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THE INTERRELATIONSHIP BETWEEN NIACIN IN LARGE DOSES AND VITAMIN B_6 STATUS AS REFLECTED BY AN ALTERED METHIONINE METABOLISM IN RATS.

BY

SARABJIT S. MANN

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

IN

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DEPARTMENT OF AGRICULTRAL, FOOD AND NUTRITIONAL SCIENCE

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T6L 2Y4

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The undersigned certify that they have read, and recommended to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled "**The Interrelationship between niacin in large doses and vitamin B_6 status as reflected by an altered methionine metabolism in rats**" submitted by **Sarabjit S. Mann** in partial fulfillment of the requirement for the degree of **Master of Science** in **Nutrition and Metabolism.**

Dr. T. K. Basu (Supervisor)

Dr. M. T. Clandinin

Dr. W. Sauer

Dr. V. Dias

Date: Septemeber 26, 1995.

ABSTRACT

Objective: This study investigates the interrelationships of pharmacologic doses of nicotinic acid used for the treatment of hyperlipidemia with sulphur amino acids, vitamin B_{12}, folic acid and vitamin B_6.

Design: Two experiments were conducted in which nutritionally adequate semi-synthetic diets were given to male Sprague-Dawley rats. In the first experiment the effect of large doses of niacin either at a level of 400 mg or 4000 mg/kg diet (compared to control level of 46.7 mg/kg diet) on lipid, sulphur amino acid, vitamin B_{12} and folic acid levels was monitored. Because vitamin B_6 play an important role in the metabolism of sulphur amino acids, the effect of concurrent supplementation of niacin (4000 mg/kg diet) and vitamin B_6 (10 mg/kg diet) on these parameters was also examined.

Results: Pharmacologic doses of niacin resulted in a significant decrease in plasma and liver lipid levels. However, an increase in plasma and urinary methionine associated with decreased plasma and urinary cysteine concentrations was observed following niacin administration. Similar results were found in the second experiment along with increased concentrations of homocysteine in plasma and urine in the niacin treated group. No difference in plasma and urine levels of these sulphur amino acids was observed, when animals were fed concurrent supplementation of niacin and vitamin B_6 together. Vitamin B_{12} and folic acid concentrations in plasma were not effected following niacin and/or vitamin B_6 treatment. However, plasma PLP (pyridoxal-5'-phosphate) levels were significantly decreased in niacin treated

animals. Plasma and liver lipids levels were not affected due to simultaneous administration of niacin and vitamin B_6 when compared with niacin treated animals.

Conclusion: The present study concludes that niacin in pharmacologic dose levels may lead to vitamin B_6 deficiency resulting in altered methionine metabolism. However, concurrent supplementation with both niacin and vitamin B_6 can successfully reverse metabolic abnormalities resulting from feeding high doses of niacin without effecting hypercholesterolemic properties of niacin. Therefore, treatment of dyslipidemia with simultaneous administration of niacin and vitamin B_6 could further improve niacin therapy.

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LIST OF ABBREVIATIONS

ACAT	Acyl conenzyme A:cholesterol transferase
AMP	Adenosine 5'-monophosphate (adenylic acid)
ATP	Adenosine triphosphate
CHD	Coronary heart disease
DNA	Deoxyribonucleic acid
GTP	Guanosine triphosphate
HDL	High density lipoprotein
HDL-C	High density lipoprotien cholesterol
HMG CoA	3-Hydroxy-3-methylglutaryl coenzyme A
IDL	Intermediate density lipoprotein
LDL	Low density lipoprotein
LDL-C	Low density lipoproteine cholesterol
Lp(a)	Lipoprotein(a)
MNA	N^1-Methylnicotinamide
NA	Nicotinic acid
NAD	Nicotinamide adenine dinucleotide
NADase	Nicotinamide adenine dinucleotide hydrolase
NADH	Nicotinamide adenine dinucleotide (reduced form)
NADP	Nicotinamide adenine dinucleotide phosphate
NADPH	Nicotinamide adenine dinucleotide phosphate (reduced form)
NCEP	National cholesterol education program
PL	Pyridoxal
PLP	Pyridoxal-5'-phosphate
PRPP	5'-phosphoribosyl 1-pyrophosphate
RNI	Recommended nutrient intake
SAM	S-Adenosyl methonine
SAH	S-Adenosyl homocysteine
T-C	Total cholesterol
T-G	Triglycerides
t-RNA	transfer (soluble) ribonucleic acid
VLDL	Very Low density lipoprotein
VLDL-Tg	Very low density lipoprotein triglycerides

1. INTRODUCTION

Epidemiological, clinical, genetic and laboratory-animal studies all indicate that hypercholesterolemia, associated with increased levels of low density lipoprotein (LDL) and very low density lipoprotein (VLDL) remnants, is related to an increased risk of coronary heart disease (CHD) (1-4). Clinical studies have shown that interventions to reduce serum lipid levels may lead to a reduction in total mortality from cardiovascular diseases (5-7) and regression of atherosclerotic plaques in hyperlipidemic patients (8-11). Considerable research effort has thus been dedicated towards understanding the etiology and potential treatments for high serum cholesterol and atherogenic lipoprotein concentrations. Consensus Conference on lowering blood cholesterol to prevent heart disease, recommended dietary modifications and potential drug therapy for the treatment of hypercholesterolemia (1).

1.1. DIETARY TREATMENT

The first step in the treatment of hypercholesterolemia is diet therapy and reduction in weight for overweight subjects (13). According to the National Cholesterol Education Program (NCEP), the general goal of dietary therapy is to lower elevated cholesterol levels while maintaining a nutritionally adequate diet (13). Dietary treatment involves the modification of diet to reduce the intake of total fat and cholesterol (12). "Step one diet" recommends total fat less than 30% of calories, saturated fatty acids <10% of

calories and cholesterol less than 300 mg/day. At the end of three months of period starting the step-one diet, serum total cholesterol and LDL-cholesterol are measured. If this dietary regime fails to lower cholesterol level, the hypercholesterolemic subjects are put on a step-two diet, where saturated fatty acids intake is further reduced to <7% of caloric intake and cholesterol <200 mg/day (12, 13). If the serum cholesterol remains unchanged at the end of three month on the lipid restricted diet, drug therapy is seriously considered.

1.2. DRUG TREATMENT

Dietary treatment is usually carried out for at least six months before initiating drug therapy. Usually drug therapy is added to dietary therapy, not as a substitute. The drugs of first choice include bile acid sequestrants (cholestyramine and colestipol), 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors (lovastatin, pravastatin, simvastatin), fibric acid derivatives (gemfibrozil, clofibrate), acyl coenzyme A:cholesterol acyl transferase (ACAT) inhibitors (SaH57118, SaH58035), lipid oxidation inhibitor (probucol) and nicotinic acid.

1.2.1. Bile Acid Sequestrant

The bile acid sequestrants are primarily effective in lowering-LDL-cholesterol (14, 15). The two drugs in this group are cholestyramine and colestipol. The primary action of

bile acid sequestrant is to bind bile acids in the intestinal lumen, with a concurrent interruption of the enterohepatic circulation of bile acids and a markedly increased faecal excretion of acidic steroids (12, 16). The bile acid pool is depleted and there is a compensatory increase in synthesis of bile acids from cholesterol with the enzyme 7-alpha hydroxylase (17). Decreased hepatic cholesterol content stimulates an increase in LDL receptors, which increases the uptake of LDL; therefore catabolism and removal of LDL from the blood, resulting in decreased concentrations of these lipoproteins in plasma (18).

Adherence is a problem with the bile acid sequestrants (19), the most frequent side effects include nausea, constipation, vomiting, heartburn, abdominal pain, belching and bloating (14). Bile acid sequestrants can also interfere with the absorption of warfarin, thyroxin, digoxin, and thiazide diuretics (20), and may decrease the absorption of folic acid and fat-soluble vitamins (16). Biochemical side effects include modest increase in plasma triglycerides, and mild increases in alkaline phosphatase and transaminases (16).

1.2.2. HMG-CoA Reductase Inhibitors

Recently, development of inhibitors of HMG-CoA reductase, the rate limiting enzyme in cholesterol biosynthesis, has provided a new therapeutic approach to the treatment of hypercholesterolemia (19). Lovastatin, pravastatin and simvastatin are major agents

in this category in the market (21). These agents block the synthesis of cholesterol in the liver (22) and lower the LDL cholesterol by as much as 40% (23). The resultant decrease in hepatic cholesterol stimulates the production of LDL receptors, resulting in increased catabolism of LDL and decreased circulating LDL levels (14). Thus the beneficial reduction in plasma LDL is achieved.

Headache, myalgia, insomnia, and gastrointestinal complaints are the most frequently reported side effects of these hypocholesterolemic agents (24). Marked and persistent serum transaminase elevations requiring discontinuation of these drugs are seen in 0.1-1.5% of subjects treated with these agents (23). The most serious toxic effects of the drugs of this category relate to the liver and skeletal muscle. Muscle weakness, markedly elevated creatine kinase (23), jaundice (25, 26) may develop (23).

1.2.3. Fibric Acid Derivatives

Fibrates are an established class of drugs for the treatment of hyperlipoproteinemias. The parental compound, clofibrate (p-cholorophenoxyisobutyrate) is the ethyl ester of p-cholorophenoxyiso bytyrate. Most commercially available fibrates have a Cl substitute at the *para* position of the aromatic ring. The fibric acid derivatives, such as gemfibrozil and clofibrate currently available in the United States (24), are mainly T-G lowering drugs. According to the World Health Organization (27) and Helsinki Heart (7) trials, these drugs reduce the risk of nonfatal CHD. The mechanisms of action of

the fibric acids are not well understood. Many researchers consider stimulation of lipoprotein lipase to be the mechanism of action (28), but others have maintained that the lipid-lowering activity is due to the enhanced rate of receptor-mediated clearance of LDL from plasma (29-31). The treatment with fibric acid derivatives may cause nausea, abdominal pain, weight gain and drowsiness (20). These drugs increase lithogenicity of bile (32) and cholelithiasis (20, 33). They also displace warfarin from binding sites and can cause myositis and ventricular dysrhythmias (20). Other side effects include rash, urticaria, pruritus, headaches, insomnia, increased aminotransferases and decreased alkaline phosphatase (12).

1.2.4. ACAT Inhibitors:

In recent years, the inhibition of acyl coenzyme A:cholesterol acyl transferase (ACAT), for the prevention of high cholesterol levels is under consideration. Because ACAT is the enzyme that esterifies cholesterol in the body, a process believed to be the key for cholesterol absorption (21). Inhibition of this enzyme resulted in beneficial effects on plasma cholesterol levels in rats due to a decreased absorption of dietary cholesterol (34). Block of cholesterol esterification in the liver could decrease synthesis of VLDL as esterification is required for VLDL synthesis (35). Blockage of ACAT activity in the arterial wall will decrease the accumulation of cholesterol esters and prevent foam cell formation in the artery wall (36). The free cholesterol thus generated may then be removed by HDL or other acceptors, back to the liver (36).

However, the possibility remains that these inhibitors could lead to an accumulation of cholesterol in the liver and therefore down regulation of LDL receptor uptake (36). There is an inverse relationship between biliary cholesterol output and ACAT activity (37). Patients with gallstones have a reduced ability to esterify free cholesterol due to inhibition of hepatic ACAT (38). It is therefore possible that block of ACAT in the liver may result in a lithogenic bile (36).

1.2.5. Lipid Oxidation Inhibitor

Probucol, the best studied agent of this class is modestly effective drug for the treatment of hypercholesterolemia (12). It is a bis-phenol (24), that lowers LDL cholesterol concentrations by 8% to 15% (39). The mechanism by which probucol lowers LDL cholesterol is not fully understood, but the drug appears to increase nonreceptor mediated catabolism of LDL (40). Oxidation of LDL with in the arterial wall is a critical step leading to foam cell formation (41), anti-oxidant properties of probucol may decrease the deposition of lipids and lipoproteins in the arterial wall (42,43).

Probucol is also thought to decrease HDL-cholesterol by 20 to 25 % (44,45) by decreasing apolipoprotein A1 synthesis and lipoprotein lipase activity (46). Side effects of probucol include diarrhea, flatulence, abdominal pain, and nausea (20). Probucol causes prolongation of the QT interval (14). This lipid soluble drug persists in adipose tissue for at least six months, should be discontinued well before any attempted

pregnancy (14, 24).

1.2.6 Untoward Aspects of The Use of Conventional Hypolipidemic Agents

Enormous progress has been made in the development and testing of pharmacological agents towards the control of hypercholesterolemia and for the treatment and prevention of atherosclerosis complications. Of the agents discussed above, lovastatin, colestipol and cholestyramine produce a maximum reduction in LDL-C level, where as niacin and gemfibrozil produce a maximum increase in HDL-C level. Although probucol also produces a substantial reduction in total cholesterol levels, much of its effects is due to a reduction in HDL-C levels. Lovastatin and niacin are drugs that produce the greatest reduction in LDL-HDL cholesterol ratio. Increased attention has been focused on potential harmful side effects during treatment with these agents. Gastrointestinal symptoms are the most common side effects. Potential harmful adverse effects include malabsorption of vitamins, drug interaction (bile acid resins), hepatic injury (HMG-CoA reductase inhibitors), increased gallstone formation and venticular arrhythmias (fibrates).

Selecting efficient treatment strategies requires consideration of both the effectiveness and the cost of therapy. In view of the high cost of lipid lowering drugs, the cost effectiveness of therapy for preventing or treating coronary heart disease is becoming increasingly important. Using cost-effective analysis, Schulman et. al. (47) compared the cost of treatment of hyperhcolesterolemia with different pharmacologic agents. The

medications studied include cholestyramine, colestipol, niacin, gemfibrozil, lovastatin and probucol. Annual costs for therapy ranged from $327 (niacin) to $1181 (lovastain).

