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UNIVERSITY OF ALBERTA

The effects of miglitol and metformin on vitamin status in Type 2 Diabetes

by

Lila Assiff 

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

in

Nutrition and Metabolism

Department of Agricultural, Food and Nutritional Science

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ABSTRACT

Anti-diabetic drugs such as miglitol and metformin have been shown to effectively reduce postprandial glucose levels in patients with Type 2 diabetes. The potential side effects of these drugs on vitamin status have not been considered. The objectives of this study were to determine the biochemical status of a select group of vitamins, including A, B_1, B_{12}, and folate, in Type 2 diabetes patients, and to determine if the status of these vitamins is affected by anti-diabetic drugs. A total of 324 patients from across Canada participated in this 48-week double-blind, placebo controlled study. Subjects were randomized into one of 4 treatment groups: placebo, miglitol, metformin, or the combination of miglitol and metformin. The diabetic patients were found to be at risk of deficiency of vitamins A, B_1, B_{12}, and folate. Plasma levels of vitamins A and B_{12} were significantly decreased ($p<0.05$) in the metformin treated group, while vitamin B_1 was significantly ($p<0.05$) decreased in the combination treatment group. No significant effects were observed in the miglitol treated group. These results suggest that Type 2 diabetes patients treated with metformin only may require vitamin A and B_{12} supplementations. Miglitol may be the agent of choice, either alone or as an adjuvant to oral anti-diabetic therapy, since it has not been shown to adversely affect the biochemical status of the vitamins studied.

To my dad, Kamal,
and mom, Fatima,
&
my sisters Hanna, Wafa, and Kathy,
&
my brothers Nasry, Monire and Nooraldein.

I love you all.

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LIST OF ABBREVIATIONS

μg	microgram
μmol	micromole
5-CH_3-THF	5-methyl-tetrahydrofolate
α	alpha
β	beta
BMI	body mass index
CDA	Canadian Diabetes Association
DCCT	Diabetes Control and Complications Trial
DM	Diabetes Mellitus
ETKA	erythrocyte transketolase activity
FPG	fasting plasma glucose
GDM	gestational diabetes mellitus
HbA_{1C}	glycosylated hemoglobin
HCl	hydrochloric acid
HPLC	high performance liquid chromatography
I_f	intrinsic factor
IU	international units
PPG	post-prandial glucose
RBC	red blood cell
RBP	retinol binding protein
RNI	recommended nutrient intake
S-7-P	sedoheptulose-7-phosphate
SCFA	short chain fatty acid
SEM	standard error of the mean
t.i.d.	three times per day
T1DM	type 1 diabetes
T2DM	type 2 diabetes
TPP	thiamin pyrophosphate
WHO	world health organization

1. GENERAL INTRODUCTION

Diabetes Mellitus (DM) is a disease that affects up to 7% of the global population (World Health Organization, 1994). The prevalence of the disease in people of Aboriginal, Hispanic, and African descent, living in North America can reach more than 50 percent (Jiwa, 1997). In Canada, over 1.5 million people (5% of Canadians) have been diagnosed with DM, and by the year 2010, the prevalence of the disease in Canada is expected to double (Tan et al, 1997). Moreover, another 750 000 individuals are thought to have the disease but remain undiagnosed. The prevalence of DM among First Nation Canadians is at least 2-3 times greater than the rest of Canada's population.

DM is metabolically characterized by hyperglycemia associated with abnormal metabolism of carbohydrates, fats and protein. The metabolic aberrations of DM are the result of a lack of insulin either because it is not produced in the body, or it is produced but the body is resistant to it. As a chronic metabolic disorder, DM can affect all of the body's major organ systems. For example, the risk of cardiovascular disease and stroke is increased two-fold in men, and three to four-fold in women with DM (Lerner & Kannel, 1986; Stamler et al, 1993).

Not only are patients with diabetes at increased risk of atherosclerosis, but this disease is the most common condition leading to non-trauma related lower limb amputations. DM is the leading cause of adult vision loss (retinopathy), accounting for 12% of all new cases of blindness in Canada. Moreover, DM among Canadians

accounts for 25% of all new cases of serious kidney disease (nephropathy), and is a major cause of end-stage renal failure, resulting in dialysis or kidney transplantation. DM is thus, a major health problem which can severely compromise quality of life and can lead to premature mortality.

These complications are a source of substantial morbidity related costs and suffering. The World Health Organization (WHO,1994) estimates that up to 5% of health budgets are spent on diabetes-related illness. A person with DM incurs two to five times higher medical costs than a person without the disease. In Canada alone, direct and indirect costs of treating the disease reach $5-$6 billion annually (Melchior and Jaber, 1996; Jiwa, 1997). A large proportion of this cost is attributed to treating the highly associated microvascular (small blood vessel) and macrovascular (large blood vessel) complications of the disease (Huse et al, 1989).

Research has shown the cost effectiveness of careful management of the disease in order to alleviate the repercussions of DM. The Diabetes Control and Complications Trial (DCCT, 1993) showed that implementing intensive therapy (close monitoring and management of blood glucose levels) to normalize blood glucose levels in insulin dependent patients with DM slowed the development and progression of retinopathy, nephropathy and neuropathy by up to 70%, and lowered the incidence of atherosclerosis compared to conventional therapy (Canadian Diabetes Association, 1989). In addition, the long-term costs associated with this therapy were lower compared to those of traditional non-intensive therapeutic methods of control.

Diabetes mellitus comprises a set of heterogeneous diseases which differ in their etiological, clinical, and epidemiological characteristics. It can be classified into 3 major categories: Type 1 diabetes (T1DM); Type 2 diabetes (T2DM), and gestational diabetes mellitus (GDM).

T1DM is an autoimmune condition where the pancreatic β-cells are completely destroyed and thus endogenous insulin cannot be produced. The lack of insulin leads to excessive levels of glucose in the body, which requires strict dietary control and injections of insulin to prevent ketoacidosis and death. This form of the disease accounts for about 10% of all cases of DM, and typically manifests in the early teen years.

Type 2 diabetes is by far the most prevalent form of diabetes, accounting for nearly 90% of all diabetics. It is characterized by a genetic predisposition and interaction between insulin resistance and decreased β-cell function. This form of the disease usually manifests after the age of 45 years, and often in obese patients. Obesity is associated with insulin resistance (decreased binding of insulin to cell membranes accompanied by decreased numbers of receptors) (Huang Z, et al, 1999).

GDM develops in 4% of pregnant women in the second or third trimester. It is usually a transient condition with mild symptoms which usually disappear after delivery. Cesarean sections are three to 4 times more likely for pregnant women with diabetes. Women who have previously been diagnosed with GDM have a 40% chance of developing T2DM within 10 years of their pregnancy.

The primary method for the management of T2DM includes changes in lifestyle, particularly dietary modifications as recommended by the CDA (1998) and increased physical activity in order to achieve glycemic control. However, since T2DM patients are diverse, not all respond effectively to diet and exercise modifications. Therefore, alternate methods such as drug therapy often need to be considered in addition to the lifestyle recommendations.

Adjuvant anti-diabetic drug therapy includes sulphonylureas, biguanides, and α-glucosidase inhibitors. These drugs exert their beneficial effects on hyperglycemia through different mechanisms of action. Sulfonylureas act by stimulating the pancreas to release more insulin, while the biguanides attempt to improve the action of the insulin that is present in order to manage hyperglycemia. Alpha-glucosidase inhibitors are a relatively new class of drugs which work by delaying carbohydrate absorption in order to control hyperglycemia. Although these anti-diabetic agents have been shown to be effective at managing glucose levels, side effects with respect to vitamin status has not been considered.

1.1 PATHOGENESIS OF TYPE 2 DIABETES

Hyperglycemia refers to increased plasma glucose due to decreased insulin action, and is the main feature of uncontrolled diabetes. In order to maintain

glucose homeostasis, the pancreas normally secretes insulin after a carbohydrate meal. Insulin alleviates hyperglycemia by promoting cellular uptake of glucose, and synthesis of glycogen in the liver and muscle. These metabolic effects of insulin therefore lead to decreased blood glucose levels, thereby adequately controlling hyperglycemia.

In the T1DM patient, the insulin secreting pancreatic β-cells are completely destroyed. In the T2DM patient, however, there are at least two pathological defects. One is a decreased ability of insulin to act on peripheral tissues to stimulate glucose metabolism or inhibit hepatic glucose output (i.e.: insulin resistance). The other is the inability of the pancreas to compensate for this insulin resistance. The heterogeneous nature of T2DM leads to the assumption that these fundamental defects may be caused by both genetic (Warram et al, 1990; Martin et al, 1992) and different environmental factors (Figure 1.1).

Race, ethnicity and family history have been linked to the development of the disease (Bogardus et al, 1991). These genetic factors have been attributed to a malfunctioning of the β-cells characterized by a decreased capacity of the islet cells to sense glucose (Kahn, 1994). This decreased sensitivity leads to a decline in insulin secretion with a resultant increase in hepatic glucose production, causing hyperglycemia. Insulin sensitivity is inherited. Decreases in insulin sensitivity precede and predict the development of T2DM (Kahn, 1994). Ultimately, chronic hyperglycemia may result in the progressive impairment of β-cell secretion of insulin (Lee, 1996).

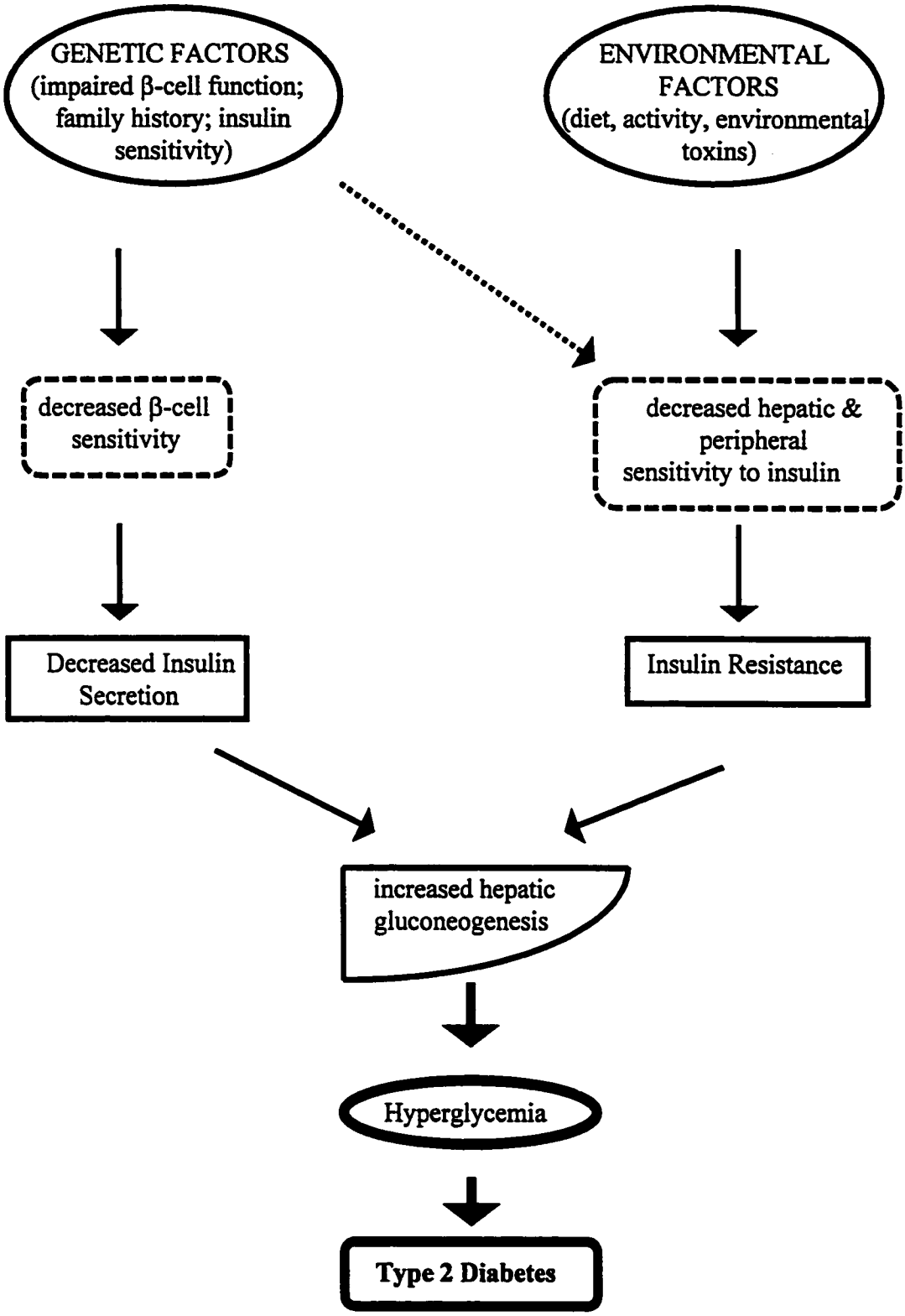

Figure 1.1: Simplified diagram of the pathogenesis of Type 2 diabetes (T2DM). Both genetic and environmental factors result in insulin resistance and hyperglycemia, and hence, T2DM. Insulin sensitivity is primarily inherited (adapted from Lee, 1996).

The genetic predisposition is also supported by the fact that the concordance rate of T2DM is up to 90% in identical twins (Lee, 1996). Furthermore, if both parents suffer from T2DM, the risk for their children to develop insulin resistance is around 80% (Martin et al, 1992; Kuehnle, 1996).

Studies of indigenous populations such as Pima Indians of Arizona (Lefebvre, 1992) or the Lac Simon Algonquin Community of Quebec (Delisle et al, 1995), whose incidence of T2DM is as high as 50% in the adult population suggest evidence of an environmental nature. The development of a sedentary lifestyle coupled with obesity leads to a marked increase in the prevalence of T2DM due to insulin resistance (Zimmet, 1997). Normally, hepatic gluconeogenesis is suppressed in the presence of insulin. As shown in Figure 1.1, however, hepatic insensitivity to insulin results in an increased hepatic production of glucose, leading to both fasting and postprandial hyperglycemia and ultimately, T2DM (Defronzo et al, 1992; Consoli, 1992; Lee, 1996).

Basualdo and colleagues (1997) observed a higher prevalence of T2DM in a First Nations population in Alberta, attributed to the development of a sedentary lifestyle, further supporting environmental conditions. In a study of the Sandy Lake community, Harris and his associates (1997) observed that high unemployment, lack of exercise and consumption of high fat diets (>30% of total energy intake) have replaced a traditional nomadic "hunter-gatherer" existence. This "Westernization" has been hypothesized to lead to obesity, which is a strong

predictor of T2DM in that it has been associated with a decreased number of insulin receptors on peripheral cells (Saad, et al, 1991).

1.2 MANAGEMENT OF TYPE 2 DIABETES

If left untreated, the chronic hyperglycemia of diabetes mellitus may ultimately result in tissue damage which is clinically manifested as microvascular (retinopathy, nephropathy, and neuropathy) and macrovascular (cardiac heart disease and hypertension) complications. The recommendations of the Expert Committee of the Canadian Diabetes Advisory Board (1992, 1999) for optimum metabolic control of T2DM are outlined in Table 1.1.

TABLE 1.1: Optimum values for metabolic control of various parameters in patients with Type 2 Diabetes (based on recommendations of the Expert Committee of the Canadian Diabetes Advisory Board, 1992 and 1999)

PARAMETER	TARGET VALUE
Fasting plasma glucose (mmol/L)	4-7
Postprandial* plasma glucose (mmol/L)	5-11
Glycosylated Hemoglobin (HbA_{1C})	<8.5%
Total cholesterol (mmol/L)	<5.2
Low density lipoprotein cholesterol (mmol/L)	<3.5
High density lipoprotein cholesterol (mmol/L)	>1.1
Triglyceride (mmol/L)	<2.0
Body Mass Index (kg/m^2)	<27

*** 1-2 hours after meal.**

Intensive management of T2DM refers to the extent to which all aspects (diet, drugs, and education) of diabetes care are applied to achieve near-normal

glycemic control. The Diabetes Control and Complications Trial Research Group (DCCT, 1993) demonstrated that by implementing intensive glycemic control, the onset of the subsequent complications of DM may be delayed or prevented. Similarly, the results of the Wisconsin Epidemiology Study of Diabetic Retinopathy in T1DM (Klein et al, 1995) suggests a benefit of glycemic control in the incidence and progression of diabetic retinopathy which may be applicable to T2DM. In the UK Prospective Diabetes Study (UKPDS) on more that 4200 patients with Type 2 DM, intensive therapy using metformin, sulfonylureas, or insulin was more effective in reducing FPG and HbA_{1C} levels than diet alone (Turner et al, 1996; Baliga and Fonseca, 1997).

Fasting hyperglycemia results primarily from accelerated hepatic gluconeogenesis due to insulin resistance at the level of the liver. Insulin resistance also results in postprandial hyperglycemia due to poor glucose disposal to the muscle cell. Achieving goals of fasting plasma glucose (FPG) and postprandial plasma glucose (PPG) between 4-7 mmol/L and 5-11 mmol/L respectively, should control hyperglycemia in T2DM patients (Table 1.1).

Plasma glycosylated hemoglobin (HbA_{1C}) is a measure of the degree of hyperglycemia to which the red blood cells have been exposed over the last three months. The average normal value of HbA_{1C} should be less than 8.5%. Since atherosclerosis is a major cause of mortality in DM, hypertension and atherogenic lipid profiles, which often co-exist with hyperglycemia (Krentz et al, 1994; Toeller, 1994), should be effectively managed. These recommendations should be followed

in order to alleviate symptoms and minimize the risk for the aforementioned long-term complications associated with T2DM. The methods of management of T2DM can be classified as either non-pharmacological or pharmacological plus non-pharmacological.

1.2.1 NON-PHARMACOLOGICAL MANAGEMENT: DIET & EXERCISE

The management of T2DM requires an integrated approach with dietary modifications and recommendations to increase physical activity. According to the CDA (1997), effective self-management training of diabetes requires individualized approaches, tailored to the personal lifestyle and management goals of the patient. In T2DM, dietary management and patient education are of crucial importance if the desired metabolic control is to be achieved (Gerich, 1989; CDA, 1998). After the start of a hypocaloric diet regimen for T2DM, a rapid and large decrease in serum glucose level is often observed, primarily due to reduced liver glucose production. With prolonged caloric restriction and weight loss, both lean and fat mass are reduced and tissue insulin sensitivity is enhanced (Henry, 1996). The recommended diabetic diet is outlined in Table 1.2. The CDA (1998) recommends avoiding fast digestible carbohydrates such as sucrose or refined starch to prevent excessive postprandial hyperglycemia. A diet high in complex carbohydrates and soluble dietary fiber helps to achieve glycemic control (Johnson, 1996; Scott &

Marliss, 1991). It is also desirable for the diabetic to decrease dietary intakes of saturated fatty acid and cholesterol in order to minimize hypercholesterolemia and hypertriglyceridemia, and hence atherosclerotic risk (CDA, 1989).

