et al., 1988, Marsman and McBurney, 1995; Reimer and McBurney, 1996; Tappenden et al, 1997b). SCFA are also associated with hypertrophy of the small intestine as SCFA supplemented total parenteral nutrition (TPN) significantly increased total, mucosal and submucosal mass, ileal DNA and RNA and proglucagon mRNA after both 3 and 7 d after massive small bowel resection (Tappenden et al., 1996).

3.3. SCFA and Immunity

There is little information available on the effects of SCFA and immunity. The work that has been done has produced interesting results suggesting a positive effect on immune function. Recently, SCFA have been implicated in modulation of the immune system as SCFA supplemented TPN significantly increased the relative percent of T-cells, decreased the relative percent of macrophages, and increased NK cell cytotoxicity from rats 3 d after massive small bowel resection (Pratt et al., 1996). SCFA lower pH in the

gut, which can affect bile acid metabolism (Jacob 1988), and bile acids are known to affect immunoglobulin production (Lim et al., 1994).

4. Incretins

Many of the beneficial effects involving dietary fiber could be mediated by gastrointestinal hormones or incretins. The term "incretin" was coined by Zunz and La Barre (1929) for a humoral factor released from the gastrointestinal tract that releases insulin or potentiates the glucose-induced insulin release. Because of incretins, oral glucose augments insulin secretion to a greater extent than intravenous glucose infusion in healthy individuals (Perley and Kipnis, 1967; Nauck et al., 1986). It is estimated that >50% of post- prandial insulin secretion is triggered by intestinal peptide hormones, or incretins (McIntyre et al., 1964; Hampton et al., 1986).

4.1. Glucose-Dependent Insulinotropic Polypeptide (GIP)

Glucose-dependent insulinotropic polypeptide (GIP) is a 42 amino acid peptide hormone produced by the K cells of the proximal intestine. GIP is released into the circulation in response to the ingestion of glucose, fat and some amino acids (Cataland et al., 1974; Falko et al., 1975; Pederson et al., 1975; Thomas et al., 1978). Kieffer et al (1994) demonstrated a dose dependent GIP release by glucose from isolated canine intestinal duodenal and jejunal epithelial cell preparations enriched for GIP endocrine cells maintained in short term culture indicating glucose acts directly on the GIP cell. GIP is recognized as being an established incretin able to induce the glucose-mediated release of insulin via the enteroinsular axis (Brown et al., 1989; Dupre et al., 1973). In patients with NIDDM, the overall incretin effect is reduced due primarily to GIP which loses much of its insulinotropic activity (Nauck et al., 1993b; Elahi et al., 1994).

4.2. Glucagon-Like Peptide-1(GLP-1)

4.2.1. Proglucagon. Proglucagon is a 180 amino acid precursor containing the glucagon sequence and two glucagon-like polypeptides arranged in tandem. Proglucagon is produced in the pancreatic alpha-cells and the intestinal L-cells which are found in an increasing gradient from the proximal intestine to distal colon (Holst 1994). In both the intestine and pancreas, proglucagon mRNA is identical and diversification of proglucagon gene expression occurs during post-translational processing (Mojsov et al., 1986; Orskov et al., 1987). In the pancreas, the main products are glucagon, glicentin-related pancreatic peptide and the major proglucagon fragment. The main products in the small intestine are glicentin, glucagon-like peptide-1 (GLP-1) and glucagon-like peptide-2 (GLP-2), (see **Figure 1**). Proglucagon is processed in the large intestine in the same manner as the small intestine and results in the formation of fully processed biologically active GLP-1 (Deacon et al., 1995b). Northern blot analyses of mRNA transcripts for the prohormone convertases (PC), PC1 and PC2, in cell lines demonstrated correlations between PC2 and the presence of glucagon and PC1 and the production of intestinal GLP-1 and GLP-2 providing support that prohormone convertases play roles in tissue specific post-translational processing of proglucagon (Tucker et al., 1996).

4.2.2. Glucagon-Like Peptide-1. Glucagon-like peptide-1 (GLP-1) is a 30 amino acid peptide with a 50% sequence homology to glucagon (see **Figure 2**). GLP-1 is physiologically present in 2 forms, amidated GLP-1 (GLP-1(7-36)NH_2) which corresponds to 80% of the GLP-1 immunoreactivity (Orskov et al., 1994) and glycine extended GLP-1 (GLP-1(7-37)) which corresponds to 20% (Orskov et al., 1994). Amidation at the arginine residue of GLP-1(7-37) occurs during post-translational processing to form GLP-1(7-36)NH_2 (Kreymann et al., 1988). GLP-1(7-36)NH_2, corresponding to proglucagon 78-107NH_2 (Orskov et al., 1989) is the gut derived insulinotropic hormone. GLP-1(7-36)NH_2 and GLP-1(7-37) equipotently stimulate integrated insulin and c-peptide response and lower the plasma glucose and glucagon response (Orskov et al., 1993) thereby indicating both as insulinotropic peptides (Mojsov

et al., 1990; Weir et al., 1989). GLP-1 also stimulates proinsulin gene expression and proinsulin biosynthesis (Fehmann and Habener, 1992) thereby not causing a depletion of insulin stores. In non-diabetic subjects and in the presence of elevated glucose concentrations, GLP-1 stimulates insulin secretion, inhibits glucagon secretion and acts as a physiological inhibitor of gastric acid secretion and emptying resulting in significant decreases in fasting and postprandial plasma glucose concentrations (Van Dijk et al., 1996; D'Alessio et al., 1994; Hvidberg et al., 1994; Wettergren et al., 1993; Willms et al., 1996; O'Halloran et al., 1990; Komatsu et al., 1989). GLP-1 may also have a separate peripheral insulin-independent glucose clearing effect (Van Dijk et al., 1996; D'Alessio et al., 1995). Although GLP-1 is a potent and glucose-dependent insulin secretagogue (Hendrick et al., 1993) when plasma glucose concentrations are in the normal fasting range, GLP-1 is unable to stimulate insulin secretion to a degree that causes hypoglycemia (Qualmann et al., 1995). With NIDDM, the overall incretin effect is reduced. However, GLP-1 retains much of its insulinotropic activity (Nauck et al., 1993b; Elahi et al., 1994).

4.2.3. GLP-1 Receptor. Consistent with the role of GLP-1 on insulin secretion, GLP-1 receptor (GLP-1R) mRNA is highly expressed on both human and rat pancreatic islets (Thorens 1992; Dillon et al., 1993; Thorens et al., 1993; Bullock et al., 1996). Radioligand binding studies have localized GLP-1R to several extrapancreatic tissues, including the brain (Uttenthal et al., 1992; Shimizu et al., 1987; Kanse et al., 1988), lung (Kanse et al., 1988; Richter et al., 1990), gastric glands of the stomach (Uttenthal and Blazquez 1990), rat hepatocytes (Villaneuva-Penacarrillo et al., 1995) and skeletal muscle (Delgado et al., 1995; Villaneuva-Penacarrillo et al., 1994). The GLP-1R is a specific receptor (Thorens 1992) that belongs to a subfamily of seven-transmembrane spanning G protein-coupling receptors. The mechanism of action of GLP-1 has not been elucidated, but GLP-1 stimulates adenylate cyclase and activation of protein kinase A seems essential (Fehmann and Habener, 1992). There is a significant elevation in intracellular Ca^{2+} which is probably the mechanism by which insulin secretion is stimulated (Gromada et al.,

1995; Holz et al., 1995). Therefore, the GLP-1 receptor, like the structurally related glucagon and parathyroid hormone receptors, can activate multiple intracellular signaling pathways. Important to the study of GLP-1R specific responses are two peptides isolated from the venom of the lizard *Heloderma suspectum*, exenden-4 and exenden 9-39 which are an agonist and antagonist to GLP-1R respectively (Goke et al., 1993; Thorens et al., 1993). Exenden-4 is a 39 amino acid peptide which shares 53% homology to GLP-1(7-36)NH_2 (Eng et al., 1992).

4.2.4. Satiety. Previously, many peptides initially thought to be specific to the gastroenteropancreatic system were later found to be present in the mammalian brain, modulating appetite, energy balance and body weight. Among these hormones are cholecystokinin, bombesin, and glucagon (Gibbs 1985; Leibowitz 1992; Shimizu et al., 1993). GLP-1 has recently been thought of as one of these gut-brain peptides (Calvo et al., 1995). GLP-1 may play a role as a neurotransmitter, controlling food and water intake. Immunohistochemical evidence suggests that proglucagon-like immunodeterminants are present in specific areas of the brain known to be involved in neuroendocrine and autonomic regulation of homeostatic mechanisms including carbohydrate metabolism and fluid balance (Jin et al., 1988). High densities of GLP-1 binding sites have been found by receptor autoradiographic experiments in specific areas of the brain (Goke et al., 1995a; Goke et al., 1995b). Cells in the subfornical organ and the area postrema could even be responsive to blood borne GLP-1. Both these areas have close neuroanatomical connections with hypothalamic areas involved in water and appetite homeostasis (Orskov et al., 1996a). There is a highly specific effect of intraventricularly administered GLP-1 to profoundly inhibit food intake in rats (Tang-Christensen et al., 1996; Turton et al., 1996), which is completely blocked with exendin 9-39 administration.

4.3. Degradation

Dipeptidyl peptidase IV (DPIV) is an enzyme that circulates in plasma and is associated with the plasma membrane of a variety of cells, including capillary endothelial cells, enterocytes, hepatocytes and cells of the kidney brush border (Mentlein 1988). DPIV preferentially cleaves peptides and proteins having either X-Pro, X-Hyp, or X-Ala at the end terminus (Walter et al., 1980) and to a lesser extent, X-Ser, X-Thr and X-Val (Martin et al., 1993). Both intact GIP and GLP-1 serve as substrates for DPIV since both have alanine residues at the penultimate *N*-terminal position. Mentlein et al (1993) reported that incubation of GIP(1-42) or GLP-1(7-36)amide with either serum or purified DPIV resulted in the production of GIP(3-42) or GLP-1(9-36)amide. Removal of these two *N*-terminal amino acids is significant in GIP (Brown et al., 1981; Moody et al., 1981) and GLP-1 (Adelhorst et al., 1994) as it renders these peptides biologically inactive. DPIV has been identified as the primary mechanism by which GLP-1 is degraded in human plasma in vitro (Deacon et al., 1995a; Pauly et al., 1996; Keiffer et al., 1995). The half-life of GLP-1 and GIP have not yet been fully established, however, in vivo studies show that 50% of ^{125}I-GLP-1 and ^{125}I-GIP are converted to their N-terminally truncated forms within 2 minutes in the rat (Keiffer et al., 1995). It appears as though the kidney is the major site of GIP (Hanks et al., 1984; Chap et al., 1987) and GLP-1 (Orskov et al., 1992; Ruiz-Grande et al., 1993) catabolism.

4.4. GLP-1 and GIP Interactions

Both GIP and GLP-1 concentrations increase significantly and in parallel with insulin in response to meals (Orskov et al., 1996b; Elliot et al., 1993). Kreymann et al (1987) reported that infusion of GLP-1(7-36)NH_2 significantly elevated plasma insulin values and significantly lowered glucose and glucagon concentrations whereas infusion of GIP was less effective in stimulating insulin release. These authors concluded that GLP-1(7-36)NH_2 is a more powerful incretin than GIP. Dose response studies of the insulinotropic activity of GIP and GLP-1 indicate that lower plasma concentrations of GLP-1 (~10-100 pmol / L) than GIP (~ 40-240 pmol / L) are necessary to enhance insulin

secretion (Holst et al., 1987; Mojsov et al., 1987; Weir et al., 1989) especially at elevated glucose concentrations (Nauck et al., 1989; Creutzfeldt, 1979). This last feature is of particular interest in regards to NIDDM, because when plasma glucose concentrations are in the normal fasting range, GLP-1 is unable to stimulate insulin secretion to a degree that causes hypoglycemia (Qualmann et al., 1995). This is in contrast to the oral sulfonureas currently in use. The proximal location of intestinal K cells would make GIP the perfect incretin as it could produce an early signal for the release of insulin upon the oral ingestion of nutrients. In spite of the distal location of the intestinal L cells, GLP-1 (along with GIP) are both seen to rise early and rapidly in response to a mixed meal. It has been suggested that these two important incretins interact significantly in an additive manner (Nauck et al., 1993a), probably through an enteroendocrine pathway and this may account for the early release of GLP-1 (Plaisancie et al., 1994; Roberge et al., 1993).

4.5. NIDDM & Incretin Effect

In patients with NIDDM, the overall incretin effect is reduced. This is due to a decreased insulinotropic activity of GIP in contrast to GLP-1 which retains much of its insulinotropic activity (Nauck et al., 1993b; Elahi et al., 1994). At equimolar concentrations and infusion rates, GLP-1 is more potent than GIP in stimulating the pancreatic B-cell to secrete insulin (Andersen et al., 1990; Shima et al., 1988). Many studies have demonstrated a beneficial effect of GLP-1 in NIDDM patients, including lowered fasting and postprandial blood glucose, increased glucose induced insulin and c-peptide secretion, suppressed glucagon release and delayed gastric emptying (Ahren et al., 1997; Gutniak et al., 1996; Willms et al., 1996; Gutniak et al., 1994; Nauck et al., 1993c). Controversy exists over circulating GLP-1 concentrations in NIDDM patients. Orskov et al (1991) reported significantly elevated fasting and postprandial GLP-1 values in NIDDM versus non-diabetic controls. The authors reported significantly elevated fasting levels of GLP-1 in NIDDM subjects when compared to a control group (47 ± 7 vs 37 ± 3 pmol / L). These rose to approximately 100 pmol / L (NIDDM) vs approximately 50 pmol / L (control) after an oral glucose load peaking at 30 min. This resulted in a significantly

larger area under the curve for GLP-1 (5776 $\pm$ 1674 vs 2015 $\pm$ 644 pmol / L•hr, NIDDM vs control respectively, $p \leq 0.05$). In contrast to this finding, Vaag et al (1996) reported decreased plasma concentrations of GLP-1 in NIDDM twins after an oral glucose tolerance test and hypothesized that decreased intestinal GLP-1 secretion may contribute to the abnormal insulin secretion seen with NIDDM. These authors reported fasting plasma GLP-1 levels in healthy control subjects of 7.1 $\pm$ 0.7 pmol / L and 6.1 $\pm$ 0.9 pmol / L in NIDDM subjects. GLP-1 concentrations measured 30 min after a test meal were not found to be significantly different between healthy normal subjects and NIDDM patients (22.4 $\pm$ 5.0 pmol / L vs 18.1 $\pm$ 1.7 pmol / L, $p > 0.05$). The area under the curve for GLP-1 in healthy subjects was significantly greater than NIDDM patients (1.17 $\pm$ 0.25 nmol / L*min vs 0.55 $\pm$ 0.14 nmol / L*min, $p < 0.05$).

5. Intestinal Adaptation

There are a number of physiological and pathological conditions which alter the absorptive capacity of nutrients in the small intestine. These include developmental age, pregnancy and lactation, disease states such as diabetes and thyrotoxicosis, starvation and alterations in the type and quantity of diet (reviewed in Philpott et al., 1992). Published studies have shown that high fiber diets stimulate hypertrophy of the distal small and large intestine (Goodlad et al., 1987b; Jacobs and Lupton, 1984) which are the primary sites of proglucagon mRNA and GLP-1 secretion (Larsson et al., 1975; Mojsov et al., 1986; Tappenden et al., 1996). It was only recently shown that GLP-2 may play a role in intestinal adaptation. GLP-2 is released in equimolar amounts with GLP-1 from the posttranslational processing of intestinally derived proglucagon (Orskov et al., 1986). Cheeseman and Tsang (1996) reported that vascular infusion of GIP or GLP-2 significantly increased D-glucose maximal transport rate in rat jejunum. GLP-2 has been shown to stimulate crypt cell proliferation and consistently induced a marked increase in bowel weight and villus growth in the jejunum (Drucker et al., 1996). These observations suggest a biological role of GLP-2 as an intestinal-derived stimulator of small intestinal

epithelial mass. In NIDDM, there is an increase in total hexose transport due primarily to a premature expression of transporters along the crypt-villus axis, causing a cumulative increase in enterocyte transporter protein during maturation (Burant et al., 1994). These changes suggest an adaptive response to increase nutrient absorption in a perceived state of tissue starvation (Burant et al., 1994), which is most likely due to increased DNA synthesis and crypt cell production rates (Bloom and Polak 1982b; Miazza et al., 1985).

6. Glucose Transporters & Upregulation Effects

SCFA supplemented TPN has been reported to significantly increase total, mucosal and sub-mucosal weights after major bowel resection in rats. (Tappenden et al., 1996). There are multiple mechanisms by which glucose enters and exits enterocytes (see **Figure 3**). SGLT-1 and GLUT5 are the transport proteins associated with the brush border membrane. Intestinal brush border glucose uptake is affected by dietary carbohydrate levels (Cheeseman and Harley, 1991). GLUT2 is the transport protein responsible for moving both fructose and glucose out of the enterocyte across the basolateral membrane under basal conditions (Cheeseman, 1993). The activity of this transporter is rapidly upregulated by the presence of hexoses in the intestinal lumen (Cheeseman, 1993) via increasing the number of carriers in this membrane (Cheeseman and Harley, 1991). In response to glucose infusion, D-glucose transport in the basolateral membrane is regulated by subsequent changes in carrier site density (Cheeseman and Maenz, 1989). Ferraris and Diamond (1992) concluded that this signal for glucose upregulation is perceived in the crypts and the observed lag time is due largely to cell migration times. Therefore, substrate-dependent upregulation of intestinal glucose transport is reported to involve increased numbers of transporters along the crypt-villus axis (Ferraris et al., 1992). It is not known whether or not the fermentability of carbohydrates is able to alter nutrient uptake, however, Tappenden et al., (1997b) demonstrated that intravenous SCFA facilitate intestinal adaptation after resection by increasing basolateral intestinal nutrient transport in which ileal D-glucose uptake and

GLUT2 mRNA were significantly elevated. Reimer (1997) reported consumption of a diet supplemented with a physiologically relevant concentration of fermentable dietary fiber significantly increased the passive permeability coefficient for D-glucose as estimated by L-glucose, and significantly increased the estimated values for the apparent Michaelis affinity constant when compared to a cellulose-supplemented diet.

7. Immunology Review:

7.1. Background

The immune system is a defense mechanism which is capable of adapting to protect the host from external (invading pathogenic microorganisms) and internal (cancer) destructive forces, thereby maintaining body integrity. There are limitless numbers of pathogens in the environment, and the immune system is able to adapt to specifically recognize and eliminate most of these components.

There are two types of immunity all healthy individuals possess. Innate immunity is the branch of the immune system present at all times, whereas adaptive immunity requires induction by antigen and adapts over time. The former is phylogenetically older with some forms present in all multicellular organisms, whereas the latter evolved approximately 400 million years ago and is found only in cartilaginous and bony fish, amphibians, reptiles, birds and mammals (Thompson, 1995). These two constituents of the immune system do not function individually, rather an integrated response mediated through cytokines (reviewed in Kuby 1994c) is essential for combating invading pathogens and maintenance of health.