Niacin (3 g/day) and lovastatin (20 mg/d) are thought to be most efficient for reducing cardiovascular risk. Lovastatin, the first HMG-CoA reductase inhibitor to be released for the treatment of hypercholesterolemia is, however, an expensive drug, not recommended for first-line therapy (13). The cost for 20 mg lovastatin is $37.50 compared to $ 4.68/g for niacin. Niacin still holds first line position in the treatment of the hyperlipidemia not only because of its low cost, but also for reasons that niacin is the only lipid lowering drug shown to reduce total mortality (48, 49).

1.3. NIACIN (NICOTINIC ACID)

Nicotinic acid is an effective and inexpensive drug for the treatment of patients with hypercholesterolemia. Niacin therapy has been found to be effective in lowering serum total cholesterol, LDL-cholesterol and VLDL-cholesterol as well as triglycerides (49-52). The use of niacin as a drug was established when the ability of nicotinic acid to significantly lower serum cholesterol in man was discovered in the early 1950s (30). Although usually considered an essential nutrient, niacin exerts pharmacological effects to lower serum lipid concentrations when consumed in large amounts. The Canadian Recommended Nutrient Intake (RNI) for niacin is 7.2 mg (or niacin equivalent) per 1000 kcal per day. The doses necessary to achieve pharmacologic effects are usually

in the range of 2-4 g daily. This drug is particularly important in increasing serum HDL concentrations (50-52). These changes are known to reduce the risk for the development of coronary heart disease. The treatment with niacin has also been shown to significantly lower the incidence of definite nonfatal myocardial infarction (53). Furthermore, the lipid lowering action of niacin has been clinically tested in conjunction with diet and other drugs including cholestyramine, colestipol, clofibrate and lovastatin (54-57). A detectable synergistic effect has been observed in these studies.

In view of its beneficical effects, niacin has been increasingly the choice of drug by many clinicians and patients for the treatment of hypercholesterolemia. However, the potential risks of the use of high doses of niacin has not been critically considered in the same way as has the use of other lipid-lowering drugs. Niacin is excreted as methylated pyridones (58, 59,). With these metabolites, methylation is formed by a simple methyl transfer reaction in which S-adenosylmethionine is the methyl donor. Niacin is water soluble, it is not stored in the body beyond its tissue saturation level. Since niacin excretion is dependent upon methionine, an essential amino acid, an excess intake of niacin may increase the requirement of methionine. Therfore, it is important that the interaction between niacin and the amino acid is examined.

1.3.1. Biosynthesis of Niacin

Niacin (Nicotinic acid) is a generic description for pyridine 3-carboxylic acid (nicotinic

acid) and pyridine 3-carboxamide (nicotinamide). In addition to dietary intake, niacin is available through synthesis in the body from tryptophan, an essential amino acid (Fig 1.1). The conversion factor of 1 mg of niacin for 60 mg of tryptophan has been suggested (60, 61). The first step in the metabolic pathway is the conversion of tryptophan to N'-formylkynurenine. The conversion of tryptophan to N'-formylkynurenine is catalyzed by an enzyme called tryptophan pyrrolase (L-tryptophan 2,3,-dioxygenase). This enzyme is harmoniously induced by glucocorticoids and tryptophan itself (62). It is the rate limiting enzyme in the sequence when not induced. As shown in the Figure 1.1, there are a number of competing pathways for the formation of nicotinic acid. 3-hydroxyanthranilic acid is the most immediate precursor for nicotinic acid. The enzyme quinolinate phosphoribosyltransferase converts quinolinic acid to nicotinic acid mononucleotide by simultaneous decarboxylation (63).

The pathway from tryptophan to niacin is sensitive to a variety of nutritional, hormonal, and pathological alterations, as well as the iatrogenic effects of drug therapy. The most important of these is vitamin B_6. When animals are depleted of this vitamin, a disturbance in the tryptophan pathway after tryptophan loading has been demonstrated in rats (64-68). Quinolinic acid excretion increases after a tryptophan load in vitamin B_6-deficient humans (68) and 3-hydroxyanthranilic acid is also said to be elevated in humans on a vitamin B_6-deficient diet (69). Cases of pellagra have been reported in subjects with isoniazid therapy, which responded to combined vitamin B_6 and niacin

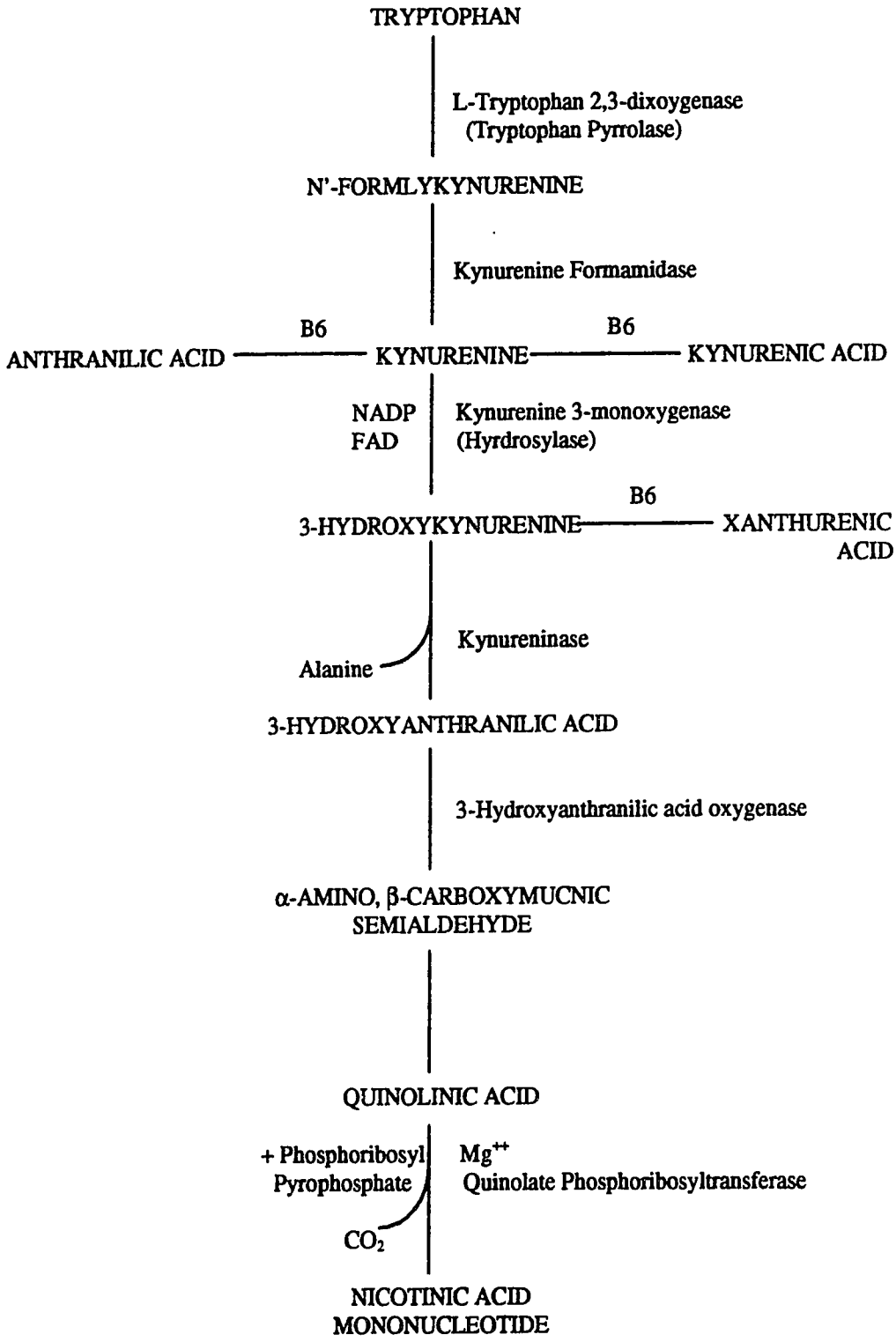


Figure 1.1 THE PATHWAY OF THE CONVERSION OF TRYPTOPHAN TO NIACIN

treatment (70). Penicillamine has been demonstrated to inhibit the tryptophan niacin pathway in humans (71, 72), and this may be due to the copper chelating effect, though vitamin B_6 binding could also be responsible.

Nicotinic acid functions in the body after conversion to either nicotinamide adenine dinucleotide (NAD) or nicotinamide adenine dinucleotide phosphate (NADP). Condensation of nicotinic acid and phosphoribosyl pyrophosphate forms nicotinate ribonucleotide, a key intermediate in the pathway of NAD biosynthesis. Nicotinate ribonucleotide subsequently acquires an adenylic acid group from ATP in a reaction catalyzed by NAD pyrophosphorylase to form desamide-NAD. Addition of an amide group from glutamine to the nicotinic acid portion of the molecule completes the synthesis of NAD. The later can be phosphorylated at the 2 position of the ribose ring to NADP. Both NAD and NADP can be reduced to give NADH and NADPH, respectively, in oxidation-reduction reactions (73).

Nicotinamide is produced from hydrolysis of excess NAD by NADase (NAD hydrolase). Nicotinic acid can be formed from nicotinamide by the action of a deaminase. An important fact about the reactions involved in mammalian NAD biosynthesis is that no known enzyme can convert nicotinic acid directly to nicotinamide. The amide form of niacin (nicotinamide) arises only from degradation of NAD. Thus although nicotinamide is the form of niacin biologically active in the coenzyme function of NAD, both nicotinic acid and nicotinamide can function as vitamins for NAD biosynthesis.

However, nicotinamide must be converted back to nicotinic acid prior to incorporation into NAD because no pathway exists for direct incorporation of nicotinamide into an NAD precursor or into NAD itself (73).

The NAD participates in a wide array of oxidation-reduction reactions catalyzed by dehydrogenase or oxido-reductase enzymes. Virtually every aspect of cellular metabolism involves NAD/NADH or NADP/NADPH-linked systems. In the absence of sufficient supplies of niacin precursors for NAD biosynthesis, cellular functions and life itself would be impaired. Thus niacin is a critical nutrient for living species (73).

1.3.2. Metabolism

When dietary sources are pyridine nucleotide, NAD is quickly digested by the action of a pyrophosphatase present in the intestinal contents. The resulting nicotinamide ribonucleotide is then rapidly hydrolysed to nicotinamide riboside which accumulates in the lumen of upper small intestine (74-77). The accumulated amide is hydrolysed to nicotinic acid within hours by the action of bacterial nicotinamidase (78). The nicotinic acid is then transported to the liver, where it is converted to NAD (79). The rate limiting digestion step is the release of nicotinamide from nicotinamide riboside (NR) by hydrolysis or phosphorolysis or both under the influence of mucosal enzymes.

Biosynthesis of nicotinic acid from tryptophan does not result in free nicotinic acid, but

rather is utilized directly to form nicotinic acid nucleotide. Preformed nicotinic acid is converted to nicotinic acid mononucleotide which reacts with ATP to form nicotinic acid adenine dinucleotide plus pyrophosphate. The nicotinic acid adenine dinucleotide is then converted to nicotinamide adenine dinucleotide with ATP and glutamine (79). The NAD further degrades to form AMP & nicotinamide mononucleotide and nicotinamide adenosine diphosphate ribose. The pyrophosphatase yield nicotinamide mononucleotide and nicotinamide mononucleotide glycohydrolase yields free intracellular nicotinamide (80). The NAD glycohydrolases, which convert NAD to nicotinamide and adnosine diphosphate ribose, are a group of enzymes that have assumed major importance in molecular biology. These enzymes form poly-ADPR and in so doing influence metabolism of DNA widely.

Mostly tissues take up nicotinic acid by simple diffusion and metabolic trappings except the facilitated diffusion into erythrocytes (81). The level of free nicotinic acid in plasma reaches a peak value between 30 and 60 minutes after ingestion of a single dose of 1 g (82). Plasma nicotinic acid is rapidly taken up by the liver & erythrocytes and is converted to NAD (79). NAD glycohydrolase then releases the pyridine nucleus as nicotinamide, which is the precursor of NAD in other tissues (83). Most mammalian cell lines can utilize either nicotinic acid or nicotinamide for synthesis of NAD, though some can utilize only nicotinamide (83).

All tissues can incorporate nicotinamide into NAD and actually prefer nicotinamide to

nicotinic acid. Nicotinamide is main circulating form of the vitamin (84, 85). In the liver nicotinic acid is incorporated into NAD, which is then broken down into nicotinamide. It is been postulated that nicotinamide in extracellular fluid controls tissue levels of NAD and plasma nicotinamide is regulated by the release of NAD from storage of NAD in liver. The nicotinamide phosphoribosyl transferase, which catalyzes the first reaction of this sequence is influenced positively by ATP and negatively by NAD (86). None of these controls is very effective in the liver and this may account in part for the observed toxicity of nicotinamide. The toxicity could be the result of depletion of cellular ATP and/or PRPP used in NAD synthesis (87). Suppression of DNA synthesis and inhibition of t-RNA methylation in the liver have been suggested as explanations for the toxicity of nicotinamide at levels of 5-10 m mol per kg of body weight, the dose required to stimulate NAD synthesis in rats and mice (88).

Metabolites of NA found in the urine are mainly the products of catabolism of the pyridine nucleotide which are stored forms of the vitamin (59). The primary source of metabolism is via methylation to N^1-methylnicotinamide (MNA) which is further oxidized to N^1-methyl-2-pyridone-5-carboxamide, and N^1-methyl-4-pyridone-3-carboxamide (59, 60). For doses of 1 g or less, the major product is N^1-methyl-2-pyridone-5-carboxamide. At increasing doses, nicotinuric acid and N^1-methylnicotinamide become the dominant excretory products (89). At a dose of 100 mg nicotinamide/kg, relatively higher amounts of metabolites are recovered in urine as N^1-methylnicotinamide, N^1-methyl-2-pyridine-5-carboxamide and N^1-methyl-4-pyridine-3-

carboxamide (90).