TABLE 1.2: Nutrition Recommendations for patients Type 2 diabetes*

DIETARY COMPONENT	COMPOSITION IN DIET
Carbohydrate** (Complex/High Fiber)	45-55%
Fat**	30% (10% each SFA, MUFA, PUFA)
Protein	0.8 g/kg ideal body weight
Fiber	40 g/day

*** Adapted from the Canadian Diabetes Association, 1998**
**** Percent of total energy in kcal (SFA=saturated fatty acids; MUFA=mono-unsaturated fatty acids; PUFA=polyunsaturated fatty acids).**

In addition to the dietary modifications, regular exercise is of benefit to all individuals with DM as exercise reduces the need for exogenous insulin. Aside from the obvious cardiovascular benefits, increased physical activity is often accompanied by weight loss which can improve the body's sensitivity to insulin (Olefsky, 1981), and thereby control hyperglycemia (Toeller, 1992). It has long been recognized that obesity causes insulin resistance an predisposes to the development of T2DM (Kissebah et al, 1982; Campbell et al, 1993; Felber et al, 1993; Kahn, 1994). Exercise has been shown to have a glucose lowering effect, while the lack of exercise has been linked to an increasing risk of developing T2DM (CDA, 1989; Bonen, 1995). In animal studies, Bonen et al (1984, 1992) have shown that exercise can markedly increase glucose uptake by the muscle. Similar results were observed in humans (Jandrain et al, 1984). Exercise has also

been reported to improve insulin action (Henry, 1986; Horton, 1991) and hence glucose tolerance (Searle and Ready, 1991). Physical training has also been demonstrated to reduce plasma triglycerides and cholesterol, accompanied by increased levels of high density lipoprotein cholesterol (HDL-C) (Lefebvre and Scheen, 1992).

Sometimes dramatic effects of even modest amounts of weight loss on reducing glucose output and improving insulin secretion or improving peripheral insulin sensitivity are observed (Henry, 1996). However, this form of therapy alone is often not sufficient to maintain desired glycemic goals in obese T2DM patients. Therefore, pharmacological intervention may be desirable.

1.2.2 PHARMACOLOGICAL MANAGEMENT

There are situations where dietary measures, either alone or in combination with exercise, may not be adequate for control of the hyperglycemic state. Such a situation may warrant the addition of drug therapy. Over the years, a variety of oral anti-diabetic agents have been developed. These agents are often used as adjuvants to the dietary management of diabetes.

Oral anti-diabetic agents can be classified into two groups. One group (e.g. sulfonylureas) lowers plasma glucose by inhibiting endogenous hepatic glucose production, and increasing insulin action. The second group (e.g., biguanides, alpha-glucosidase inhibitors) reduces elevated circulating glucose levels toward the

normal range by increasing peripheral glucose uptake or delaying carbohydrate absorption to control hyperglycemia.

1.2.2.1 SULPHONYLUREAS

Sulphonylureas, such as glibenclamide, glipizide, and tolbutamide (Figure 1.2), are generally considered in the first-line of drug treatment in T2DM patients who are not obese (BMI $\leq 30 kg/m^2$), since these drugs may promote weight gain (Henry, 1996). This mode of therapy has been available since the early 1950s, and is recommended in T2DM patients who cannot be adequately treated by diet and exercise alone (Lebovitz, 1992, Krentz et al 1994).

Figure 1.2: Structure of tolbutamide, a commonly prescribed sulphonylurea.

In order to be effective, sulfonylureas require some degree of β-cell function. They act primarily by stimulating the pancreatic β-cells to secrete more endogenous insulin in both the fasting and post-prandial state (Lebovitz, 1992). This reduces hepatic glucose output and facilitates glucose disposal. Although

sulfonylureas can be effective in improving glycemic control, a frequent complication of this therapy is the risk of hypoglycemia. The antidiabetic agents promote the release of insulin by β-cells, even after plasma levels of glucose have decreased, creating the potential for episodes of severe hypoglycemia. This sulfonylurea-induced hypoglycemia can be exacerbated by interaction with alcohol and some drugs, such as aspirin, and some anti-depressants (Lefebvre and Scheen, 1992), since they all have a hyperglycemic effect and may lead to a loss of blood glucose control.

Furthermore, the presence of a hepatic disorder reduces inactivation of sulfonylureas, prolonging their half-lives and hence the risk of hypoglycemia. As a result, sulfonylureas are often contraindicated in elderly people with poor nutritional status, hepatic disorders, and renal insufficiency. It is estimated that as many as 20% of diabetic patients treated with sulfonylureas experience hypoglycemia (Lee, 1996). The dose of sulfonylureas is typically 20-500 mg/day and the cost of this therapy ranges from $30 to $90 per month (Goo et al, 1996).

1.2.2.2 BIGUANIDES: METFORMIN

This class of drugs, including metformin, phenformin and buformin, was first introduced for the management of T2DM in 1957. Metformin therapy is recommended for the obese T2DM patient since it has been found to be associated with a loss of weight or no weight gain (Bailey, 1992). In contrast to sulfonylureas,

metformin appears to have no effect on the β-cell and endogenous insulin secretion (Jackson et al, 1987; Johnson, 1993), and hence the risk of hypoglycemia is reduced. Like the sulfonylureas, however, metformin requires the presence of insulin to exert its pharmacologic effects (Lee, 1996; Melchior and Jaber, 1996). The mechanism of anti-diabetic action of metformin is thought to be mediated by decreasing hepatic glucose output (Stumvoll et al, 1995) by inhibiting gluconeogenesis (Defronzo, 1991; Perriello et al, 1994; Yu et al, 1994), and increasing peripheral glucose uptake (Nosadini, 1987; Lee, 1996). Metformin has also been shown to exhibit a favorable effect on plasma lipids by decreasing triglycerides as well as total and LDL-cholesterol levels (Rains et al, 1988; Defronzo and Goodman, 1995) while increasing HDL-cholesterol (Goo et al, 1996).

Lactate is a gluconeogenic precursor that stimulates glucose production. When glucose is taken up into cells by insulin, anaerobic glucose degradation increases, yielding more pyruvate. Pyruvate is then reduced to lactate, resulting in accumulations of this acid, which may precipitate lactic acidosis. Since biguanides decrease liver glucose output by inhibiting hepatic gluconeogenesis (Lee, 1996), lactic acidosis is a concern when using this class of drugs (Bloomgarden, 1994).

Symptoms of lactic acidosis include somnolence, confusion, nausea, fatigue and muscle pain. Treatment includes hydration and correction of the metabolic acidosis (Goo et al, 1996). Due to the side effects associated with biguanides, phenformin and buformin have been withdrawn from the North

American market. In contrast to other glucose-lowering biguanides, metformin does not increase the production of lactate from muscle and therefore minimizes the risk of lactic acidosis (Krentz et al, 1994; Goo et al, 1996). Decreased risk may also be attributed to the shorter half-life and poor hepatic metabolism of metformin.

Although the risk of lactic acidosis is decreased with metformin, it is still capable of causing this condition. Therefore, it is contraindicated in patients with renal insufficiency, which may allow the biguanide to accumulate (Krentz et al, 1994), or hepatic impairment which leads to decreased lactate extraction (Tucker et al, 1981).

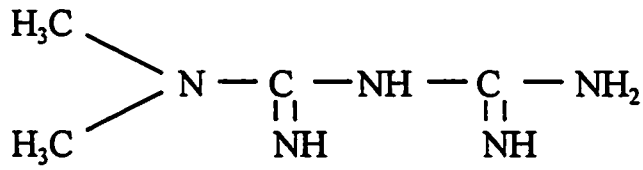

Figure 1.3 Structure of the biguanide, metformin.

Metformin (Figure 1.3) is readily available and often prescribed in Canada, Europe, and Mexico to treat T2DM patients who cannot achieve adequate glycemic control with diet and/or sulfonylureas. In 1994, metformin was approved in the United

States, when sulfonylureas were the only available oral anti-diabetic agents (Melchior and Jaber, 1996).

1.2.2.3 ALPHA-GLUCOSIDASE INHIBITORS

The potential shortcomings of sulfonylureas and biguanides as manifested by hypoglycemia and lactic acidosis, have stimulated interest in developing alternative drug therapies for T2DM. In search of this alternative, an α-amylase inhibitor was isolated from wheat flour (Puls and Keup, 1973; Donckier and Williams, 1994). This derivative eventually led to the production of an α-glucosidase inhibitor (Donckier and Williams, 1994).

Starch and sucrose are the most important dietary carbohydrate components in human diets, which comprises 50-60% of the average Canadian diet. In the gut, these saccharides are broken down by α-glucosidases to glucose and fructose which are then absorbed. Alpha-glucosidases, such as glucoamylase, sucrase, dextrinase, maltase and isomaltase, are enzymes which are located in the brush border of the intestinal mucosa. The breakdown of di- and poly- saccharides to monosaccharides takes place rapidly in the upper parts of the small intestine. As a result, a rapid and high postprandial rise in blood glucose following a carbohydrate load is observed. In recent years, there have been attempts to develop competitive inhibitors of α-glucosidases.

The inhibition of α-glucosidases is effective in managing T2DM because this inhibition prevents the late postprandial rise in blood glucose by inhibiting the intestinal disaccharidases (Cauderay et al, 1985). The slow absorption of glucose over a prolonged period of time is thought to decrease insulin secretion and improve insulin sensitivity (Lefebvre and Scheen, 1992, Krentz et al, 1994). It also lowers serum total cholesterol, and LDL-C levels, implying improvements in hyperglycemia and hyperlipidemia (Leonhardt et al, 1994). Furthermore, α-glucosidase inhibitors act to decrease postprandial hyperglycemia without stimulating insulin secretion and therefore hyperinsulinemia is not a concern (Hoffman, et al, 1994).

Overall, α-glucosidase inhibitors are effective in improving glycemic control and have theoretical advantages over conventional oral anti-diabetic agents. Alpha-glucosidase inhibitors do not promote weight gain, and therefore, the state of insulin resistance in T2DM is not worsened (Baliga and Fonseca, 1997). These drugs have been shown to decrease plasma triglyceride and insulin levels, thereby decreasing the risk of atherogenesis (Leonhardt et al, 1994). They are highly effective during high carbohydrate intake, which is consistent with the recommendations to those with diabetes. The only disadvantage reported is the gastrointestinal side effects which result from the fermentation of unabsorbed carbohydrates. These effects, however, can be minimized by gradual introduction of the drug to the patient.

1.2.2.3.1 ACARBOSE

Acarbose (Figure 1.4) was the first commercially available alpha-glucosidase inhibitor for the management of T2DM. It is a complex pseudo-oligosaccharide which competitively and reversibly inhibits α-glucosidases and thus delays the digestion of starch and sucrose into glucose and fructose units (Caspary et al, 1971; Martin & Montgomery, 1996).

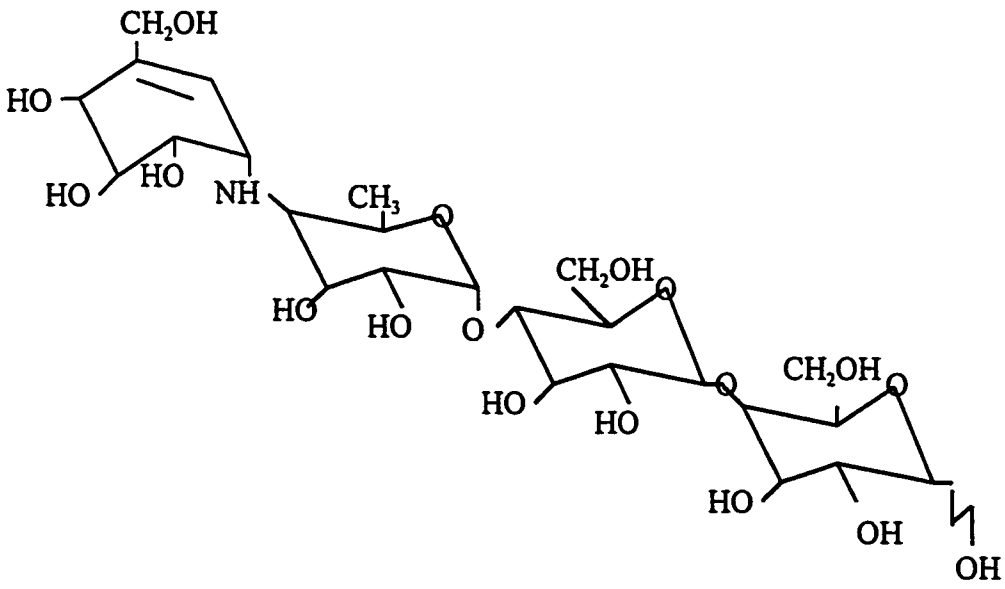

Figure 1.4: Structure of Acarbose

As a result, carbohydrates not digested in the upper parts of the small intestine are transported and absorbed in the more distal regions of the small intestine (Bischoff, 1994), resulting in the desired late postprandial rise of blood glucose concentration. Some carbohydrates reach the large intestine and are fermented by colonic bacteria, yielding short chain fatty acids (Ladas et al, 1992).

The clinical efficacy of acarbose in the treatment of T2DM, as monotherapy or in combination with other oral anti-diabetic agents has been reviewed previously (Campbell et al, 1996; Bressler and Johnson, 1997; DeFronzo, 1999). Numerous studies have shown that α-glucosidase inhibition reduces postprandial hyperglycemia in T2DM . Compared to placebo (Coniff, 1994, Hotta et al, 1993) or sulfonylurea (Hoffman, 1994, Coniff et al, 1995), acarbose treatments have been shown to reduce HbA_{1c}, FPG, and PPG levels, and exhibit better lipid profiles in T2DM patients (Martin & Montogomery, 1996). Acarbose is not absorbed, and its half-life is 2.7-9 hours, which is increased in the presence of renal dysfunction. Acarbose does not affect glucose, lactose or fructose absorption because these saccharides are not dependent on alpha-glucosidases for absorption. The suggested dose of acarbose therapy is 100mg, three times per day.

1.2.2.3.2 MIGLITOL

Miglitol (Figure 1.5), another α-glucosidase inhibitor, has been developed in more recent years. It is a semi-synthetic alpha-glucosidase inhibitor with a

structural similarity to glucose and is rapidly and completely absorbed in the jejunum (Lefebvre and Scheen, 1992). As a result, miglitol may enter circulation and might exert systemic effects when taken up by cells of various organs (Reuser and Wisselaar, 1994). Miglitol's action is anti-hyperglycemic in that it prevents glucose levels from rising, but does not increase insulin secretion. Joubert et al, (1987) showed that blood glucose decreases significantly after a glucose load due to enhancement of insulin effects or depression of counter-regulatory hormones, such as glucagon. The blood glucose peak was unaffected by miglitol, therefore it is unlikely that miglitol has an effect on blood glucose absorption (Joubert et al, 1987; 1990).

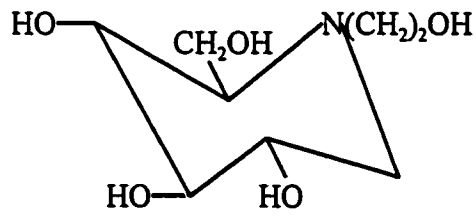

Figure 1.5: Structure of Miglitol

Miglitol is a potent, reversible, competitive inhibitor of several α-glucosidases of the small intestinal brush border (Bischoff, 1994). Carbohydrates not digested in the duodenum and jejunum are, as a result, transported to the ileum.

Therefore, the generation and absorption of glucose is delayed without stimulating insulin secretion and the postprandial blood glucose peaks characteristic of T2DM are attenuated (Johnston et al, 1994; Samad et al, 1988).

Preliminary studies of miglitol in normal volunteers have shown that blood glucose and serum insulin levels were lower in individuals receiving metformin when given in combination with miglitol compared with patients receiving metformin alone (Schnack, et al, 1986). The beneficial effect of miglitol on glycemic control by reducing HbA_{1c} levels was also evidenced in T2DM patients who were poorly controlled on sulfonylureas (Johnston et al, 1994; Schnack et al, 1986). However, Johnston et al (1994) noted that the reductions in HbA_{1c} levels were due primarily to reductions in PPG, not FPG. Similarly, in a short term crossover study investigating the effect of miglitol on metabolic control in T2DM patients, Samad and associates (1988) found postprandial blood glucose levels to be significantly ($p<0.05$) lower compared to placebo. However, no effect was observed on FPG and cholesterol levels.

In T2DM patients treated with sulfonylureas, 50mg of miglitol with each meal resulted in a significant decrease in blood glucose concentration, beyond the reduction observed with sulfonylureas alone (Joubert et al, 1986; Hillebrand and Rudolf, 1985). For patients with T1DM, a 50 mg dose of miglitol was also shown to improve insulin efficacy and effectively decreased postprandial glucose levels (Wing et al, 1990; Dimitriadis et al, 1991; Pagano et al, 1995).

Miglitol must be administered at the first bite of the main meal, and in doses which allow all digestible carbohydrates to be digested (Hara et al, 1996). Otherwise, the undigested carbohydrates are not completely absorbed by the small intestine and consequently they will enter the colon. The latter would result in bacterial fermentation resulting in gastrointestinal side effects, including flatulence, abdominal discomfort and diarrhea. These adverse drug reactions are common at the beginning of treatment, but become more infrequent after a few weeks on the drug due to an adaptation effect (Krentz et al, 1994).

1.3 VITAMINS AND DIABETES

Diabetes mellitus is a metabolic disorder. Patients with this condition, particularly T2DM, have an increased prevalence of other chronic diseases, such as hypertension, renal failure, and congestive heart failure. Furthermore, these patients are prescribed with drugs to control primary as well as secondary conditions. Both the disease and the use of drugs may affect nutritional status. Despite the increasing interest in the nutritional needs of diabetic subjects, the literature on the role of micronutrients in the management of diabetes has grown at a relatively slow pace. Vitamins are essential micronutrients involved in the fundamental functions of the body including growth, metabolism, and maintenance of health. Consequently, a deficiency in these nutrients may lead to serious disease states.