7.2. Innate Immune System

When antigens overcome the initial physiological barriers (skin, mucous membranes, etc.), a wave of innate immune responses occur. Innate immunity is known as such because it develops early in life and it does not need to adapt during an immunologic challenge. The most important function of this system is to provide the

early phase of host defense which will protect the host during the time required for the adaptive immune response to occur. It is the first line of defense against invading pathogens. Besides some soluble factors (complement, lysozymes, interferons and heat shock proteins), the innate immune system is comprised of various cells including macrophages, neutrophils, eosinophils, basophils, mast cells and natural killer (NK) cells.

NK cells were originally identified through the natural cytotoxic activity found in peripheral lymphoid organs against different tumor cell lines (Kiessling et al., 1975a; Kiessling et al., 1975b). Typically, NK cells are defined as lymphoid cells able to kill altered self-cells (virally infected and tumor cells) to which they have not been previously sensitized (Timonen et al, 1979; Trinchieri 1989; Yokoyama et al., 1995). How this recognition occurs has not yet been fully elucidated, however cells which have downregulated or absent major histocompatability class I (MHC I) molecules are known targets for NK cells (Trinchieri 1989; Yokoyama et al., 1995). As well, certain lectin receptors (killer cell inhibitory receptors, KIRs) are now thought to play a role (Olcese et al., 1997; Binstadt et al., 1997; Yokoyama and Seaman 1993).

Monocytes (CD14+ cells) circulate in the blood for 1-2 days and then migrate to the tissues and differentiate into macrophages. Macrophages phagocytose foreign material and express these antigenic peptides on their cell membrane by way of an MHC II complex (Unanue 1978; Unanue and Allen 1987; Rosenthal 1978) for recognition by cells of the adaptive immune system. Macrophages can become activated by T-helper / inducer cells (CD4+ T-cells) which recognize this material as foreign and secrete IFN-γ. Activated macrophages have enhanced killing ability due to the production and secretion of toxic chemical mediators such as nitric oxide and TNF-α.

7.3. Adaptive Immunity

Unlike innate immunity, adaptive immunity exhibits unique characteristics such as specificity, memory and diversity. Immunological memory is probably the most important property of adaptive immunity and is defined as the ability of the immune system to react with increased efficiency to an antigen to which it has been previously

encountered (Colle et al., 1988). This secondary exposure to the same antigen induces a quicker and more intensive response with higher affinity (Colle et al., 1988) resulting in rapid elimination of the offending antigen. Roitt et al (1969) initially defined the terms T-cell and B-cell to emphasize that these two types of cells are of distinct lineages. T is used to denote thymus-dependent lymphocytes and B for Bursa-dependent lymphocytes, after the Bursa of Fabricus in birds (Roitt et al., 1969). It is now accepted that T-lymphocytes develop in the thymus and B-lymphocytes are derived from the bone marrow. Both bear clonally distributed antigen receptors whose specificities are generated by somatic mechanisms (Medzhitov and Janeway Jr. 1997). These two categories of lymphocytes comprise both the cell-mediated and humoral class of immunity respectively (reviewed in Janeway and Travers 1994b).

T-cells carry out a wide variety of functions that are specific to the different subsets of T-cells which can be defined by cell surface structures. These subsets can be identified by the use of specific monoclonal antibodies (mAb) for these markers. All T-lymphocytes have a T-cell receptor (TCR) in association with CD3, a cell surface molecule and all peripheral T-cells have CD5 molecules. The T-cells can then be subdivided into two major groups, the CD4+ and CD8+ T-cells.

Helper / inducer T-cells (CD4+ T-cells) have a T-cell receptor (TCR) associated with CD3 and interacts with foreign peptides bound to MHC II molecules on antigen presenting cells (APC) (Rudensky 1995). Helper / inducer T-cells orchestrate an acquired immune response by promoting intracellular killing by macrophages, antibody production by B-cells and clonal expansion of cytotoxic T-cells (Kuby 1991c). CD4+ cells can be further subdivided based on their secretory cytokine profiles into T_{H1} and T_{H2} cells (Romagnani 1992). T_{H1} cells mediate delayed hypersensitivity reactions and activate macrophages. They secrete IL-2, IFN-γ, and TNF. T_{H2} cells help B-cells to produce antibodies. When activated, T_{H2} cells secrete IL-4, IL-5, IL-6 and IL-10. All these cytokines influence B-cell growth and development. A third CD4+ T-cell has been identified and classified as T_{H0} (Firestein et al., 1989) which has both T_{H1} and T_{H2} cytokine secretory profiles.

The second type of T-cell is the cytotoxic / suppressor cell (CD8+ T-cell) which has a TCR associated with CD3 and interacts with foreign peptides bound to MHC I molecules on target cells (Kuby 1991a). Cytotoxic T-cells kill infected cells by delivery of a cytotoxic hit (release of destructive agents) and suppressor T-cells turn off the immune system once a pathogen is cleared. As with CD4+ T-cells, CD8+ T-cells can be classified in regards to their cytokine secretory profiles, but still have similar functions. Tc_1 T-cells secrete γ-IFN and Tc_2 T-cells secrete IL-4 and IL-5 (reviewed in Mosmann et al., 1997). Recently, a third CD8+ subset known as Tc_0 (Maggi et al., 1997) has been identified which secretes both IL-4 and γ-IFN.

CD45, the common leukocyte antigen, includes a family of isoforms characterized by the use of alternatively spliced exons creating alternative external domains (Trowbridge 1991) and is expressed on all T-cells, including CD4+ and CD8+ T-cells. The high molecular mass isoform CD45RA is preferentially expressed on T-cells throughout childhood and on antigen-inexperienced T-cells while the low molecular mass isoform, CD45RO is expressed after childhood on antigen-experienced memory cells (Sanders et al., 1988; Deans et al., 1989, Akbar et al., 1988; Deans et al., 1991; Deans et al., 1992).

When T-cells become activated by proper signals, they are able to carry out one or more of the following functions: proliferation, differentiation, production of cytokines and development of effector function. Effector cells are those lymphocytes that can mediate the removal of pathogens from the body without the need for further differentiation and proliferation (Janeway and Travers, 1994b). The delay seen with adaptive immune responses is a result of this activation process. As with all lymphocytes, T-cells requires a second signal for activation (Croft and Dubey, 1997) which can come from a variety of sources including macrophages, B-cells or dendritic cells. T-cells can be activated specifically (antigen and MHC from antigen-presenting cells interact with TCR and CD3 on T-cells) or non-specifically (polyclonal activators such as mitogens). Mitogens stimulate a large number of T-cells by interacting with receptors on the T-cell surface that are different from the antigen specific TCR, therefore inducing a polyclonal

(all T-cells) response. An individuals response to such a mitogen is an estimate of T-cell capacity.

B-cells, like T-cells, can be stimulated from their resting state to enlarge and develop synthetic machinery, divide, mature and become antibody-secreting cells, known as plasma cells. The second signal for activation for B-cells are secreted by T-cells (Jones 1996). Proper signals for stimulation depend on the triggers and again can be specific (complimentary antigen to surface Ig) and non-specific (polyclonal activation via B-cell mitogens). B-cells can be identified by immunoglobulins (Ig) present on the cell surface. There are five different Ig isotypes, including IgM, IgG, IgA, IgE, and IgD. IgG is the most abundant isotype in serum constituting approximately 80% of total serum Ig (Kuby 1991b). IgM accounts for 5-10% of total serum Ig (Kuby 1991b). Although IgA represents only 10-15% of total serum Ig, it is the predominant Ig class in peripheral tissues and their secretions, such as that from the gut-associated lymphoid tissue (GALT) (Kagnoff 1993b). IgA enhances barrier function by preventing the attachment of bacteria to mucosal cell surfaces (Pabst 1987).

7.4. Peripheral Lymphocytes and Secondary Lymphoid Organs

Lymphocytes are very mobile *in vivo*, migrating from one lymphoid (and non-lymphoid) organ to another via the lymph and blood (Yednock and Rosen 1989; Pabst and Binns 1989). Secondary lymphoid organs include the spleen, lymph nodes, Peyers' patches, tonsils, skin, and the gut-associated lymphoid tissue (GALT). These lymphatic tissues are not static collections of lymphocytes. On the contrary, lymphocytes are continually entering and exiting these sites. Since most lymphoid (and non-lymphoid) organs are included in the migration routes of lymphocytes, alterations in lymphocyte composition within these organs may be detected by studying lymphocytes in the blood (Westermann and Pabst 1990). However, measures made in peripheral lymphocytes should not be extrapolated to estimate responses in the secondary lymphoid organs (Westermann and Pabst 1990). Blood only represents approximately 2% of the total lymphocyte pool in the healthy adult human (Trepel 1974). It would be incorrect to

assume that these lymphocytes are truly representative of the other 98% of the lymphocytes distributed throughout the rest of the body. However, the peripheral blood itself is a distinct compartment in terms of lymphoid composition and can be examined to understand the immune system.

7.5. Gut Associated Lymphoid Tissue (GALT)

The gut mucosa is protected by a complex lymphoid system. The gut associated lymphoid tissue (GALT) represents a considerable lymphoid mass, quantitatively exceeding that of peripheral lymphoid organs (Cerf-Bensussan and Guy-Grand, 1991). The immune cells of GALT (intraepithelial lymphocytes, lamina propria lymphocytes, and the cells found in Peyers patches and the mesenteric lymph nodes) are continually migrating back and forth between the periphery and the intestine (Cerf-Bensussan and Guy-Grand, 1991). In the gut, immune cells are in constant exposure to dietary antigens, therefore it could be predicted that changes in dietary nutrients or digestion/fermentation products could directly impact immune function.

7.6. Diet and Immunity

Nutrition is essential in maintaining all normal physiological functions including defense of self. Changes in dietary intake logically should affect certain physiological functions, and the immune system is no exception. It has been known for centuries that malnutrition predisposes patients to infection and recent research suggests many individual dietary components can exert profound effects on the body's immune system (Bower 1990). For example, deficiency in vitamin A and the carotenoids results in depressed antibody responses and decreased cellular-mediated (Nauss et al., 1979). Vitamin C can affect both cell-mediated and humoral immunity (Panush and Delafuente 1985) and both excessive and deficient levels of linoleic acid (a polyunsaturated fatty acid) affects neutrophil and monocyte function (Endres et al., 1993). Because of the importance of fiber in modulating the structure and function of the gut, attention has

focused on the effects of dietary fiber and its effects on GALT in modulating the immune system (Lim et al., 1997; Field et al., submitted).

7.7. Dietary Fiber and Immunity

Dietary fiber helps to maintain normal gut microflora (Crowther et al., 1973) which protects against colonization by pathogenic bacteria (Fleming and Arce 1986). Indeed, changes in the gut microflora have been hypothesized to affect immune function (Finegold et al., 1974). Recently, Lim et al (1997) reported that the type of dietary fiber modified the proportion of CD4+ and CD8+ lymphocytes in the mesenteric lymph nodes. Fiber type can also affect mucous secretion which influences barrier function (Lee and Ogilvie 1982; Iatskovskii et al., 1989). The type of dietary fiber is reported to influence the anaerobic microbial production of short chain fatty acid (SCFA) composition in the colon (Zhang and Lupton, 1994) which could then affect immune function. SCFA have been reported to prevent gut atrophy (Tappenden et al., 1997; Koruda et al., 1988) and gut atrophy is related to an increase in gut-barrier dysfunction and the translocation of bacteria and toxins (Deitch and Berg 1987; Wilmore et al., 1988). Butyrate, a SCFA, accounts for approximately 17% of all SCFA produced endogenously and is an important energy source for colonic epithelial cells (Roediger 1980). Butyrate concentrations from 1 to 10 mM have profound effects on the phenotype and proliferation of cultured human cells (Kruh 1982). Acetate is another SCFA produced in the colon and has been reported to activate the immune system in mice (Ishizaka et al., 1990) and enhance peripheral blood antibody production (Ishizaka et al., 1993). This has been suggested to be due to the involvement of acetate in phospholipid synthesis by lymphocyte membranes (Huber et al., 1968). SCFA lower pH (Lupton et al., 1988) which inhibit the conversion of primary bile acids to secondary bile acids (Jacob 1988), and bile acids have been reported to influence immune function (Lim et al., 1994).

Lymphocytes in the intestinal mucosa first interact with antigens (or other factors, i.e. SCFA) in the organized lymphoid tissues (Peyer's patches and lymphoid follicles in the colon) and further differentiate and mature in the germinal centers (Stephen and

Martin 1994). They leave the mucosa and migrate through the mesenteric lymph nodes and thoracic duct to reach systemic circulation (Stephen and Martin 1994). Therefore it is reasonable to suggest that dietary fiber type and fermentation end-products via their effect in GALT may affect lymphocytes in the periphery.

7.8. Immunity and NIDDM

Although there are a number of long term complications associated with diabetes mellitus (Diehl 1995), infection is associated with significant morbidity and mortality in the diabetes population (McMahon and Bistrian 1995; Rayfield et al., 1982). Indeed, the leading cause of death in people with diabetes mellitus at the Kaohsiung Medical College hospital was infection (25.8%) followed by cardiovascular disease (18.5%) (Wei et al., 1996).

Alexiewicz et al, (1995) reported that patients with NIDDM have elevated cytosolic levels of polymorphonuclear leukocytes and that this abnormality is probably induced by hyperglycemia and may be responsible for the impaired phagocytosis seen in these subjects. In another study there were no differences in peripheral blood T-cells between pan T-cells (CD3), CD4, CD8 or the CD4:CD8 ratio in NIDDM compared to healthy subjects (Chang and Shaio 1995). There was however a decreased expression of interleukin-2 receptors (IL-2R) on activated lymphocytes, despite a higher production of TNF-α, resulting in decreased lymphocyte proliferation in patients with NIDDM (Chang and Shaio 1995). TNF-α is part of a family of soluble immune mediators known as cytokines. Another cytokine, IL-1 in combination with TNF-α, increases net glucose flux and oxidation (Ling et al., 1994) thereby indicating a possible role of cytokines in glucose metabolism. Traditionally, it was thought that the disease played a role in the increased occurrence of infections with diabetes mellitus. However, hyperglycemia itself is thought to influence immune function (Kwoun et al., 1997) impairing key steps which may promote the virulence of certain microorganisms (Rayfield et al., 1982).

7.9. Functional and Phenotypic Assays

7.9.1. Mitogen-induced cellular proliferation: The polyclonal activation of lymphocytes provides a useful model for studying the metabolic events associated with antigen stimulation (Field 1995). Cellular proliferation is measured as [^{3}H]thymidine uptake by cultured lymphocytes in the presence or absence of mitogens and is expressed as a stimulation index, that is the response of stimulated cells compared to unstimulated cells. [^{3}H]thymidine provides an alternative nucleotide to be incorporated into the replicating DNA of the lymphocytes, and it is this retained [^{3}H]thymidine DNA that is measured to estimate proliferation.

7.9.2. Natural killer cell cytotoxicity: The activity measured as % specific lysis by NK cells provides a useful measure of the capacity for lysis by these cells and indirectly provides information on the innate immune system. Specific lysis (%) is determined by lysis of ^{51}Cr-incorporated target cells which is non-specifically taken up but not released from live cells. Upon lysis, the ^{51}Cr is released into the media and an aliquot of this is counted. Specific lysis (%) is expressed by experimental lysis compared to complete lysis and is corrected for spontaneous lysis. The NK sensitive target cells which are chosen are normally allogeneic tumor cells due to a higher degree of specific lysis.

7.9.3. Flow Cytometry: A suspension of cells is passed through a laser beam in a flow cytometer. The cells, attached to mAb which are conjugated to fluorescent markers (fluorochromes). The resulting fluorescent emission and scattered light from the cells are collected by photodetectors and converted to electronic data signals. Light scatter can occur either as forward scatter (related to cell size) and side scatter (corresponding to cell shape and granularity). There are different fluorochromes which can be used including fluorescein isothiocyanate (FITC), phycoerythrin (PE) and peridinin-chlorophyll a complex (PerCP). These fluorescent dyes can all be excited at one wavelength (488 nm) but emit light in different regions, therefore are distinguished based on colour. Single colour immunofluorescence allows for a mAb conjugated to a fluorochrome, to attach to a specific cell surface molecule thereby distinguishing classes of lymphocytes. Two

colour immunofluorescence uses two different mAb each conjugated to a different fluorochrome thereby allowing further classification into subsets of lymphocytes (e.i., CD4+CD5+ T-cells vs CD8+CD5+ T-cells). This assay determines percentages of lymphocytes by quantifying the amount of fluorescence each population and/or subset of these lymphocyte populations emits.

8. Rationale & Hypotheses

Two primary goals in the treatment of NIDDM are to prevent hyperglycemia (Diabetes Control and Complications trial, 1993) and prevent major complications involved with NIDDM, i.e. the occurrence of infections (Nichols and Crenshaw, 1995; Diehl, 1995). Dietary intervention is a major component in achieving blood glucose control and preventing the complications associated with diabetes (Ihle, 1995) such as infection (Wei et al., 1996).

Reimer and McBurney (1996) demonstrated that a diet supplemented with fiber is able to significantly alter proglucagon gene expression and modulate GLP-1 and insulin secretion 30 min after an oral glucose gavage in rats. Fermentable fiber has recently been reported to significantly increase ileal proglucagon mRNA and modulate intestinal glucose uptake in rats (Reimer 1997). Tappenden et al (1996) demonstrated that SCFA supplemented TPN upregulates proglucagon expression. Dogs are a useful animal model for several reasons. This larger animal model allows for greater sample volume thereby allowing a complete characterization of response curves to an OGTT, not just a one time blood sample and larger gut samples. The dog, although not as extensively studied as the rat, has been used as the animal model in many GLP-1 and glucose tolerance studies (Knudsen and Pridal 1996; Pridal et al., 1996; van der Burg et al., 1995; Wen et al., 1995; Sugiyama et al., 1994; Ohneda et al., 1991; Kawai et al., 1990; Kawai et al., 1989).

The gastrointestinal tract is a large lymphoid organ containing approximately 25% of the immune cells in the body. Although the effects of fermentable dietary fiber on the immune system are not fully known, Lim et al (1997) reported different dietary fibers

modulated the mesenteric lymph node cells in rats and Pratt et al (1996), reported that SCFA supplemented TPN improved components of the non-specific immune system after major bowel resection in rats. Whether any of these components modulate the immune system in the periphery is unknown.

Based on this previous work, our hypotheses were: The consumption of a highly fermentable fiber diet will:

1) increase proglucagon mRNA abundance which will be associated with:
    a) increased postprandial GLP-1 and insulin secretion and,
    b) increased intestinal glucose transport capacity,
    with a net improvement in glucose homeostasis and,
2) improve components of the non-specific immune system (i.e. NK activity) without causing deleterious effects on other components of the specific immune system (cellular response to mitogens and lymphocyte population distributions of peripheral blood).