1.3.3. Hypolipidemic Actions of Nicotinic Acid

Nicotinic acid is used as the first line of treatment for most lipidemias after dietary measures fail. Nicotinic acid, with its beneficial effects on VLDL-Tg, LDL-C and HDL-C levels could be used for most forms of hyperlipidemia. Significant decreases in serum total cholesterol, triglycerides and LDL-cholesterol have been observed in hyperlipidemic patients following nicotinic acid therapy (49, 91, 92). It is particulary useful in patients who have elevated plasma VLDL-Tg levels and depressed levels of HDL-Cholesterol. Niacin treatment decreases VLDL-triglycerides and LDL-cholesterol along with an increase in the levels of HDL-cholesterol (91-93). Prolonged niacin therapy also mobilizes cholesterol from peripheral tissues. In the Coronary Drug Project, a long-term, nationwide double blind placebo-controlled study, nicotinic acid significantly lowered the incidence of definite nonfatal MI as well as fatal or nonfatal cerebrovascular events than placebo group. Further more niacin has been shown to significantly decrease serum total cholesterol (15%), triglycerides (27%), and LDL-C (36%) along with significant increase in HDL (23%) in five healthy subjects (48).

Niacin has been reported to be more effective for cholesterol management when used in conjunction with other lipid-lowering drugs. A combination of nicotinic acid and bile acid-binding resin, such as colestipol, reduced total cholesterol by 34%-45% and LDL-C

by 45% to 55% in patients with heterozygous familial hyperlipidaemia (94-96). In another clinical trial, addition of 3 g nicotinic acid daily to type II hyperlipidemic patients treated with 2 g neomycin, further lowered total cholesterol and LDL-C by 18% and 25% respectively and raised the levels of HDL-C by 32% (97). Also a triple regimen of colestipol-lovastatin-Nicotinic acid was even more effective in lowering total cholesterol, triglyceride and LDL-C levels (98).

The lipid-altering ability is not shared by nicotinamide and seems to be unrelated to the role of nicotinic acid as a vitamin in the NAD and NADP coenzyme system. Nicotinic acid is available in 100, 125, 250 and 500 mg tablets, as well as in a time release form (53). The typical dosage of nicotinic acid is 3-7 g daily; therapeutic effects of the drug usually are not manifested until the patients reaches a total daily dose of at least 3 g (53). Once the initial maintenance dose is reached, it is important to evaluate the therapeutic effects by measuring plasma lipoprotein values. If the therapeutic effects are unsatisfactory, the dose should be increased by a further 1.0 to 1.5 g per day, to a maximum of 7-8 g daily as needed (53).

1.3.4 Mechanisms of Hypocholesterolemic Action of Nicotinic acid

The beneficial effects of nicotinic acid in the treatment of hyperlipidemia have been suggested as a result of (i) inhibition of lipolysis in adipose tissue, (ii) Inhibition of VLDL Secretion, (iii) Increased Biliary Lipid Output, (iv) Increase in HDL_2

concentrations, and (v) Lowering of lipoprotein(a)

Inhibition of Lipolysis in Adipose Tissue : Pharmacological doses of nicotinic acid decreases mobilization of fatty acids from adipose tissue by inhibiting the breakdown of triglycerides through lipolysis (99). Adipose tissue lipolysis is regulated by the c-AMP system. Elevated levels of c-AMP activate a protein kinase that phosphorylates a hormone-sensitive lipase to catalyze triglyceride hydrolysis (73). Guanine nucleotide-linked G-protein system regulates cellular c-AMP levels by interacting with adenyl cyclase. In fat cells, G-proteins function as transducing elements for receptors that are either stimulatory (R_s) or inhibitory (R_i). Antilipolytic agents such as adenosine, prostaglandins E_2 and nicotinic acid bind inhibitory receptors, which interact with the inhibitory G-protein. These interactions inhibit adenyl cyclase and therefore, decreased mobilization of fat from adipose tissue (100, 101). A molecular mechanism of antilipolytic effect of nicotinic acid has also been proposed (102, 103). Nicotinic acid binds to specific inhibitory receptor on plasma membrane adipocytes, which forms a complex with the inhibitory G-protein. In the presence of GTP, the protein dissociates into alpha and beta-gamma subunit moieties, as well as dissociates from the receptor. The resulting G-protein decreases c-AMP by inhibiting adenyl cyclase. Decreased c-AMP activity leads to deceased lipase activity and therefore decreased breakdown of fat.

Nicotinic acid can also interact with adenylate cyclase directly, rather than indirectly through G-protein receptor complex (102, 103). Measurements of nicotinic acid binding

to adipocyte plasma membrane fractions and characterization of the hypothesized protein receptor are needed to clarify the mode of action of nicotinic acid on lipolysis (73).

Inhibition of VLDL Secretion : Nicotinic acid decreases synthesis of VLDL production in hyperlipidemic patients (49). The antilipolytic effect of nicotinic acid in adipose tissue is thought to be due to reduced hepatic VLDL synthesis, thereby limiting overall assembly and secretion of VLDL from the liver (73). Free fatty acids released from adipose tissue serve as the major precursors for the synthesis of VLDL-Tg, a major carrier of endogenous triglycerides (53, 104). Nicotinic acid decreases fatty acid release from adipose tissue and therefore reduction in hepatic synthesis of VLDL due to decreased uptake of free fatty acids by the liver (104). Since the majority of LDL is produced by the catabolism of VLDL (53). A decreased synthesis and secretion of VLDL by the liver (73) may lead to the lack of availability of VLDL for catabolism, thus reduced production of LDL.

However, variations in the extent of inhibition of lipolysis by nicotinic acid suggest that this is not the only mechanism of reduction of VLDL by nicotinic acid (99). Other possible explanations are increased hepatic clearance of IDL, therefore decreased conversion of IDL to LDL and decreased VLDL-independent synthesis of LDL (53). A number of studies in animals and humans have shown that nicotinic acid inhibits endogenous synthesis of cholesterol (105). In humans, incorporation of radioactive acetate into mevalonic acid was decreased during nicotinic acid therapy, suggesting

inhibition of cholesterol biosynthesis at the level of HMG-CoA reductase (106).

Results also support the inhibition of synthesis of apolipoprotein-B during nicotinic acid treatment. Apolipoprotein B-100 (apo B) is the major protein component of VLDL. In monkeys, the incorporation of labelled amino acids into the protein fraction of VLDL was decreased after nicotinic acid treatment (107). Isotope of studies in humans have demonstrated the decreased turnover of protein moiety of LDL following nicotinic acid treatment (108, 109). These results support an inhibition of the synthesis of apolipoprotein-B during nicotinic acid treatment. The mechanism by which this inhibition is accomplished has not been studied (73).

Increased Biliary Lipid Output : Nicotinic acid increases biliary cholesterol output (49, 110, 111). Biliary cholesterol is derived from both de novo synthesis in the liver and a pre-formed hepatic cholesterol pool (112). It is possible that niacin increases biliary cholesterol output either by increasing HMG-CoA reductase or by inhibition of ACAT activity, but this is unlikely as such an effect would increase serum cholesterol concentrations as well (112). It is been suggested that biliary cholesterol and phospholipid output is controlled by intracellular second messenger (112). Membrane-permeable cyclic AMP analogues and forskolin (adenylate cyclase stimulator) produce a sustained reduction in biliary bile acid output (113). As niacin reduces cyclic AMP concentrations in adipose tissue (73, 114, 115), it is tempting to speculate that niacin similarly decreases cyclic AMP concentrations in the liver (110). Such an effect would

be expected to increase the biliary output of cholesterol from pre-formed sources.

Increase in HDL_2 concentrations : The HDL (gge) subclasses were recently shown to be strong determinants for the severity as well as the progression of coronary atherosclerosis in survivors of myocardial infraction (116). A highly significant correlation was found between plasma levels of HDL-2b protein and the degree of coronary atherosclerosis. In contrast, HDL-3b correlated positively with progression of coronary atherosclerosis. Blum et. al. (117) and Shepherd et. al. (50) have indicated that nicotinic acid raises the HDL_2/HDL_3 ratio. In a recent study, it has been found that 4 g nicotinic acid daily after 6 weeks had increased the concentration of HDL_2 cholesterol on an average by 180%, while that of HDL_3 remained unchanged.

The exact mechanism related to the increase in HDL_2 is not known, but it is speculated that nicotinic acid inhibit catabolism of HDL_2 by decreaseing hepatic lipase activity. It has been suggested that hepatic lipase is instrumental in coverting HDL_2 to HDL_3 (118) and marked accumulation of HDL_2 along with virtually no HDL_3, was observed in two subjects with hepatic lipase deficiency (119). The findings of Shepherd et. al. (50) support the probability that nicotinic acid, in fact, reduces the catabolism of HDL. Acipimox [5 methylpyrazine carboxylic acid], closely related compound to NA, has been shown to decrease hepatic lipase activity after long term treatment (120).

Lowering of lipoprotein(a) : Elevated plasma levels of Lp(a) are considered to be an

independent risk factor for the development of cardiovascular disease (121, 122). Lipoprotein(a) is regarded as a particularly atherogenic lipoprotein and has been identified in atherosclerotic lesions by immunocytochemical techniques (123). The ability of nicotinic acid therapy to decrease serum levels of Lp(a) is distinctive among antihyperlipidemic drugs (73). Recent clinical studies have indicated that nicotinic acid therapy decreased serum levels of lipoprotein(a) (124). Nicotinic acid at a dose of 4 g per day administered to hyperlipidemic patients for 6 weeks decreased serum Lp(a) levels by an average of 38% (124). There was a linear relationship between the percent decrease of Lp(a) and the diminution in LDL cholesterol levels. The authors suggested that nicotinic acid may act by inhibiting the synthesis of apo(B), since this is the common component of both Lp(a) and LDL (124). By contrast a study with new HMG-CoA reductase inhibitors lovastatin and simvastatin showed average 33% elevation of serum Lp(a) levels (125).

1.3.5. Adverse Effects of Nicotinic Acid

Niacin is a well-established agent for lowering circulating lipid concentrations and has been associated with a reduced risk of cardiovascular morbidity in clinical trials. However, the potential risks of use of large doses of niacin have not been critically considered in the same way as far the use of other lipid-lowering drugs. Its only consistent deleterious effect in high doses has been cutaneous flushing and/or itching. Flushing occures in most people with as small as 100 mg orally (126). Previous studies

have shown that niacin-induced vasodialtion can be greatly attenuated by pretreatment with cycloxygenase inhibitors (127), implicating a role for prostaglandins. However prostaglandins responsible for the vasodilation were not clearly established until it was shown that ingestion of niaicn in humans selectively resulted in 400 to 800 times increases in the endogenous release of prostaglandins (PG)D_2, a potent vasodilator (126). These findings implicated that PGD_2 as the mediator responsible for niacin induced vasodilation. The site from which PGD_2 is released was not known (126). Authors suggested that the origion of PGD_2 is unlikely to be mast cells because the release of PGD_2 after ingestion of niacin is not accompanied by a release of histamine.

Furthur, Morrow et. al. (126) investigated whether nicotinic acid activates a cell or cells in human skin to release PGD_2 from the site of application. They found that indeed topical methylnicotinate evoked a release of large quantities of PGD_2 from the site of application in a dose dependent fashion. Therefore they concluded that some cell(s) in skin is activated by niacin to produce PGD_2. Further examination revealed that extremely high concentratios of PGD_2 (several thousand pg/ml) were detected in superficial venous blood draining skin following oral ingestion of niacin; comparatively trivial quantities (14 to 1200 times less) were present in arterial circulation supplying the skin. These results indicated that the skin is a major site from which PGD_2 is released following administration of niacin and that the flush reaction following oral ingestion of nicoinic acid results form a local release of PGD_2 in skin . Although the cell in the skin that is activated by nicotinic acid to release PGD_2 is not known. High

selectivity of the cell present in skin for the release of PGD_2 after oral niacin would suggest that cell is unique to skin and is not found in other tissues or cell in skin is funtionally different than its counterparts present in other organs (126).

This unpleasant side effect is often diminished with long-term use and can be partly mitigated by slowly incrementing the dose, ingesting the drug with food, avoiding hot drinks at the time of ingestion. The use of nicotinic acid in diabetic patients often necessitates adjustments in dosage of hypoglycaemic agents and requires close monitoring of diabetic status (53). Because nicotinic acid may produce hyperglycaemia and impaired glucose tolerance, even in nondiabetic patients, and a loss of diabetic control may counteract the lipid-altering effects of the drug (128). Isolated reports (129-131) have indicated that niacin in large doses may be hepatotoxic. The signs include clinical cholestatic jaundice association with delayed bromosulphthalein clearance.

1.4. CONCLUSION AND OBJECTIVES OF THE PRESENT STUDY

As explained earlier the main urinary metabolites of niacin are N^1-methylnicotinamide (MNA), N^1-methyl-2-pyridone-5-carboxamide, and N^1-methyl-4-Pyridone-3-Carboxamide (60). With these metabolites the N^1-methylation is formed by a simple methyl transfer reaction in which S-adenosylmethionine is the methyl donor. The transfer of methyl group is catalyzed by methyl transferase for which cobalamin (vitamin B_{12}) as methylcobalamin is a cofactor.

Niacin is water soluble, it is not stored in the body beyond its tissue saturation level. Since its excretion is dependent upon methionine and vitamin B_{12}, an excess intake of niacin may raise the requirement of these nutrients. Consequently, the increased requirement of methionine for niacin excretion, may lead to deficiency of cysteine, as cysteine is a metabolic product of methionine metabolism via transsulfuration pathway (Figure 1.2). The sequences of methionine catabolism produces cysteine. Methionine is metabolized by transmethyation and transsulfuration pathways (132). Methionine, as S-adenosylmethionine is converted to S-adenosylhomocysteine. Cleavage of adenosylhomocysteine leads to the formation of homocysteine. Homocysteine is either remethylated to methionine or is condensed with serine to form cystathionine. The salvage of homocysteine to methionine is either catabolyzed by betaine-homocysteine methyl transferase or 5-methyltetrahydrofolate-homocysteine methyltransferase (methionine synthase) (132). Methionine synthase requires 5-methyltetrahydrofolate as methyl donor and vitamin B_{12} as cofactor (133).