1.3.1 VITAMIN A (RETINOL)

Vitamin A is a fat-soluble vitamin which is stored in the liver in combination with fatty acids as retinyl esters (Basu, 1988). The α-globulin, retinol binding protein (RBP), is the carrier of retinol from the liver. RBP binds in a 1:1 molar ratio with retinol. Retinol, bound to RBP, is released into the circulation and subsequently transported to the target tissues.

The biochemical functions of vitamin A include vision, reproduction, immune function, tissue differentiation and growth (Olson, 1984). Vitamin A may also have an important role in the regulation of insulin secretion by β-cells. Evidence obtained from *in vitro* experiments and *in vivo* animal models suggest that an excess, or lack of vitamin A can alter insulin secretion (Krempf et al, 1991; Baker and Campbell, 1992). Thus, vitamin A at low concentrations stimulates insulin release, while at high concentrations, it inhibits its release (Mooradian and Morley, 1987).

There appears to be a substantial amount of evidence suggesting that diabetes may affect vitamin A metabolism. In several studies involving patients with T1DM, the level of plasma retinol has been reported to be significantly decreased when compared with that of the non-diabetic, age- and sex-matched controls (Basu et al,1989; Krempf et al 1991, Martinoli et al, 1993). Reduced

plasma vitamin A is accompanied by a decrease in plasma RBP concentration (Basu et al, 1989).

The biochemical evidence of vitamin A deficiency in subjects with T2DM, however, is controversial. According to Wako and his associates (1986), the plasma retinol level in T2DM patients are consistent with levels reported in individuals diagnosed with T1DM. On the other hand, there are many studies in patients demonstrating no reductions in the plasma vitamin A levels (Straub et al, 1993; Sasaki et al, 1995, Basualdo et al, 1997). In a study involving 100 T2DM patients and 112 healthy subjects the mean concentration of plasma retinol were within the normal range and not statistically diefferent from non-diabetic subjects. A greater number of these patients, however, had the biochemical evidence of vitamin A deficiency compared with their matched controls (Havivi et al, 1990).

In poorly controlled diabetes the metabolic availability of vitamin A from the liver has been suggested to be of concern because of the unavailability of RBP synthesis (Tuitoek et al, 1996). This has been suggested by the fact that experimentally induced diabetic rats are accompanied by decreased plasma but elevated hepatic concentrations of vitamin A, compared to the non-diabetic control animals. The reduced plasma vitamin A levels in diabetic animals remained unaltered while their hepatic levels were further elevated when these animals were supplemented with vitamin A at a daily dose 10 times the physiological level (Tuitoek, et al, 1996).

1.3.2 VITAMIN B_1 (THIAMIN)

Vitamin B_1 is a water-soluble vitamin which is a cofactor for many enzyme systems involved in the metabolism of energy, and hence its requirement is dependent in part by the amount of carbohydrate consumed. In order to be activated, thiamin must be converted to thiamin pyrophosphate (TPP). The activation sites for this conversion are generally in the small intestine, liver, and/or kidney.

Erythrocyte transketolase activity (ETKA) is frequently a measure of thiamin status based on the conversion of intracellular pentose to sedoheptulose. In this reaction, transketolase (TK) acts as an apoenzyme and thiamin functions as a coenzyme (Basu and Dickerson, 1996). Kjosen and Seim (1977) found ETKA in DM (both T1DM & T2DM) to be significantly reduced when compared to healthy controls (37 IU vs. 46 IU, respectively). The reduced ETKA could not be corrected by the supplementation of TPP suggesting that the low ETKA in the DM patients was due to a reduced apoenzyme level, rather than a deficiency of thiamin (TPP), the cofactor for ETKA.

1.3.3 VITAMIN B-12

Vitamin B_{12} is a water soluble vitamin which is absorbed from the distal third of the ileum. It's absorption is regulated by the gastric secretion of intrinsic

factor (I_f). Vitamin B_{12} deficiency has been associated with T1DM in the context of polyglandular autoimmune diseases (Trence et al, 1984). The concurrent presence of T2DM and vitamin B_{12} associated pernicious anemia is not uncommon as these two conditions increase independently with age (Reed and Mooradian, 1990). Although the cause and effect relationship between diabetes and vitamin B_{12} deficiency has not been established, it is noteworthy that the secretion of both I_f + HCl is stimulated by insulin (Mathews and Van Holde, 1989). Since many T2DM patients have malfunctioning pancreatic β-cells and sub-normal secretion of insulin, poor absorption of vitamin B_{12} is a logical consequence.

1.3.4 FOLIC ACID

The function of folic acid in its reduced form, 5-methyl-tetrahydrofolate (5-CH_3-THF) is in the transfer of one-carbon fragments in many biosynthetic and catabolic reactions and as a cofactor in nucleic acid synthesis (Cooper, 1984). Within the intestinal lumen, folate conjugates are hydrolyzed by pteroyl-polyglutamate hydrolase (conjugase). Free folate is absorbed in the jejunum. 5-CH_3-THF is the main folate in plasma, which reflects its recent dietary intake. Red cell folate reflects body folate stores and is a more accurate and less variable indicator of folate status than its plasma level.

Although folate deficiency is not a common problem in diabetes (Davis et al, 1976; Carpentier et al, 1976), elderly individuals with DM are at a high risk of folate deficiency (Mooradian et al, 1994). Furthermore, age-related trends were

observed in the NHANES II survey where adults in the 45-74 yr. age group had the lowest levels of serum and red blood cell folate compared to all other age groups (Gibson, 1990).

1.4 VITAMINS AND ORAL ANTI-DIABETES DRUGS

Administration of drugs in patients with superimposed disease processes can result in hypovitaminosis. The use of therapeutic drugs for the management of DM may therefore affect vitamin status by interfering with their bio- and metabolic availability. The interrelationship between oral anti-diabetic agents and vitamins has received very little attention. The α-glucosidase inhibitors in relation to diabetes are only recent issues. Only isolated studies relating these inhibitors and vitamin status have been reported. There have been two studies (VanGall et al, 1991; Tuonilehto et al, 1994) showing minimal adverse effects of acarbose, an α-glucosidase inhibitor, on serum B_{12} and folate in T2DM patients.

Unlike acarbose, miglitol is an absorbable α-glucosidase inhibitor. Since the site of absorption of miglitol is the same as that for many nutrients (Figure 1.6), it is possible that the α-glucosidase inhibitor may affect nutrient status. Vitamins act as coenzymes and function as integral parts of membranes. The availability of vitamins for cellular function depends not only on their intake, but also on absorption, metabolic activation and transport to target tissues from their storage sites. These mechanisms may be affected by therapy with α-glucosidase inhibitors.

Hence miglitol may cause not only malabsorption of vitamins by altering the intestinal lumen environment, or by inhibiting digestive enzymes, but it may also affect their metabolic availability.

Miglitol has been shown to exhibit gastrointestinal side effects including diarrhea, which in turn may cause not only the fecal loss of vitamins, but also their absorption (Wolever, et al, 1994). Vitamin A, thiamin, and folic acid status may be directly affected by administration of an α-glucosidase inhibitor to T2DM patients, because the site of absorption for these vitamins is in the duodenum and jejunum which are the same sites of miglitol's anti-hyperglycemic action (Figure 1.6).

Vitamin B_{12} status may be indirectly affected by the action of miglitol due to a possible inhibition of the secretion of intrinsic factor, the transport protein for vitamin B_{12} , located in the stomach. Moreover, the delayed absorption of carbohydrates as a result of α-glucosidase inhibition results in the undigested carbohydrates to be transported to the ileum. These carbohydrates are then digested into short chain fatty acids (SCFAs) by bacterial fermentation in the colon (Wolever et al, 1994). These fatty acids may undergo oxidation to succinyl CoA, and require the vitamin B_{12}-dependent isomerase enzyme. Higher production of SCFAs may therefore affect vitamin B_{12} status by increasing its utilization.

There have been some reports suggesting that metformin can potentially interact with vitamin B_{12}. In a study of 46 randomly selected T2DM patients on metformin therapy (1-3g daily), Adams et al (1983) observed that 30% of these patients had reduced absorption of vitamin B_{12}.

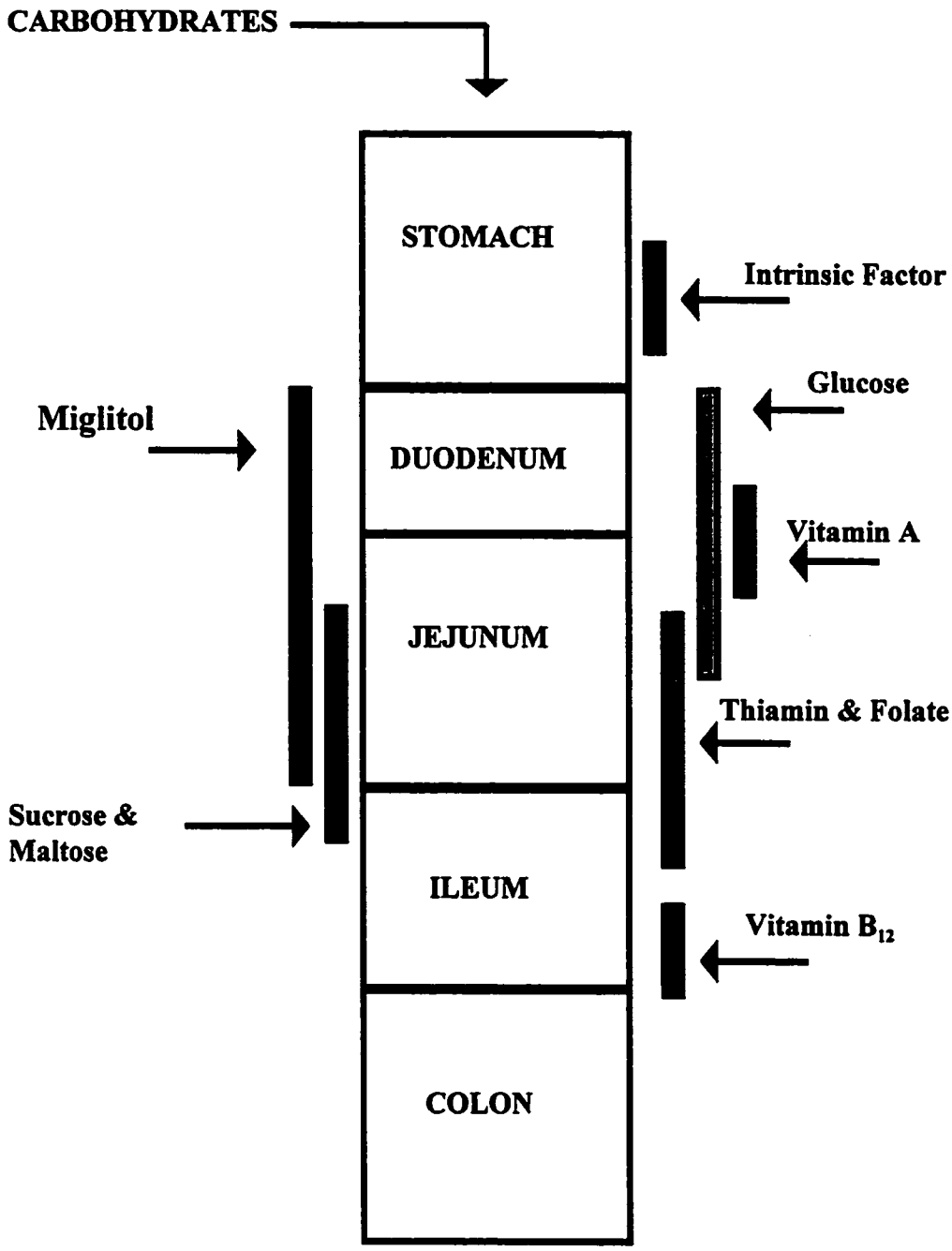

FIGURE 1.6: Sites of nutrient and miglitol absorption in the gastrointestinal tract.

Withdrawal of metformin resulted in an increased absorption in half of these patients, without interfering with intrinsic factor. However, the other half still exhibited malabsorption, even after metformin therapy was discontinued, perhaps indicating a permanent, though unsubstantiated, suppression of I_f secretion and therefore, vitamin B_{12} status. The metformin associated malabsorption of vitamin B_{12} was also observed by others (Tomkin et al, 1971; Shaw et al, 1994;; Defronzo et al, 1995).

In view of the clinical significance of miglitol and metformin in diabetes, their respective interactions with vitamins and possible side effects warrant further investigation. The untoward effects of these drugs on vitamins are probable since the site of action of these anti-diabetic drugs are concurrent with the sites where vitamins, such as A, B_1, B_{12}, and folate are absorbed (Figure 1.6). The rationale for choosing these vitamins is that they all have a unique mode of absorption and transport, thereby making these vitamins potentially affected by α-glucosidase inhibitors, such as miglitol.

1.5 HYPOTHESIS AND OBJECTIVES OF THE PRESENT STUDY

It is hypothesized that patients with T2DM are associated with biochemical evidence of deficiency of a select group of vitamins and that this effect is further exacerbated by the treatment with metformin and miglitol. This hypothesis was tested with the following objectives:

1. To assess the biochemical status of retinol, thiamin, folate, and vitamin B_{12} in patients with T2DM.

   H_o: Patients with type 2 diabetes will have adequate biochemical status of retinol, thiamin, folate, and vitamin B_{12}.

2. To examine if treatment with either metformin or miglitol has adverse effects on these vitamins.

   H_o: Treatment with metformin will not affect vitamin status in type 2 diabetic patients.

   H_o: Treatment with miglitol will not affect vitamin status in type 2 diabetic subjects.

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2. MATERIALS AND METHODS

2.1 SUBJECTS AND STUDY DESIGN

The present study was undertaken to examine the status of vitamins A, B_1, B_{12}, and folate in Type 2 diabetic patients, and the effects of miglitol and metformin on the biochemical status of these vitamins in patients with Type 2 diabetes (T2DM). This study was part of a multi-centre, randomized, double-blind and placebo-controlled study investigating the efficacy and safety of miglitol in the treatment of T2DM.

A total of 324 subjects with Type 2 DM were recruited at 13 centres in cities across Canada, including Vancouver, North Vancouver, Edmonton, Montreal, London, Calgary, Toronto, Ottawa, Sherbrooke, Winnipeg, Hamilton, and Saskatoon. The protocol for the study was approved by the local ethics committees at each participating centre. The inclusion and exclusion criteria for the recruitment of subjects are listed in Table 2.1. All subjects who conformed to these selection criteria gave informed written consent to participate.

The subjects meeting the selection criteria first underwent a 4 week washout period where any previous oral hypoglycemic agents were withdrawn. This was followed by a single blind, 8 week placebo baseline period at entry. Following this initial 12 week period, the plasma glycosylated hemoglobin (HbA_{1c}) levels were measured and subjects with the level of this parameter falling between 7.2% and

9.1% were randomized in a double blind manner into one of 4 study arms (I, II, III or IV) as shown in Figure 2.1.

Table 2.1: Selection Criteria for Study Participants at Entry

INCLUSION	EXCLUSION
• primary diagnosis of T2DM[1] • > 40 years of age • male or female • BMI ≤ 40 kg/m^2 • able to understand and willing to comply • capable and willing to give written informed consent	• patients with T1DM • T2DM treated with insulin • unwilling to self blood glucose monitor • suffering from a major debilitating disease • taking drugs that can impair intestinal motility or carbohydrate absorption • taken investigational drugs in last 30 days • a recent cardiovascular event[2] • serum creatinine ≥130 μmol/L • non-euthyroid patients • medical/surgical stress in past 3 months • documented GI disease • on systemic glucocorticoids • hypersensitivity to miglitol or metformin • history of lactic acidosis

[1] According to "Clinical Practice Guidelines for Diabetes Mellitus in Canada" (1989)
[2] Such as myocardial infarction, cerebrovascular accident, or congestive heart failure within the last 6 months

Randomization of patients at the end of week 12 was done in a double blind manner using a random code generated by Biostatistics and Health Economics at Miles Canada Inc. (Toronto, Canada). Miglitol was supplied in tablet form (25mg, 50mg, and 100mg), while metformin was provided in 500mg encapsulated forms. The miglitol and metformin placebos were given in tablet and encapsulated forms, respectively, to match the size and shape of the active medications.

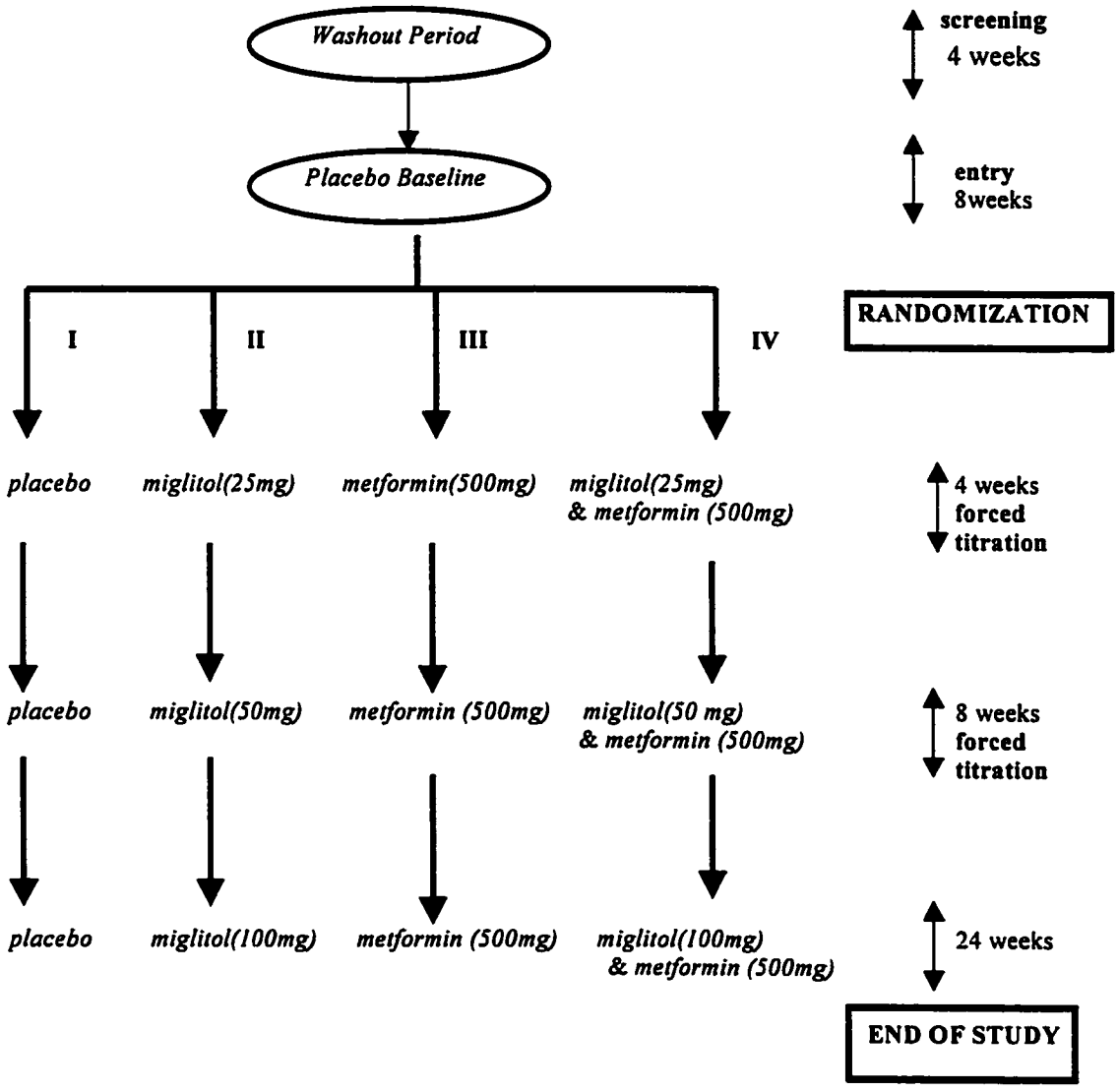

Figure 2.1: Overall study design indicating the four treatment arms and timeline. Miglitol and metformin were ingested 3 times per day. The 12 week washout and placebo baseline periods are single blind, while the 36 week randomization of treatment period is double blind.