II. Materials and Methods

1.Diets.

Experimental diets were designed to be isonitrogenous and isoenergetic providing approximately 19.5 MJ/kg diet with 35% of the energy from carbohydrate, 30% from fat and 35% from protein. The low fermentable fiber (LFF) diet contained wood cellulose as the fiber source and the high fermentable fiber diet (HFF) diet contained a mixture of more fermentable plant fibers. The total dietary fiber (TDF) content of the diets, determined by AOAC (Association of Official Analytical Chemists Official Method 985.29 under section 45.4.07) was 8.3 g/kg for the LFF diet and 7.3 g/kg for the HFF diet (**Table 1a and 1b**). Fructooligosaccharides (FOS) which are added as 15 g/kg diet, are not recovered in the AOAC method. Assuming 95% of the FOS is dietary powder, the actual fiber content of the HFF diet was calculated as (7.3 + (0.95 x 1.5 g / 100g diet) = 8.7%). The predicted fermentability of the LFF and HFF diets are 9 mmol of total SCFA / kg OM and 229 mmol of total SCFA / kg OM respectively where total SCFA concentrations were calculated from the sum of acetate, propionate and butyrate using average 24 h fermentations (Sunvold et al., 1995a; Sunvold et al., 1995b; Sunvold et al., 1995c). The pre-experimental chow diet composition is shown in **Table 2.**

2.Animals.

All procedures received ethical approval from the Health Sciences Animal Welfare Committee of the University of Alberta and are consistent with the guidelines of the Canadian Council on Animal Care.

Adult dogs (n=16) were obtained from Butler Farms USA Inc., (North Rose, NY). Upon arrival, animals were acclimatized for a 7 d period and fed a nutritionally complete diet (Can-Pro, Beaumont, AB, Canada). A crossover experimental design was used whereby animals were randomly assigned to receive one of two isoenergetic, isonitrogenous diets. Animals were weighed daily and food offerings were adjusted to ensure minimal weight gain/loss using the formula: MJ = 0.553 x $kgBW^{0.67}$ (NRC.,

1985). Eight dogs were fed the HFF diet for 14 d followed by LFF diet for 14 d whereas the other eight dogs were fed the diets in the opposite order (**Figure 4**). Because all 16 dogs could not be accommodated at one time, dogs were paired throughout the cross-over design. This is referred to as group within the statistical model. All dogs were individually fed to meet energy requirements and water was provided *ad libitum*.

3.Oral Glucose Tolerance Test.

Food was removed at 1600 h on Days 13 and 27. At 0845 - 0900 h on Days 14 and 28, the dogs were loosely restrained in a table sling and were given an oral glucose tolerance test (OGTT) using 70% (w/w) dextrose to provide 2 g glucose / kg BW. Peripheral blood was sampled at time points 0, 15, 30, 45, 60, 90 and 120 min, via the saphenous vein using a Insyte-W 20GA 2" catheter (Becton-Dickinson Vascular Access, Sandy, UT). Blood samples were also obtained after the pre-experimental period (Pre-Exp) for immune function assays.

4. Peripheral blood samples.

Blood samples for general chemistry screen and complete blood counts (2 mL) and immune assays (10 mL) were collected in 3 mL and 10 mL heparinized vacutainer™ tubes (Becton-Dickinson, Sunnyvale, CA) respectively and stored on ice until analysis. Hematological analyses were conducted using a Coulter STKS instrument (Coulter Electronics Inc., Hialeah, FA) and manual differential counts were performed by the staff at the Veterinary Pathology Laboratory (Edmonton, AB). Blood samples for insulin and GLP-1 analysis were collected into 10 mL EDTA heparinized vacutainer™ tubes (Becton-Dickinson, Sunnyvale, CA) with aprotinin (500 KIU / ml blood, Sigma Chemicals, St. Louis, MO) and stored at -70°C (GLP-1) or -35°C (insulin). Blood samples for serum glucose determinations were placed in 250 µL microcentrifuge tubes, centrifuged at 2900 x g for 10 min at room temperature, the serum was removed by pipet and stored at -35°C.

5. Intestinal samples and sacrifice.

On Day 28, the dogs were anesthetized with somnitol (MTC Pharmaceuticals, Cambridge ON) using 1 mL / 2.27 kg BW. Duodenal, jejunal, ileal and colonic samples were taken for northern blot analysis and histology. Northern blot samples were promptly placed in liquid nitrogen, and histological samples were placed directly into formalin. Jejunal and ileal samples were taken for and nutrient uptake assays and western blot analysis. Western blot samples were scraped gently to free the mucosal lining and stored on ice until analysis, and nutrient uptake samples were placed immediately into ice cold saline. Histological slide preparation was performed by the staff at the Veterinary Pathology Laboratory (Edmonton, AB). The animals were then killed by euthanol (MTC Pharmaceuticals, Cambridge ON.) using 2 mL / 4.5 Kg BW.

6.Glucose.

Serum glucose was determined using the Sigma Diagnostics Glucose (Trinder) Reagent for the enzymatic determination of glucose at 505 nm (Cat # 315-100, Sigma Chemical, St. Louis, MO).

7. Insulin.

Serum insulin concentrations were determined using the Coat-A-Count® I^{125} diagnostic radioimmunoassay (Cat # TKIN1, Diagnostics Products Corporation, Los Angeles, CA).

8. Plasma GLP-1(7-36)NH_2 Extraction.

GLP-1 immunoreactive peptides were extracted from 2.5 mL of plasma using a SEP-COLUMN containing 200 mg of C_{18} (Cat # RIK-SEPCOL 1, Peninsula - Laboratories, Belmont, CA) with Buffer A (0.1% trifluoroacetic acid (Cat # RIK-BA-1, Peninsula Laboratories, Belmont, CA)) and Buffer B (60% acetonitrile (Cat # RIK-BB 1, Peninsula Laboratories, Belmont, CA)) as elution solvents. Samples were lyophilized overnight using a speed-vac (Savant Inc., Midland, MI) and stored at -70°C.

9. Intestinal GLP-1(7-36)NH_2 Extraction.

Extraction of GLP-1(7-36)NH_2 from intestinal segments has been described by Xioyan (1996) and was carried out with modifications. Briefly, 400-500 mg of each segment (jejunum, ileum and colon) was added to a 12 x 75 mm Simport polypropylene tube (Fischer Scientific, Edmonton, AB) with 0.5 mL 2M acetic acid and boiled for 1 h. Tubes were centrifuged at 4500 x g for 10 min, the supernatant collected, transferred to a fresh tube and neutralized with 1N NaOH. For RIA purposes, the sample of supernatant was diluted 1:10 with RIA buffer (100 mM Tris, 50 mM NaCl, 200 mM Na_2-EDTA, 0.2 g/L Na azide, pH 8.5) to give a final sample volume of 100 µL.

10. GLP-1(7-36)NH_2 Radioimmunoassay.

Concentrations of GLP-1(7-36)NH_2 were measured using a competitive binding radioimmunoassay described by Xiaoyan, (1996) with modifications. Briefly, the lyophilized plasma samples were reconstituted in 250 µL of RIA assay buffer (100 mM Tris, 50 mM NaCl, 20 mM Na_2-EDTA, 0.2 g/L Na azide, pH 8.5). Polypropylene tubes (12mm x 75mm) were used for controls, standards and samples and the entire procedure was carried out on ice. GLP-1 (7-36 NH_2) standards (Peninsula Laboratories, Belmont, CA) made from serial dilutions, ranged from 4000 pg/mL to 15 pg/mL. Total counts (TC), non-specific binding (NSB), total bound (TB), standards and samples were determined in duplicate as outlined in **Table 3**. The GLP-1(7-36)NH_2 Ab (KMJ-03) (1:20000) was a generous gift from Dr. Chris McIntosh (University of British Columbia, BC, Canada). Tubes were mixed and incubated 24 hr at 4°C. Following incubation, 50Bq of ^{125}I-GLP-1(7-36)NH_2 tracer was added to the tubes, the tubes were mixed by vortexing and incubated for 48 hr at 4°C. Dextran-charcoal suspension (4 g/L dextran T70, 80 g/L charcoal in assay buffer) was added to all tubes (100 µL) except TC tubes. Tubes were mixed by vortexing and left on ice for 15 min, centrifuged at 2200 x g for 30 min and 600 µL of supernatant was transferred to new tubes which were counted using a Cobra™ Auto-Gamma counter (Packard Instrument Company, Downers Grove, IL).

11. GLP-1 (7-36)NH_2 Iodination.

GLP-1 (7-36 NH_2) was iodinated using the chloramine-T method as described by Xiaoyan (1996). Briefly, the cartridge was primed by allowing 10 mL acetonitrile with 0.1% trifluoroacetic acid (TFA) followed by 10 mL of ddH_2O with 0.1% TFA to flow through. The cartridge was dried by allowing 10 mL of air to be pushed through via a syringe. The iodination was carried out by weighing 30 - 40 µg of GLP-1, dissolving it in 30 - 40 µL of ddH_2O and transferring 10 µL to a fresh eppendorf tube. To this, 10 µL 0.5 M PO_4 (pH 7.0) was added followed by 0.5 mCi ^{125}I. Chloramine-T (10 µL) was added and the tube was tapped for exactly 30 s. Sodium metabisulfite (5 mg / mL) was added followed by 1 mL of 0.1% TFA which was then transferred to the primed column. Gentle pressured was applied to the column using a 10cc syringe. Acetonitrile with 0.1% TFA was used as the elutant to acquire 5 fractions. Acetonitrile (5 mL, 10% + 0.1% TFA) and acetonitrile (5 mL, 20% + 0.1% TFA) are the first 2 elutants used in that order and the fractions were collected into 14 mL round bottom tubes. Then 30 % acetonitrile (1 mL + 0.1% TFA, 4 times), 38% acetonitrile (1 mL + 0.1% TFA, once) and 40% acetonitrile (1 mL + 0.1% TFA, 5 times) were used as the next elutants in that order and the fractions were collected in small polypropylene tubes. Each eluted fraction was mixed well and 10 µL from each fraction was counted using a Cobra™ Auto-Gamma counter (Packard Instrument Company, Downers Grove, IL). The label usually was eluted in fraction 1, 2 and/or 3 of the 40% acetonitrile. Fractions containing the labelled GLP-1(7-36)NH_2 were pooled and stored at -35°C. The ^{125}I-GLP-1(7-36)NH_2 has a storage life of approximately 2 weeks.

12. Isolation of Total RNA.

Total RNA was isolated from each intestinal segment using Trizol™ (Gibco BRL, Burlington, ON, Canada) according to the protocol provided by the manufacturer. Briefly, 400 - 500 mg of tissue was ground in a pre-chilled sterile mortar with pestle. The ground tissue (200 mg in duplicate) was weighed and transferred in duplicate to polypropylene tubes (12mm x 75mm), 2 mL of Trizol™ solution was added and samples were

homogenized with a Polytron homogenizer for 30 s at setting 10. The homogenized sample was transferred to a 14 mL sterile polypropylene falcon™ tube and incubated for 5 min at room temperature. To each sample, 400 µL of chloroform was added, tubes vigorously hand shaken for 15 sec and incubated for another 2-5 min at room temperature. Next, samples were centrifuged at 12,000 x g for 15 min at 4°C. The aqueous phase was transferred to a fresh eppendorf tube, 1 mL isopropanol was added, tubes were vortexed, and the RNA precipitated overnight at -20°C. Samples were centrifuged at 10 000 - 12 000 x g for 10 min at 4°C, the supernatant was removed, and the pellet was washed 2 times with 75% ethanol (at least 1 mL). The sample was mixed by vortexing and pelleted by centrifuging at 7,500 x g for 10 min at 4°C. The RNA pellet was briefly allowed to air dry (no more than 10 min) and dissolved in RNAse free water (50-100 µL per 100 mg of tissue) by gentle vortexing, incubated for 5 - 10 min at 55 - 60°C and stored at -70°C. Quantity and purity of RNA were determined by ultraviolet spectrophotometry at 260, 280 and 230nm.

13. Northern Blot Analysis.

Messenger RNA was measured by Northern blot analysis as described by Zhao et al (1993). Aliquots of 15 µg total RNA were dissolved in 10 µL loading gel buffer (50% deionized formamide (vol/vol), 2M formaldehyde, 1.3% glycerol (vol/vol), 0.02M morpholinopropanesulphonic acid (MOPS), 5 mM sodium acetate, 1 mM EDTA and 0.1% bromophenol blue (wt/vol)) and boiled for 2 min to denature the RNA which was then loaded onto a 1% agarose (wt/vol) gel containing (0.66M) formaldehyde. RNA was fractionated according to size by electrophoresis in the presence of a recirculating running buffer containing 0.02M MOPS, 5 mM sodium acetate and 1 mM EDTA (5 h at 100V). After electrophoresis, the gels were soaked in two changes of 10X standard saline citrate (SSC) (1.5 M NaCl, 0.15M trisodium citrate, pH 7.0) and blotted onto a zeta-probe GT Genomi tested blotting membrane (BioRad, Mississauga ON, Canada), employing the capillary method by Southern (1975). The RNA was fixed onto membranes by baking in

vacuum at 80°C for 2 h. Prior to hybridization with the [^{32}P] CTP-labelled riboprobe, each membrane was prehybridized for 2 h at 50°C in 20 mL of prehybridization buffer (deionized formamide (60% vol/vol), 20 x SSPE (5% vol/vol), 10% blotto (5% vol/vol), 20% SDS (5% vol/vol), and 10 mg/mL sheared salmon DNA (denatured by boiling in a hot water bath for 10 min, 5% vol/vol)). Hybridization was carried out for 12-16 h at 50°C in an identical volume of fresh hybridization solution (deionized formamide (55% vol/vol), 20 x SSPE (5% vol/vol), 10% blotto (5% vol/vol), 20% SDS (5% vol/vol), and 10 mg/mL sheared salmon DNA (2.5% vol/vol mixed with an equal part of deionized formamide. To this, 16.7KBq (1 x 10^6 cpm) of labelled riboprobe was added and pre-warmed in a 70°C water bath for 5 min before being added to the pre-warmed hybridization solution. The membranes were washed with 2 x SSC at room temperature for 5 min and then in 2 x SSC / 0.1% SDS for either 10 min (GLUT2, GLUT5) or 15 min (proglucagon, SGLT-1). The membranes were transferred to a bath of 0.2 x SSC / 1% SDS as follows: proglucagon (70°C for 10 min), SGLT-1 (70°C for 20mins), GLUT5 (45°C for 3-4 min), and GLUT2 (60°C for 2-3 min). Lastly, the membranes were washed in 0.2 x SSC at room temperature for 2-3 min. Membranes were heat sealed in plastic bags and exposed to Kodak XRA5 film (Eastman Kodak, Rochester, NY) at -70°C using an intensifying screen (Dupont Canada, Mississauga, ON). For statistical analysis, the signals were quantified using laser densitometry (Model GS-670 Imaging Densitometer, BioRad Laboratories (Canada) LTD., Mississauga, ON). The 28S and 18S ribosomal bands were quantified from negatives of photographs of the membranes. These bands were used to confirm the integrity of the RNA and compensate for minor loading discrepancies.

14. Riboprobes.

A 3.8 kb radiolabeled GLUT2 antisense riboprobe was generated from Xba I-linearized plasmid DNA [pGEM4Z - HTL - 3] and T7 polymerase. The GLUT5 cDNA insert from plasmid pUC13 - phJHT - 5 was subcloned into the BAM HI and ECO RI

sites of gPEM3 plasmid and a 2.2 kb antisense riboprobe was synthesized using BAN HI - linearized plasmid and T7 RNA polymerase. The 350 kb proglucagon sense riboprobe was generated from Rsa I-linearized plasmid DNA [pGEM4Z - HTL - 3] and Sp6 polymerase. Lastly, the 2.1 kb SGLT-1 antisense riboprobe was generated from a 1.4 Kb fragment of lamb intestinal SGLT-1 clone (aa 207-664), Wood et al, (1994).

15. BBM and BLM Isolation.

All procedures were performed on ice using previously described procedures (Maenz and Cheeseman, 1986). Briefly, approximately 5 g of mucosal scrapings were added to 15 mL of membrane suspension solution, (MSS buffer, 125 mM/L sucrose. 1 mM/L Tris-HCL, 0.05 mM/L PMSF, pH 7.4) and homogenized with a Polytron homogenizer for 30 s at setting 8. Aliquots of this homogenate were then taken for enrichment assays. The samples were split into two 30 mL eppendorf tubes and 20 mL of MSS buffer was added to each tube which were homogenized twice more at setting 8 for 30 s. Samples were then centrifuged for 15 min at 2400 x g, the supernatant was collected and centrifuged at 43 700 x g for 20 min. The remaining pellet consisted of two fractions. The outer white fluffy layer comprised the basolateral membranes (BLM) and the inner dark brown pellet comprised the brush border membranes (BBM). BLM were gently resuspended in a small amount of MSS buffer and transferred to a 14 mL eppendorf tube. BBM were resuspended in MSS buffer and samples from the same animal were pooled into 1 tube and made up in 20 mL of MSS buffer. BBM were then centrifuged for 20 min at 43 700 x g. Again the fluffy white pellet was gently resuspended with MSS buffer and added to the 14 mL eppendorf tube and the dark pellet was resuspended in exactly 30 mL of MSS buffer.

16. BLM Preparation.

Isolated BLM were homogenized for 15 s on setting 8. The sample was loaded on 25 mL of 20% percoll and centrifuged for 30 min at 46 000 x g. This resulted in a fluffy band appearing in the percoll which was collected and transferred to 25mm x 89mm

polycarbonate ultracentrifuge tubes (Beckman Instruments Inc., Palo Alto, CA) which were brought up to volume (approximately 38 mL) with MSS buffer and centrifuged at 115 000 x g for 30 min. The membrane layer was removed, diluted with 20 mL of MSS buffer, and homogenized for 15 s at setting 8. $CaCl_2$ (1 M, 100 µg) was added stirred gently on ice for 10 min. Samples were centrifuged for 10 min at 7700 x g, the pellet resuspended in 20 mL MSS buffer, and homogenized for 15 s at setting 8. Samples were centrifuged another 20 min at 46 000 x g and the pellet was resuspended in 1 mL MSS buffer. Aliquots were then taken for enrichment assays.

17. BBM Preparation.

BBM samples were homogenized for 15 s at setting 8 and centrifuged for 10 min at 1900 x g. The supernatant was transferred and centrifuged another 15 min at 14 600 x g. Again, the supernatant was transferred to a tube containing 300 µL of 1 M $CaCl_2$ and stirred gently on ice for 20 min. Samples were centrifuged for 30 min at 3000 x g, the supernatant was collected, and centrifuged another 30 min at 46 000 x g. The pellet was resuspended in 1 mL of ddH_20 and aliquots were taken for enrichment assays.