The synthesis of cystathionine involves the enzyme cystathionine β-synthase. Notably this enzyme requires pyridoxal 5-phosphate, the biological active form of vitamin B_6, as cofactor (133). Cystathionine is cleaved by enzyme γ-cystathionase to cysteine. Also this enzyme requires pyridoxal 5-phosphate for its activity. γ-cystathionase completes the conversion of methionine to cysteine (134). Therefore, it is logical to examine the effect of mega doses of nicotinic acid on methionine metabolism and its consequences.

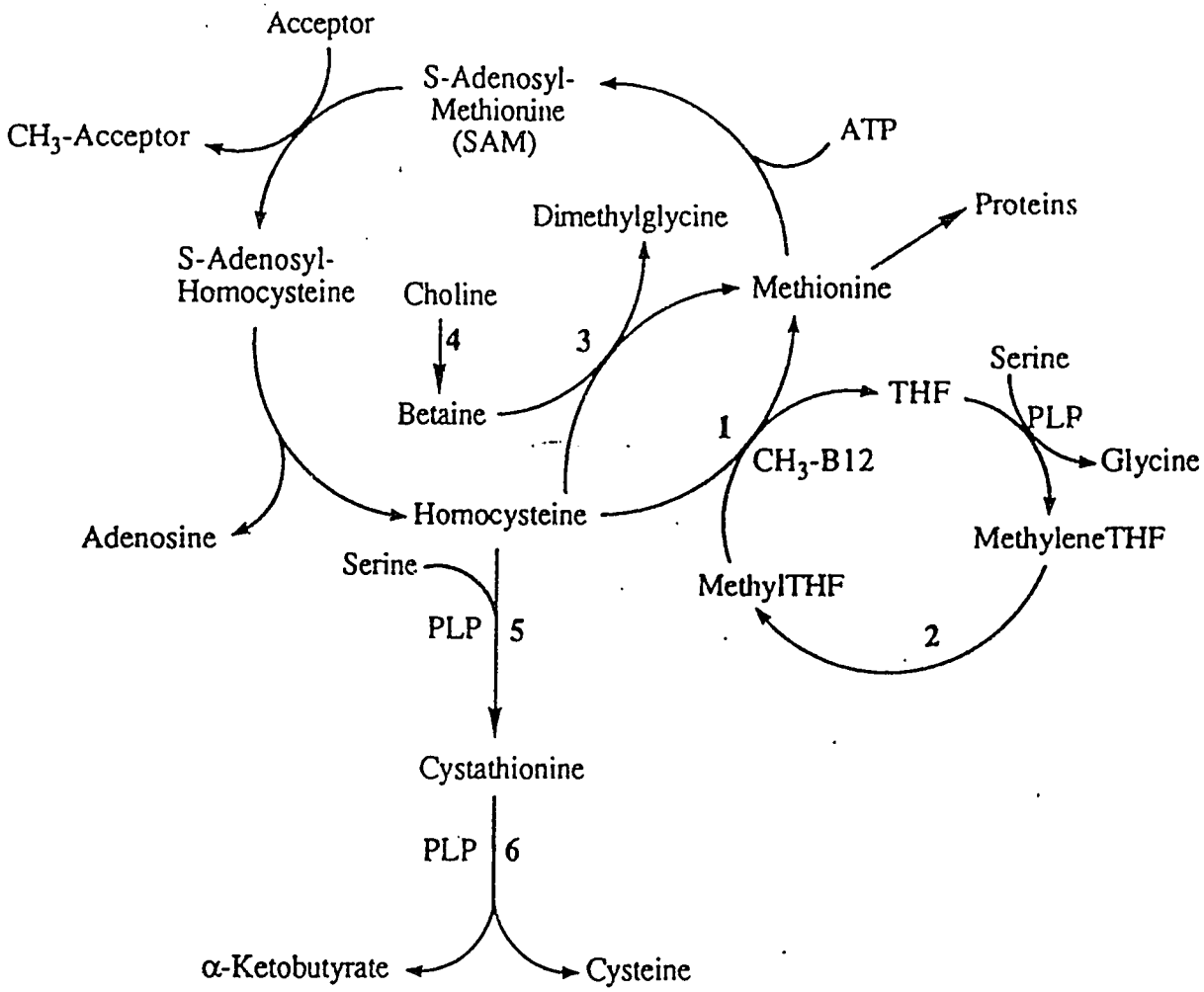


Figure 1.2 Methionine metabolism involving vitamin B12 vitamin B6 and folic acid

1.4.1. HYPOTHESIS

It is hypothesized that the consumption of large doses of niacin affects methionine metabolism, leading to a cysteine deficiency and that the concurrent supplementation of niacin and vitamin B_6 lowers blood cholesterol without causing an alteration in methionine metabolism. Using the rat as a model, this hypothesis was tested with the following objectives:

1. Does the administration of niacin in large doses lead to an interruption of methionine degradation to cysteine ?
2. Whether the alteration in methionine metabolism affects vitamins. These vitamins include vitamin B_{12} , B_6 and folic acid ?
3. If any of the vitamins is of concern, does its supplementation prevent niacin associated alteration in cysteine synthesis without affecting the hypolipidemic action of niacin.

MATERIAL AND METHODS

2.1. ANIMALS AND DIETS

Male Sprague-Dawley rats (University of Alberta), weighing 120-150 g, were used throughout the study. They were individually housed in stainless steel metabolic cages in a well-ventilated room maintained at 21 ± 2^{0}C and were on a 12-hour light-dark cycle. All animals were fed a pellet diet (wayne rat chow, Allied Mills Inc.) for a week before being fed an experimental semi-synthetic diet (Table 2.1). The protocol was approved by the animal welfare committee.

After a run-in period, the animals were randomly divided into three groups (Figure 2.1) of six each. Group 1 was fed a semi-synthetic diet containing physiological levels of niacin (Table 2.1) and used as controls (diet A). Groups 2 and 3 were fed a diet containing niacin at two dose levels : 400 mg (diet B) and 4000 mg (diet C) per kg diet in addition to control diet, respectively. All animals had free access to water and their respective diets for a period of 3 weeks. In addition, three groups of animals of six each, were fed diets A and C without or with vitamin B_6 supplementation (10 mg vitamin B6 per kg diet), respectively for a period of 6 weeks. Body weight of these animals and daily food intake were recorded once a week throughout the study periods.

At the end of the appropriate experimental period, the animals were fasted for 12 hours

Table 2.1

Composition of The Semi-synthetic diet

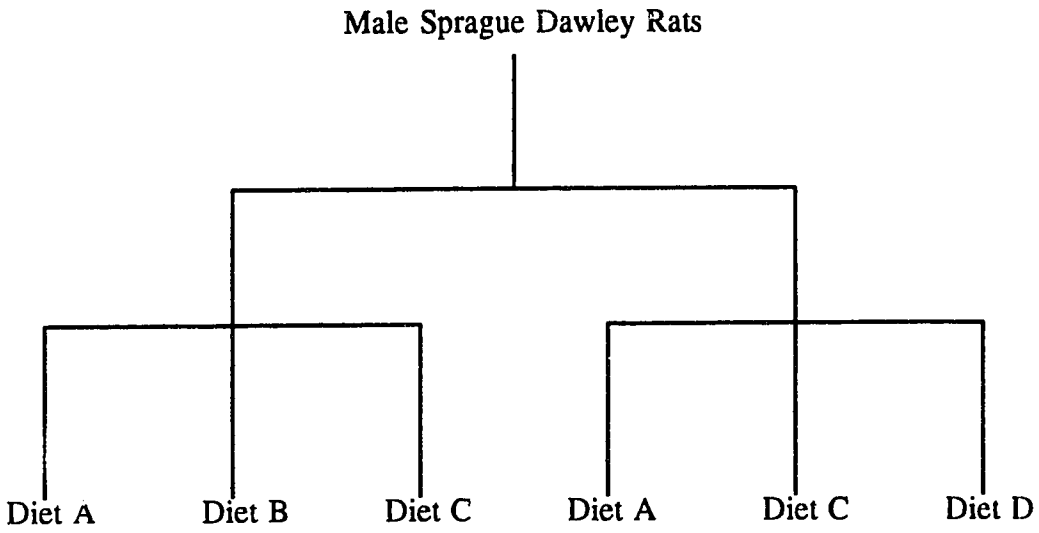
Ingredient	(g/Kg)
Casein (vitamin free)	200
Starch (corn)	648
AIN Vitamin mixture*	10
AIN Mineral mixture**	30
Cellulose	50
DL-Methionine	2
Corn oil	60

*Vitamin mixture : vitamin A acetate (500,000 IU/g) 19.8 mg; vitamin D_2 (850,000 IU/g), 1.38 mg; vitamin E acetate (500 IU/g), 110 mg; ascorbic acid 495 mg; Inositol 55 mg; choline bitrate 2227 mg; menadione 24.75 mg; p-aminobenzoic acid 55 mg; niacin 46.75 mg; riboflavin 11 mg; pyridoxine HCl 11 mg; thyamine HCl 11 mg; D-calcium pantothenate 33 mg; biotin 0.22 mg; folic acid 0.99 mg; vitamin B_{12} 0.0149 mg.

**Mineral mixture : calcium phosphate diabasic, 15 g; sodium chloride, 2.22 g; potassium citrate monohydrate, 6.6 g; potassium sulfate, 1.56 g; magnesium oxide, 0.72 g; manganous carbonate (43-48% Mn), 0.105 g; ferric citrate (16-17% Fe), 0.18 g; zinc carbonate (70% ZnO), 0.048 g; cupric carbonate (53-55% Cu), 0.009 g; potassium iodate, 0.0003 g; sodium selenite, 0.0003 g; chromium potassium sulfate, 0.0165.

Figure 2.1.

Experimental design of the study



Diet A : Control (Table 2.1)

Diet B : Control diet + 400 mg niacin/kg diet

Diet C : Control diet + 4000 mg niacin/kg diet

Diet D : Control diet + 4000 mg niacin/kg diet + 10 mg vitamin B_6/kg diet

and sacrificed using CO_2 chamber. Two twenty-four hour urine samples were collected from each animal just before killing, in both experiments. Blood was collected through cardiac puncture in heparinized tubes and plasma was separated by centrifugation (3000 rpm for 10 min at -4^{0}C) within half an hour after collection. The livers were quickly removed, excised, weighed and frozen immediately in liquid nitrogen. The separated plasma and liver samples were stored at -40^{0}C until analyses.

2.2. DETERMINATION OF PLASMA TOTAL CHOLESTEROL

Using Sigma Diagnostics kit (#352), plasma total cholesterol was determined enzymatically as described by Allain et al. (135). The test principle reactions are as follows :

$$\text{Cholesterol Esters} + H_2O \xrightarrow[\text{Esterase}]{\text{Cholesterol}} \text{Cholesterol} + \text{Fatty acids} \qquad \text{(Eq 1)}$$

$$\text{Cholesterol} + O_2 \xrightarrow[\text{Oxidase}]{\text{Cholesterol}} \text{Cholest-4-en-3-one} + H_2O_2 \qquad \text{(Eq 2)}$$

$$2H_2O_2 + \text{4-Aminoantipyrine} + \text{p-Hyrdoxybenzensulfonate} \xrightarrow{\text{Peroxidase}} \text{Quinoneimine dye} + 4H_2O \qquad \text{(Eq 3)}$$

Cholesterol esters present in the sample were hydrolyzed to cholesterol by cholesterol esterase (Eq 1). The cholesterol produced by hydrolysis is oxidized by cholesterol oxidase to cholest-4-en-3-one and hydrogen peroxide (Eq 2). The hydrogen peroxide produced is then coupled with the chromogen, 4-aminoantipyrine and p-hydroxybenzenesulfonate in the presence of peroxidase to yield a quinoneimine dye (Eq 3), which has an absorbance maximum of 500 nm. The intensity of color produced is directly proportional to the total cholesterol concentrations in the sample.

2.3. DETERMINATION OF PLASMA TRIGLYCERIDES

Triglyceride concentrations in plasma samples were measured by using an enzymatic colorimetric method Sigma Diagnostics kit (# 352) as described by Bucolo and David (136). The enzymatic reactions involved in the assay are as follows:

$$\text{Triglycerides} \xrightarrow{\text{Lipoprotein lipase}} \text{Glycerol} + \text{Fatty Acids} \qquad \text{(Eq 4).}$$

$$\text{Glycerol} + \text{ATP} \xrightarrow{\text{Glycerol Kinase}} \text{G-1-P} + \text{ADP} \qquad \text{(Eq 5).}$$

$$\text{G-1-P} + \text{NAD} \xrightarrow{\text{G-1-PDH}} \text{DAP} + \text{NADH} \qquad \text{(Eq 6).}$$

$$\text{NADH} + \text{INT} \xrightarrow{\text{Diaphorase}} \text{Formazan} + \text{NAD}^{+} \qquad \text{(Eq 7).}$$

Triglycerides were first hydrolyzed by lipoprotein lipase to glycerol and free fatty acids (Eq 4). Glycerol is then phosphorylated by adenosine-5-triphosphate (ATP) to form glycerol-1-phosphate (G-1-P) and adenosine-5-d-phosphate (ADP) in a reaction catalyzed by glycerol kinase (Eq 5). The G-1-P is oxidized to dihydroxyacetone phosphate (DAP) with a concomitant reduction of nicotinamide adenine dinuclotide (NAD) to NADH in a reaction catalyzed by glycerol-1-phosphate dehydroxygenase (G-1-PDH) (Eq 6).

In a subsequent step the NADH is oxidized with the simultaneous reduction of 2-(p-iodophenyl)-3-p-nitro-phenyl-5-phenyltetrazolium chloride (INT) to formazan (INTH) in the presence of diaphorase. The resulting formazan is highly colored and has an absorbance maximum at 500 nm. The intensity of color produced is directly proportional to the triglycerides concentration of the sample.

2.4. DETERMINATION OF PLASMA HDL-CHOLESTEROL

HDL-cholesterol was assayed enzymatically using Sigma Diagnostics kit (procedure # 352-4), in which HDL-cholesterol was measured as cholesterol after the precipitation of the LDL and VLDL fractions. Phosphotungistic acid in conjunction with $MgCl_2$ was used to precipitate LDL and VLDL fractions of serum, leaving the HDL fraction in solution. Cholesterol concentration in HDL fraction was then determined as explained earlier.