All medications, including placebos, were supplied in blister packs by Miles Canada Inc. (a Bayer Co.). Each pack was specially labeled with a visible portion indicating study and patient numbers, and a concealed portion identifying the medication that the patient was receiving.

Throughout the study, the subjects remained on their initial treatment (e.g., placebo, miglitol, metformin, or combination therapy). The metformin dose was maintained at 500 mg three times per day (t.i.d.) for the entire study period while the miglitol was titrated from 25 mg t.i.d. at week 12, to 50 mg t.i.d. at week 16, then to 100 mg t.i.d. at week 24 for the remainder of the study. The total duration of the study was 48 weeks, including the 4 week washout period, followed by the 8 week placebo baseline period, and finally the 36 week double-blind treatment period. During the total study period, subjects were seen at 4 week intervals. Fasting blood samples for routine biochemistry, complete blood count, hematocrit, and HbA_{1c} were obtained at randomization and at each study visit. Patients were counseled by a nutritionist and instructed in a diabetic diet according to the Canadian Diabetes Association guidelines (CDA, 1989). Patients were also supplied with a glucose meter and monitoring equipment and instructed on how to use them.

At any time during the study, patients with HbA_{1C} levels >140% above the upper limit of normal, or whose fasting plasma glucose (FPG) level was >13mmol/L at 2 consecutive visits were discontinued for the rest of the study. Moreover, patients who became markedly hyperglycemic or symptomatic were also discontinued at their

physician's discretion. Obvious non-compliance (less than 70% on 2 consecutive visits) and voluntary withdrawal were also criteria for dropping out of the study.

At baseline and end of study (end of weeks 12 and 48, respectively), approximately 6 mL of whole blood were collected into separate lavender top Vacutainer® (Becton-Dickinson, Rutherford, NJ) tubes containing ethylenediamine-tetraacetic acid (EDTA) at each local hospital laboratory. This whole blood, in two aliquots, and the separated plasma, were frozen at -40°C and transported by courier from each centre within 36 hours of collection packed in ice to the University of Toronto (central lab). The samples were then sorted and transported by courier on dry ice at minus 20°C from Toronto to the University of Alberta. These samples were used to determine the biochemical levels of a select group of vitamins, including retinol (for vitamin A), thiamin (for vitamin B_1) , folate, and vitamin B_{12}.

The whole blood was used to determine thiamin and folate contents, while the plasma samples were used to measure vitamin B_{12}, folate, and retinol along with its carrier, retinol binding protein (RBP). In order to minimize the light-induced oxidation of vitamin B_{12} and retinol, the plasma samples were wrapped in aluminum foil before storage. In order to lyse the erythrocytes, an aliquot of the whole blood was mixed gently with 0.4% ascorbate solution prior to storage to assess folate content of the red blood cells.

At the screening visit, each patient was seen by a nutritionist and instructed in a diabetic diet according to the Canadian Diabetes Association (CDA) Guidelines (CDA, 1989). Patients were given a diet diary; they were asked to record three days

(two working days plus one weekend day) of food intake. Diet diaries were collected throughout the study. The diary at week 4 reflects the patient's baseline diet. Review of subsequent diaries and counseling of patients were carried out by a nutritionist throughout the study in an attempt to maintain the same dietary regimen. The diary at week 48 reflects the patients diet at the end of the study.

The second National Health and Nutrition Examination Surveys (NHANES II, 1976-80) were conducted to estimate the number and percent of persons in the United States with selected diseases and risk factors and to monitor trends in the prevalence, awareness, treatment and control of selected diseases. The population base consists of 33994 people aged 2 months to adult and was used to get information on health and nutrition status of the U.S. population via surveys and physical exams. Since there was no control group in the present study, the vitamin status of our Type 2 diabetes subjects were compared to a similar age group in the NHANES II population cohort.

2.2 VITAMIN ANALYSIS

2.2.1 Vitamin A

The fasting plasma retinol concentration was determined using a modified method of Nierenberg and Lester (1985). In this method, a Varian 5000 liquid chromatograph combined with a Shimadzu Sil-9A autoinjector (Columbia, MD), and a Waters 486 turnable absorbance detector (Millipore, Mississauga ON) was used.

The detector was set at a wavelength (λ) of 300nm for maximum absorbance. The chromatograph was equipped with a 4.6cm x 50mm guard column containing Supelco LC-18 (Supelco, Ontario) reverse phase packing (20-40μm), and the retinol in plasma was separated on a 15.0cm x 4.6 mm Supelcosil LC-18 (Supelco, Mississauga, ON) reverse phase analytical column with 5μm packing.

The gradient for elution of the mobile phase was 95%:5% (acetonitirile: methanol) at time 0, 0%:100% at 4 minutes, and 95%:5% at 8.5 minutes. The flow rate was 1mL/minute and the injection volume was 100μL at ambient temperature. The total run time per sample was 12 minutes. Retinol concentration was determined using both external and internal standard methods. The external standard was all-*trans*-retinol, and the internal standard was all-*trans*-retinol acetate (Sigma, St.Louis, MO).

Extraction of retinol was performed using the solvent extraction method of Nierenberg and Lester (1985). Stock solutions of all-*trans*-retinol were prepared in ethanol and stored, covered in foil, at -20°C. Standard dilutions were prepared daily in acetonitrile with concentrations ranging from 2.5 mg/mL to 12.5 mg/mL. 100μL of acetonitrile and internal standard were added to 200μL of plasma. The retinol in the samples was extracted with 250μL of buffer (butanol:ethyl acetate, 1:1). 100μL of this organic layer was injected into the high performance liquid chromatography (HPLC) apparatus. All samples were prepared in duplicate and under dim light to prevent possible oxidation of retinol. Standards were exposed to the same extraction procedure as the samples. All solvents used were HPLC grade.

Chromatograms were recorded, integrated and reprocessed using the Shimadzu EZ Chrom Data System version 2.1 (Kyoto, Japan). The percent recovery of retinol after the extraction procedure was 95.1 ± 5.9% , and the same day coefficient of variation was less than 5% and the between day coefficient of variation was less than 7%.

2.2.2 Retinol Binding Protein (RBP)

The plasma RBP concentration was assessed using a radial immunodiffusion (RID) assay kit (The Binding Site, Birmingham, England). This method involves an antigen diffusing radially from a cylindrical well through an agarose gel containing an appropriate mono-specific antibody. Specifically, the RBP in the plasma sample binds to rabbit antiserum in the agar gel to form a precipitate ring which is measured and evaluated. The ring size will increase until equilibrium is reached between the formation and breakdown of these complexes, thereby indicating "completion". This technique is derived from the work of Mancini and colleagues (1965).

The ring diameters produced by a gradient of standard concentrations (0.5, 3, and 5 mg/mL) were used to construct a calibration curve, using procedure two of the general instructions for BIND A RID™ NANORID™ products (The Binding Site, 1996). Plasma samples were diluted 1 in 20 before applying to the plate wells. Standards and control were applied to the plates undiluted. Plates were incubated at ambient temperature, wrapped in foil pouches for 96 hours to allow for the complete

formation of rings. Same day coefficient of variation both within day and between day was less than 4%.

The diameter of the rings were measured using Behring Diagnostics viewer, to an accuracy of 0.1mm, under dim light for easier reading. Concentration of RBP in samples was determined by extrapolating the square of the diameters off the standard curve and multiplying by the dilution factor in the final calculations. Controls were used to check for accuracy of the method and purity of the RID plates.

2.2.3 Vitamin B_1 (Thiamin)

The enzyme transketolase (TK) catalyzes the conversion of pentos-5-phosphate into heptulose-7-phosphate, part of the reactions in the hexose monophosphate shunt in the glycolytic pathway, which requires thiamine pyrophosphate (TPP) as a cofactor. Using the methods of Schouten et. al. (1964), Brin (1970), and Basu et. al. (1974), the status of thiamin in red blood cells was assessed. The biochemical status of this vitamin was determined by measuring the thiamin pyrophosphate (TPP) dependent transketolase (TK) activity, and its *in vitro* stimulating effect following an addition of TPP (TPP effect) in red blood cells.

This is a functional evaluation of vitamin B_1 adequacy using ETK and TPP effect. It is specific for thaimine depletion and is unaffected by changes in plasma enzymes. The assay reveals 2 measurements, one for TK enzyme activity, and one

for TPP effect. This allows for differentiation between a depletion of the apoenzyme and a simple coenzyme deficiency.

The procedure is a simplified colorimetric assay for samples of 50 μL of whole blood. L-cystine hydrochloride (3% w/v) was used to initiate color development. A gradient of standards of sedoheptulose-7-phosphate (S-7-P), with concentrations of 1.05 mM, 2.10 mM, and 3.15 mM were prepared by making adequate dilutions of the stock solution with buffer in volumetric flasks. Standard tubes were exposed to the same method as the sample tubes. All tubes were prepared in duplicate for a total of 6 tubes for each sample, and 6 tubes for standards.

After the final incubation in the methods previously described (Schouten et al, 1964; Basu et al, 1974), all the patient blank tubes and the TK activity tubes were a clear, light yellow. The tubes showing the TPP effect were a clear, yellow-orange color. The standard tubes were progressively darker clear orange as the concentration of S-7-P increased.

Absorbance of the tubes were measured spectrophotometrically using a Hewlett Packard 8452A Diode Array Spectrophotometer (Germany) at 510nm and 540nm, and acquired using the HP-89532A UV-Visible General Scanning Software, 1992 version (Hewlett Packard, Germany). The difference between absorbency at these wavelengths was used to calculate TK activity and TPP effect as outlined by Schouten et.al. (1964). A higher percent TPP effect indicates lower blood transketolase levels and therefore low vitamin B_1 status.

2.2.4 Vitamin B_{12} and Folic Acid (Folate)

Vitamin B_{12} in plasma as well as folate in both plasma and red blood cells were measured simultaneously using a commercially available Solid Phase No Boil Dualcount® kit (Intermedico, Markham, Ontario) which follows the method of Mollin et al (1976). During the procedure, all tubes were wrapped with aluminum foil to protect against light exposure. Vitamin B_{12} and folic acid were first released from their carrier proteins by incubation at a pH greater than 12 in the presence of dithiothreitol (DTT) and potassium cyanide (KCN). This allows inactivation of intrinsic factor (I_f) and conjugase antibodies. Purified porcine intrinsic factor and folate binding protein (conjugase) are used as binders for vitamin B_{12} and folic acid, respectively. The vitamin B_{12} tracer was radioactively labeled with ^{125}Iodine while that of folate was labeled with ^{57}Cobalt. The unlabeled vitamin B_{12} and folate in the patient sample compete with the respective labeled species for a limited number of available binding sites on their specific binders. The greater the concentration of the vitamin in the patient samples, the less binding there will be of the labeled species, and thus the lower the radioactive counts.

Isolation of the bound fraction was achieved by centrifugation and aspiration. Simultaneous counts of the isotopes were achieved using a Cobra Auto-Gamma dual-channel gamma counter (Canberra Packard Co., Canada). The kit has a detection limit of approximately 35 pg/mL for vitamin B_{12} and 0.3 ηg/mL for folate. Anemia controls supplied in the kit were used to determine accuracy of the method. Seven calibrators were used to obtain a standard calibration curve off which quantities of

the vitamins in the patient samples were calculated using a software program (Canberra Packard Co., Canada).

Whole blood folate was calculated by multiplying the "raw" result by 11 to account for the dilution of the blood with ascorbate. To get the red cell (rbc) folate in ng/mL, the value calculated for whole blood folate is multiplied by 100 and divided by the hematocrit (in percent).

Serum vitamin B_{12} < 148pmol/L, serum folate <13.6 nmol/L and rbc folate <363 nmol/L were considered low based on NHANES II (1976-80) males and females 50-74 years. The proportion of subjects in each treatment group with low values were compared using chi-squared test.

2.3 STATISTICAL ANALYSIS

Statistical analysis of the data was performed using Statistical Analysis Systems (SAS version 6.03, 1996). Means and standard error of the means (SEM) were determined for all patients. Comparisons were made within and between treatments using a paired t-test and linear regression analysis. Significant differences within treatments were determined using one-way analysis of variance (ANOVA). Significant differences between treatments were determined by comparison of least square means. The level of significance (α) considered was 0.05 with a confidence interval of 95%.

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3. EFFECT OF DIABETES ON THE STATUS OF A SELECT GROUP OF VITAMINS

Most studies involving metabolic derangements in DM are limited to macronutrients such as carbohydrates, proteins, and lipids. Micronutrients such as vitamins often act as cofactors in the metabolism of macronutrients, and yet very little reports are available on these essentials in the context of diabetes.

Reduced levels of retinol and its carrier retinol-binding protein (RBP) have been reported in patients with T1DM (Basu et al, 1989; Krill et al, 1997). It has been suggested that the reduced serum vitamin A is a result of impaired mobilization of retinol from the liver (Basu and Basualdo, 1997). Vitamin A has also been reported to be an essential factor for insulin secretion (Cherton, 1987) and therefore improves the hyperglycemic state. This is further supported by a study demonstrating that insulin treatment given to STZ-induced diabetic rats normalizes the metabolic availablility of vitamin A (Tuitoek et al, 1996). Vitamin A plays an essential role in vision, and a deficiency of this vitamin may cause blindness (Flodin, 1980). DM has also been shown to be a major cause of retinopathy leading to blindness in adults (Moss et al, 1989).

Other vitamins that have been mentioned in the literature, specifically in the context of diabetes, include vitamins B_1 and B_{12}. In a study involving both T1DM and T2DM patients, vitamin B_1 deficiency was observed, as evidenced by decreased erythrocyte transketolase activity (ETKA) compared to healthy controls (Kjosen and

Seim 1977). Vitamin B_{12}-associated pernicious anemia and the concurrent presence of T2DM have also been reported (Tomkin et al, 1971; McCarty and Rubin, 1984; Mooradian et al, 1994). A causal relationship between DM and vitamin B_{12} status has not been elucidated. However, the secretion of intrinsic factor, the vitamin B_{12}-dependent carrier glycoprotein synthesized in the parietal cells of the stomach, is stimulated by insulin (Mathews van Holde, 1989), and hence, the absorption of vitamin B_{12} may be adversely affected in the presence of T2DM.

The present study was undertaken to assess the nutritional status of a select group of vitamins in patients with T2DM. The vitamins included are retinol, thiamin, vitamin B_{12}, and folate.

3.1 METHODS AND MATERIALS

3.1.1 SUBJECTS AND SAMPLE COLLECTION

Recruitment of subjects, randomization, and sample collection and storage were undertaken as previously described in Chapter 2.

3.1.2 VITAMIN ANALYSIS

The analysis of vitamins was undertaken as described in Chapter 2.

3.1.3 STATISTICAL ANALYSIS

Unless otherwise specified, data was analyzed as previously described in Chapter 2.

3.2 RESULTS

A total of three hundred and twenty-four T2DM patients participated in this study. The demographic characteristics at baseline are outlined in Table 3.1. The age range of all patients (male and female) was 38 to 80 years and the overall body mass index (BMI) ranged from 20.4 to 43.3 kg/m^2. The overall male to female ratio was about 3:1 (240:84, n/n). The duration of diabetes ranged from newly diagnosed to 39 years.

Table 3.1: Characteristics of T2DM Patients at the Time of Random Selection

CHARACTERISTIC	T2DM Patients at Baseline
Number (n)	**324**
Age, years	**57.9 ± 0.5 ***
Gender	
male, n	**240**
female, n	**84**
Weight, kg	**88.6 ± 0.9***
BMI[†] kg/m^2	**30.6 ± 0.2 ***
Duration of Diabetes, years	**5.9 ± 0.3***
HbA_{1c}[‡], %	**8.0 ± 0.1***

*** Means are expressed ± SEM**

[†] Body mass index

[‡] Glycosylated hemoglobin

Dietary intakes of the vitamins were measured and were found to meet the RNI in all of the study subjects. The individual distributions of plasma vitamin B_{12} and folate as well as the red blood cell folate in patients with T2DM are depicted in Figure 3.1.