18. BLM Enrichment.

The enrichment assay described by Esmann M (1988) was used for the basolateral membrane enzyme Na^+K^+ ATPase. Briefly, total ATPase activity was assayed by incubating mucosal homogenates and membrane preparations in the presence of ATP and Mg^{2+} and measuring the liberated inorganic phosphate using the classic molybdenum reaction. Ouabain insensitive ATPase activity was assayed as described above in the presence of ouabain. Na^+K^+ ATPase activity is ouabain sensitive, therefore the difference between total and ouabain insensitive ATPase activity is the Na^+K^+ ATPase activity. Results are expressed as percent-fold enrichment.

19. BBM Enrichment.

The enrichment assay for the brush border membrane enzyme alkaline phosphatase was measured using the Alkaline Phosphatase kit from Sigma (Cat # 245-10, Sigma Diagnostics, St. Louis, MO). Briefly, the procedure is based on the hydrolysis of p-nitrophenyl phosphate to p-nitrophenol and inorganic phosphate by alkaline phosphatase. The p-nitrophenol which is formed is yellow in colour and shows a maximum absorbance at 405 nm.

20. Western Blot Analysis.

The Western blot analysis protocol as described by Tappenden et al (1997) was used for the quantification of brush border membrane (BBM) and basolateral membrane (BLM) glucose transporters. BLM (60 µg isolated protein) samples were diluted at 1:4 with 1x sample buffer (0.5M Tris-HCl pH 6.8 (13.2% vol/vol), glycerol (10.5% vol/vol), 0.05% (w/vol) bromophenol blue and 10% SDS (0.21% w/vol)) and BBM (60 µg isolated protein) samples were diluted 3:1 with 4x sample buffer (0.24M Tris-HCL, 40% glycerol, 8% vol/vol of 10% w/vol SDS, 0.5 mL bromophenol blue). BBM were boiled for 10 min but BLM were not. The stacking gel (4.1 M acrylamide / 21 mM N'N-bis methylene-acryl (10.7% vol/vol), 0.5 M Tris-HCL, pH 6.8 (0.24% vol/vol), 10% (w/vol) SDS (0.97% vol/vol), 10% APS w/v (4.86% vol/vol) and 0.4% TEMED (vol/vol)) was placed on top of the separating gel (4.1 M acrylamide / 21 mM N'N-bis methylene-acryl (32.1% vol/vol) , 1.5 M Tris-HCL, pH 8.8 (32.1% vol/vol), 10% (w/vol) SDS (1.3% vol/vol), 10% (w/vol) APS (0.66% vol/vol) and 0.16% (vol/vol) TEMED) . Electrophoresis was carried out in running buffer (0.3% Tris (w/vol), 1.44% glycine (w/vol) and 0.1% SDS)) at 100 - 200 V for 1 - 2 h until the dye front reached the end of the gel. Proteins were then transferred to a nitrocellulose membrane (MSI Laboratories, Houston, TX), using a transfer unit (BioRad, Mississauga, ON, Canada) and transfer buffer (Tris-base (0.189% w/vol), glycine (0.9% w/vol), methanol (20% vol/vol), SDS (0.02% w/vol)) for 1.5 - 2 h at 200 V. Following the transfer, the membranes were placed immediately into TBST (1M Tris pH 7.5 (2% vol/vol), NaCl (0.88% w/vol), 0.05% Tween-20 (0.05% vol/vol)).

Membranes were blocked in TBSTM (TBST with 5% (w/vol) powdered milk) for at least 1 h with gentle agitation and incubated with primary antibodies to SGLT-1 (Cat # AB1352, Chemicon International Inc., Temecula, CA.) at a dilution of 1:1000 or GLUT2 (Cat # AB1342, Chemicon International Inc., Temecula, CA.) at a dilution of 1:500 overnight at 4°C. Membranes were washed 3 x 10 min in TBST with gentle agitation, followed by an incubation with the secondary antibody (anti-rabbit IgG HRP-conjugate, Signal Transduction, PDI Bioscience, Inc., Aurora, ON) at a dilution of 1:4000 for at least 2 h with gentle agitation. Blots were covered completely and incubated with Supersignal CL-HRP (Cat # 34080, Pierce, Rockford, IL) working solution for 5 min before being exposed to KODAK XRA5 film (Eastman Kodak, Rochester, NY). Loading consistency and protein transfer was confirmed by staining the blots with Ponceau S (0.1% w/vol Ponceau S (BDH), 5% acetic acid). Statistical analysis was performed on the relative intensities of the bands. For statistical analysis, the signals were quantified using laser densitometry (Model GS-670 Imaging Densitometer, BioRad Laboratories (Canada) LTD., Mississauga, ON).

21. Measurement of Transport Kinetics.

Transport kinetics were measured as previously described by Thomson and Rajotte (1983). Briefly, a 12 cm segment of intestine was removed from each animal, opened along the mesenteric border and carefully washed with ice-cold saline to remove visible mucus and debris. Pieces of intestine (1 cm^2) were cut out and the tissue was mounted as flat sheets in incubation chambers containing oxygenated Kreb's bicarbonate buffer (pH 7.4) at 37°C. Tissue discs were preincubated in this buffer for 15 min to allow equilibration at this temperature. After preincubation, the chambers were transferred to beakers containing [^{3}H]-inulin and various [^{14}C]-probe molecules in oxygenated Kreb's bicarbonate buffer (pH 7.4) at 37°C. The concentration of solutes was 4, 8, 16, 32 and 64 mM for *D*-glucose and 16mM for *L*-glucose. The preincubation and incubation solutions were mixed using circular magnetic bars which were adjusted with a strobe light to achieve a stirring rate of 600 rpm and a low effective resistance of the intestinal unstirred

water layer (Thomson and Dietschy, 1980). The experiment was terminated by removing the chambers, quickly rinsing the tissue in cold saline for approximately 5 s and cutting the exposed mucosal tissue from the chamber with a circular steel punch. The tissue was dried overnight in an oven at 55°C to determine the dry weight of the tissue and then saponified with 0.75 N NaOH. Scintillation fluid (Beckman Ready Solv HP) was added to the sample and radioactivity was determined using an external standardization technique to correct for variable quenching of the two isotopes (Beckman Beta LS-5801, Beckman Instruments Inc, Mountain View, CA). The weight of the mucosa in the samples used to measure uptake was determined by multiplying the dry weight of the intestinal sample by the percentage of the intestinal wall comprised of mucosa. The uptake of nutrients was expressed as nmol • 100mg tissue^{-1} • minute^{-1}.

22. Villi height and Crypt Depth Measurements

Intestinal segments were sectioned by staff at the Veterinary Pathology Laboratory (Edmonton, AB). Intestinal villi height and crypt depths were measured under a light microscope using Northern Exposure Image Analysis software (Empix Imaging Inc., ON). A total of 10 recordings were made for each animal and each segment, with the average used for statistical analysis.

23. Obtaining Viable Lymphocytes.

Lymphocytes were obtained from 10 mL peripheral blood by separation by density centrifugation through a 1.077 Ficoll-Hypaque (Sigma Diagnostics, St. Louis, MO) gradient at 450 x g at 22°C for 30 min. The lymphocyte layer was located at the interface of the serum and Ficoll-Hypaque. The cells were gently removed by suction and diluted upto 2 mL with sterile PBS. Once cells were obtained, a 20 µL sample was added with 20 µL of trypan blue and were counted using a hemocytometer under a light microscope. Cell viability was determined using the trypan blue (Sigma Chemical Co., St. Louis, MO) exclusion test by counting cells in which the dye had not permeated the membrane and was not less than 95%.

24. Mononuclear Cell Phenotyping.

Peripheral blood mononuclear cells were characterized by immunofluorescence assay using monoclonal antibodies specific to canine cell surface molecules. Monoclonal antibodies CD4, CD5 and CD14 were purchased from VMRD Inc, Pullman, WA. CD8, CD45R and Ig (G+A+M) were purchased from Serotec Ltd, Toronto, ON, Canada. CD5 (DH3B) recognizes all peripheral T-lymphocytes, CD4 (DH29A) recognizes MHC-II restricted T- lymphocytes (helper/inducer T-cells), CD8 (YCATE55.9) recognizes MHC-I restricted T- lymphocytes (cytotoxic / suppressor T-cells), CD14 (CAM36A) recognizes monocytes, CD45R (YKIX753.22) is a maturity marker for all T-lymphocytes and IgG_1 (K992E3) recognizes the immunoglobulins IgG + IgA + IgM on canine B-lymphocytes. Aliquots of 5 x 10^5 lymphocytes from individual dogs were incubated for 20 minutes at 4°C with each antibody, washed three times in 200 µL of phosphate buffered saline containing fetal calf serum (40 g/L), and incubated for another 20 minutes at 4°C in 50 µL of a 1:300 dilution of fluoroscein isothiocyanate (CedarLane® Laboratories Limited, Hornby, ON, Canada). Cells were washed three times and fixed in phosphate buffered saline containing paraformaldehyde (10 g/L) and relative fluorescence was measured using a FACScan® (Lysis II, Becton-Dickinson, Sunnyvale, CA). Background fluorescence (1-3%) was determined by incubating the cells with fluoroscein isothiocyanate only. For double label immunofluorescence, cells were washed three more times in PBS after the addition of fluoroscein isothiocyanate and then a second antibody was added. Cells were washed three times in PBS and were incubated in 10 µl of a 1:25 dilution of R-phycoerythrin (CedarLane® Laboratories Limited, Hornby, ON, Canada). Background fluorescence was measured by incubating the lymphocytes with both fluoroscein isothiocyanate and R-phycoerythrin. Relative fluorescence was measured using a FACScan®.

25. Natural Killer (NK) Cell Cytotoxicity.

NK cell cytotoxicity was measured using a 4 h ^{51}Cr release assay. NK sensitive Canine Thyroid Adenocarcinoma cells (CTAC) were a generous gift from Dr. Sandmaier,

Seattle, WA. CTAC were incubated with 18.5 MBq ^{51}[Cr] sodium chromate / well (Amersham Canada, Oakville, ON, Canada) and seeded into 96 well v-bottom microtiter plates (Becton-Dickinson Labware, Lincoln Park, NJ). Lymphocytes were added in triplicate to the wells to achieve effector:target ratios between 10:1 and 150:1. Following a 4 h incubation at 37 °C, plates were centrifuged at 157 x g and an aliquot of the supernatent (75 µL) was counted in a Gamma counter (Beckman Gamma 8000®, Beckman Instruments Inc, Mississauga, ON, Canada) to determine the extent of target cell lysis. Spontaneous release was determined from target cells incubated in the absence of effector cells. Maximum release was determined from detergent lysis (1:10 Triton-X 100, BDH Chemicals, Toronto, ON) of labelled target cells. Cytotoxicity was determined as follows:

$$\% \text{ Specific lysis} = 100 \times \frac{\text{(experimental release - spontaneous release)}}{\text{(experimental release - spontaneous release)}}$$

26. Mitogenic Responses of immune Cells.

Peripheral lymphocytes (2 x 10^5 cells / well) were cultured in 96 well microtiter plates (Corning Glass Works, Corning, NY) without mitogen or with either 5 mg / L Concanavalin A (ICN, Montreal, Que, Canada), 5 mg / L Phytohemagglutinin (ICN, Montreal, Que, Canada PHA), 55 mg / L pokeweed mitogen (Sigma Chemical Co., St. Louis, MO) or 40 µg / L Phorbol Myristate Acetate (ICN, Montreal, Que, Canada) plus 0.5 µg / L ionomycin (Sigma Chemical Co., St. Louis, MO) for 48 and 72 hours. Cells were incubated in humidified 5% CO_2 atmosphere at 37°C. Six hours before harvesting the cells, each well was pulsed with 37 KBq [^{3}H] thymidine (Amersham Canada, Oakville, ON, Canada). Cells were harvested on glass fiber filters using a multiwell harvester (Skatron, Lier, Norway) and counted using Ecolite® (ICN, Montreal, Canada) in a Beckman betacounter (LS 5801®, Beckman Instruments Inc., Mississauga, ON,

Canada). All assays were performed in triplicate and stimulation indices (SI) were calculated as follows:

$$SI = \frac{([^3H]\text{thymidine SC}^1 - [^3H]\text{thymidine UC})^2}{[^3H]\text{thymidine UC}^2}$$

[1][^{3}H] thymidine SC = thymidine incorporation of stimulated cells measured in kBq/min

[2][^{3}H] thymidine UC = thymidine incorporation of unstimulated cells measured in kBq/min

27. Statistical Analysis.

All statistical analyses were performed using the Statistical Analysis System (SAS) statistical package (version 6.10, SAS Institute, Cary, NC). For proglucagon and SGLT-1 mRNA abundance, and SGLT-1 and GLUT2 transporter abundance, data was analyzed using proc GLM and significant differences were identified by one-way ANOVA. The model included diet, gel, period, group and diet•period. Both period and diet•period were found to be non-significant and subsequently excluded. Villi height, crypt depth and intestinal GLP-1 concentrations were analyzed using proc GLM and the one-way ANOVA which included diet and group. Again both period and diet•period were non-significant and excluded from the model. Plasma AUC for GLP-1, insulin and glucose were analyzed using paired T-tests within proc GLM. Repeated measures ANOVA was used to analyze for differences between animal weights, cell phenotypes, lymphocyte proliferation and NK cytotoxicity, and effect of period of feeding was tested but not significant ($p > 0.05$). Differences between diets were identified by least squares means for cell phenotype, lymphocyte proliferation and NK cytotoxicity data. Data presented are means $\pm$ SEM. Significant differences were identified when $p < 0.05$.

III. RESULTS

1. Effect of diet on body weight.

Energy requirements were individually calculated and dietary portions were adjusted accordingly such that animal weights did not differ by experimental diet (23.4 ± 1.8 kg, 22.9 ± 1.8 kg, 23.5 ± 1.8 kg for pre-experimental, HFF and LFF respectively, **Figure 5**, $p > 0.05$) or by period (23.4 ± 1.8 kg, 23.4 ± 1.8 kg, 23.4 ± 1.8 kg for day 7, 21 and 35 respectively, $p > 0.05$).

2. Effect of OGTT on plasma GLP-1, insulin and glucose.

Plasma GLP-1 concentrations were higher ($p < 0.05$) at 30 and 90 min for dogs when fed the HFF diet vs the animals fed the LFF diet (**Figure 6a**). Insulin concentrations were statistically higher ($p < 0.05$) at 90 min for dogs when fed the high fermentable fiber (HFF) diet vs dogs fed the low fermentable fiber (LFF) diet (**Figure 6b**). Dietary fiber type did not influence blood glucose concentrations at any time points during the OGTT (**Figure 6c**). The incremental area under the curve was significantly higher for GLP-1 (**Figure 7a**, 988 ± 92 vs 648 ± 92 pmol / L*h, $p \leq 0.05$) and insulin (**Figure 7b,** 15781 ± 1371 vs. 11209 ± 1371 pmol / L*hr, $p < 0.05$) for dogs fed the HFF diet vs LFF diet. The area under the curve for glucose was significantly lower for dogs fed the HFF diet vs LFF diet (219 ± 22 mmol / L*hr vs 291 ± 22 mmol / L*hr, $p \leq 0.05$, **Figure 7c**).

3. Effect of diet on intestinal proglucagon and GLP-1 concentration.

Consumption of HFF vs LFF resulted in significantly greater proglucagon mRNA abundance in the ileum (1.13 ± 0.04 vs. 0.83 ± 0.04 densitometer units, $p < 0.001$) and the colon (1.45 ± 0.05 vs. 0.78 ± 0.05 densitometer units, $p \leq 0.01$) (**Figure 8**). Proglucagon mRNA expression was not detected in the duodenum. Of the three intestinal samples analyzed, only the ileal mucosal scrapings were significantly higher in GLP-1 for dogs fed the HFF diet vs LFF diet (41 ± 4 pmol GLP-1 / mg protein vs. 25 ± 4 pmol GLP-1 / mg protein, $p < 0.05$), (**Figure 9**). GLP-1 concentrations in whole ileum tended

to be higher in dogs fed the HFF diet (43 $\pm$ 4 pmol GLP-1 / mg protein vs 33 $\pm$ 4 pmol GLP-1 / mg protein, $p = 0.09$). There was no significant effect of diet on colonic GLP-1 concentrations (40 $\pm$ 7 pmol GLP-1 / mg protein vs. 36 $\pm$ 7 pmol GLP-1 / mg protein, $p > 0.05$).

4. Histology.

Dietary effects on intestinal villi height and crpyt depth are shown in **Figure 10.** Duodenal villi height tended to be higher on the HFF diet (1505 $\pm$ 83 vs 1294 $\pm$ 83 μm, $p = 0.096$), and there were no significant differences in duodenal crypt depth (289 $\pm$ 28 vs 262 $\pm$ 28 μm, $p = 0.488$). Jejunal villi height was significantly higher on the HFF diet vs LFF diet (1517 $\pm$ 43 vs 1343 $\pm$ 43 μm respectively, $p \leq 0.05$) but no significant differences were noted in crypt depth (277 $\pm$ 19 vs 234 $\pm$ 19 μm, $p = 0.142$). Ileal villi height and crypt depth were not significantly different on either HFF or LFF diet (1035 $\pm$ 45 vs 993 $\pm$ 45 μm, $p = 0.517$ and 251 $\pm$ 46 vs 357 $\pm$ 46 μm, $p = 0.125$ respectively). Colonic crypt depth was not significantly different (724 $\pm$ 33 vs 727 $\pm$ 33 μm, $p = 0.943$, HFF vs LFF respectively).

5. Nutrient uptake.

The effect of dietary fiber source on nutrient uptake is shown in **Table 4.** Consumption of HFF resulted in a significantly higher Vmax for D-glucose uptake in the jejunum ($p \leq 0.05$). This is also represented in **Figure 11.** A significant diet effect was also noted in fatty acid-12 uptake in the jejunum ($p \leq 0.05$). The Michaelis affinity constant (Km) was not affected by diet ($p > 0.05$). The estimation of paracellular D-glucose uptake, as determined by L-glucose uptake at 16 mM normalized to 1 mM, was not significantly affected by diet ($p > 0.05$). Kd for D-fructose was not affected by diet ($p > 0.05$).

6. Glucose Transporters.

Diet did not affect SGLT-1 mRNA in any of the intestinal segments measured (**Figure 12,** $p > 0.05$). The consumption of HFF vs LFF was associated with higher jejunal SGLT-1 transporter abundance (22.2 ± 3.7 vs 6.6 ± 3.7 densitometer units, $p \leq 0.01$). SGLT-1 transporter abundance tended to be higher in the ileum (13.4 ± 0.7 vs 10.4 ± 0.7 densitometer units, $p = 0.09$, **Figure 13**). Significant differences due to diet are seen in both jejunum and ileum GLUT2 transporter abundance (**Figure 14**) which showed an increase with consumption of HFF (1.9 ± 0.2 vs. 0.9 ± 0.1 densitometer units $p \leq 0.05$ and 4.2 ± 0.2 vs. 1.5 ± 0.2 densitometer units $p \leq 0.01$, respectively).

7. Hematology.

Blood hematology (**Table 5a**) and chemistry (**Table 5b**) were not found to be significantly different ($p > 0.05$) by diet.