2.5. DETERMINATION OF LDL CHOLESTEROL

The cholesterol content of LDL was determined indirectly using the method described by Frildwald et al. (137). This method requires the measurement of plasma total cholesterol, triglycerides and HDL-cholesterol concentrations. The following formula was used to calculate the plasma content of LDL-Cholesterol in mmol/litres (Eq 8)

$$\text{LDL -Cholesterol} = \text{Total-C} - \frac{\text{Triglycerides}}{2.2} - \text{HDL-cholesterol} \qquad \text{(Eq 8)}$$

This formula is based on the assumption that VLDL-C approximates triglycerides in plasma divided by 2.2. This equation, however cannot be applicable to plasma samples from subjects with plasma triglycerides concentration exceeding 400 mg/100 ml. This restriction appeared not to be present in the plasma samples meaured in the present study, thus the obtained values can be considered reliable.

2.6 EXTACTION OF LIVER LIPIDS

Liver lipids were extracted from approximately 0.5 g portions as described by Folch et al (138). Liver samples were homogenized with 2:1 chlorofom:methanol (v/v) solution. Filtered the homogenate and the layers were splite by the addition of adequate amount of 0.1 mM KCl solution. The lower phase containing lipids was collected, dried and diluted to required volume.

2.7. DETERMINATIONS OF PLASMA AND URINARY METHIONINE, CYSTEINE AND HOMOCYSTEINE

Plasma and urinary free methionine, cysteine and homocysteine were analyzed by the method of Jones and Gilligan (139), which utilizes o-phthaldialdehyde as a precolumn derivatizing agent. Aliquotes of standards and unknown samples were mixed 1:1 with the fluoraldehyde reagent prior to injection. The fluoraldehyde reagent was prepared by dissolving O-phthaldialdehyde in methanol followed by the addition of mercaptoethanol, sodium borate and brij 35.

Chromatography System: Separation and quantification of amino acids were achieved by using a Varian 5000 high performance liquid chromatograph and a Varian Fluorichrom detector (Exication 340 nm, emission 450 nm). Plasma and urine samples were mixed 1:1 with the fluoroaldehyde reagent prior to injection into a Supelcosil 3 micron LC-18 reverse phase column (4.6 * 150 mm; supelco) equipped with a guard column (4.6 * 50 mm) packed with supelco LC-18 reverse phase packing (20-40 um). Gradients were formed between two degassed solvents. Solvent A was tetrahydrofuran:methanol:0.1 M sodium acetate (pH 7.2) (5:95:900) and solvent B was methanol. The flow rate was maintained at 1.1 mL/min.

Peaks were identified with reference to the retention times of standard amino acids injected separatly. The peak areas of known concentrations of amino acids were

recorded and integrated using a Shimdzu Ezchrom Chromatography Data System. AGPA was used as the internal standard to account for injection variations. The linearity of response was estimated by injecting different concentrations of derivatized amino acids and constructing regression equations of fluoroscence response-concentration curves. The within run precision at different concentrations was estimated by injecting different volumes of derivatization standards in replicates and the between-run precision by analyzing aliquotes of the same standard on different days. The accuracy of measurement was tested by adding known quantities of amino acids to the plasma and calculating the percentage of recovery. All samples were analyzed in duplicate.

2.8. DETERMINATION OF PLASMA VITAMIN B_{12} AND FOLIC ACID

Plasma vitamin B_{12} and folic acid were determined by using commercially available Dualcount solid phase boil assay kit (Inter Medico, Markham, Ontario). Vitamin B_{12} and folic acid present in the sample were released from the carrier proteins by incubation at 100^{0}C in the presence of dithiothreitol and potassium cyanide to inactivate intrinsic factor antibodies and even the most extreme levels of vitamin B_{12} transport proteins. Addition of purified hog intrinsic factor and purified beta lactoglobulin served as the binders for vitamin B_{12} and folic acid respectively. The unlabeled vitamin B_{12} or folate competes with its labeled species for the limited number of available binding sites on its specific binder, thus reducing the amount of labeled vitamin B_{12} or folate

bound. Vitamin B_{12} analogs do not interfere, since the binder is free of R-protein. Moreover, the reaction takes place at a pH 9.3 where added intrinsic factor is fully active and the folic acid binder has equal affinity for metyltetrahydrofolate (MTHF), the main form of folic acid in circulation. After an adequate incubation period, isolation of the bound fraction is achieved by centrifuging and decanting. Counts in the precipitate are then converted by comparison with a calibration curve into vitamin B_{12} and folic acid concentrations. The level of radioactivity bound is inversely related to the concentration in the sample or standard.

2.9. DETERMINATION OF VITAMIN B6 (PLASMA PYRIDOXAL 5'-PHOSPHATE).

Plasma pyridoxal-5'-phosphate (vitamin B_6) was determined using a commercially avialable radioassay kit (Buhlmann Laboratories AG, Switzerland) as a modification of the method described by Shin et al (140). The principle of the assay involves the decarboxylation of ^{3}H-tyrosine by the vitamin B_6 dependent enzyme tyrosine apodecarboxylase to ^{3}H-tyramine. The activity of this tyrosine apodecarboxylase is quantitatively dependent on the amount of pyridoxal -5'-phosphate (vitamin B_6) present in the reaction mixture. The ^{3}H-tyramine thus produced is selectively extracted in the scientillation cocktail. The excess of ^{3}H-tyramine remains in the aqueous phase and is measured by liquid scintillation counting.

2.10. DETERMINATION OF CREATININE

Urinary creatinine was measured using the procedure explained by Digiorgio (141), which involves the quantitation of the red pigment alkaline creatinine picrate.

2.11. STATISTICAL ANALYSIS

Means and standard error of the means (SEM) were determined for all groups of animals. Data were analyzed by using one-way analysis of variance (ANOVA). When significant differences were detected, significant effects were furthur defined by using student-t and multiple comparison test (142) were used to determine which mean values were significantly different. In this present study, the level of significance considered was 0.05.

RESULTS

3.1 ANIMAL GROWTH AND FOOD INTAKE FOLLOWING NIACIN INTAKE

Table 3.1 shows the effects of a semi-synthetic diet (diet composition in Table 2.1) containing large doses of niacin (nicotinic acid) at two dose levels (400 and 4000 mg/kg diet) on the body and liver weights of rats. Feeding these niacin supplemented diets for three weeks resulted in a marked decrease in body weight gain compared to the animals not given niacin supplementation. The decrease in body weight gain was found to be dose-related and paralleled a reduction in food intake. Liver weight was also decreased when niacin was given in large doses.

3.2 LIPID LEVELS FOLLOWING NIACIN TREATMENT

The effect of supplemental intake of niacin for three weeks on lipid status was examined. Cholesterol, triglyceride, HDL-C and LDL-C concentrations in plasma and liver are shown in Table 3.2 and Table 3.3. Administration of niacin at a dose level of either 400 or 4000 mg/kg diet above physiological dose levels resulted in significant reductions of plasma total-cholesterol as well as triglycerides, compared with those of the animals fed a diet containing physiological levels (46.8 mg/kg diet) of the vitamin. Niacin supplementation also caused a reduction in plasma LDL-cholesterol. On the other hand the HDL-cholesterol levels were increased. The decrease in LDL-cholesterol

Table 3.1.

Effect of large doses of niacin on food intake and growth of animals.

	Control	Niacin (400 mg/kg)	Niacin (4000 mg/kg)
Body weight gain (g)	75.15 ± 3.29^{a}	66.83 ± 2.96^{ab}	59.87 ± 2.08^{b}
Food intake (g/day)	17.24 ± 0.40^{a}	15.91 ± 0.42^{b}	14.97 ± 0.33^{b}
Liver weight (g)	14.15 ± 0.16^{a}	13.90 ± 0.56^{a}	12.25 ± 0.42^{b}
Liver wt/Body wt X 100	4.78 ± 0.08^{ab}	4.93 ± 0.18^{a}	4.38 ± 0.11^{b}

Each value is the mean ± SEM of at least 6 animals. In each row values not sharing a common superscript letter are significantly different at $P < 0.05$.

Table 3.2

Effect of feeding high doses of niacin on plasma lipid profiles in rats.

TREATMENT	T-C (mmol/L)	TG (mmol/L)	HDL-C (mmol/L)	LDL-C (mmol/L)
Control	2.44 ± 0.03^{a}	1.84 ± 0.02^{a}	1.11 ± 0.01^{a}	0.48 ± 0.05^{a}
Niacin (400 mg/kg)	2.23 ± 0.02^{ab}	1.61 ± 0.03^{b}	1.29 ± 0.02^{b}	0.20 ± 0.04^{b}
Niacin (4000 mg/kg)	1.94 ± 0.05^{b}	1.30 ± 0.02^{c}	1.24 ± 0.03^{b}	0.11 ± 0.04^{b}

Each value is the mean ± SEM of 6 animals. In each column values not sharing a common superscript letter are significantly different at $P < 0.05$.

Table 3.3

Effect of Niacin on liver lipid concentrations

Treatment	Total-C (mg/liver)	TG (mg/Liver)
Control A	15.5 ± 3.61[a]	77.8 ± 4.7[a]
Niacin A (400 mg/Kg diet)	15.8 ± 3.32[a]	66.5 ± 3.9[b]
Niacin B (4000 mg/Kg diet)	18.6 ± 2.25[a]	53.2 ± 2.5[c]

Each value is the mean ± SEM of at least 6 animals. In each column values not sharing a common superscript letter are significantly different at $P < 0.05$.

respouse to niacin was dose related. This was not, however, the case with HDL-cholesterol, which was significnatly increased at a 400 mg/kg diet level and this level remained unaffected when the dose of the niacin was increased to 4000 mg/kg diet.

Similarly liver triglycerides were decreased following niacin treatment, but only at the higher dose level (4000 mg/kg diet). Liver cholesterol levels were increased following a high dose of niacin, athough difference was not statistically significant (Table 3.3).

3.3 METHIONINE AND CYSTEINE LEVELS FOLLOWING NIACIN TREATMENT

The effect of supplemental niacin on the plasma levels of the sulphur containing amino acid including methionine and cysteine is shown in Figures 3.1-3.4. There was a significant increase in methionine levels (Figure 3.1), while cysteine concentrations decreased (Figure 3.3). The niacin associated changes in circulating levels of the amino acids were reflected in their urinary metabolites. Thus, analysis of 24 hour urine samples revealed an elevated excretary level of methionine (Figure 3.2) in niacin supplemented animals, while cysteine excretion levels decreased (Figure 3.4).

3.4 PLAMA VITAMIN B_{12}, FOLIC ACID AND VITAMIN B_6 LEVELS

Since vitamin B_{12}, folate and vitamin B_6 are important factors involved in methionine

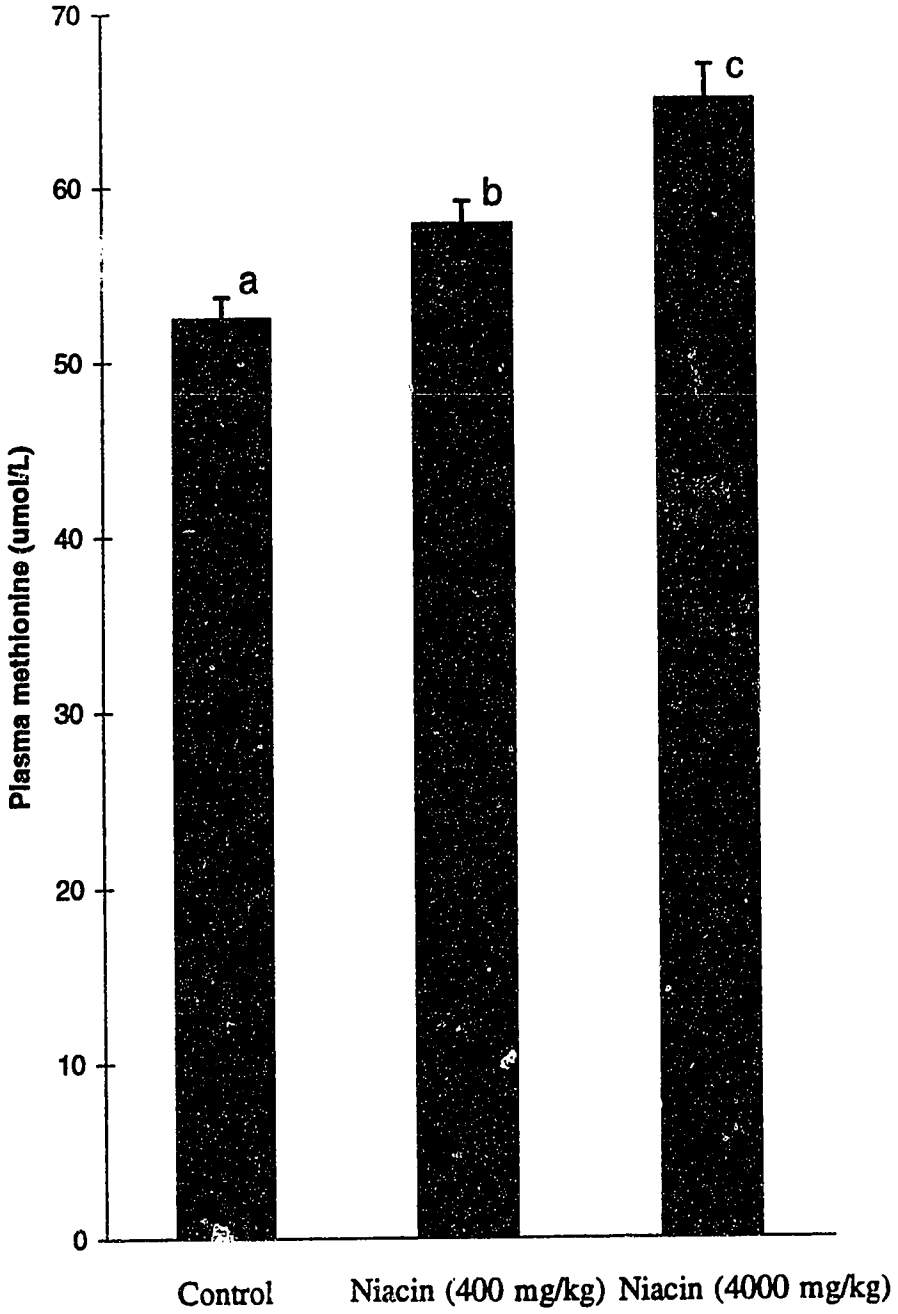


Figure 3.1 Plasma methionine levels following niacin treatment

Plasma methionine concentrations at each point are group means + SEM. Values without common superscript are significantly diffeerent ($p<0.05$).