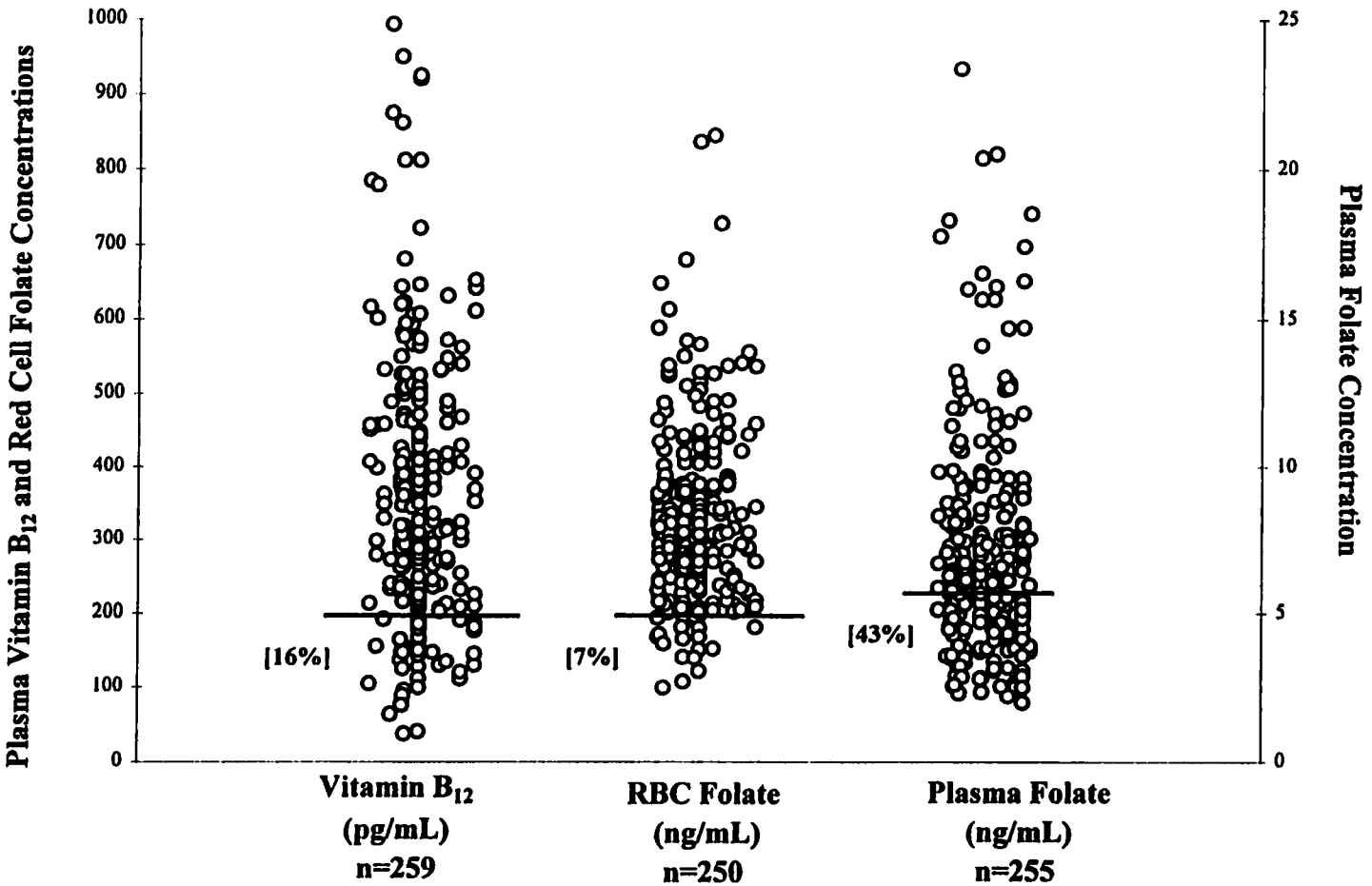

Figure 3.1: Distribution of the concentration of plasma vitamin B_{12} (200-900pg/mL) and folate (160-700ng/mL) and red cell (RBC) folate (6-17ng/mL) in type 2 diabetes (T2DM) patients relative to normal average for general population (45-74yr) in NHANES II (Gibson, 1990) as indicated by horizontal line. Numbers in square brackets indicate % of patients below the lower range.

According to the average value of the normal range of vitamin B_{12} determined by NHANES II (1976-1980), 78% of the patients had plasma levels of vitamin B_{12} below this reference value (550 pg/mL). Forty-one out of 258 (16%) of the diabetic subjects had plasma vitamin B_{12} less than 200pg/mL (Table 3.2), which is indicative of a borderline deficiency (Gibson, 1990).

The biochemical status of folate exhibited a similar pattern to that of vitamin B_{12} in that the average plasma and RBC folate levels did not achieve normal average values (11.5 ng/mL and 450 ng/mL, respectively) in almost 90% of the diabetic subjects (Figure 3.1). A folate level in plasma less than 6 ηg/mL is considered to be suggestive of a borderline deficiency in the intake of the vitamin (Senti and Pilch, 1985; Butterworth and Tamura, 1989; Gibson, 1990). According to this guideline, folate status was found to be of concern in 109 (43%) of the study subjects (Table 3.2).

Table 3.2: Biochemical Status of Folate and Vitamin B_{12} in T2DM Patients Compared to a Reference Healthy Population

VITAMINS	n	T2DM[1]	% T2DM deficient[2]
Vitamin B_{12}			
plasma, (pg/mL)	258	375 ± 12.4	16
Folate			
plasma, (ng/mL)	255	7.2 ± 0.2	43
rbc, (ng/mL)	242	329 ± 7.7	7

1 **Means expressed ± SEM**

2 **Defined as "borderline deficiency": vitamin B_{12} < 200 ρg/mL, plasma folate <6 ηg/mL, RBC folate <160 ηg/mL compared to "normal" male and female population in the 50-74yr age group as assessed by the NHANES II 1976-1980 (Gibson, 1990).**

The RBC folate is generally considered to be a marker for the body reserve of the vitamin. According to the guideline that an RBC folate level less than 160 ηg/mL is indicative of a borderline deficiency of folate body reserve, 7% of the study subjects were found to have the concern (Table 3.2). It was noteworthy that although the individual analyses of the data revealed the presence of deficiencies for both vitamin B_{12} and folate in appreciable numbers of diabetic subjects, the mean concentration for these vitamins were all within the normal ranges (Table 3.2). The normal plasma concentration for vitamin B_{12} should fall in the range of 200-950 pg/mL while that of plasma and RBC folate should be between 3-17 ηg/mL and 160-700 ηg/mL, respectively.

The correlations between plasma vitamin B_{12} and either plasma or RBC folate are shown in Table 3.3. The vitamin concentrations were all significantly positively correlated (p=0.0001). A significant positive correlation was also observed between plasma and RBC folate levels (Table 3.3).

Table 3.3: Linear Regressions* Between Vitamin B_{12} and Folate in T2DM Patients*

	n	Intercept	Slope	r	p
Vitamin B_{12} vs. Plasma Folate	254	4.2 ± 0.4	0.008 ± 0.001	0.44	0.0001
Vitamin B12 vs. RBC Folate	241	266 ± 15.5	0.17 ± 0.036	0.28	0.0001
Plasma Folate vs. RBC Folate	238	193 ± 12.7	18 ± 1.6	0.60	0.0001

* **Data expressed ± SEM**

Figure 3.2 shows the individual data for plasma retinol and its carrier protein in a total of 273 subjects with T2DM. According to the normal reference range of 1.05-7μmol/L (or 30-200 mg/dL; Gibson, 1990), 89% of the diabetic subjects had plasma retinol concentrations below the normal mean (4.03 μmol/L; Figure 3.2). Of these, 16 patients (6%) had plasma retinol less than 1.05 μmol/L (Table 3.4) the level considered to be a borderline deficiency of vitamin A (Gibson, 1990).

The plasma concentration of retinol binding protein (RBP) in the T2DM patients was normally distributed around the reference mean (Figure 3.2), and yet almost 60% of these patients had concentrations of this protein below the normal mean (2.6-7.6 mg/dL). There were 3% of the study subjects who had subnormal (<2.6 mg/dL) RBP concentrations in the plasma (Table 3.4).

Table 3.4: Biochemical Status of Plasma Retinol in Type 2 diabetes Patients Compared to a Reference Non-Diabetic Population

	n	T2DM[1]	% T2DM deficient[2]
Retinol (μmol/L)	273	2.6 ± 0.085	6
Retinol Binding Protein, (mg/dL)	273	5.3 ± 0.097	3

1 Means expressed ± SEM
2 Indicates % of T2DM patients below the deficient range for the normal population: retinol <1.05μmol/L, rbp < 2.6 mg/dL (Gibson, 1990)

There was a positive (r=0.224) and significant (p=0.0002) correlation between plasma retinol and its carrier protein RBP (molecular weight 21000 g/mol) in the T2DM patients (Figure 3.3). Normally, retinol circulates in the plasma largely

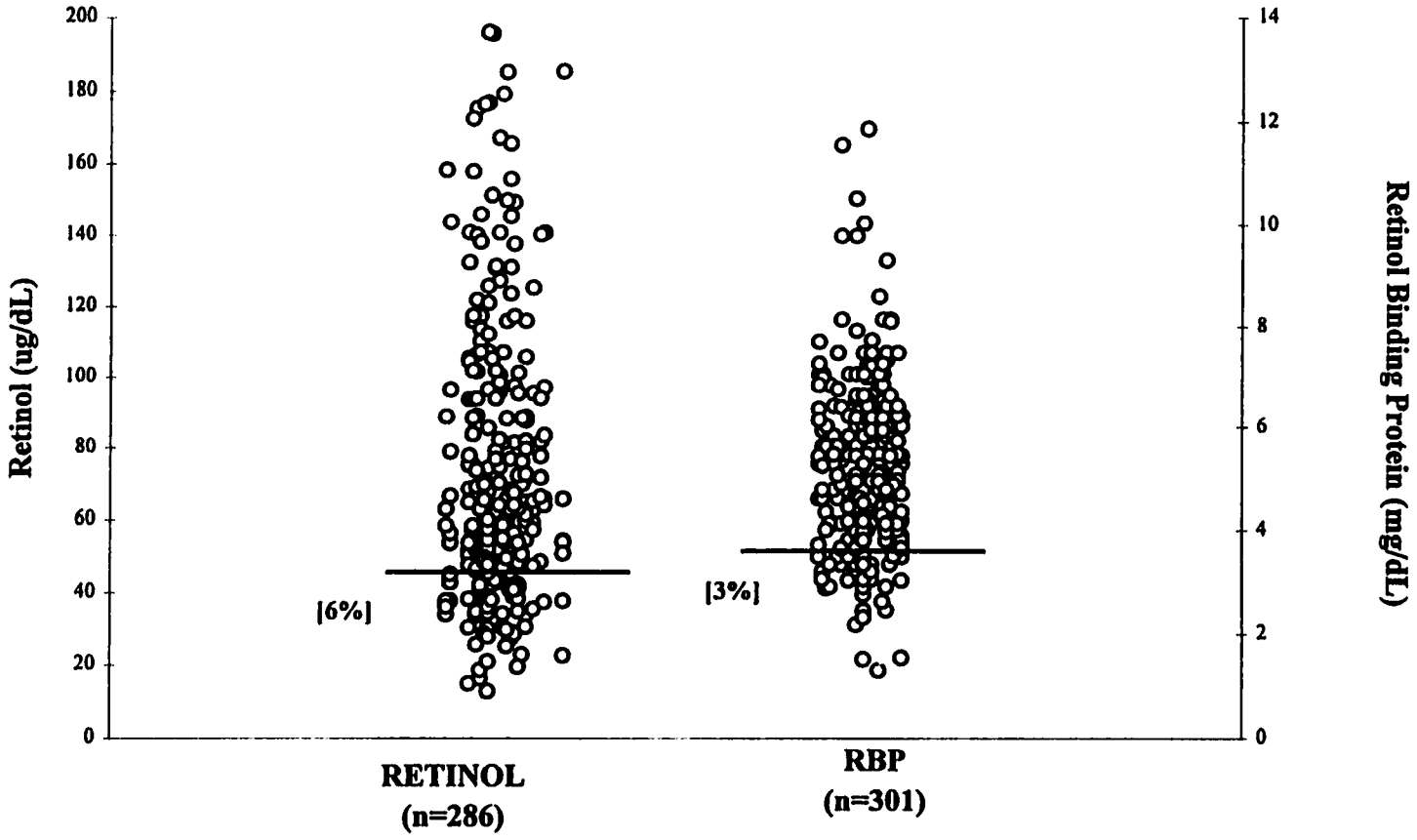

Figure 3.2: Distribution of plasma concentrations of retinol (ug/dL) and its carrier protein (mg/dL) in 'n' number of T2DM patients relative to normal average for the general population (45-74 yr) in NHANES II (Gibson, 1990) as indicated by horizontal line. Numbers in square brackets indicate % of T2DM patients below normal mean. S.I. units (umol/L)=ug/dL x 0.035.

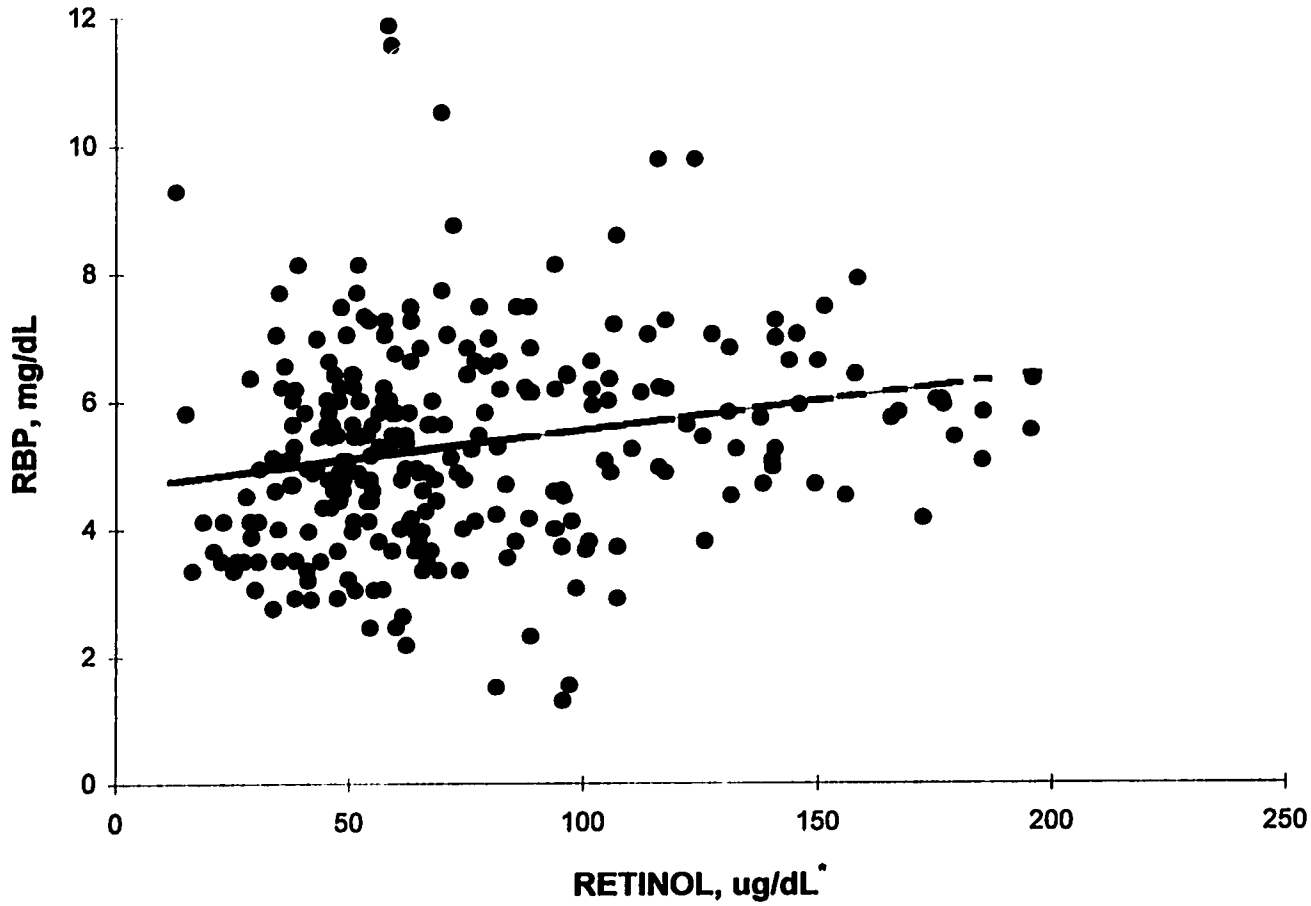

Figure 3.3: Correlation (r=0.224; p=0.0002) between plasma retinol and retinol binding protein (RBP) in type 2 diabetes (T2DM) patients (n=272). *Conversion to S.I. (umol/L)=ug/dL x 0.035.

in the form of a 1:1 complex with RBP. In this study, the ratio was found to be 1:1.1 in the diabetic subjects.

The biochemical status of thiamin in patients with T2DM, as determined by erythrocyte transketolase activity (ETKA) and thiamin pyrophosphate (TPP) stimulating effect on the ETKA (TPP Effect), is shown in Figure 3.4.

The majority of the T2DM patients (89%) had erythrocyte transketolase activity (ETKA) greater than the normal reference mean of 1.15 IU (Gibson, 1990). The higher the ETKA, the lower the concentration of thiamin in the blood. According to this criterion, sixty percent of the patients with T2DM were at risk of biochemical deficiency of the vitamin (Table 3.5). The mean ETKA for the patients was also found to be higher than the value (2.0 IU) which is indicative of reduced TK activity compared to the normal population.