8. Peripheral mononuclear cell phenotypes.

The relative proportion of peripheral mononuclear cell phenotypes identified using anti-rat and anti-mouse monoclonal antibodies was significantly affected by diet (**Figure 15**). The proportion of CD5 bearing cells were significantly ($p < 0.01$) lower in pre-experimental diet fed animals. IgG+A+M bearing cells were significantly higher in LFF fed animals vs HFF ($p < 0.05$) and tended to be higher vs pre-experimental diet fed animals ($p = 0.06$). The proportion of cells bearing the CD4 marker were significantly elevated in the HFF diet vs pre-experimental diet fed animals ($p < 0.001$) and were also significantly elevated in the LFF diet vs pre-experimental diet fed animals ($p < 0.05$). This significant elevation of % CD4 bearing cells was found to consist of normal CD4+CD8- cells determined by double label immunofluorescence (data not shown). As a result of this elevation of CD4 bearing cells in the HFF diet, there was a subsequent significant increase in the CD4:CD8 ratio over the LFF and the pre-experimental diet fed animals ($p \leq 0.01$). Lastly, % CD14 bearing cells were significantly lower in the pre-experimental diet fed animals vs HFF ($p < 0.01$) and LFF ($p < 0.05$) diet fed animals.

9. Cell proliferation by mitogenic stimulation.

Incubation of peripheral lymphocytes with pokeweed mitogen significantly depressed ^{3}[H]-thymidine incorporation at both 48 and 72 h for both HFF and LFF diets when compared to the pre-exp diet (**Figure 16**, $p < 0.05$). Diet did not significantly ($p > 0.05$) affect all other mitogen-induced stimulation indeces ($p > 0.05$) for both 48 and 72 h incubations.

10. Natural Killer (NK) cell cytotoxicity.

Peripheral NK cell specific lysis between the 3 diets is illustrated in **Figure 17** and was found to be significantly affected by diet. Effector : target ratios ranging from 50:1 to 100:1 were not significantly different between pre-experimental and HFF diets ($p > 0.05$). However, values at these ratios were found to be significantly lower in the LFF diet vs pre-experimental ($p < 0.05$).

IV. Discussion

The ingestion of high fiber diets has been shown to modulate proglucagon gene expression and postprandial GLP-1 secretion (Reimer & McBurney, 1996). Recently, Reimer (1997) reported that proglucagon mRNA abundance was increased in rats fed diets containing fermentable fiber sources. This study suggests that the ingestion of fermentable fiber supplemented diets upregulates intestinal proglucagon gene expression to modulate intestinal glucose transport capacity and GLP-1(7-36)NH_2 secretion. Fiber type also modulates the proportion of lymphocytes in peripheral blood.

The GLP-1 antibody used in the radioimmunoassay in this study is specific for the c-terminally amidated GLP-1 isomers. Therefore, it does not distinguish between GLP-1(7-36)NH_2, GLP-1(9-36)NH_2 or GLP-1(1-36)NH_2. However, an increase in insulin secretion was seen in this study, and the only two GLP-1 isomers which are known to be insulinotropic are GLP-1(7-36)NH_2 and GLP-1(7-37) (Suzuki et al., 1989). Therefore it can be assumed that there was an increase in GLP-1(7-36)NH_2 secretion. The canine plasma GLP-1(7-36)NH_2 concentrations measured in this study are in the same range reported previously in dogs (Wen et al., 1993) and humans (Vaag et al., 1996).

GLP-1 has been proposed to be an antidiabetogenic agent because of its biological functions as an inhibitor of gastric acid secretion and emptying (Willms et al., 1996; Nauck et al., 1996; Wettergren et al., 1994; Layer et al., 1995; Schlodager et al., 1989), glucagon secretion (Hvidberg et al., 1994; Orskov et al., 1993; Wettergren et al., 1993; Komatsu et al., 1989) and as a potent insulin secretagogue (Ahren et al., 1997; Qualmann et al., 1995; Orskov et al., 1993; Wettergren et al., 1993; Mojsov et al., 1987; Holst et al., 1987). The current study demonstrates that consumption of the HFF diet results in a significant increase in intestinal proglucagon mRNA expression. The incremental area under the curve for plasma GLP-1(7-36)NH_2 was significantly higher after an OGTT, thereby resulting in significantly elevated glucose-induced plasma insulin secretion and consequently, significantly lower plasma glucose concentrations.

In support of these results, Van Dijk et al (1996) reported a significantly reduced rate of glucose appearance and an increased rate of glucose clearance with GLP-1 administration. These effects appear to be mediated by an increased insulin:glucagon ratio and the increased rate of glucose clearance was dependent on insulin (Van Dijk et al., 1996). D'Alessio et al (1995) reported similar findings and attributed the promotion of glucose assimilation to increased insulin secretion.

The mechanism whereby diet modulates proglucagon expression and GLP-1 secretion remains unknown. The L-cell has a pyramid shape with the apical process found in the microvilli of the intestinal lumen and the base, rich in endocrine granules near the basal lamina, suggests that the cell is able to respond to luminal contents with a basal discharge of granular contents (Holst, 1997). However, it is unknown if the L-cell responds directly to nutrients and absorption or if other signals are involved. Colonic infusions of various fibers and SCFA do not affect GLP-1 release in fasted rats (Plaisancie et al., 1995) but SCFA administration in rats receiving parenteral nutriton does (Tappenden et al., 1997a) and this effect is seen within 3 d of SCFA administration (Tappenden et al., 1996). Therefore, the 14 d experimental periods used in this study should have been more than adequate to elicit a response to diet. It was not determined in this study whether there was an increase in L-cell number or in proglucagon expression and GLP-1 production per L-cell. However, Hoyt et al (1996) showed that proglucagon mRNA per cell, as determined by in situ hybridization, increased with refeeding after food was withheld suggesting more proglucagon production and GLP-1 secretion per L-cell.

Long term consumption of fiber-supplemented diets is associated with changes in intestinal motility, mass and length (Bornet 1994; Jacobs 1983; Johnson et al., 1984; Pond et al., 1989; Savory 1992; Sigleo 1984), and the rates of intestinal cell turnover, enterocyte migration along the crypt-villus axis, enterocyte life span, and villus appearance (Brown et al., 1979; Chiou et al., 1994; Johnson et al., 1984). Dogs which consumed the HFF had significantly longer jejunal villi and D-glucose transport capacity. Karasov and Diamond (1983) reported protein mediated transport is predominantly

altered by changes in Vmax. The mechanistic basis for an alteration in the absorption rate of a single nutrient is usually the result of a change in the number of transport sites per enterocyte arising from an altered rate of synthesis or degradation of that transport site (reviewed in Ferraris and Diamond, 1997). Consumption of the HFF diet was significantly associated with upregulated jejunal SGLT-1 and jejunal and ileal GLUT2 glucose transporter levels. Since an effect of both transporter quantity and activity upregulation was seen on the HFF diet, a specific moiety of HFF must be involved in both the non-specific and specific mechanisms of adaptation seen in this study. Intravenous supplementation of SCFA significantly increases ileal D-glucose uptake (Tappenden et al., 1997b). As well, SCFA have been reported to significantly increase functional adaptation by increasing total, mucosal and submucosal weight, and increasing ileal DNA, RNA and protein concentrations (Tappenden et al., 1996; Koruda et al., 1988). GLP-2 which is co-secreted with GLP-1 (Orskov et al., 1986), may also play a role. The biological actions of GLP-2 have only been recently proposed to include an intestinally-derived stimulator of small bowel epithelial proliferation (Drucker et al., 1996; Tappenden et al., 1997a) and a modulator of basolateral glucose uptake (Cheeseman and Tsang, 1996). Since a significant increase in GLP-1 secretion was noted on the HFF diet, a resulting significant increase in GLP-2 may also have occurred. This would also explain the increase in transporter abundance and Vmax seen in dogs which consumed the HFF diet.

The observed improvements in glucose homeostasis in dogs which consumed the HFF diet suggests that enhanced insulin secretion or tissue sensitivity must occur to compensate for greater glucose transport capacity seen with the HFF diet. GLP-1(7-36)NH_2 is known to inhibit gastric emptying (Willms et al., 1996; Nauck et al., 1996; Layer et al., 1995) which would slow glucose delivery to the small intestine where it is transported into the systemic circulation. Thus, GLP-1(7-36)NH_2 mediated effects on gastric emptying and glucose absorption in glucose homeostasis cannot be excluded from this study. In other words, the relative importance of GLP-1(7-36)NH_2 action on gastric

emptying and pancreatic insulin secretion in maintaining glucose homeostasis remains to be determined.

Blood chemistry or total and differential white blood cell counts were not affected by fiber fermentability. The current study reports no diet differences in the absolute numbers of the major white blood cell (WBC) types.

Dogs which consumed the HFF diet had a significantly elevated proportion of CD4+ T-cells, thereby resulting in an elevated CD4:CD8 ratio. This ratio has been used to monitor immune system status (Hansbrough et al., 1984). CD4+ cells generally provide stimulatory signals to other cell subsets (Maes et al., 1992). For example, they co-operate with B-cells in the production of antibodies, they are involved in the maturation of cytotoxic T-cells, and they release cytokines which help macrophages kill microorganisms (Kuby 1994c). CD8+ T-cells include cytotoxic T-cells which play an important role in the elimination of virally infected cells, and suppressor T-cells which suppress the function(s) of other T and B-cells (Janeway and Travers, 1994a). A decreased CD4:CD8 ratio has been associated with an immunosuppressive state (Stagnaro-Green et al., 1992). Specifically, previous studies have reported a parallel loss of innate immunity with decreasing CD4:CD8 ratios (Hanlon et al., 1993), a loss of proliferative T-cell responses (Kneitz et al., 1993) and immunoglobulin secretion (Farrant et al., 1994). Although these other studies were conducted in disease states, the effect of increasing the CD4:CD8 ratio in healthy models is unknown. Despite changing the CD4:CD8 ratio, fiber fermentability did not affect peripheral lymphocyte proliferation or NK cell mediated cytotoxicity. Recently it was shown that fiber type was able to modulate GALT (Lim et al., 1997; Field et al., 1997 submitted).

Although the two experimental diets (HFF and LFF) were similar in composition except for dietary fiber type, the pre-experimental diet was not (**Tables 1a, 1b, 2**) and nutrient intakes were probably different during the pre-experimental period when the dogs were also adapting to their new environment. Dog weights were unaffected by diet or period, and all macronutrients and micronutrients met known nutrient requirements for dogs (Kallfelz 1989). The composition of the pre-experimental chow diet and the

experimental fiber diets were not identical in macronutrient content which can modulate immune function. For example, dietary protein type (Corman 1985; Wong and Watson, 1995; Field et al., 1995), protein concentration (reviewed in Daly et al., 1990) and amino acid concentrations modulate immune responsiveness (reviewed in O'Riordain et al., 1996; Heberer et al., 1996; Sax 1994), as do dietary fat intakes (Kelley and Dauda, 1993). Omega-3 fatty acids, which were higher in the experimental diets suppress lymphocyte proliferation (reviewed in Blok et al., 1996). Dietary vitamin (Chandra 1997; Bendrich 1996; Bendrich 1992) and mineral (Scrimshaw 1990) intakes are known to affect immune function. Since the pre-experimental diet was not balanced with the experimental diets for any of these macronutrients or micronutrients, any or all of these nutrients may have contributed to the differences seen in this study in lymphocyte proliferation and NK cell mediated cytotoxicity. However, no differences in these functions were noted between HFF and LFF supplemented diets which were balanced for all the above nutrients.

Both psychological and physical stress has been reported to alter immune function in both humans (Bartrop et al., 1977; Kiecolt-Glaser et al., 1984) and animals (Keller et al., 1983; Maier and Laudenslager 1988). Anxiety has been reported to suppress immune function (Cunnick et al., 1988; Cunnick et al., 1990) and is a learned response that does not necessarily require an aversive stimulus. This may explain why the pre-experimental diet, which was always the first exposure of the dogs to laboratory conditions, was different in terms of lymphocyte proliferation and NK cell mediated cytotoxicity, whereas the HFF and LFF experimental diets which were randomized to control for order, did not. Stress hormones or glucose tolerance were not measured on Day 0, so the role that stress may have played cannot be determined.

To summarize the immunology data, the fermentability of dietary fiber does not appear to affect lymphocyte proliferative responses to mitogenic stimulation, NK cell mediated cytotoxicity nor total lymphocyte numbers, but it does result in a higher CD4:CD8 ratio in peripheral blood. It is important to realize that only two measures of lymphocyte function were used in this study. Many assays are available to study lymphocyte responses (i.e., cytokine production, macrophage nitric oxide production, B-

cell function) and the effect of dietary fiber type on the outcome of these responses is unknown.

In conclusion, the addition of highly fermentable fiber sources vs low fermentable fibers significantly increases proglucagon mRNA expression, intestinal GLP-1(7-36)NH_2 concentration and pancreatic insulin secretion. The net effect is a lower integrated glucose response to an OGTT in healthy dogs despite an increased capacity to absorb glucose and increased abundance of brush border and basolateral glucose transporters. Dietary fiber type does not affect peripheral lymphocyte proliferation in response to mitogenic stimulation or NK cell cytotoxicity, but the CD4:CD8 ratio was increased with the ingestion of fermentable fibers. Based on these findings in healthy dogs, fermentable fiber may be important in the dietary management of non-insulin dependent diabetes mellitus. Future work should determine if the ingestion of fermentable fibers elicits similar improvements in glucose homeostasis in diabetic individuals with residual pancreatic function and to determine if changes in the CD4:CD8 ratio improves overall health. As well, other assays to assess cell-mediated and humoral immunity should explored.

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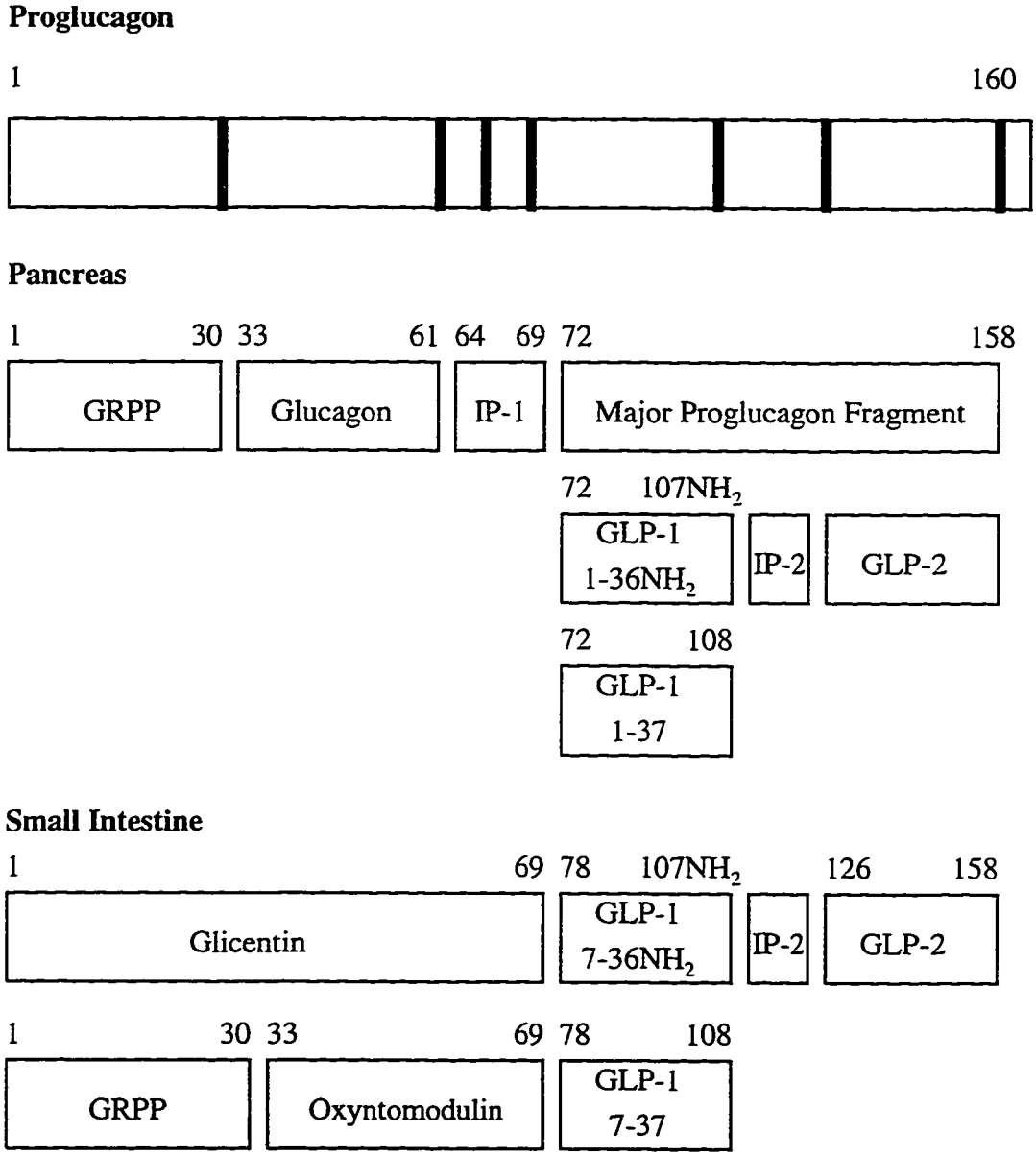

Figure 1. Post-translational processing of proglucagon in the pancreas and intestinal L-cells. Enzymatic cleavage occurs at positions indicated by numbers. Adapted from Fehmann & Habener, 1992.

His - **Ala** - **Glu** - Thr - Phe - Thr - Ser - Asp - **Val** - Ser - **Ser** - Tyr -

Leu - **Glu** - **Gly** - **Ala** - Ala - **Lys** - **Glu** - Phe - **Ile** - **Ala** - Trp - Leu -

Val - **Lys** - **Gly** - **Arg** - NH_2

Figure 2. The amino acid structure of mammalian glucagon-like peptide-1(7-36)NH_2. Amino acids shown in bold occur at the same position in the sequence of glucagon. Adapted from Orskov, 1992.