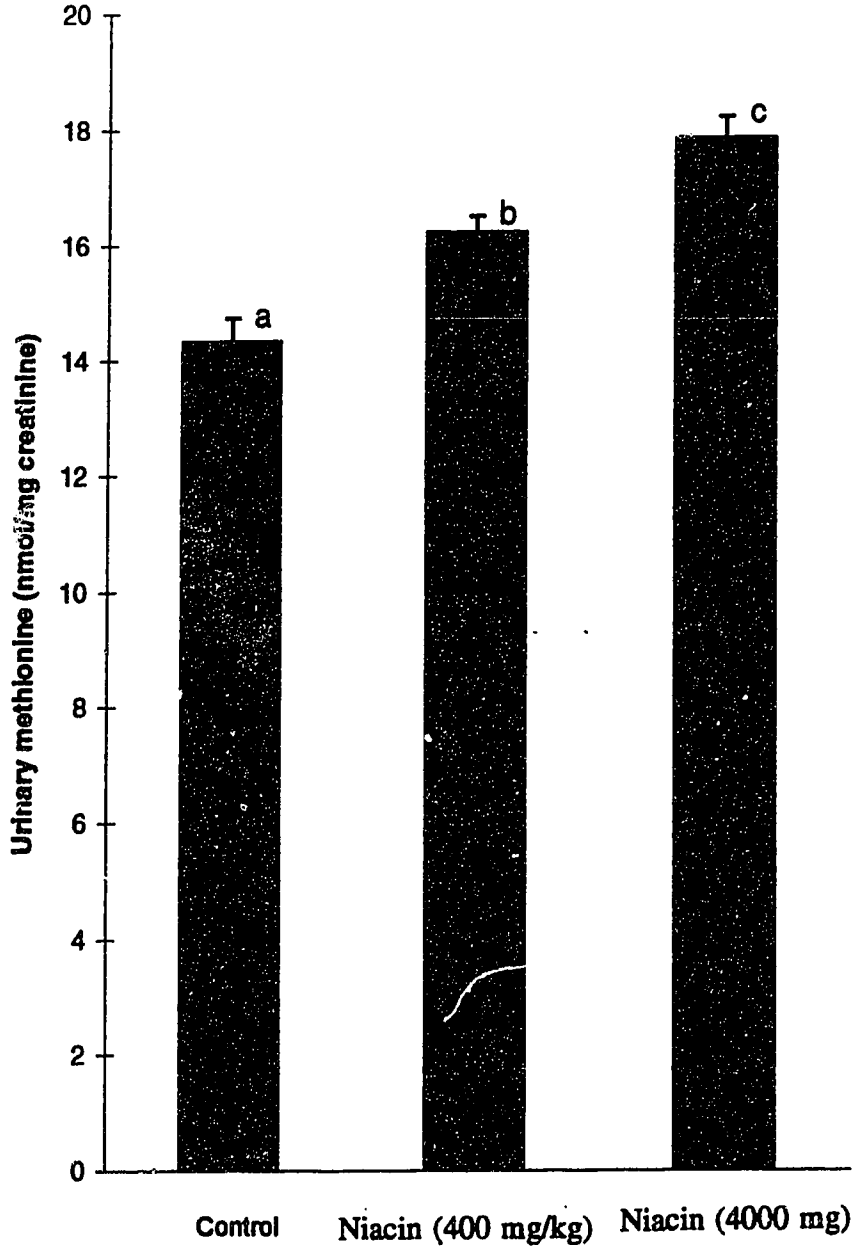


Figure 3.2 Urinary methionine excretion following niacin treatment

Urinary methionine concentrations at each point are group means + SEM.
Values without common superscript are significantly different ($p<0.05$).

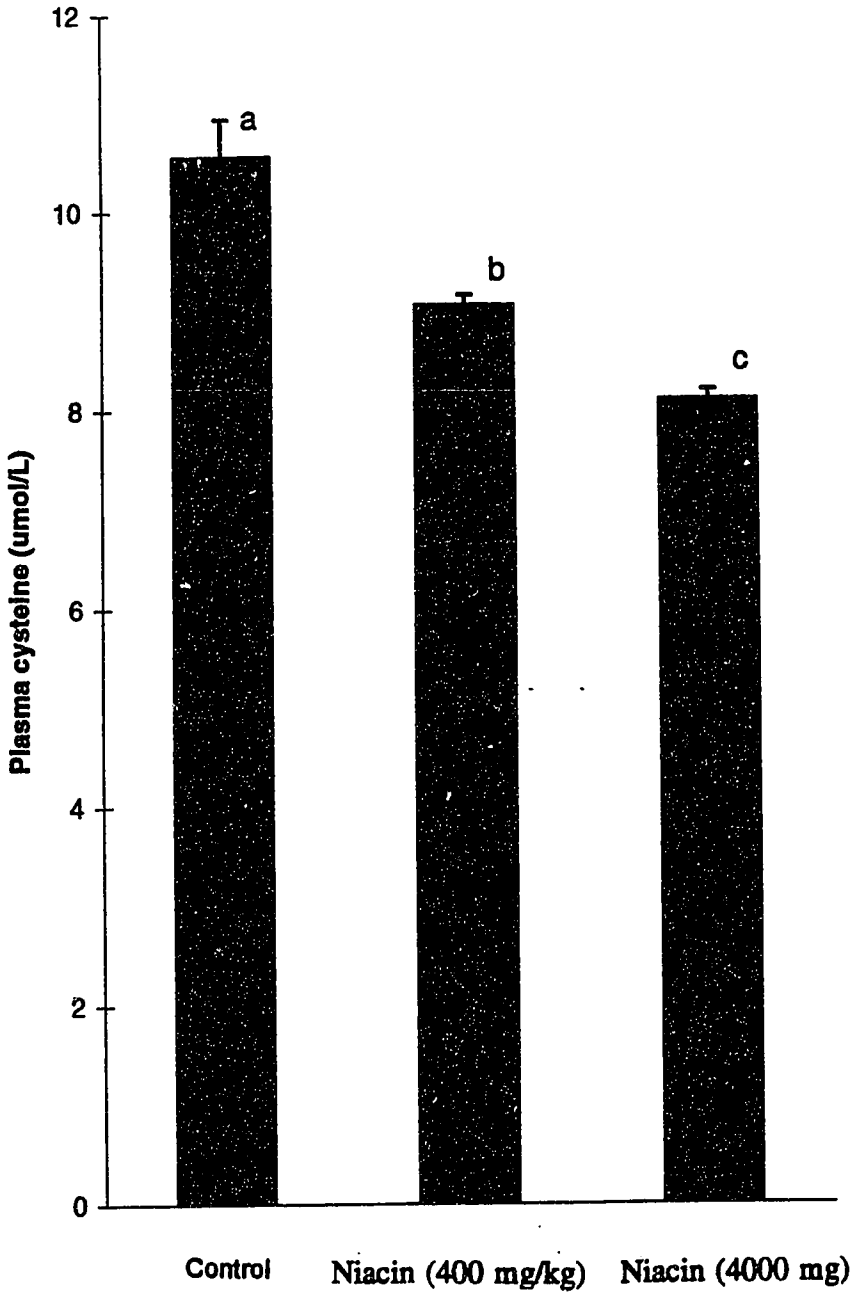


Figure 3.3 Plasma cysteine concentrations following niacin treatment

Plasma cysteine concentrations at each point are group means + SEM.
Values without common superscript are significantly different ($p<0.05$)

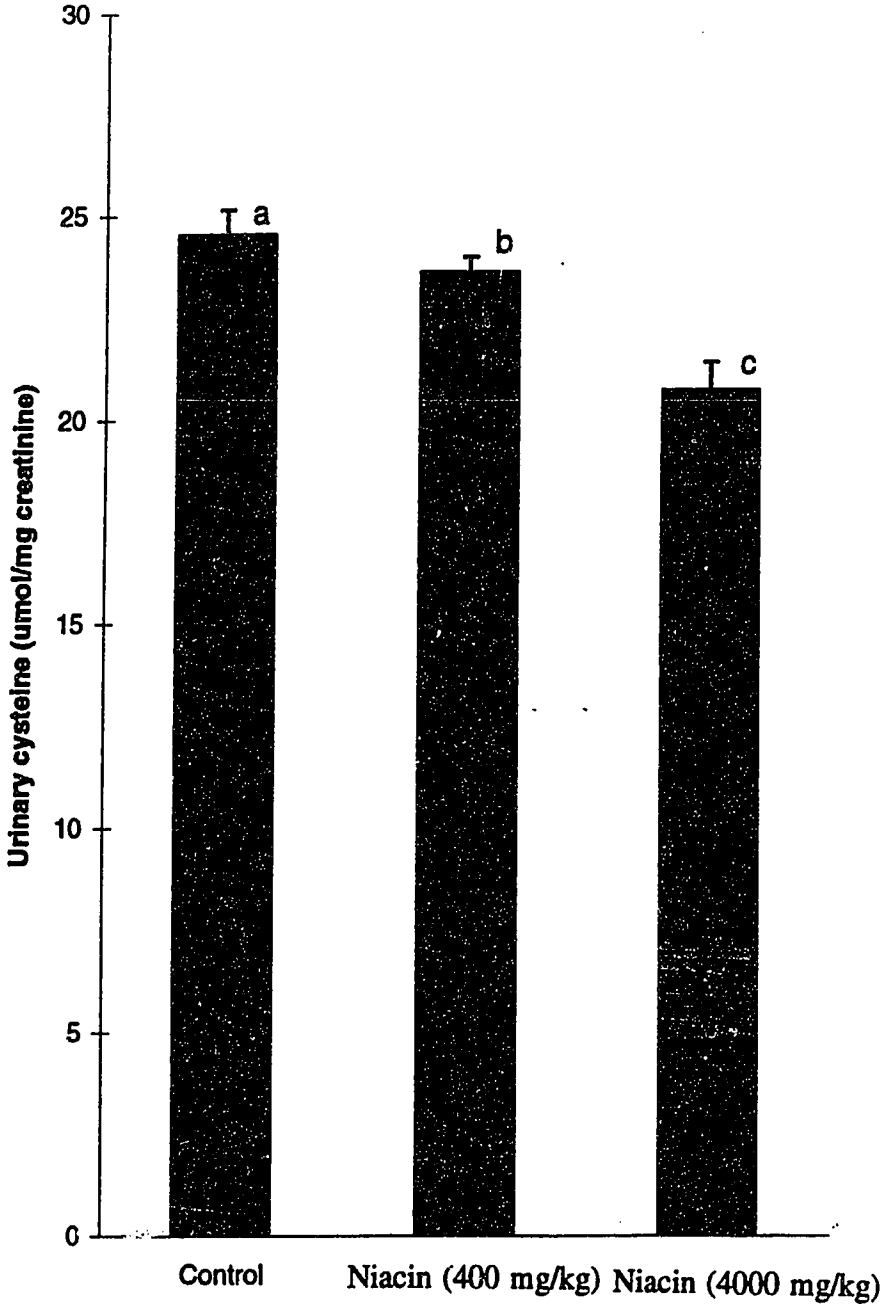


Figure 3.4 Urinary Cysteine excretion following niacin treatment

Urinary cysteine concentrations at each point are group means + SEM.
Values without common superscript are significantly different ($p<0.05$)

metabolism (Figure 1.2), and the responses of these vitamins in plasma to niacin supplementation were also examined. Administration of niacin did not induced any applicable changes in vitamin B_{12} and folic acid status. However vitamin B_6 was decreased significantly following niacin treatment, but only at high dose levels (Table 3.4). Subseqently another experiment was designed to examine, if vitamin B_6 supplementation reverses the niacin-associated altered methionine metabolism. This was rationalized by the fact that methionine is catabolized to cysteine through a series of reactions involving cystathionine β-synthase and γ-cystathionase enzymes, for which PLP (pyridoxal-5'-phosphate), an active form of vitamin B_6 is a cofactor (Figure 1.2).

3.5 SULPHUR AMINO ACID STATUS AFTER FEEDING NIACIN AND VITAMIN B_6 TOGETHER.

The effect of giving niacin (4000 mg/kg diet) together with vitamin B_6 (10 mg/kg diet) for a period of six weeks on the plasma levels of suphur amino acids is shown in Figures 3.5-3.10. Supplementation with vitamin B_6 reversed the effects of niacin on the sulphur amino acid status. Thus methionine concentrations were significantly elevated (Figure 3.5) while cysteine concentrations (Figure 3.7) were reduced in plasma of the animals receiving only 4000 mg niacin/kg diet). These values were normalized when vitamin B_6 was administered along with niacin. The excretary values of the amino acids paralleled that found in the plasma (Figure 3.6 and Figure 3.8).