Table 3.5: Vitamin B_1: Distribution of T2DM Patients into risk groups based on Erythrocyte Transketolase Activity (ETKA) and the *in vitro* stimulation of this enzyme by Thiamin Pyrophosphate (TPP Effect)

	ETKA (I.U.)[2] n=309	TPP Effect [3] (%) n=302
Mean Value for T2DM[1]	2.2 ± 0.035	12 ± 0.83
Low Risk[4]	40%	63%
High Risk[5]	60%	25%

[1] Means expressed ± SEM
[2] I.U.=International Units = µmol/min/L
[3] Calculated as: [(ETKA - TPP Activated ETKA)/TPP Activated ETKA] x 100 (%) (Takeuchi et al, 1989)
[4] % of patients at low risk of deficiency (defined as < 2.0 IU and < 15% for ETKA and TPP Effect, respectively)
[5] % of patients at high risk for thiamin deficiency (defined as >2 I.U. and >20%, for ETKA and TPP Effect, respectively)

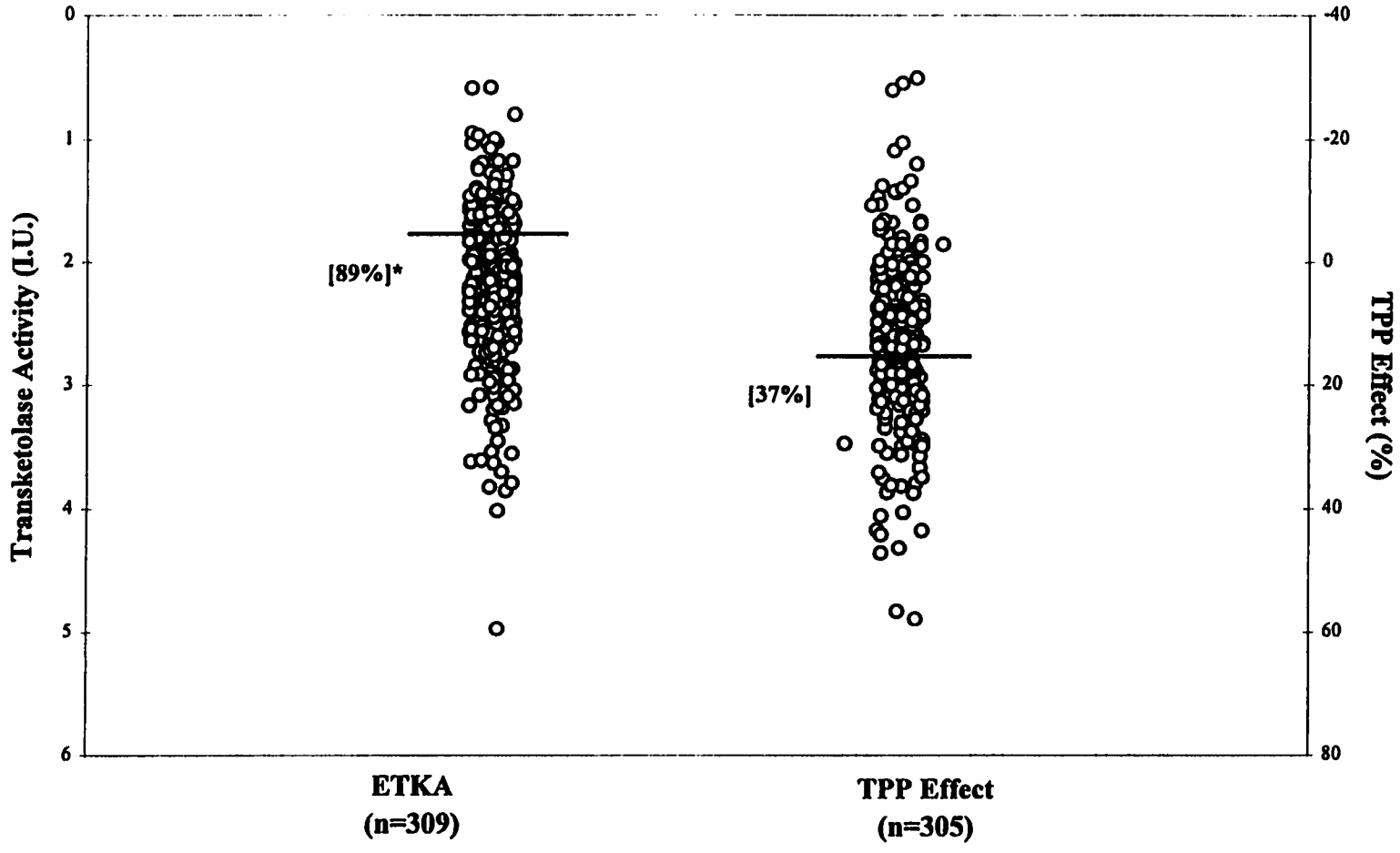

Figure 3.4: Erythrocyte transketolase activity (ETKA) and thiamin induced transketolase activity (TPP Effect) in type 2 DM (T2DM) patients. Mean for general population (45-74yr) as indicated by horizontal line (Gibson, 1990). *Numbers in square brackets indicate % of patients below normal mean. International Units = umol/min/L (Vuilleumier et al, 1983).

The degree of thiamin deficiency was also assessed using thiamin pyrophosphate (TPP) induced TK activity expressed as the "TPP effect". The higher the percentage, the lower the thiamin concentration in the blood. Thirty-seven percent of the T2DM patients had a "TPP effect" greater than the normal mean of 15% (Figure 3.4). One quarter of the population studied appears to be at high risk of a biochemical deficiency as evidenced by a TPP effect > 20 % (Table 3.5).

Linear regression analysis of ETKA and TPP induced TK activity shows that these parameters are significantly ($p=0.0001$) and highly positively ($r=0.88$) correlated (Figure 3.5). The mean value for TPP induced TKA was 2.4±0.036IU.

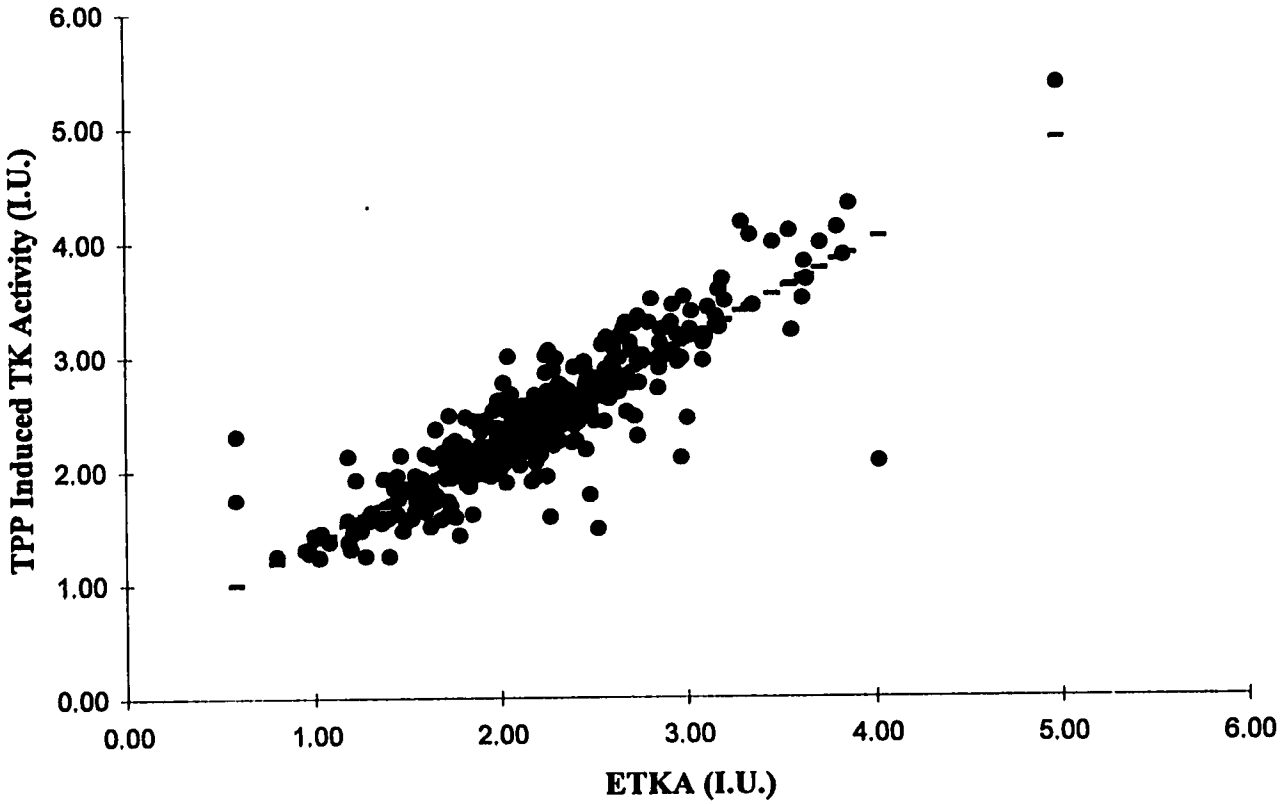

Figure 3.5: Correlation (r=0.88, p<0.0001) between erythrocyte transketolase activity and TPP effect in T2DM patients (n=309). International units (I.U.)=umol/min/L.

3.3 DISCUSSION

According to the present study all study subjects consumed adequate amounts of thiamin, 75% consumed the recommended nutrient intake (RNI) for vitamin A and folate, and the RNI for energy was met by only 55% of the T2DM patients. Overall, all subjects met two-thirds RNI for all of the nutrients, which was expected (Schmidt et al, 1994). Despite adequate intake of the vitamins, and appreciable proportion of the T2DM patients displayed biochemical evidence of the vitamin deficiencies.

In humans, plasma concentration of retinol below 0.70 μmol/L (20μg/dL) is normally associated with clinical signs of vitamin A deficiency, while values less than 1.05 μmol/L are indicative of borderline deficiency of vitamin A, requiring dietary supplementation (Gibson, 1990). According to this criterion, 6% of the present study subjects are considered to be marginally deficient in vitamin A, as evidenced by their plasma vitamin A levels below 1.05 μmol/L.

A clinical deficiency in vitamin A is rare in the normal adult population of developed countries with an incidence of <0.1% (Pilch, 1987; Euronut, 1991; Maiani et al, 1993). It is also noteworthy that 89% of 286 T2DM patients had plasma retinol levels below the average for the 45-74 year population (Gibson, 1990; Olmedilla et al, 1997). These results are in parallel with the plasma concentrations of the vitamin A carrier protein (RBP). Approximately 60% of the study subjects with T2DM had RBP levels less than the average values in the population. There were, however, only 3% of the study population with plasma RBP levels below 2.6 mg/dL, the level

that is indicative of vitamin A deficiency (Pilch, 1987; Gibson, 1990). The dietary intake of vitamin A (in RE) was found to meet RNI in over 90% of the T2DM patients.

The reported normal range for plasma RBP levels is 2.6-6 mg/dL (Gibson, 1990) for populations of all ages. According to Basu et al (1994), the lower limit of the normal range relates more to younger, rather than older age groups. If this is so, the cut off point for the normal range of the plasma RBP levels for the age group of the study subjects could be considerably higher than 2 mg/dL. In this case, a greater proportion of the diabetic subjects would have been found to be associated with a below normal level of the plasma protein. The present study did not include age-matched diabetic control subjects.

The plasma retinol level is largely regulated by the synthesis of RBP in the liver (Hunt and Groff, 1992). Retinol is present in the plasma in a 1:1 molar ratio with RBP. The plasma retinol and RBP concentrations in patients with T2DM were significantly ($p=0.0002$) correlated (Figure 3.3), and their molar ratio was approximately 1:1. These results are in agreement with others (Basualdo et al, 1997), who made a similar observation in subjects with insulin resistance T2DM.

Thiamin plays an important role in energy metabolism, with particular reference to carbohydrate. It is thus involved in decarboxylations of pyruvate and α-ketoglutarate, the two metabolites in Kreb's cycle. Thiamin nutriture, therefore, becomes an important issue in patients with diabetes. The blood thiamin levels in diabetic subjects have been reported to be increased or unaffected (Finglas, 1993).

The majority (>60%) of the present study population with T2DM was found to have a reduced thiamin-dependent erythrocyte transketolase activity (ETKA). These results are in agreement with earlier studies (Kjosen and Seim, 1977).

Adequacy of thiamin nutriture is perhaps more accurately determined by measuring the thiamin pyrophosphate (TPP)-stimulating effect on ETKA (Graudal et al, 1985; Doolman et al, 1995). In this procedure, the TKA before and after the addition of TPP is measured, and an increase in activity of over 20% is indicative of a thiamin deficiency (Brin, 1970; Cromer, 1989; Gibson, 1990). According to this criteria, 37% of 305 T2DM patients were thiamin deficient.

In addition to vitamin A and thiamin, the present study determined folate and vitamin B_{12} status in patients with T2DM. The plasma or red blood cell (RBC) folate was significantly positively correlated with the plasma concentration of vitamin B_{12}. However, according to the criteria that vitamin B_{12} less than 200 pg/mL and plasma folate <6ηg/mL are considered to be borderline deficiency levels (Gibson, 1990). Sixteen and 43% of the study subjects were marginally deficient in vitamin B_{12} and folate, respectively. In contrast, a clinical deficiency in vitamin B_{12} is very rare among the non-diabetic population in developed countries (McCarty and Rubin, 1994), while approximately 9% of the population may exhibit a deficiency in plasma folate (Euronut, 1991; VanDenBerg, 1993).

Plasma folate levels generally reflect dietary intake while RBC folate is more indicative of long-term folate status. While up to 12% of the general population can exhibit RBC folate deficiency (Senti and Pilch, 1985; Mooradian et al, 1994), it was

of interest that only 7% of the diabetic patients in this study had RBC folate levels less than 160 ηg/mL, an indication of borderline deficiency of the vitamin (Butterworth and Tamura, 1989; Maree et al, 1989).

In conclusion, a substantial number of patients with T2DM recruited from seven provinces across Canada appear to be at risk of deficiency of vitamins A, B_1, B_{12}, and folate, as determined by their biochemical status. In most cases, the average mean values for the biochemical index fall within the normal range, but it is only the individual analysis that has revealed the concern. On average, these diabetic subjects had dietary intakes of the vitamins meeting the recommended nutrient intake (RNI). There are, however, many individuals whose nutrient intakes should be improved. The sub-normality of the nutriture of vitamins was more pronounced in their biochemical states than in their dietary intakes. It is possible that the impaired biochemical status is a reflection of an altered metabolism of the nutrients due to the presence of diabetes mellitus.

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4. THE EFFECT OF ORAL ANTI-DIABETES DRUGS ON THE BIOCHEMICAL STATUS OF VITAMINS IN TYPE 2 DIABETES

Biguanides and alpha(α)-glucosidase inhibitors are classes of oral anti-diabetic agents for the clinical management of T2DM. Metformin, a widely used biguanide, reduces glucose levels without stimulating insulin secretion. Its mechanism of action is thought to be mediated through decreased hepatic glucose production as a result of inhibition of hepatic gluconeogenesis, and increased peripheral glucose uptake, and decreased gastrointestinal absorption of glucose (Lee, 1996).

Alpha-glucosidase inhibitors are a new class of drugs which delay carbohydrate absorption by reversibly inhibiting the α-glucosidase enzymes in the brush border of the small intestine. This inhibition leads to a delayed and reduced postprandial blood glucose rise. The positive effect on glucose levels of miglitol (an α-glucosidase inhibitor) has already been shown in normal subjects and in T2DM patients (Wolffenbuttel and Graal, 1996).

The interactions between oral anti-diabetic drugs and vitamins have not been adequately investigated. There have been some isolated reports suggesting a prevalence of megaloblastic anemia in T2DM patients treated with metformin (Tomkin et al, 1971; Lee, 1996). The treatment with this biguanide has also been found to be associated with decreased plasma vitamin B_{12}, but not folate (Carpentier et al, 1976; DeFronzo et al, 1995).

One study reports that treatment with acarbose, an α-glucosidase inhibitor, has no appreciable effects on a select group of vitamins as evidenced by their biochemical status (Chiasson et al, 1994). Miglitol, however, is a new α-glucosidase inhibitor which, unlike acarbose, is absorbable. Furthermore, miglitol's site of action in the small intestine overlaps with the sites at which the absorption of vitamins A, B_1, and B_{12}, as well as folate, take place.

It seems possible that the treatment with oral anti-diabetic drugs on a long-term basis may affect the status of certain vitamins. This, in turn, may be linked to the aggravation of metabolic derangements in diabetic patients. It is therefore, important that the interactions between the drugs and vitamins are further examined. The present study was undertaken to examine the effects of miglitol and metformin therapy on the status of a select group of vitamins including vitamins A, B_1, B_{12}, and folate in T2DM patients.

4.1 METHODS AND MATERIALS

4.1.1 Subjects and Sample Collection

Recruitment, randomization of subjects and sample collection and storage were as previously described in Chapter 2.

4.1.2 Vitamin Analysis

Blood and plasma samples were analyzed for retinol, thiamin, vitamin B_{12}, and folate concentrations as described in Chapter 2. Three-day diet records were obtained at randomization and at four times throughout the study. Subjects were asked to record every item of food and drink consumed for 2 weekdays and 1 weekend day into booklets provided for this purpose. These records were reviewed for accuracy by a dietitian in consultation with the subject. The dietitian then coded the diets using a standardized computer program that was provided for each center for this purpose. The database for this program was based on the Canadian Condensed Nutrient File (Health and Welfare Canada, 1987) which does not contain data for vitamin B_{12}. The diet records and HbA_{1c} values obtained at baseline and at the end of study were included in the statistical analysis. Means and standard errors of the means were calculated for each parameter.

4.1.3 Statistical Analysis

Unless otherwise specified, statistical analyses were performed using the method described in Chapter 2.

4.2 RESULTS

Three hundred and twenty-four patients with Type 2 DM participated in the study and were randomly assigned to receive either placebo (n=83), miglitol (n=82), metformin (n=83) or the combination of miglitol and metformin (n=76). Data for 199 of the 324 randomized subjects were included in the statistical analysis. Blood

samples for vitamin analyses were not obtained from 56 subjects. Of the 268 remaining subjects, 69 dropped out before the end of the study due to poor glycemic control, intolerable side effects, and lack of compliance. The demographic characteristics at baseline were similar among the four treatment groups (Table 4.1).

Table 4.1: Characteristics of T2DM Patients at the Time of Random Selection[1]

Variable	Placebo	Miglitol	Metformin	Combination[2]
Number (n)	45	45	62	47
Age, years	58.5 ± 1.6	56.8 ± 1.2	58.7 ± 1.1	59.5 ± 1.1
Gender				
male, n	27	39	45	42
female, n	18	6	17	5
BMI, kg/m^2	30.8 ± 0.5	30.7 ± 0.5	30.5 ± 0.6	29.9 ± 0.4
Duration of Diabetes, years	5.1 ± 0.5	5.2 ± 0.5	7.2 ±0.8	6.1 ± 0.6
HbA_{1c}, %	7.8 ± 0.1	7.9 ± 0.1	8.2 ± 0.1	8.2 ± 0.1

[1] Means are expressed ± SEM
[2] Miglitol + Metformin (Combination Therapy)

The mean age for all the patients was 59 ± 0.50 years and the overall mean body mass index (BMI) was 30.5 ± 0.2 kg/m^2. There were significantly more males than females in the miglitol and combination groups than in the placebo or metformin groups. The duration of DM was longer in the metformin group compared to the other groups, but this difference was not significant. Among the 199 subjects included, some samples for vitamin analysis were either lost or not obtained.

Only 126 of the patient diet diaries were available at the time of analysis, and the proportion of missing records was similar in the different groups (31 to 42%).