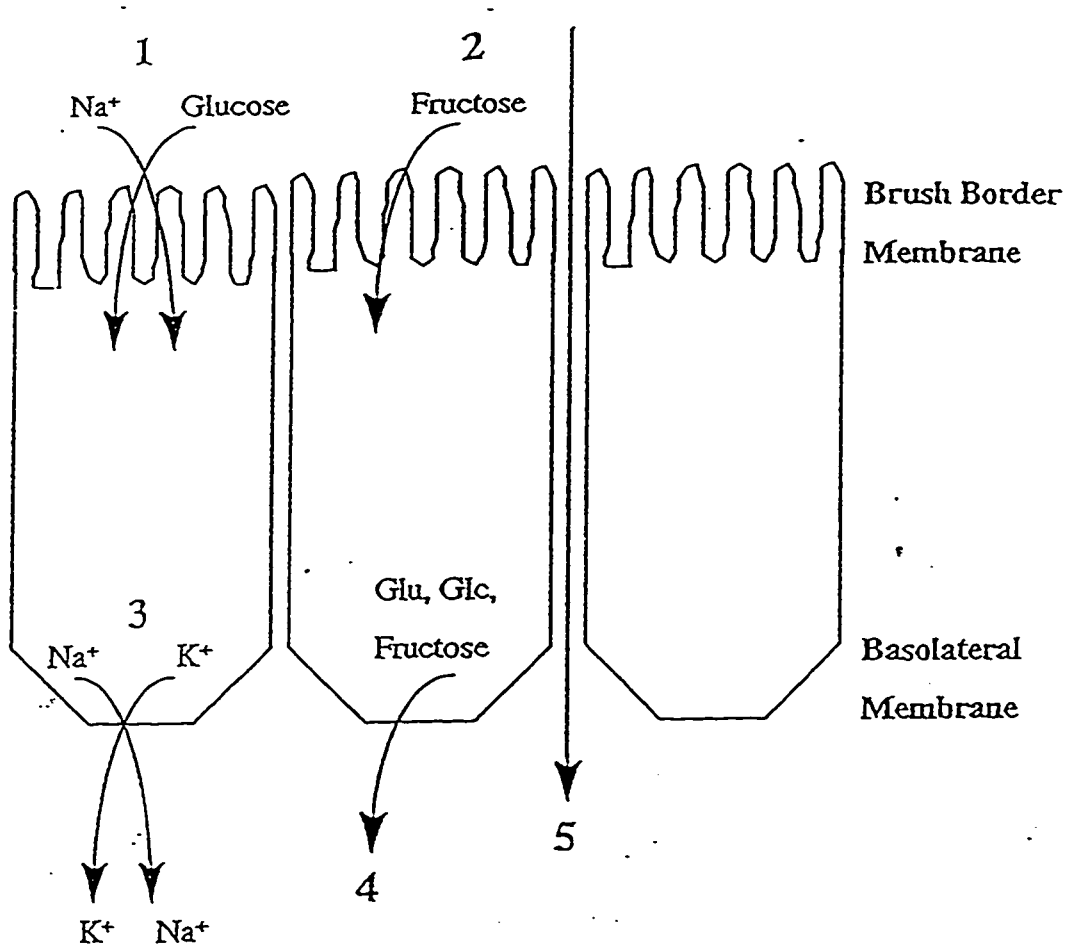

Figure 3. Mechanisms of intestinal hexose transport. 1) Na+–dependent glucose transporter SGLT-1; 2) facilitative glucose transporter GLUT5; 3) Na+K+ ATPase; 4) Na+–independent glucose transporter GLUT2; 5) paracellular route of absorption. Adapted from Philpott et al., 1992.

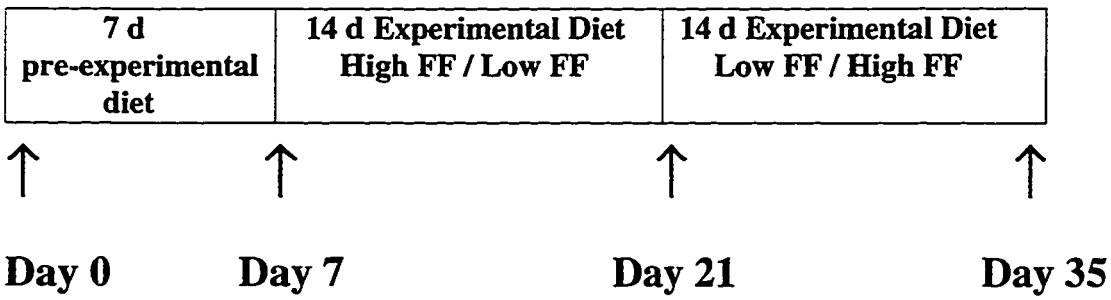

Figure 4. Experimental design illustrating the feeding regime for dogs (n=16). After food was withheld for 16 h, oral glucose tolerance tests were conducted on days 21 & 35 providing 2 g glucose / kg BW and samples taken at 0, 15, 30, 45, 60, 90 and 120 min glucose, insulin and GLP-1 concentrations. Blood samples were obtained for hematological and immune data on days 7, 14 and 28 at 0 min.

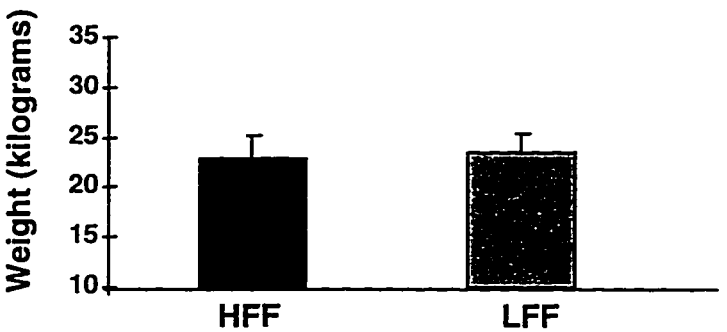

Figure 5. Effect of diet on dog weight. Each bar represents the means ± SEM of 16 dogs. No significant difference between diet was found ($p > 0.05$) as determined by repeated measures anova. HFF = high fermentable fiber, LFF = low fermentable fiber.

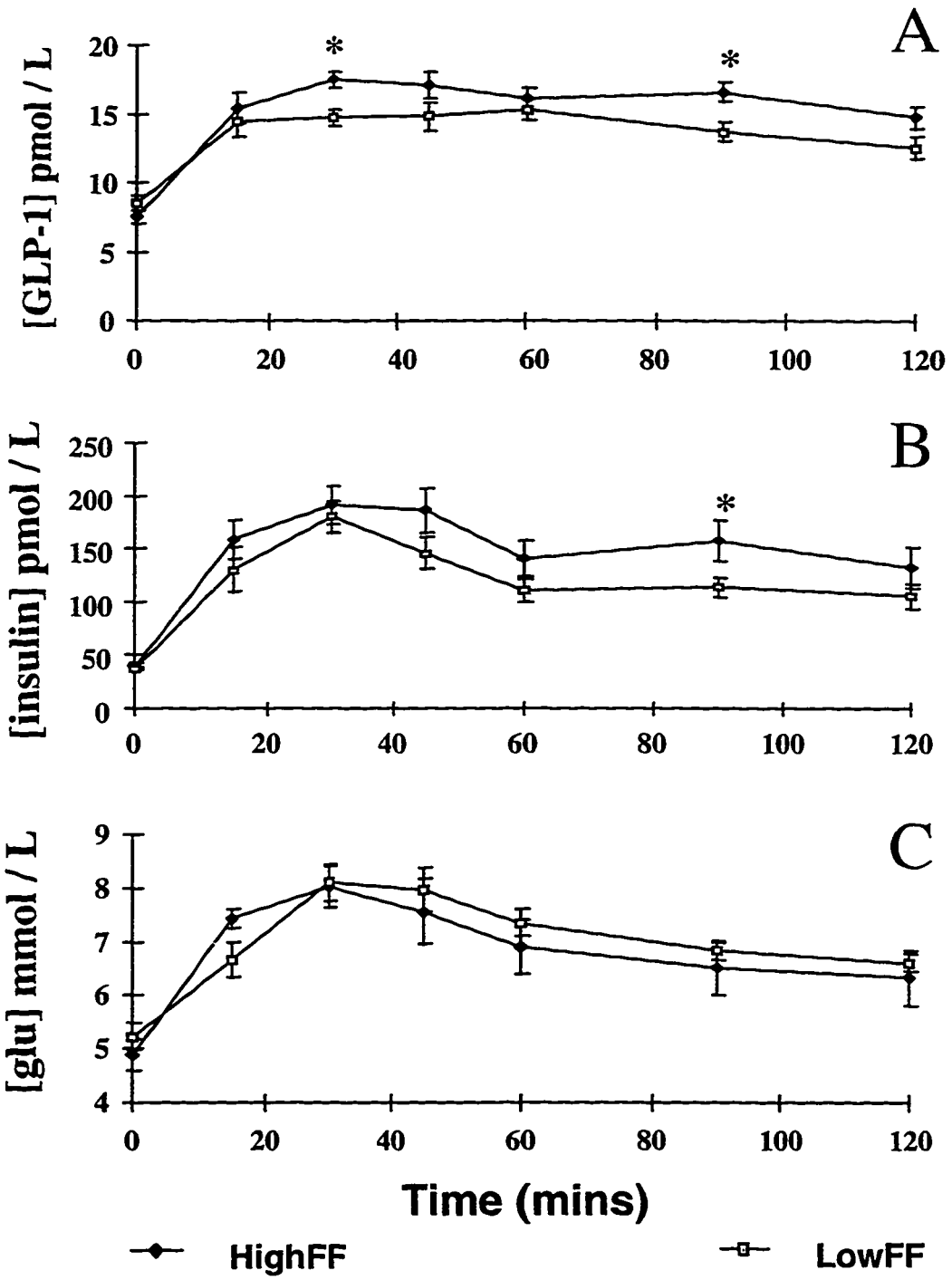

Figure 6. Plasma GLP-1 (A), insulin (B) and glucose (C) concentrations in dogs after an OGTT. Peripheral blood samples were taken at 0, 15, 30, 45, 60, 90 and 120 min after an overnight fast followed by an oral glucose tolerance test supplying 2g glucose / kg BW. Values are means ± SEM, n = 13 / diet (glucose), n = 14 / diet (insulin and GLP-1). Significantly different time points ($p < 0.05$) are indicated by " * ". HFF = high fermentable fiber, LFF = low fermentable fiber.

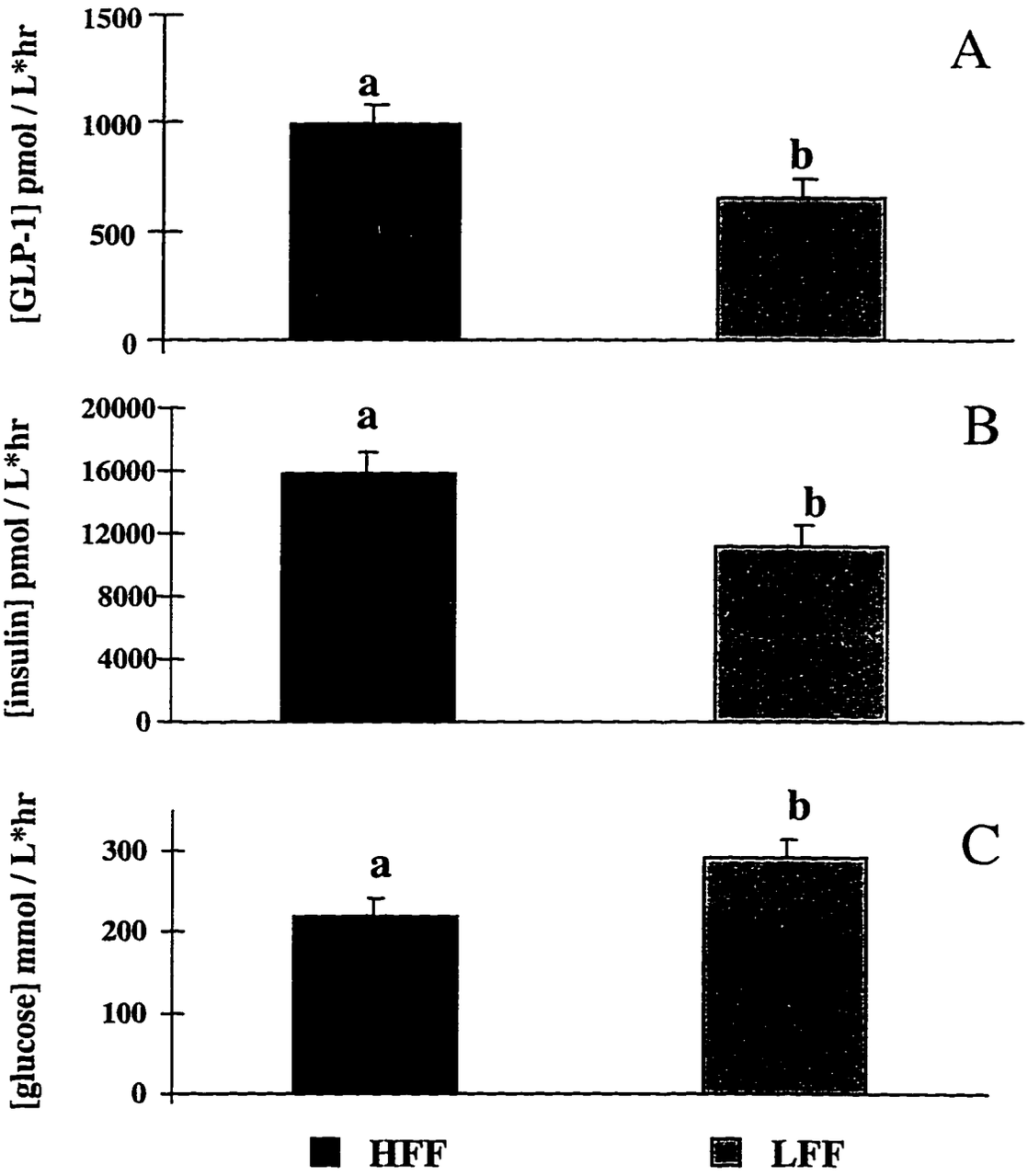

Figure 7. Incremental area under the curve for plasma (A) GLP-1, insulin (B) and glucose (C) in dogs after an OGTT. Samples were obtained after an overnight fast followed by an oral glucose tolerance test (OGTT) supplying 2 g glucose / kg BW. Peripheral blood was collected at 0, 15, 30, 45, 60, 90 and 120 min. Values are means ± SEM. n = 13 / diet (glucose) and n = 14 / diet (insulin and GLP-1). Bars with different letters are significantly different ($p < 0.05$). HFF = high fermentable fiber, LFF = low fermentable fiber.

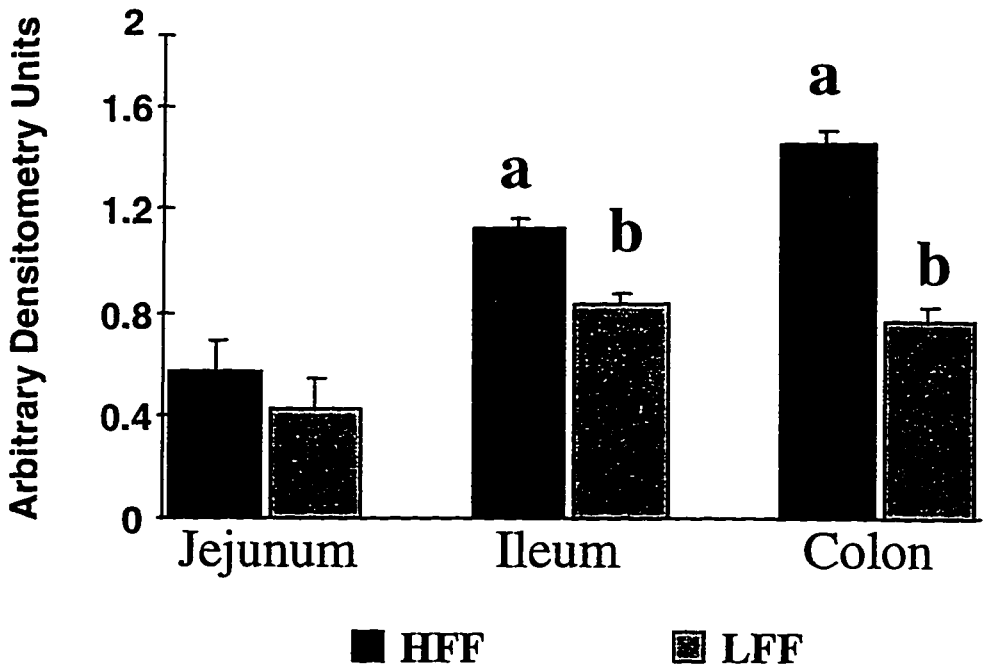

Figure 8. Effect of diet on intestinal proglucagon mRNA.
Values are means ± SEM, n=16 / diet. Bars with different letters are significantly different ($p<0.05$). Bars are not comparable between intestinal sections. Each lane was loaded with 15 ug of total RNA. HFF = high fermentable fiber, LFF = low fermentable fiber.

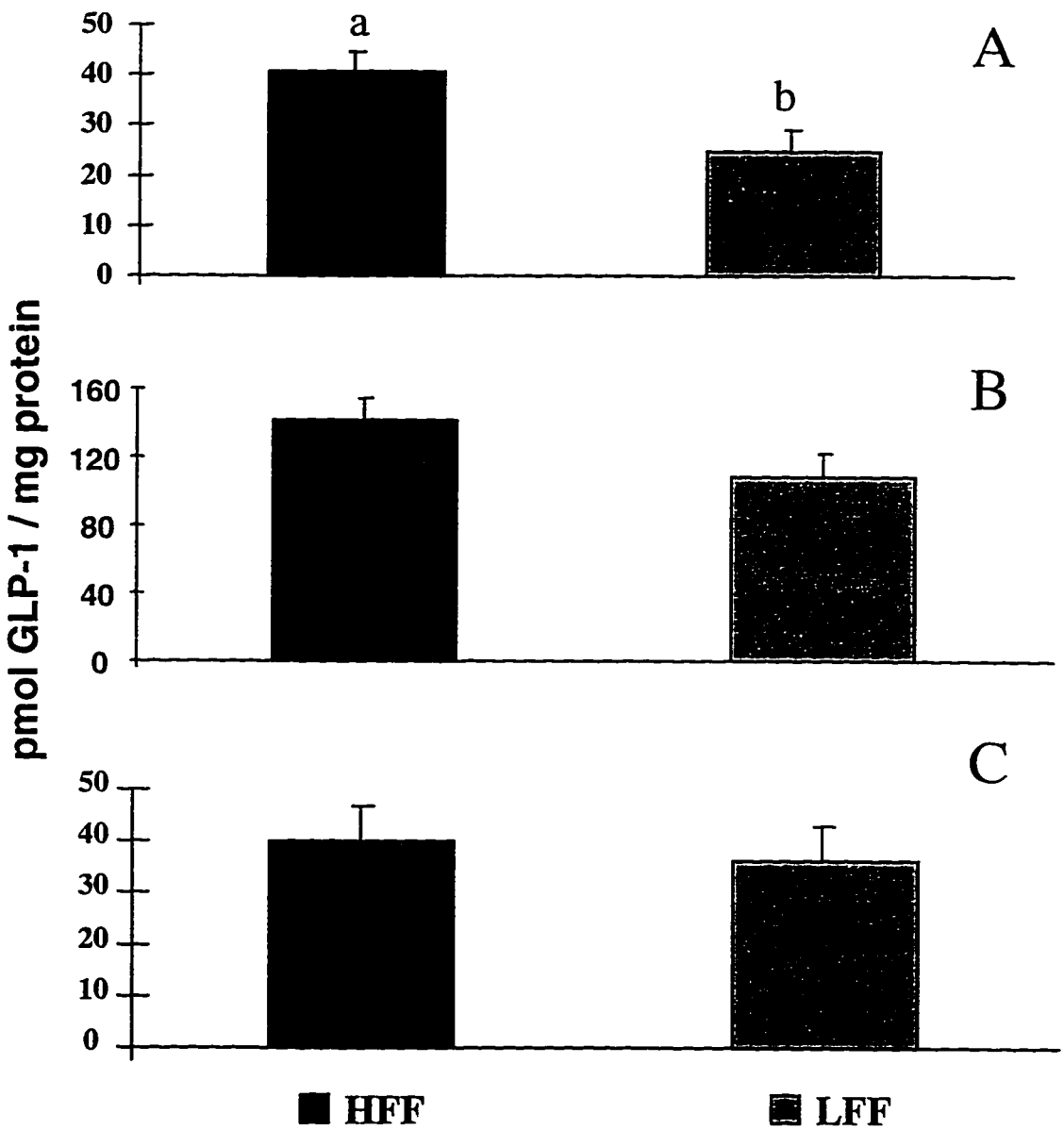

Figure 9. Effect of diet on ileal mucosal scrapings (A), whole ileum (B) and colonic (C) GLP-1(7-36)NH_2 concentrations after an OGTT in dogs. Values expressed are means ± SEM, n = 8 / diet (scrapings and whole ileum), n = 7 / diet (colon). Units are in pmol GLP-1 / mg protein. Bars with differing letters are significantly different ($p < 0.05$). HFF = high fermentable fiber, LFF = low fermentable fiber.