Table 3.4

Effect of Niacin on plasma levels of vitamin B_{12}, folic acid and vitamin B_6

Treatment	Control	Niacin (400 mg/kg)	Niacin (4000 mg/kg)
Vitamin B12 (pg/ml)	959.33 ± 12.91[a]	956.17 ± 9.37[a]	958.31 ± 10.23[a]
Folic acid (ng/ml)	31.83 ± 2.11[a]	29.50 ± 2.48[a]	30.83 ± 3.36[a]
Vitamin B6 (ng/ml)	533.94 ± 6.21[a]	507.30 ± 12.80[a]	398.97 ± 8.09[b]

Each value is the mean SEM of at least 6 animals. In each row values not sharing a common superscript letter are significantly different ($p<0.05$)

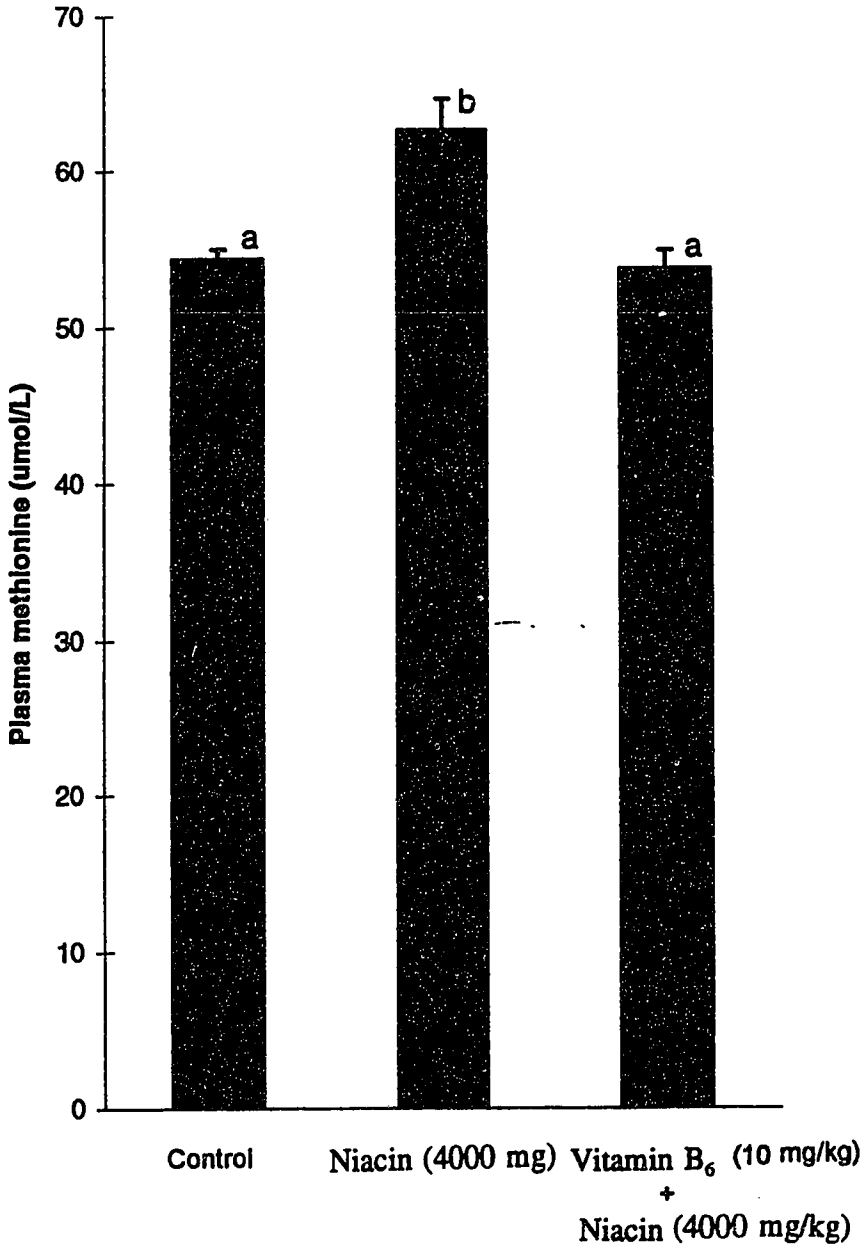


Figure 3.5 Plasma methionine following vitamin B_6 and niacin treatment

Plasma methionine concetrations levels at each point are group means + SEM. Values without common superscripts are significantly different ($p<0.05$)

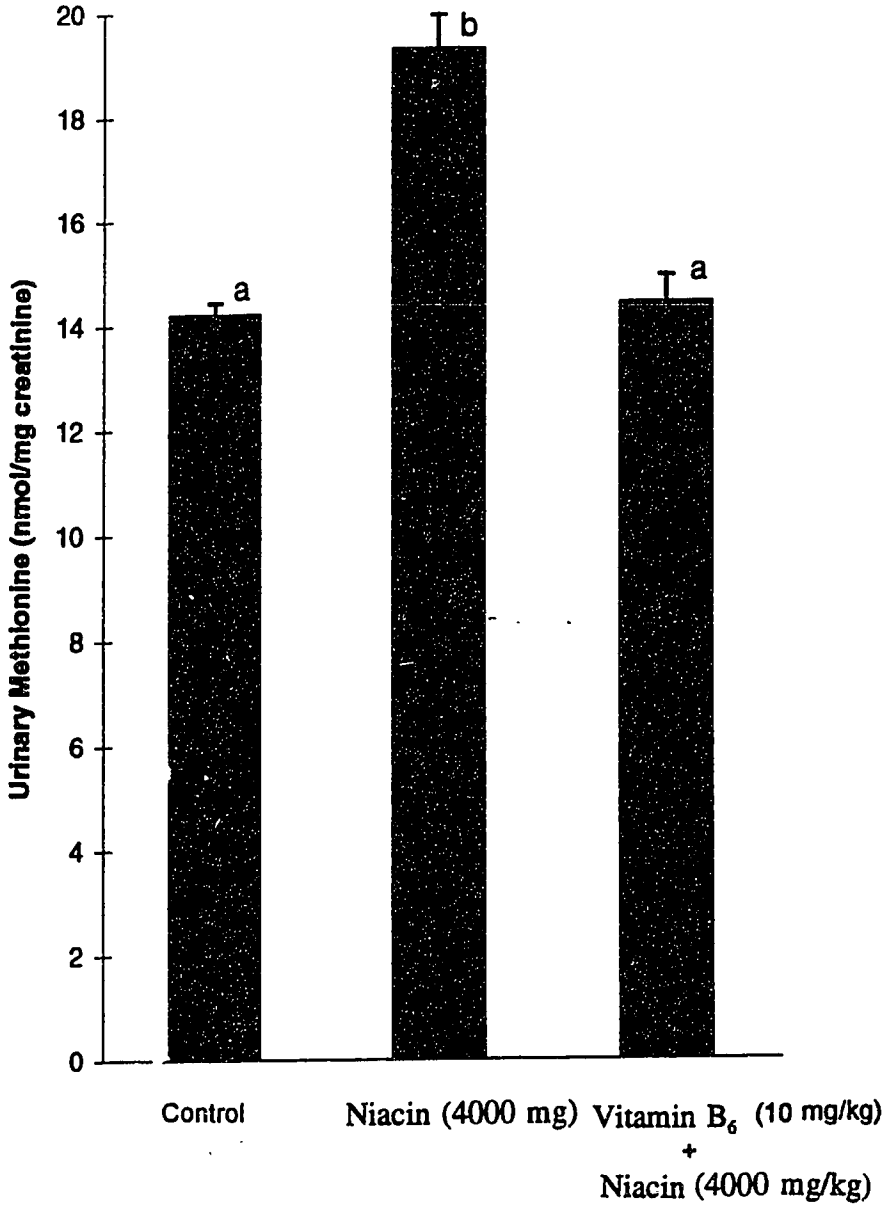


Figure 3.6 Urinary methionine excretion following vitamin B_6 and Niacin treatment.

Urinary methionine concentrations at each point are group means + SEM. Values without common superscripts are significantly different ($p<0.05$).

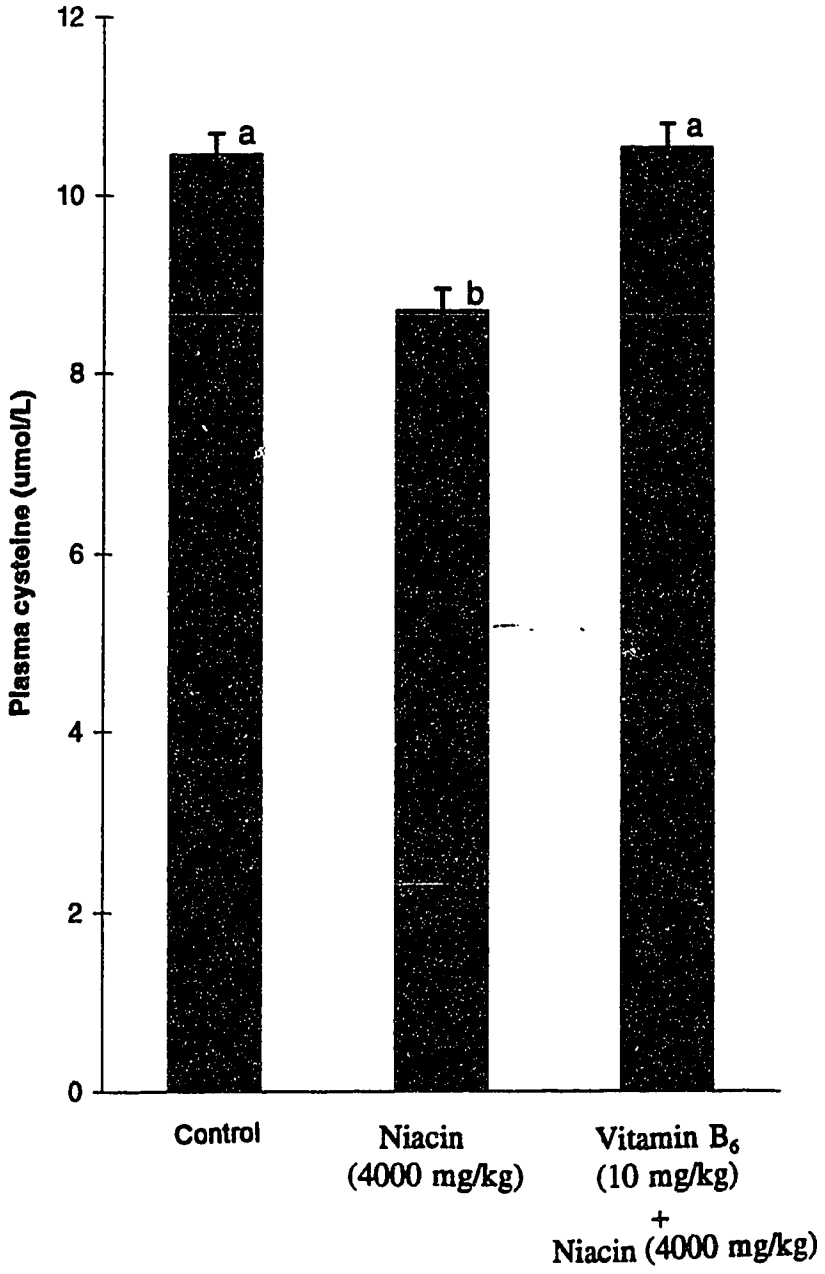


Figure 3.7 Plasma cysteine levels following vitamin B_6 and niacin treatment.

Plasma cysteine concentrations at each point are group means + SEM. Values without common superscript are significantly different ($p<0.05$)

Figure 3.8 Urinary cysteine excretion following vitamin B_6 and Niacin treatment.

Urinary cysteine concentrations at each point are group + SEM. Values without common superscript are significantly different ($p<0.05$)

Feeding a large dose of niacin for six weeks also resulted in significantly elevated plasma and urinary homocysteine concentrations, compared with of the control animals (Figure 3.9 and Figure 3.10). However this was normalized when vitamin B_6 and niacin were given simultaneously.

3.6. LIPID STATUS OF ANIMALS FOLLOWING NIACIN AND VITAMIN B_6 TREATMENT

Additon of vitamin B_6 to the diet (10 mg/kg diet) containing 4000 mg niacin/kg did not affect the hypocholesterolemic action of niacin. Thus, supplementation with niacin plus vitamin B_6 lowered plasma total cholesterol, triglycerides and LDL cholesterol to the same extent as niacin alone. The niacin associated increase in plasma HDL-cholesterol concentrations also remained unaffected following concurrent administration of vitamin B_6 (Table 3.5).

Furthermore, simultaneous administration of vitamin B_6 reversed the effects of large doses of niacin on body weight gain and food intake (Table 3.6). Both the body weight gain and the food intake were significantly decreased following administration of niacin but this effect was prevented when animals were also given vitamin B_6. Similarly niacin induced loss of liver weight was prevented when vitamin B_6 was also given.