The averages of three day dietary intakes of energy, vitamins A, B_1, and folate for each treatment group are were all within the recommended nutrient intakes (RNI, Health and Welfare Canada, 1990) for the non-diabetic 50-74 year male and female population group.

The nutrient intakes at baseline and after 36 weeks of treatment did not significantly differ between the four treatment groups. Similarly, intakes of vitamin A were not significantly different within each group from baseline to the end of the study. The difference in folate intake from baseline to end of study in the placebo group was significantly different ($p<0.05$) when compared to the miglitol treatment group. This observation was also true for the thiamin intakes where the placebo group differed from miglitol, but not from the metformin or combination groups .). Thiamin intake was adequate in all four treatment groups with almost all patients meeting 2/3 RNI and less than 5% not meeting the RNI. The overall energy intakes were within the average RNI, and the intakes did not differ between the 4 groups.

Compared to the healthy population reference group of males and females, 50-74 years old (Health and Welfare Canada, 1990), mean daily intakes of macronutrients over 3 days were met. There was no significant change in the intake of any nutrient from the beginning to the end of the study in any of the treatment groups. Overall, these Type 2 DM patients appear to have normal, micro- and macronutrient consumption. According to the recent NHANES II survey (Gibson, 1990), the normal values for plasma folate should be greater than 6 ηg/mL. The

mean concentration of plasma folate was above this value for each of the four treatment groups (Table 4.2).

No significant differences were observed in plasma folate status within or between the treatment groups. The number of patients with borderline plasma folate status decreased by nearly one-half after 36 weeks of miglitol treatment (from 42% at week 0 to 23% at week 36). In contrast, the number of T2DM patients with plasma folate concentration below 6 ng/mL increased by 14% in the metformin treated group (38% to 52%).

Table 4.2: The Effects of Metformin and Miglitol on the Mean[1] Folate Concentration in Type 2 DM Patients

TREATMENT GROUP	n	FOLATE, plasma (ηg/mL) *Week 0*	*Week 36*
Diet Alone	43	6.92 ± 0.59 **(42)**[2]	7.08 ± 0.49 **(42)**
Miglitol	43	7.36 ± 0.52 **(42)**	7.58 ± 0.44 **(23)**
Metformin	55	6.91 ± 0.42 **(38)**	6.56 ± 0.51 **(52)**
Miglitol + Metformin	42	6.37 ± 0.41 **(48)**	6.85 ± 0.50 **(43)**
		FOLATE, red blood cells (ηg/mL)	
	n	*Week 0*	*Week 36*
Diet Alone	34	296.5 ± 13.5[a] **(2)**	339.5 ± 17.4[b] **(0)**
Miglitol	39	317.0 ± 17.6[a] **(5)**	345.2 ± 20.4[b] **(2)**
Metformin	50	346.2 ± 18.5 **(2)**	351.3 ± 18.0 **(4)**
Miglitol + Metformin	39	326.2 ± 18.8 **(2)**	337.6 ± 21.0 **(2)**

[1] Data are expressed as means ± SEM. Conversion factor to SI units (nmol/L) = ng/mL x 2.266.
[2] Numbers in parentheses ( ) indicate the percent of Type 2 DM patients in each group whose plasma and RBC folate concentrations were less than 6ng/mL and 160 ng/mL, respectively, indicating a risk for deficiency. [ab]Different alphabetic superscripts within each row indicate significant differences ($p<0.05$) from baseline to the last visit within the treatment group.

Like plasma folate, red blood cell folate levels were within the normal range (>160ηg/mL, Gibson, 1990). All four groups had higher RBC folate concentrations at the end of study compared to baseline (Table 4.2). The differences were statistically significant only in the placebo (diet alone) and the miglitol groups. RBC folate concentration was adequate in nearly all of the T2DM patients, with only up to 5% with RBC folate below 160 ηg/mL

The mean plasma concentration of vitamin B_{12} in all four groups of T2DM patients is shown in Figure 4.1. All were within the range of 200-700 pg/mL, which is considered to be physiologically normal (Gibson, 1990). This was true either at the onset of the study (baseline) or at 36 weeks after treatment (end of study). The concentration of vitamin B_{12} between the two study periods remained unaltered in the groups treated with either diet or miglitol. However, metformin treatment for 36 weeks resulted in a significant decrease in plasma vitamin B_{12} levels. This difference disappeared when metformin was administered in combination with miglitol (Figure 4.1).

The effect of metformin on plasma vitamin B_{12} concentration was significantly different ($p<0.05$) than the placebo or miglitol groups (Figure 4.2). This decrease in vitamin B_{12} concentration as a result of metformin therapy did not significantly differ in comparison to the decrease in vitamin B_{12} caused by the combination of miglitol plus metformin therapy (Figure 4.2).

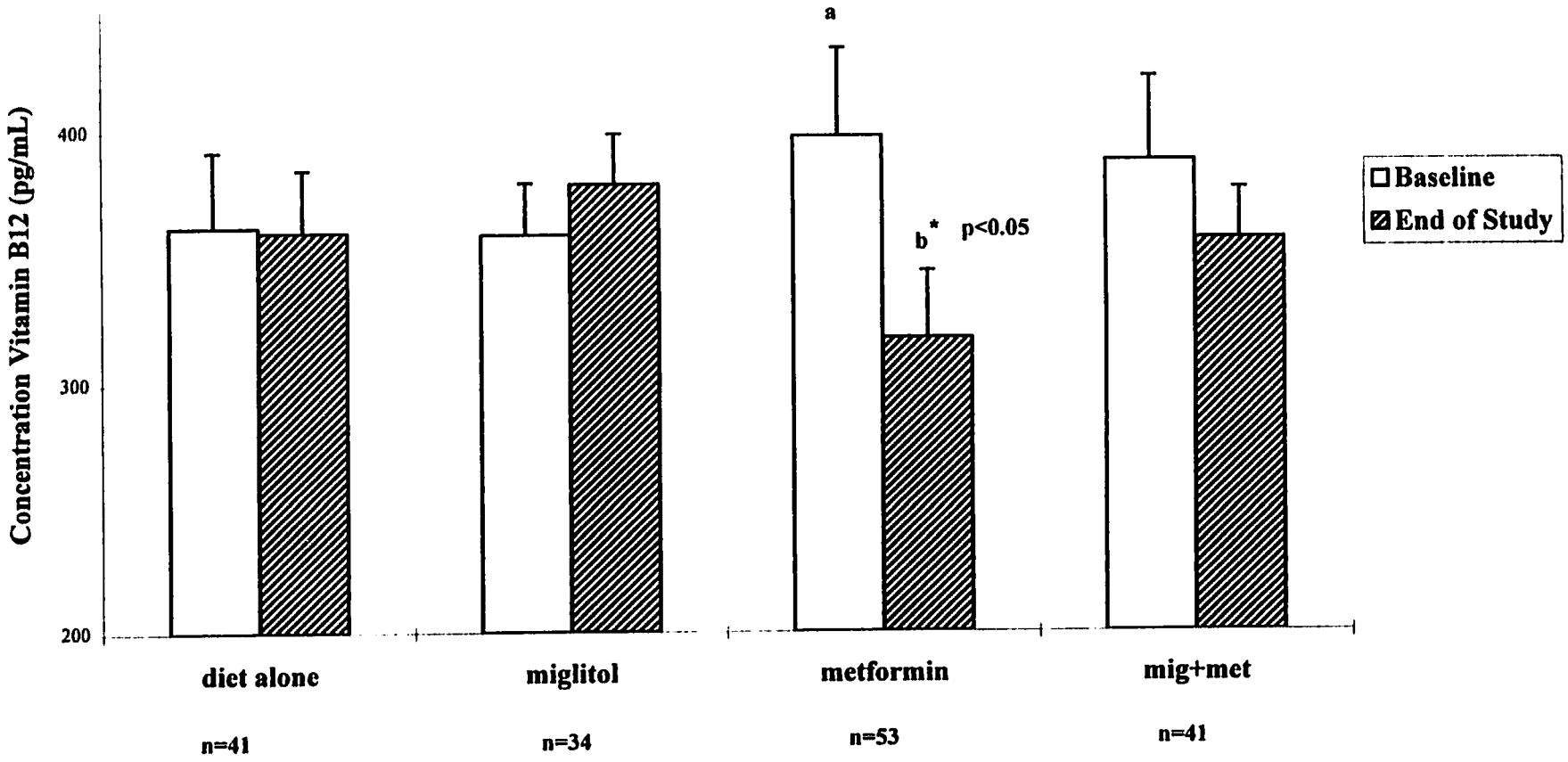

Figure 4.1: Effect of drug treatment on the mean biochemical status of vitamin B_{12} from baseline (week 0) to end of study (week 36) in patients with Type 2 DM. [ab] Difference *within* treatment significant at p<0.05.

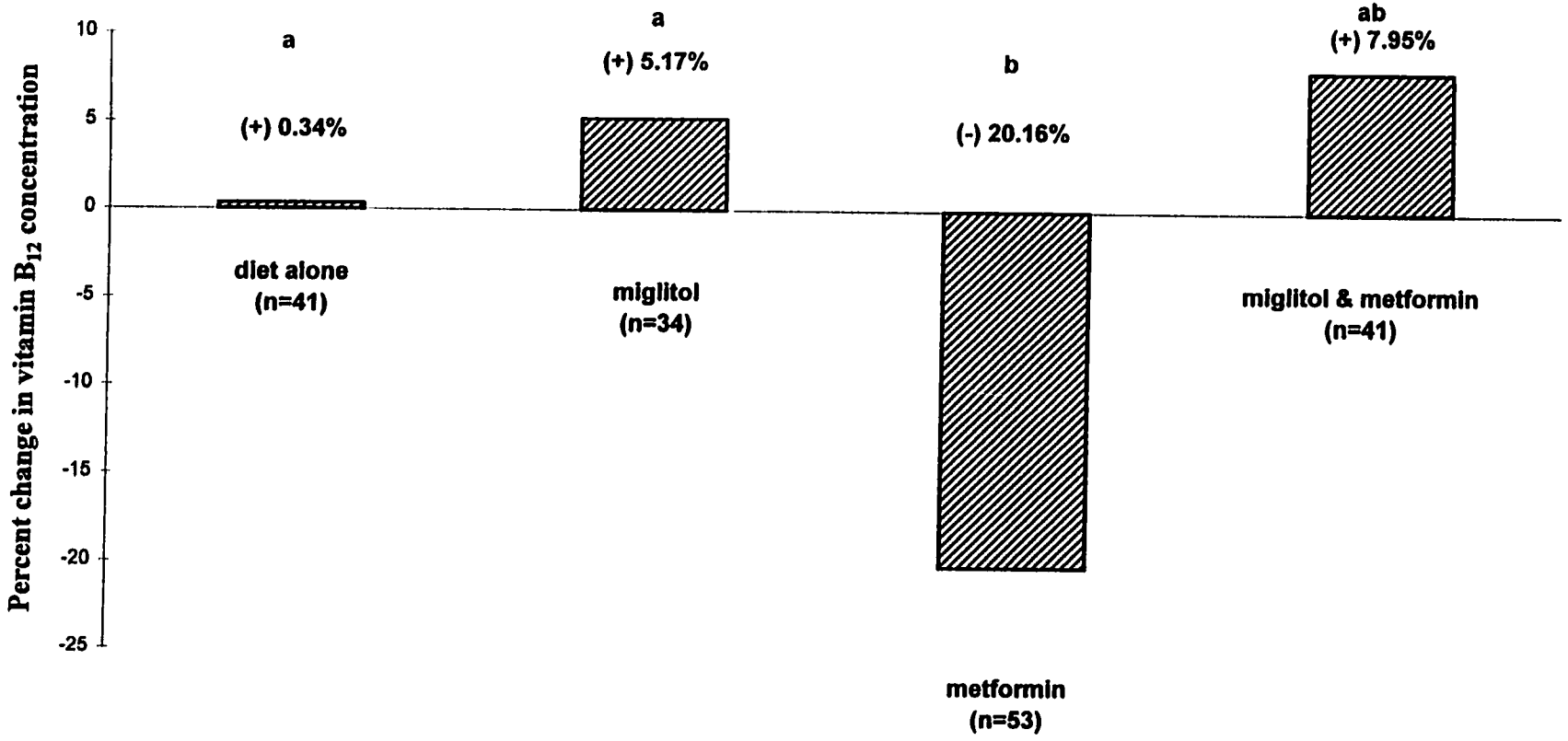

Figure 4.2: Percent difference in mean plasma vitamin B_{12} concentration in Type 2 diabetes mellitus patients after 36 weeks of metformin or miglitol treatment. (+) indicates the plasma B_{12} concentration increased; (-) indicates the plasma vitamin B_{12} concentration decreased. Different letters indicate significant differences between treatments ($p<0.05$).

The plasma concentrations of retinol remained unaffected in the T2DM patients treated with miglitol alone for 36 weeks (Table 4.3). The treatment with metformin, however, resulted in a significant decrease ($p<0.05$) in the plasma retinol level, and the effect disappeared when metformin was administered in combination with miglitol.

Table 4.3: The Effect of Miglitol and Metformin on the Mean[1] Plasma levels of Retinol in T2DM Patients

TREATMENT GROUP	*n*	RETINOL, plasma (μmol/L) *Week 0*	*Week 36*
Diet Alone	36	2.27 ± 0.21 **(8)**[4]	2.18 ± 0.23 **(14)**
Miglitol	41	2.52 ± 0.24 **(7)**	2.38 ± 0.25 **(10)**
Metformin	53	2.58 ± 0.19[a] **(4)**	1.88 ± 0.20[b] **(21)**
Miglitol + Metformin	38	2.94 ± 0.24 **(0)**	2.25 ± 0.26 **(15)**

[1] Data are expressed as means ± SEM
[2] Numbers in parentheses ( ) indicate percent of T2DM patients with plasma retinol concentrations <1.05μmol/L, levels of which are indicative of deficiency.
[ab]Values with different superscripts in the same row are significantly different at $p<0.05$ within the treatment group.

After 36 weeks of metformin therapy, 21% of the T2DM patients had concentrations of plasma retinol less than 1.05 μmol/L, compared to only 4% before treatment. Similarly, in the miglitol and metformin combination group where none of the patients exhibited low retinol concentrations at the beginning of the study, 15% had concentrations of retinol below 1.05 μmol/L by the end of the study (Table 4.3). Treatment with miglitol and diet alone showed only a 3% and 6% increase in the percent of patients with low retinol concentrations, respectively.

It was of interest that the plasma concentration of RBP was significantly elevated ($p<0.05$) in T2DM in all of the treatment groups, excluding placebo (Figure 4.3). These results were reflected in the R:RBP molar ratio which was lower at the end of the study in all four groups. The ratios were thus decreased in the presence of the anti-diabetic drugs.

Table 4.4: The Effect of Miglitol and Metformin on Erythrocyte Transketolase Activity (ETKA) and Thiamin Induced ETKA (TPP-ETKA) in T2DM Patients*

TREATMENT GROUP	*n*	ETKA (μmol/min/L) *Week 0*	*Week 36*	*p value*
Diet Alone	41	2.13 ± 0.10	2.26 ± 0.069	0.2547
Miglitol	38	2.19 ± 0.11	2.31 ± 0.076	0.2186
Metformin	56	2.26 ± 0.072	2.23 ± 0.066	0.4867
Miglitol + Metformin	44	2.11 ± 0.10	2.29 ± 0.086	0.0932
		TPP-ETKA (μmol/min/L)		
	n	*Week 0*	*Week 36*	*p value*
Diet Alone	41	2.36 ± 0.10	2.51 ± 0.078	0.1753
Miglitol	38	2.29 ± 0.12	2.52 ± 0.079	0.0559
Metformin	56	2.51 ± 0.83	2.54 ± 0.072	0.7482
Miglitol + Metformin	44	2.36 ± 0.091	2.45 ± 0.10	0.4424

*Data are expressed as means ± SEM

Using ETKA and TPP induced-ETKA, the thiamin status was determined in T2DM patients treated with metformin and miglitol for 36 weeks. The concentration of ETKA remained unaffected in the presence of the anti-diabetic drugs (Table 4.4). These results were in parallel with the response of the agents to TPP-ETKA. No significant differences between the treatment groups were observed in the ETKA assay (Table 4.4). There were slight, though not significant, increases in thiamine

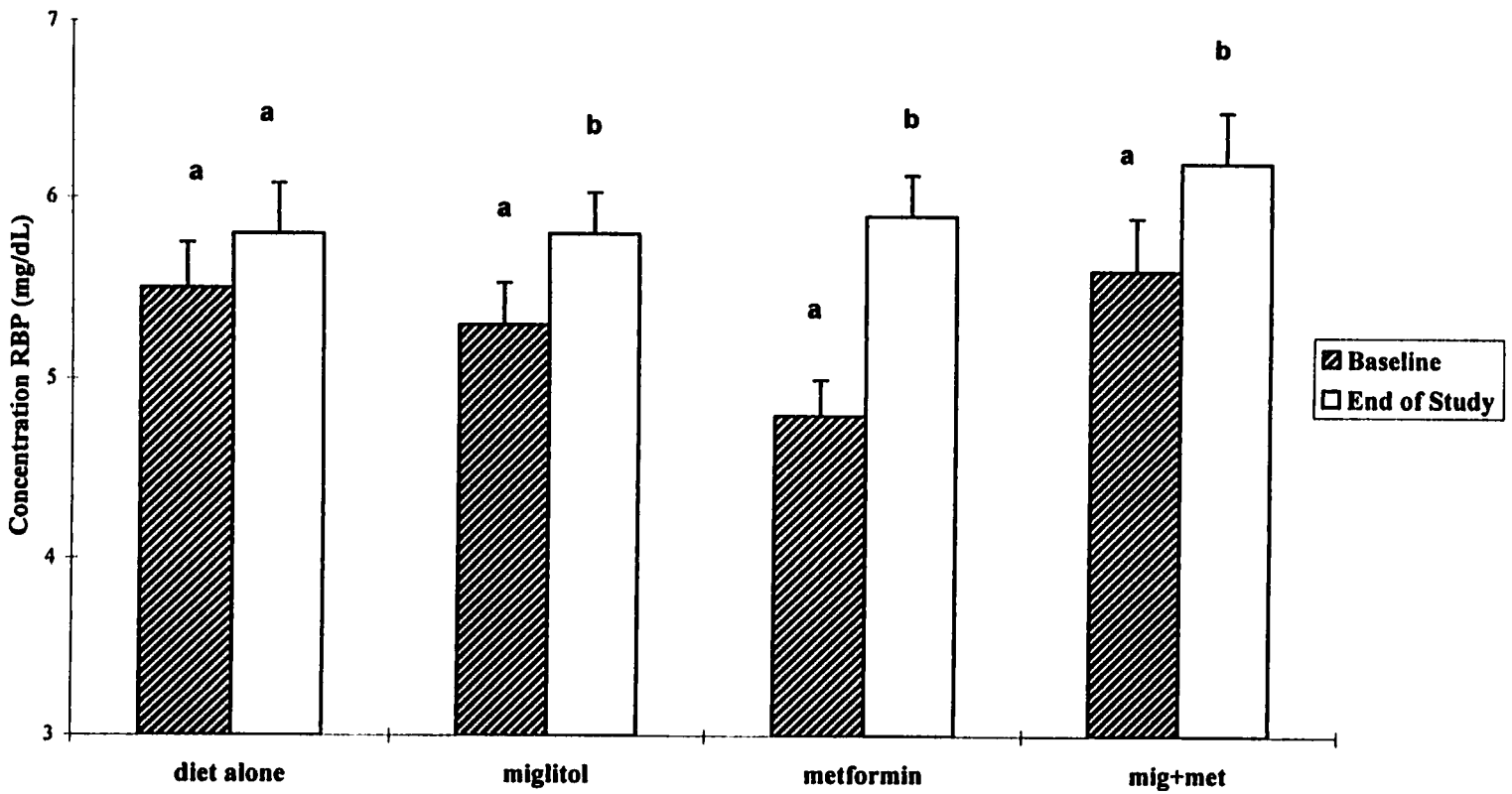

Figure 4.3: Effect of metformin and miglitol on plasma retinol binding protein (RBP) concentrations (mg/dL) in type 2 diabetes (T2DM) patients. Data are expressed as mean +/- SEM. Different superscripts indicate difference within treatment from baseline to end of study is statistically significant ($p<0.05$).

induced transketolase activity (TPP-ETKA) from baseline (Week 0) to end of study (Week 36) in all the groups, which suggests the level of thiamine in the blood has been decreased.