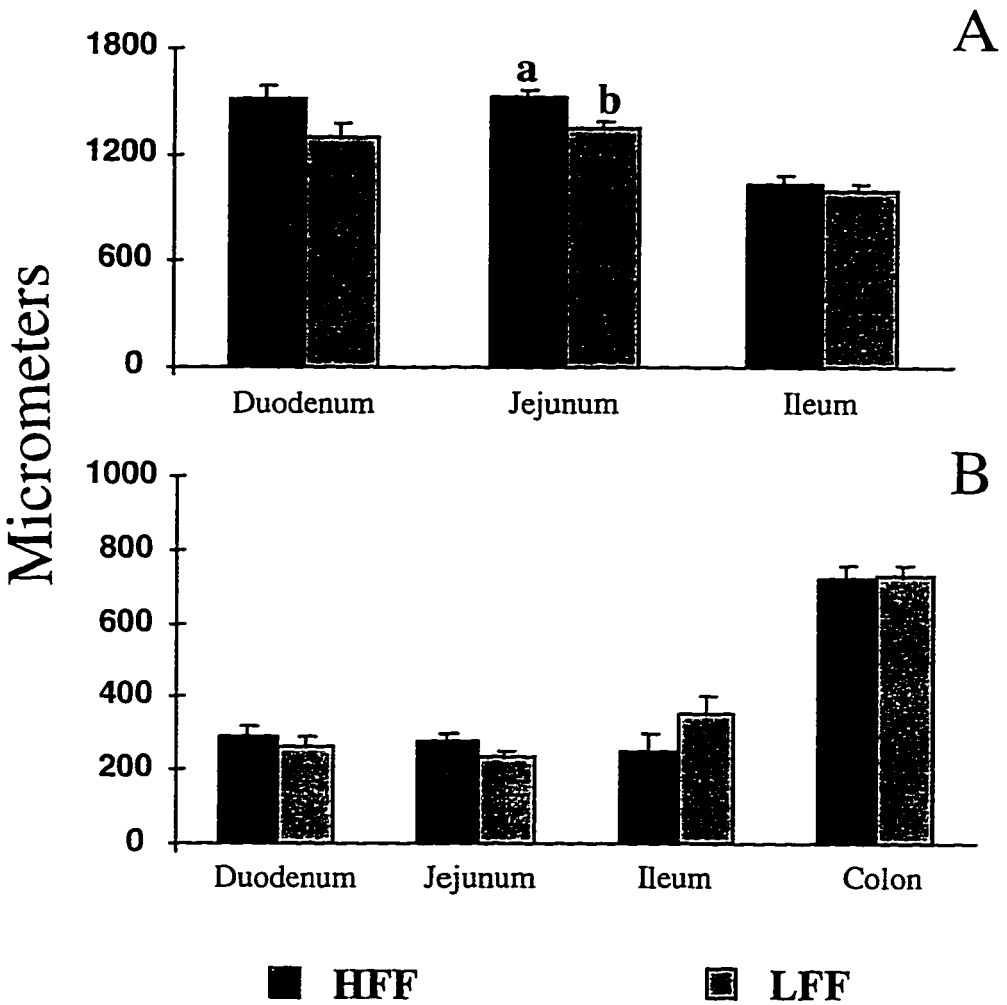

Figure 10. Villi height (A) and crypt depth (B) in canine intestinal sections. Values are in micrometers and are mean ± SEM, n = 8 / diet. Villi measured / animal / section (n=10) were averaged to deduce a single value, and those values were averaged for each diet value. Values with different letters are significantly different ($p \leq 0.05$). HFF = high fermentable fiber, LFF = low fermentable fiber.

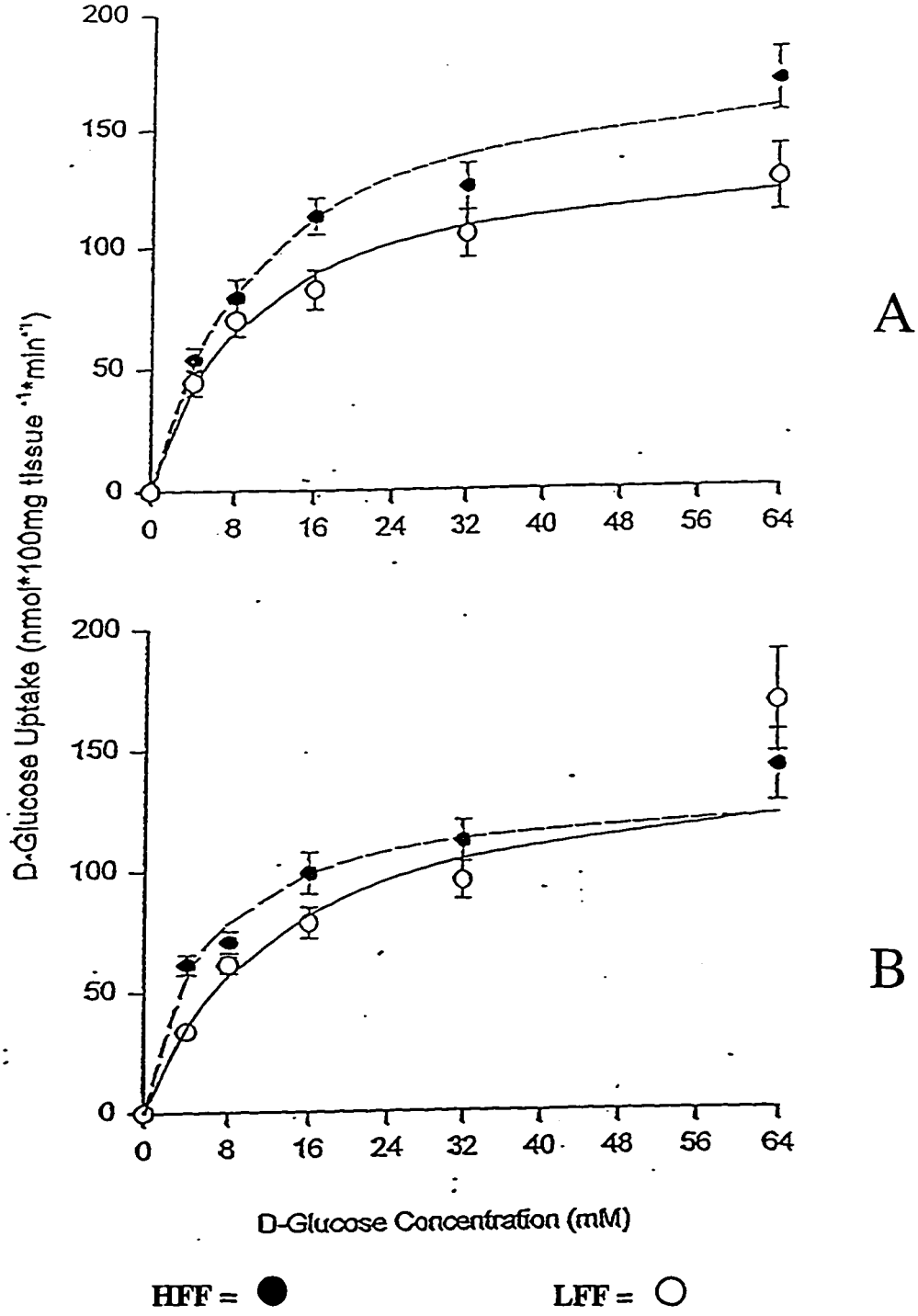

Figure 11. The effect of diet on D-glucose uptake in jejunum (A) and ileum (B) in dogs. Values are means ± SEM, n = 8 / diet. HFF = high fermentable fiber, LFF = low fermentable fiber.

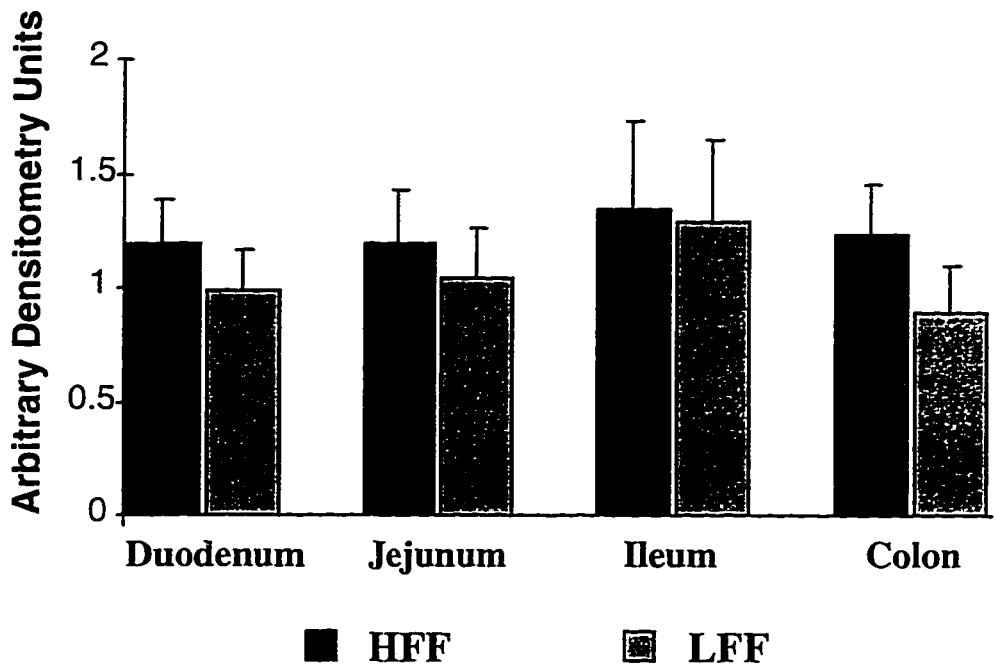

Figure 12. Effect of diet on intestinal SGLT-1 transporter mRNA. Values are means ± SEM (n = 16 dogs per dietary treatment) and are measured as relative abundance using arbitrary densitometry units. No significant differences between diets was observed. Each lane was loaded with 15 ug of total RNA. HFF = high fermentable fiber, LFF = low fermentable fiber.

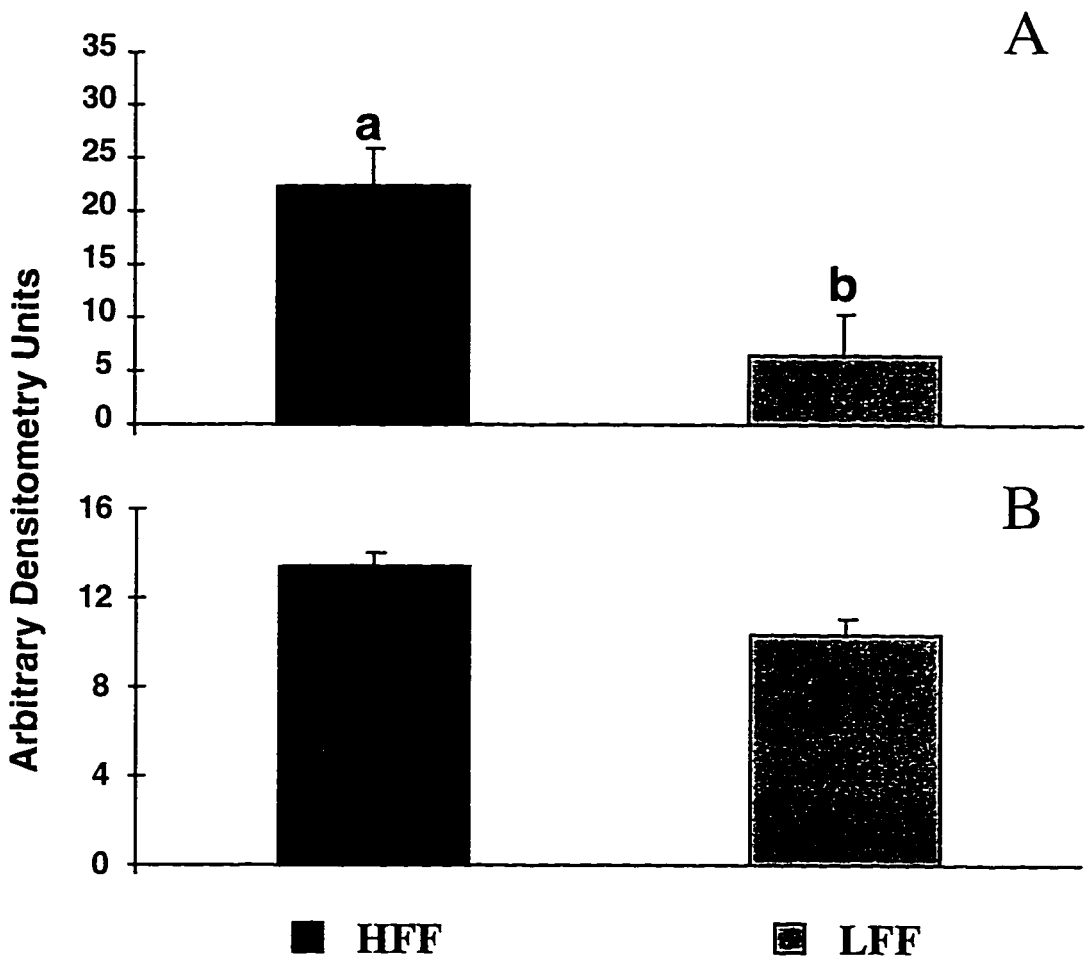

Figure 13. Effect of diet on jejunal (A) and ileal (B) SGLT-1 transporter abundance in dogs. Values are means ± SEM. n = 7 / diet (ileum) and n = 6 / diet (jejunum). Bars with different letters are significantly different ($p<0.05$). Each well was loaded with 60 ug of total protein and membranes were stained with ponceau S to confirm equal protein loading. HFF = high fermentable fiber, LFF = low fermentable fiber.

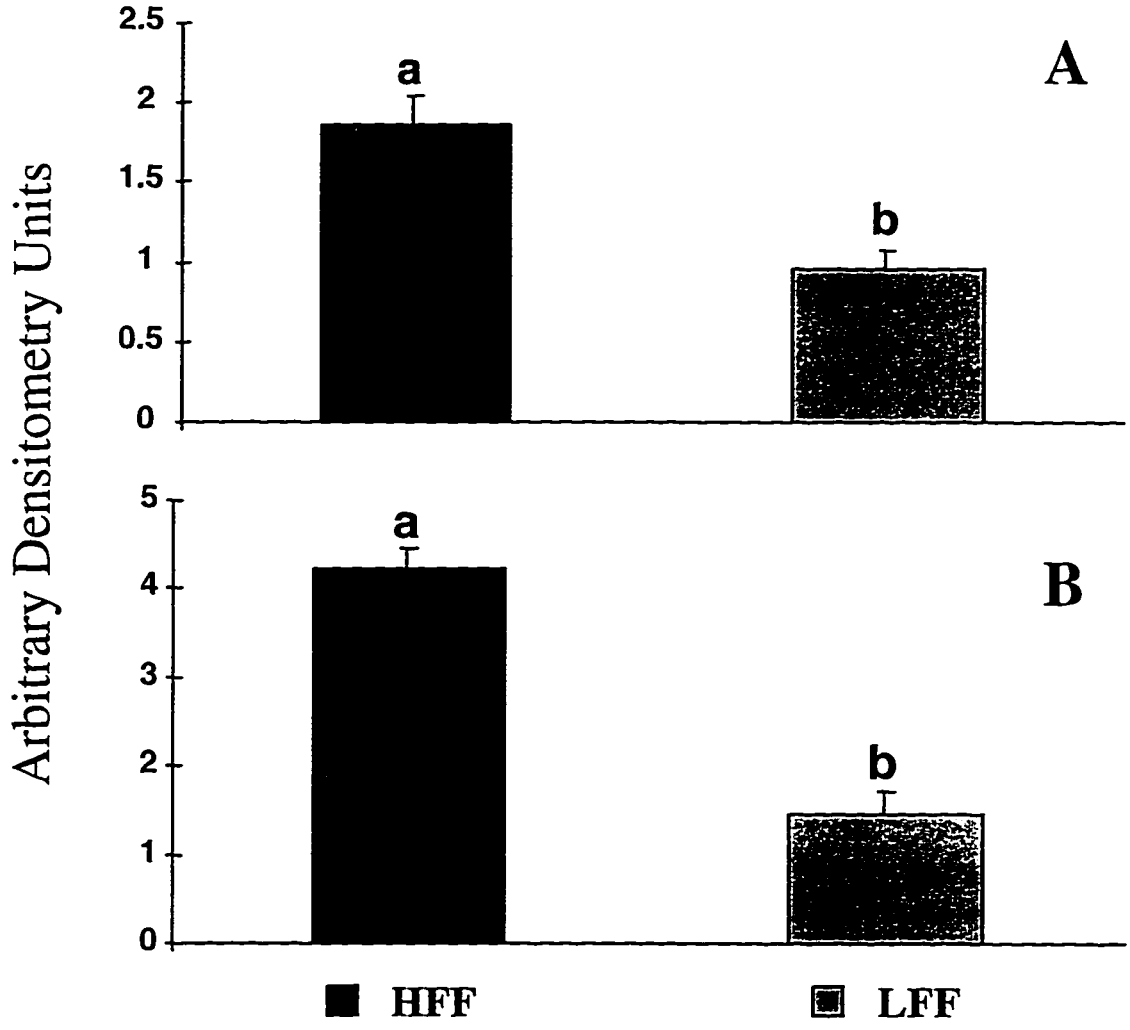

Figure 14. Effect of diet on intestinal GLUT2 transporter abundance in jejunum (A) and ileum (B) in dogs. Values are means ± SEM, n = 6 / diet (jejunum, ileum HFF) and n = 7 / diet (ileum LFF). Bars with different letters are significantly different ($p<0.05$). Each well was loaded with 60 ug of total protein and membranes were stained with ponceau S to confirm equal protein loading. HFF = high fermentable fiber, LFF = low fermentable fiber.

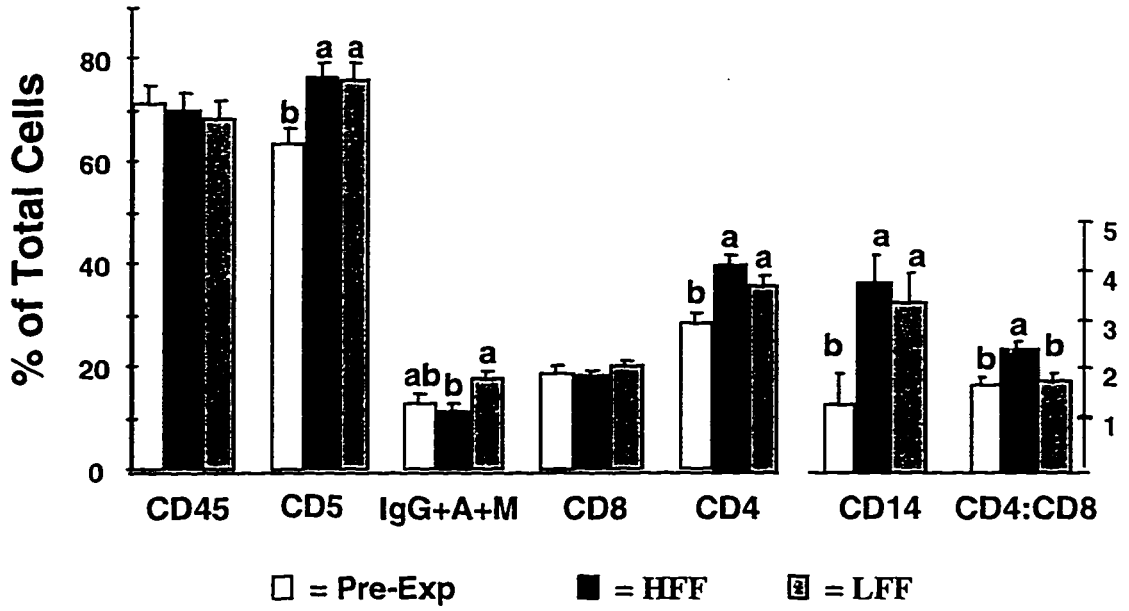

Figure 15. Effect of diet on distribution of mononuclear cell phenotypes in peripheral blood. Values are means ± SEM and are expressed as % cells as measured by relative fluorescence. n = 12 / diet (CD45, CD8, CD4, CD4:CD8), n = 11 / diet (IgG+A+M), n = 10 / diet (CD14). For each antibody, bars with different letters are significantly different ($p<0.05$). HFF = high fermentable fiber, LFF = low fermentable fiber.

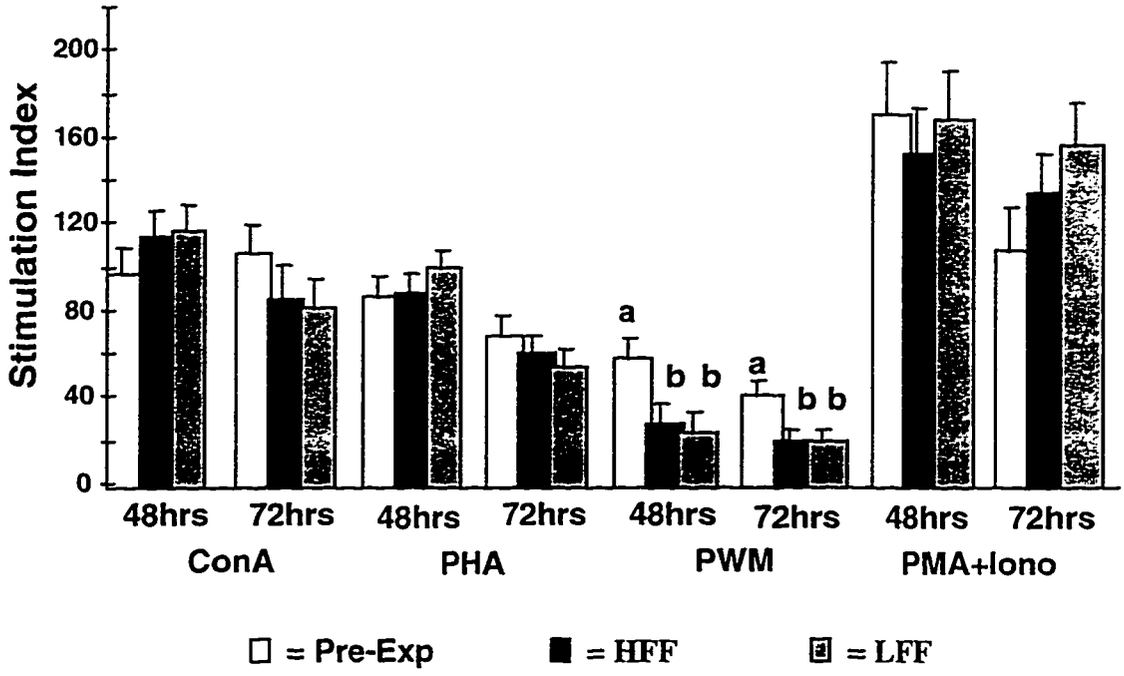

Figure 16. Effect of diet on mitogenic response of peripheral lymphocytes from dogs. Values are means ± SEM. n = 11 / diet for all groups. Mitogenic response is expressed as the stimulation index = (amount of [^{3}H] thymidine incorporated by stimulated cells - amount of [^{3}H] thymidine incorporated by unstimulated cells) / amount of [^{3}H] thymidine incorporated by unstimulated cells. Cells were incubated with mitogens for 48 and 72 hr. Bars with different letters are significantly different ($p<0.05$) within a mitogen*time interaction. ConA = concanavalin A, PHA = phytohemataggluinin, PWM = pokeweed mitogen, PMA = Phobol Myrsitate Acetate, Iono = ionomycin, HFF = high fermentable fiber, LFF = low fermentable fiber.

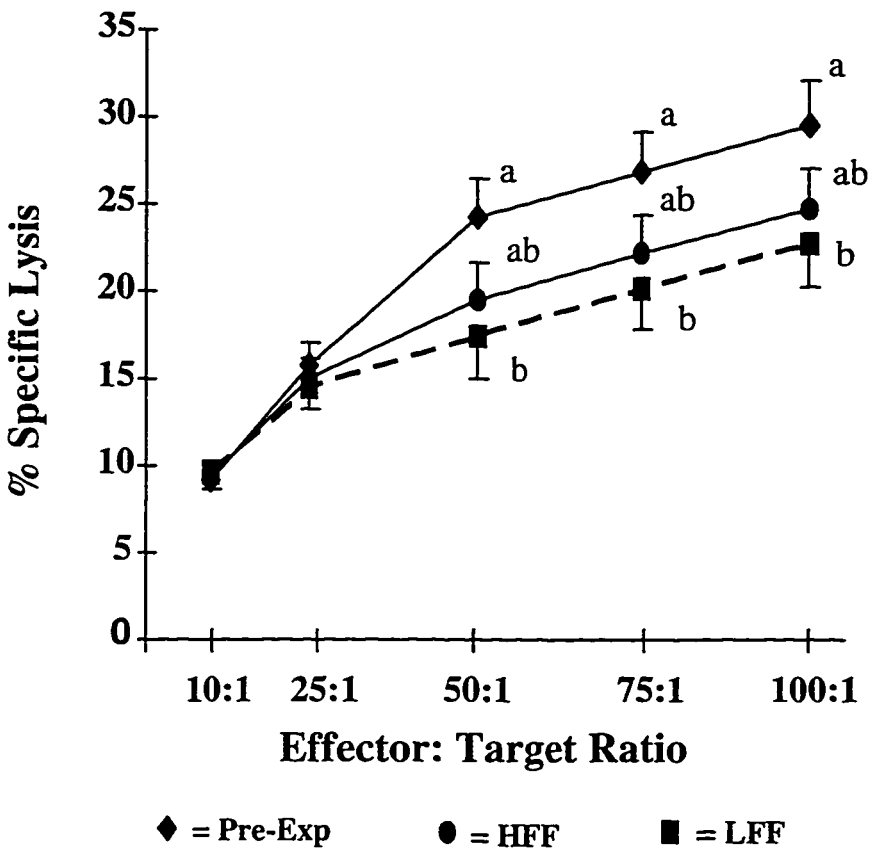

Figure 17. The effect of diet on NK cell specific lysis in dogs as measured by repeated measures. Values are means ± SEM. n = 16 / diet (HFF 50:1 75:1 100:1, LFF 50:1, pre-experimental 50:1), n = 15 / diet (LFF 75:1 100:1, pre-experimental 75:1), n = 13 / diet (pre-experimental 100:1). Different letters at a time point indicate significantly different ($p \leq 0.05$) effects. HFF = high fermentable fiber, LFF = low fermentable fiber.

	Low Fermentable Fiber (g/kg diet as fed)	High Fermentable Fiber (g/kg diet as fed)
poultry by-product meal	460	460
poultry fat	164	164
fishmeal	122	121
pre-gelled cornstarch	110	80
Menhaden oil	3	3
dried whole egg	40	40
Biodigest[1]	25	25
IAMS vitamin premix[2]	3.2	3.2
IAMS mineral premix[3]	2.4	2.4
cellulose	70	---
beet pulp	---	60
gum arabic	---	20
fructooligosaccharides	---	15
Potassium chloride	2.2	2.1
Calcium chloride	1.9	1.1
Choline chloride	1.1	----
Sodium chloride	0.3	0.3

Table 1a. Ingredient composition of experimental diets.

[1]Biodigest™ is a palatability enhancer.

[2]Vitamin premix provided the following per kg diet: 25 KIU vitamin A, 124 IU vitamin E, 1561 IU vitamin D_3, 14 mg thiamin, 59 mg riboflavin, 90 mg niacin, 32 mg d-pantothenic acid, 10 mg pyrodoxine, 0.6 mg biotin, 1.9 mg folic acid, 2 067 mg choline, 23 mg inositol, 0.31 vitamin B_{12}.

[3]Mineral premix provided the following per kg diet: 41 mg manganese, 217 mg zinc, 168 mg iron, 47 mg copper, 4 mg iodine, 0.08 mg magnesium, 4.8 mg sulfur, 0.62 mg selenium.

Nutrient	Low Fermentable Fiber	High Fermentable Fiber
Protein (g/kg)	418	427
Fat (g/kg)[1]	268[1]	270[1]
Calcium (g/kg)	14.0	14.1
Phosphorus (g/kg)	9.9	9.7
Moisture (g/kg)	80	80
Ash (g/kg)	68	71
Total Dietary fiber (g/kg)[2]	83	73
Short Chain Fatty Acids (mmol/kg OM)[3]	9	220
Metabolizable Energy (MJ/kg)	19.5	19.5

Table 1b. Nutrient composition of experimental diets.

[1]Contains 41 g/kg n-6 fatty acids and 6.6 g/kg n-3 fatty acids.

[2]TDF was determined by the AOAC Official Method 985.29 listed under section 45.4.07. The AOAC method does not recover FOS powder which was added at 15 g/kg diet. Assuming that 95% of the FOS powder is dietary fiber, then the actual fiber content of the HFF diet is approximately 7.3 + (0.95% x 1.5 g/100g diet) = 8.7%.

[3]Using 24 h fermentation results as described by Sunvold et al. (1995a), Sunvold et al. (1995b), Sunvold et al. (1995c).

Nutrient	g / kg diet
Crude protein	260
Digestible protein	190
Crude fat	160
Added fat	114
Crude fiber	27
Ash	75
Moisture	89
Calcium	17
Phosphorus	12
Metabolizable energy (kcal / kg)	3203
Vitamin / mineral mix[1]	see below

Table 2. Nutrient composition of pre-experimental chow diet.
[1]Vitamin / mineral mix, as determined by the manufacturer, provided: 130 mg/kg magnesium, 580 mg/kg potassium, 126 mg/kg manganese, 377 mg/kg zinc, 17 mg/kg copper, 3 mg/kg cobalt, 0.3 mg/kg selenium, 444 mg/kg iron, 3.6 mg/kg iodine, 16.1 KIU/kg vitamin A, 1.5 IU/kg vitamin D, 200 KIU/kg vitamin E, 1.5 mg/kg vitamin K (mena), 13.2 mg/kg thiamin, 25 mg/kg riboflavin, 87 mg/kg niacin, 32 mg/kg pantothenic acid, 11 mg/kg pyrodoxine, 1.4 g/kg choline, 1.8 mg/kg biotin, 234 μg/kg vitamin B_{12}.

Day	Day 1	Day 1	Day 1	Day 2
Tube #	Assay Buffer (μL)	Sample / Standard	anti-GLP-1 (μL)	^{125}I-GLP-1 tracer
(1/2) Total counts (TC)	--------	--------	--------	100
(3/4) Non specific binding (NSB)	700	--------	--------	100
(5/6) Total bound (TB)	600	--------	100	100
(7/8) - (23/24) Standards 1-9	500	100	100	100
(25/26 onward) Samples	400	100	100	100

Table 3. Tube protocol for the GLP-1(7-36)NH_2 radioimmunoassay. All counts were recorded in duplicate. Bracketed are tube numbers alongside which counts they represent. Although not stated in table, 100 μL of charcoal solution was added on day 4 of the protocol (refer to methods section).

	HFF		LFF	
	JEJUNUM	ILEUM	JEJUNUM	ILEUM
D-GLUCOSE				
Vmax (nmol/mg tissue/min)	182 ± 15 [a]	132 ± 11	133 ± 13 [b]	146 ± 15
Km (mM)	10 ± 1.9	5.5 ± 1.2	8.0 ± 2	12.7 ± 2.2
L-GLUCOSE				
(nmol/mg tissue/min)				
at 16 mM	21.7 ± 1.2	33.7 ± 5.3	21.5 ± 3.3	27.8 ± 3.5
at 1mM	1.4 ± 0.1	2.1 ± 0.3	1.4 ± 0.2	1.7 ± 0.2
D-FRUCTOSE				
Kd	1.96	2.43	1.61	2.28
FA 12 Uptake				
(nmol/mg tissue/min)	2.4 ± 0.2 [a]	3.6 ± 0.5	1.7 ± 0.2 [b]	4.2 ± 0.2

Table 4. Effects of diet on nutrient uptakes. Values are mean ± SEM, n = 8 / diet. Differing superscripts indicate $p < 0.05$ between HFF vs LFF. Kd = slope of the line describing the uptake data for D-glucose. Uptake of L-glucose is normalized to 1 mM which is representative of the Kd for D-glucose uptake. Fatty acid 12 uptake is a measure of unstirred water layer resistance. HFF = high fermentable fiber diet, LFF = low fermentable fiber diet.

	Pre-Experimental	HFF	LFF
Hemoglobin (g / dL)	16.6 ± 0.2	16.2 ± 0.4	16.9 ± 0.4
PVC (%)[1]	46.8 ± 0.7	46.7 ± 1.2	47.7 ± 1.2
RBC (x 10^{12} cells / L)[2]	6.8 ± 0.1	6.8 ± 0.2	7.0 ± 0.2
MCV (fL)[3]	68.4 ± 0.2	68.3 ± 0.4	68.2 ± 0.4
MCH (pg)[4]	24.3 ± 0.2	23.7 ± 0.3	24.0 ± 0.3
MCHC (g / dL)[5]	35.5 ± 0.3	34.8 ± 0.6	35.4 ± 0.6
WBC (x 10^9 cells / L)[6]	9.4 ± 0.7	8.0 ± 1.2	8.0 ± 1.2
Neutrophils (x 10^9 cells / L)	5.9 ± 0.49	5.2 ± 0.8	5.5 ± 0.8
Monocytes (x 10^9 cells / L)	0.42 ± 0.9	0.5 ± 0.2	0.5 ± 0.2

Table 5a. The effects of diet on the canine complete blood count. Values are means ± SEM, n = 16 ($p > 0.05$). [1]PVC = packed cell volume, [2]RBC = red blood cells, [3]MCV = mean corpuscular volume, [4]MCH = mean corpuscular hemoglobin, [5]MCHC = mean corpuscular hemoglobin concentration, [6]WBC = white blood cell count.

	Pre-Experimental	HFF	LFF
ALT[1] (GTP)[2] (IU / L)	27.8 ± 5.5	34.8 ± 5.5	37.9 ± 5.5
Alkaline phosphatase (IU /L)	35.5 ± 2.7	31.1 ± 2.7	31.2 ± 2.7
Total bilirubin (μmol/L)	6.9 ± 0.8	6.5 ± 0.8	6.7 ± 0.8
Glucose (μmol/L)	3.6 ± 0.3	3.3 ± 0.3	3.3 ± 0.3
Total protein (g/L)	63.2 ± 1.5	63 ± 1.5	64.1 ± 1.5
Albumin (g/L)	32.2 ± 1.6	33.4 ± 1.6	33.8 ± 1.6
Globulin (g/L)	30.7 ± 1.3	29.6 ± 1.3	30.3 ± 1.3
BUN[3] (mmol/L)	4.29 ± 0.9	4.7 ± 0.9	4.6 ± 0.9
Creatinine (μmol/L)	87.2 ± 3.1	91.0 ± 3.1	90.9 ± 3.1
Amylase (IU /L)	674 ± 51	601 ± 51	610 ± 51
Lipase (IU /L)	314 ± 37	344 ± 37	355 ± 37
Creatine kinase (IU /L)	273 ± 48	287 ± 48	248 ± 48
Osmolality (mOsm/kg)	296 ± 7	291 ± 7	292 ± 7
Anion gap (mEq/L)	18.5 ± 4	13.6 ± 4	13.8 ± 4
Na (mmol/L)	143 ± 4	141 ± 4	142 ± 4
K (mmol/L)	4.4 ± 0.4	4.5 ± 0.4	4.6 ± 0.4
Cl (mmol/L)	110 ± 3	112 ± 3	111 ± 3
CO_2 (mmol/L)	19.7 ± 3	21.4 ± 3	21 ± 3
Ca (mmol/L)	2.3 ± 0.3	2.4 ± 0.3	2.4 ± 0.3
P (mmol/L)	1.1 ± 0.3	1.1 ± 0.3	1.2 ± 0.3

Table 5b. The effects of diet on the canine general chemistry screen. Values are means ± SEM, n = 16 ($p > 0.05$). [1]ALT = serum alanine aminotransferase, [2]GTP = serum glutamic pyruvic transaminase, [3]BUN = blood urea nitrogen.