An increase in TPP effect indicates lower levels of thiamin in the blood (Figure 4.4). No significant difference in TPP effect was observed in the miglitol or metformin groups. However, a 42% decrease in TPP effect was observed after 36 weeks of combination treatment.

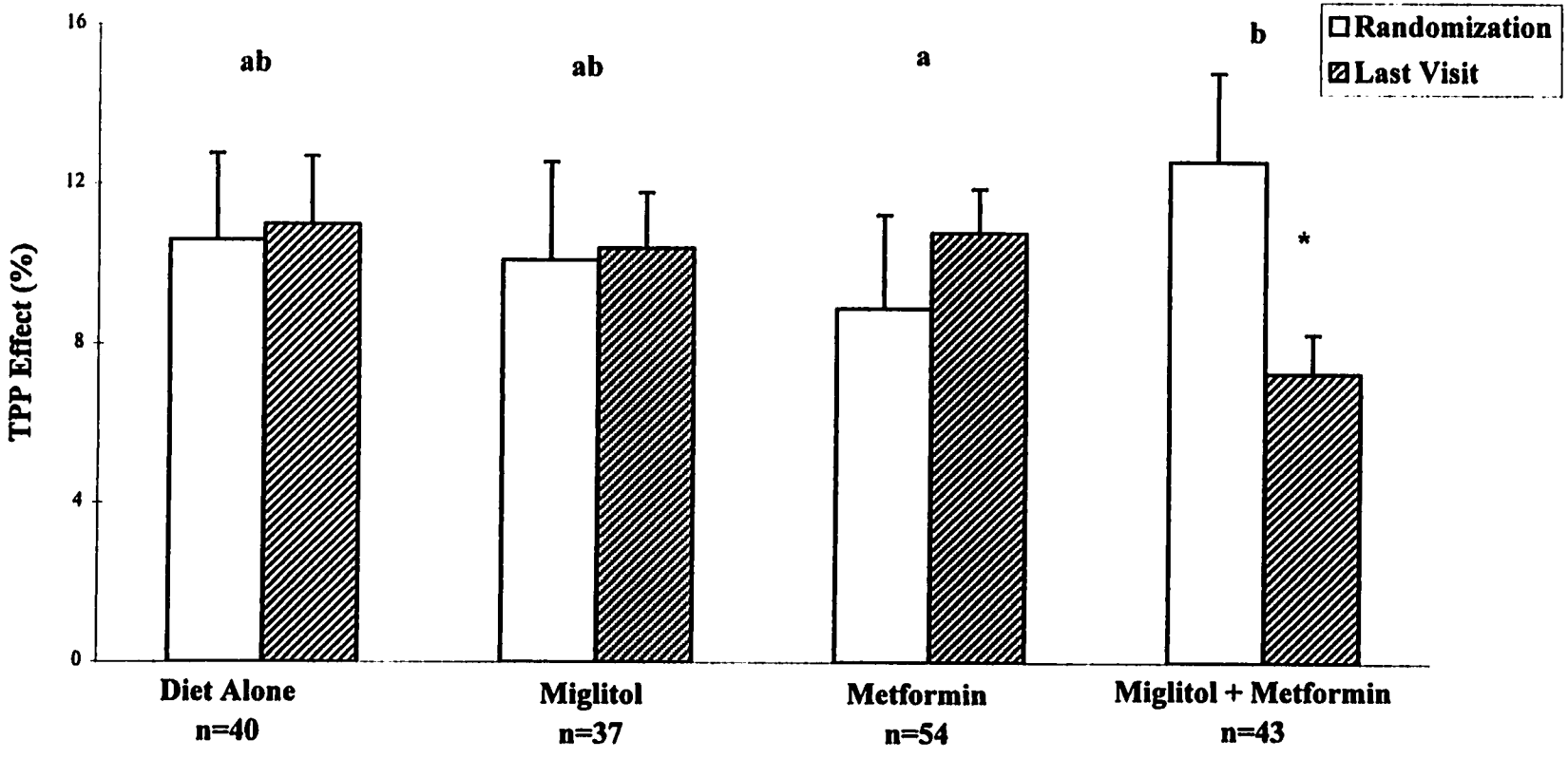

Figure 4.4: Thiamin Pyrophosphate (TPP) Effect (%) from baseline to end of study in T2DM patients in four treatment groups. Data are expressed as means +/- SEM for 'n', number of cases. Statistical significance: *p<0.05 in T2DM *within* treatment group. Letters indicate significant difference *between* treatments (p<0.05).

4.3 DISCUSSION

While the inhibition of glucose absorption is helpful in the management of type 2 diabetes (T2DM), other alterations in the function of the intestinal mucosa by biguanides or α-glucosidase inhibitors may result in adverse side effects. We have investigated the influence of metformin and miglitol on the status of vitamins A in the form of retinol, B_1 in the form of thiamine , B_{12}, and folate in T2DM patients during a 36 week treatment period. A limitation of this study is that supplemental intake of vitamins was not recorded with the dietary intakes.

Compared to placebo and miglitol treatment, plasma retinol concentration was significantly decreased in the present study after 36 weeks of metformin treatment, with a 17% increase in the number of T2DM patients with plasma retinol concentration below 1.05 μmol/L (considered low) after treatment with metformin alone. Retinol is formed either from the hydrolysis of dietary retinyl esters or from the reduction of retinal cleaved from β-carotene (Figure 4.5). It is re-esterified with retinol binding protein (RBP) in the intestinal mucosa.

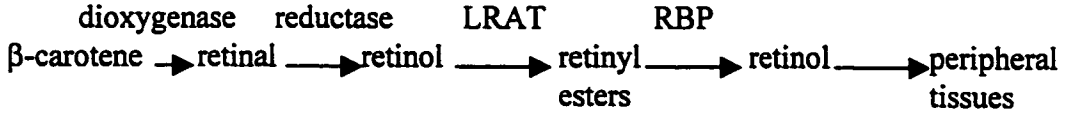

Figure 4.5: Intestinal Metabolism of vitamin A

Retinol binding protein (RBP) is a low molecular weight protein located in the enterocytes of the jejunum which in essence transports retinol to the liver for storage (Basu, 1988). Although the average concentration of retinol was high when the combination treatment was used, still 15% of the patients had plasma retinol concentrations indicative of deficiency of vitamin A after 36 weeks of the combination treatment. This effect may be thought to be reflective of inadequate dietary intake of retinol equivalents since the percent of Type 2 DM patients with low vitamin A intakes increased from 20% to 33% in the combination miglitol/metformin treatment group (Table 4.3). However, this does not explain the fact that even though the percent of Type 2 DM patients with low dietary intakes of vitamin A decreased from 32% to 27% during metformin treatment, there was still a significant decrease in plasma retinol concentration after treatment with metformin alone.

The secretion of RBP from the liver is regulated in part by vitamin A status, protein, and zinc status. Deficiencies of each of these will markedly reduce RBP secretion and thus reduce the circulating levels of plasma retinol (Goke et al, 1994). The intake of protein was significantly ($p<0.05$) decreased in the metformin treated group which may have had an effect on the decreased levels of plasma retinol. However, the intake of protein is still above the recommended nutrient intakes for non-diabetic 50-74 year males and females of 54-63 g/day (Health and Welfare Canada, 1990). Therefore, it is unlikely that the low protein intake contributed to the lower levels of plasma retinol, and it is more likely due to the effect of metformin

itself. Zinc status was not measured in the current study, so attributing the deficiency of retinol to a deficiency of this mineral is not possible.

The reason why metformin reduces the plasma retinol concentration is not clearly understood. However, it is suggested that the effect of metformin on plasma retinol may alter the binding of retinol to RBP for its transport to peripheral tissues, since metformin is postulated to inhibit hepatic glucose output through an undetermined mechanism (Coniff et al, 1994; Yee and Fong, 1996; Krill et al, 1997). β-carotene is converted to retinol in the wall of the intestine. The efficiency with which dietary β-carotene can be absorbed and converted to retinol is important in the assessment of the vitamin. Metformin may also decrease plasma retinol levels due to decreased conversion of carotene to retinol due to inhibited intestinal absorption of carotene (Connolly and Kesson, 1996). The high levels of RBP observed in the treatment groups indicates that retinol is not available to be transported to the peripheral tissues.

Miglitol likely does not inhibit the conversion of carotenes to retinol since the plasma retinol levels did not decrease significantly after 36 weeks of treatment. Miglitol did not significantly affect plasma retinol status, but plasma levels of RBP were increased. The results of the present study suggest that miglitol ameliorates the decrease in plasma retinol caused by metformin treatment in Type 2 DM. The alpha-glucosidase inhibitor may enhance the binding of RBP to retinol in metformin-treated patients.

The results of this study also suggest that miglitol prevents the fall in the serum concentration of vitamin B_{12} and folate, which is occurs in Type 2 diabetics with metformin treatment (Berger et al, 1972; Adams et al, 1983). The absorption of vitamin B_{12} was altered due to biguanide treatment in this study. Compared to diet alone and miglitol treatment, the plasma concentration of vitamin B_{12} was significantly decreased after 36 weeks of treatment with metformin. A similar decrease was also observed in a study by Carpentier et al (1976) where levels of vitamin B_{12} were significantly lower in the metformin treated group, compared with insulin and sulfonylureas. In the current study, serum folate concentration was also decreased by metformin treatment, though not significantly. The lowered vitamin B_{12} level was not in agreement with the findings of Berger et al (1972) who did not find a difference in vitamin B_{12} levels in metformin-treated patients. This may perhaps be due to a difference in duration of the study. After oral administration, biguanides reach a high concentration in the distal ileum (Carpentier et al, 1976). This concentration has been postulated to be high enough to interfere with mitochondrial membranes (Carpentier et al, 1976) and thereby inhibit oxidative decarboxylation and thereby reduce ATP production needed for the active intestinal transport of vitamin B_{12}.

It is also suggested that the reduced serum vitamin B_{12} and folate levels may be related to increased colonic fermentation (Taylor et al, 1986; Nakamura et al, 1993). The reason why metformin decreases the absorption of vitamin B_{12} could be due to colonic bacteria affecting the formation of the vitamin B_{12}-intrinsic factor (I_F)

complex, and thereby inhibiting its absorption (Goke et al, 1994; Wolever et al, 1996). It is plausible that metformin alters the colonic environment by changing pH, and increasing the number of short chain fatty acids in such a way that the growth and activity of the gut flora which bind the vitamin to I_F are inhibited (Wolever et al, 1992).

Miglitol inhibits the digestion of carbohydrates and therefore increases the amount of carbohydrates entering the colon, thereby providing increased amounts of substrate for colonic bacterial growth (Goke et al, 1994; Kawagishi et al, 1997). Folate is synthesized by colonic bacteria and this may explain why an increase in plasma and rbc folate was observed in the miglitol treated group.

In plants, thiamin occurs predominantly as thiamine. In contrast, thiamin occurs in all animal tissues, primarily in the coenzyme form of thiamin pyrophosphate (TPP). This is the metabolically active form of vitamin B_1. Thiamin is absorbed by an active carrier-mediated process at low concentrations in the intestinal lumen, but at higher concentrations, it is also absorbed by passive diffusion. The active transport mechanism is mainly in the jejunal and ileal regions of the small intestine (Figure 1.6).

Several enzymes use TPP as an essential co-factor for the cleavage of the C-C bond of alpha-ketoacids (ie: pyruvate and 5 carbon sugars) by erythrocyte transketolase (ETK) in the Pentose Phosphate Pathway. Although the synthesis of the TK enzyme is not affected by thiamin status, its catalytic activity depends on its binding to TPP. In subjects with adequate thiamin, that binding is at least 85% of

saturation, while in thiamin deficiency, the percent of TK bound to TPP is much less (Finglas, 1993).

The increase in the activity of ETK *in vitro* upon addition of exogenous TPP can be used to determine the percent TPP-saturation of the TK enzyme and hence thiamin status (Dreyfus, 1962; Schouten et al, 1964; Graudal et al, 1985). This percent stimulation of ETK activity by the addition of TPP is called the "TPP effect". Coefficients <1.15 are considered to be at low risk for vitamin B_1 deficiency, while those with >1.25 are considered to be at high risk for deficiency of the vitamin (Brin, 1970).

The present study revealed no significant effect of miglitol and metformin treatment alone on thiamin status, but the trend was towards increased TPP-induced ETK activity. When the two drugs were used in combination, the percent TPP activity was significantly decreased ($p<0.05$) indicating that thiamin status was improved.

Since intakes of thiamine were adequate in the treatment groups, the decrease in thiamin-induced transketolase activity cannot be attributed to diet. It is suggested that the delayed carbohydrate absorption by miglitol in the duodenal and jejunal region of the small intestine may inhibit the jejunal absorption of thiamin (Goke et al, 1994). This is supported by the observation that treatment with miglitol alone caused a nearly significant ($p=0.0559$) increase in TPP-ETK activity, indicating low thiamin levels in the red blood cells. It is interesting to note that the inhibitory effect

of miglitol on thiamin status was corrected when patients were treated with the combination therapy.

In conclusion, it appears as though Type 2 DM patients managing the disease with metformin may be at risk of vitamins A, B_{12}, and folate deficiencies (based on the number of people in the deficient range), while using miglitol alone puts the patient at further risk of developing a vitamin B_1 deficiency (though not significant). The status of these vitamins may be corrected when using a combination of miglitol and metformin. However, since Type 2 DM typically manifests in older individuals, the concept of taking more medications is not reasonable if it can be avoided when using biguanides or alpha-glucosidase inhibitors to manage the disease. Type 2 diabetics being treated with these drugs should be instructed on modifying their diets to include foods rich in vitamins A, B_1, B_{12}, and folate.

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5. GENERAL DISCUSSION AND CONCLUSIONS

According to the Canadian Diabetes Association guidelines (1989), vitamin supplementations are not recommended in diabetics. The present study has, however, indicated that Type 2 diabetes (Type 2 DM) patients may be at risk of developing vitamin deficiencies. Plasma retinol concentrations have been found to be less than the value of 1.05umol/L, among 16% of the 324 study subjects. Nearly 25% of the study population appears to be at high risk for vitamin B_1 deficiency, and 10% exhibited deficient plasma concentrations of vitamin B_{12} and folate.

Metformin is a conventional anti-diabetic agent. While this drug improves glycemic control and lipid concentrations it affects vitamins A and B_{12} status as evidenced by their reduced circulatory levels (Tomkin et al, 1971; Berger et al, 1972; Carpentier et al, 1976; Adams et al, 1983). These effects could be due to either metabolic interactions or gastrointestinal disturbances (Kingma et al, 1992; Goke et al, 1994; Wolever et al, 1994; Kawagishi et al, 1997). The latter include abdominal bloating, anorexia, diarrhea, flatulence and nausea.

Marginal vitamin A status may impair the immune response, vision and delay in wound healing (Wako et al, 1986; Basu and Basualdo, 1997). Since individuals with DM may have a high prevalence and severity of infection, metformin-induced vitamin A deficiency may exacerbate these secondary consequences (Consoli et al, 1990; Connolly and Kesson, 1996). Supplementation with vitamin A has been shown to enhance wound healing in STZ-induced diabetic rats (Tuitoek et al, 1996).

Miglitol is a new drug whose efficacy in T2DM has been established (Joubert et al, 1986; Heinz et al, 1989; Kingma et al, 1992; Debouno et al, 1993; Bischoff et al, 1994). This alpha-glucosidase inhibitor acts in the small intestine to inhibit several enzymes that digest carbohydrates and thereby delay the absorption of sugars and helps reduce the requirement for rapid insulin secretory responses postprandially (Goke et al, 1994). The side effects of miglitol also include flatulence, bloating and diarrhea. This alpha-glucosidase inhibitor allows undigested carbohydrates to pass into the large bowel where they are digested by colonic bacteria (Joubert et al, 1990; Nakamura et al, 1993; Kawagishi et al, 1997). Unlike metformin, the treatment with miglitol does not seem to have an effect on any of the vitamins studied. Acarbose, a precursor of miglitol, has also been reported having no effect on the status of vitamins and minerals in T2DM patients (Chiasson et al,1994).

In conclusion, patients with T2DM, treated with metformin should be encouraged to include good sources of vitamin A in their diets or take supplements. It was noteworthy that miglitol did not affect plasma retinol concentrations, and was in fact shown to ameliorate the metformin-associated decrease when given in combination with metformin. It may also be wise to monitor vitamin B_{12} levels in metformin treated Type 2 DM patients so that vitamin B_{12} hypovitaminosis and its complications can be prevented.

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