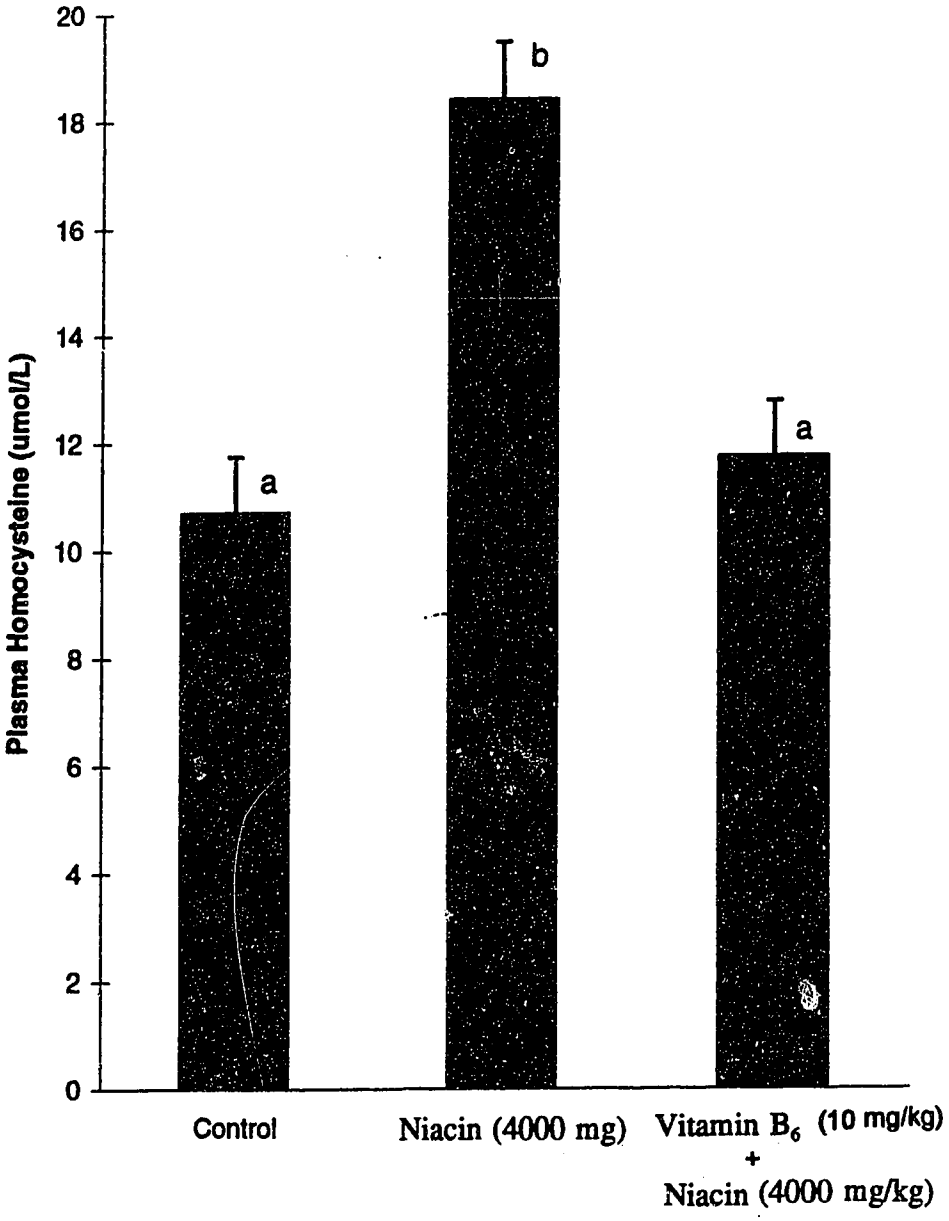


Figure 3.9 Plasma homocysteine concentrations in control, niacin and vitmain B_6 treated animals

Plasma homocysteine bars are group Means + SEM. Values without superscript are significantly different ($p<0.05$)

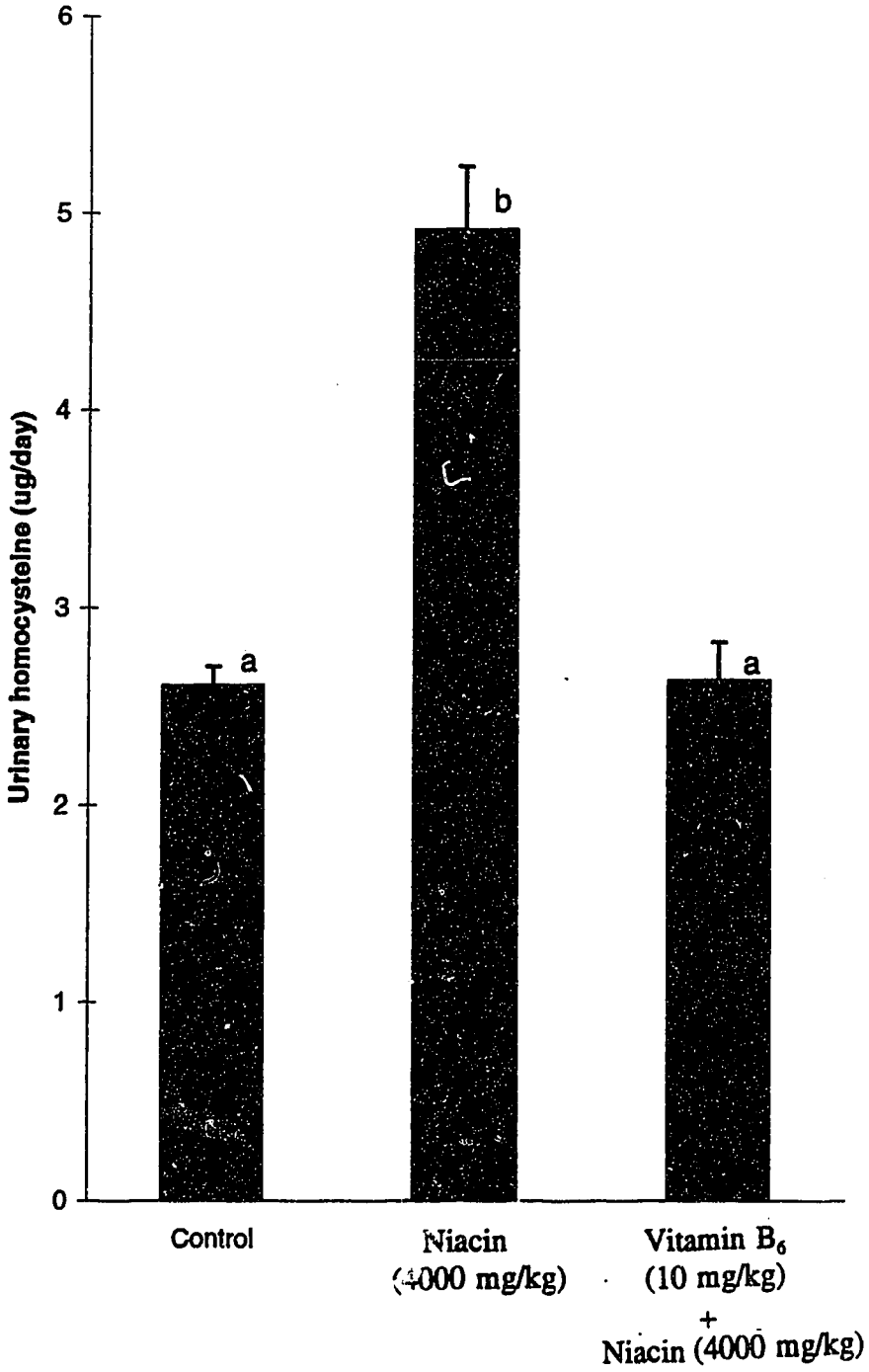


Figure 3.10 Urinary homocysteine excretion following vitamin B_6 and niacin treatment

Urinary homocysteine concentrations at each point are group means + SEM. Values without common superscript are significantly different ($p<0.05$)

Table 3.5

Effect of concurrent supplementation of niacin and vitamin B_6 on plasma Lipid profiles.

Treatment	T-C	TG	HDL-C	LDL-C
Control	2.38 ± 0.02^a	1.80 ± 0.03^a	1.15 ± 0.07^a	0.41 ± 0.05^a
Niacin (4000 mg/kg diet)	1.94 ± 0.03^b	1.30 ± 0.02^b	1.25 ± 0.02^b	0.11 ± 0.01^b
Vitamin B_6 (10 mg/kg) + Niacin (4000 mg/kg)	1.95 ± 0.02^b	1.33 ± 0.02^b	1.21 ± 0.04^b	0.12 ± 0.02^b

Each value is the mean ± SEM of 6 animals. In each column values not sharing a common superscript letter are significantly different at $P < 0.05$.

Table 3.6

Growth of animals following simultaneous intake of niacin and vitamin B_6.

Treatment	Control	Niacin (4000 mg/kg)	Vitamin B_6 (10 mg/kg) + Niacin (4000 mg/kg)
Body weight gain (g)	164 ± 3.5^a	147 ± 5.9^b	160 ± 3.9^a
Food intake (g/day)	19.36 ± 1.0^a	16.26 ± 0.5^b	19.04 ± 0.6^a
Liver weight (g)	14.59 ± 0.4^a	11.59 ± 0.6^b	14.49 ± 0.2^a
Liver wt/Body wt X 100	3.54 ± 0.1^a	2.95 ± 0.1^b	3.60 ± 0.1^a

Each value is the mean SEM of at least 6 animals. In each row values not sharing a common suberscript letter are significantly different at $P < 0.05$.

DISCUSSION

Both nicotinic acid and nicotinamide are currently used in pharmacological doses to treat human disease. The more commonly used forms of this vitamin is nicotinic acid, which is in widespread use to treat hypercholesterolemia and to prevent cardiovascular diseases (1). Previously, administration of nicotinic acid (3 g/day or more) to normal subjects has shown to decrease both cholesterol and triglyceride, while increasing HDL-cholesterol levels (48). Similarly , the present study demonstrated that feeding niacin at the rate of either 400 mg or 4000 mg per kg diet to rats for three weeks reduced plasma cholesterol significantly. The greatest decrease in plasma cholesterol was seen in the LDL fraction, on the other hand the HDL fraction increased. It was of interest to note that, a low dose of niacin (400 mg per kg diet) produced a superior effect on HDL cholesterol than a higher dose (4000 mg per kg diet) fed animals. This effect of niacin on HDL-cholesterol concides with the findings of Keenan et al (143), who reported that subjects on reduced doses of niacin had a better HDL cholesterol response (+13%) than those who had very large dose levels of the vitamin (-0.6%). These results support the theory of Knopp and co-workers (54) that there are two separate mechanisms of action of niacin on cholesterol. One action raises HDL cholesterol and seems to be more effective at lower doses, while a second lowers LDL cholesterol and increases in effect with increasing dose. In addition to decreasing plasma cholesterol and triglyceride levels, niacin is the most effective drug for elevating HDL cholesterol. The increase in HDL cholesterol after niacin treatment has been attributed to the

substantial rise in circulating HDL_2, with a simultaneous fall in HDL_3 (48).

The versatile action of niacin on lipoprotein metabolism, as well as its low cost, should make it the drug of choice in many patients with dyslipidemia and/or coronary artery disease, but unfortunately these beneficial effects are accompanied by some adverse effects. The most prominent of them is intense flushing (54, 144) which occurs in most people with as small as 100 mg orally. However, this unpleasant side effect is often diminished through maintaining the patients on a stable dose for several days (145). It has been suggested that administration of each dose of niacin with food or aspirin may alleviate this side effect (146). The incidence of cutaneous flushing after nicotinic acid ingestion varies between 82 and 100 % (54, 144). Several less common but more serious side effects may also preclude the use of niacin in some patients; these include hyperglycemia, hyperuricemia, gastrointestinal disturbances e.g., abdominal pain, bloating, diarrhea, constipation and abnormalities in hepatic function. Long-term administration of niacin may result in impaired glucose tolerance in non-diabetic patients and in decompensation of previously subclinical diabetic patients (147). It can also induce hyperuricaemia that, with glucose intolerance, is associated with an increased risk of coronary artery disease (54). Most of these effects are found to be dose related, occuring more commonly with a daily dose exceeding 3 gram. Some of these adverse effects, especially gastrointestinal problems and hepatotoxicity have been reported to be more frequent and serious with the use of sustained-release niacin preparations (95, 148).

Apart from these well-known side effects, the present study is the first to report the interrelationship between large doses of niacin and vitamin B_6. Supplementation of either 400 mg or 4000 mg niacin per kg diet to rats resulted in impaired synthesis of cysteine from methionine due to vitamin B_6 deficiency. Methionine is catabolized to cysteine through series of reactions involved in the transsulfuration pathway. Methionine is converted to S-adenosyl-L-methionine (SAM) in the presence of ATP by the enzyme S-adenosyl-L-methionine synthetase (149). S-adenosyl-L-methionine, which gives the methyl group to cellular acceptors, is converted to S-adenosylhomocysteine which is further cleaved to homocysteine catalyzed by the enzyme AdoHcy hydrolase (150, 151). The homocysteine is either remethylated to methionine (transmethylation pathway) or is combined with serine to form cystathionine, which is further cleaved by the enzyme γ-cystathionase to cysteine (transulfuration pathway).

In the transmethylation pathway homocysteine is remethylated to methionine in the presence of the enzyme methionine synthase. Folate and vitamin B_{12} are cofactors for methionine synthase, and are therefore neccessary for removal of homocysteine by transmethylation. Homocysteine acquires a methyl group from N5-methyltetrahydrofolate or from betaine to form methionine (152) in a reaction catalyzed by the vitamin B_{12} containing enzyme, N5-methyltetrahydrofolate:homocysteine methyltransferase (152). Methyl group of N5-tetrahydrofolate is synthesized *de novo* when a carbon unit is transferred from a carbon source such as serine or glycine, to tetrahydrofolate producing methylenetetrahyderofolate. Methylenetetrahydrofolate is

then reduced to methyltetrahydrofolate by methylenetetrahydrofolate reductase. The reaction with betaine is also B_{12}-dependent and is catalyzed by betaine-homocysteine methyltransferase. In the transsulfuration pathway, homocysteine condenses with serine to form cystathionine. Conversion of homocysteine to cystathionine is catalyzed by the enzyme cystathionine β-synthase, which requires pyridoxal 5'-phosphate, the biological active form of vitamin B_6 as cofactor (152). Cystathionine is cleaved by the enzyme γ-cystathionase to cysteine and ketobutyrate. This enzyme also requires pyridoxal 5'-phosphate for activity. Cystathionase completes the conversion of methionine to cysteine. The results of the present study showed that there was an increased methionine concentration associated with decreased cysteine concentration in plasma following administration of large doses of niacin. The impairment of methionine caused by niacin is entirely explained by the elevated plasma and urinary homocysteine concentrations.

Although abnormal elevations of homocysteine in plasma and urine can be induced by several congenital and nutritional disorders that directly affect homocysteine metabolism, it would be expected that a deficiency of vitamin B_6, B_{12} and/or folate may lead to elevated homocysteine concentrations. But no significant change was observed in plasma vitamin B_{12} or folate concentrations following niacin administration. However, vitamin B_6 levels in plasma were significantly lowered in niacin treated animals. These results suggest that elevated homocysteine levels in niacin treated animals are associated with vitamin B6 deficiency. This hypothesis is consistent with the important

observation that concurrent supplementation of vitamin B_6 (10 mg/Kg diet) and niacin (4000 mg/Kg diet) normalized elevated homocysteine and reversed the effect of niacin on methionine and cysteine. Similarly, Ubbink et al (153) explained that deficiencies of cofactors required for homocysteine metabolism may result in hyperhomocysteinemia, which could be successfully treated with a modest daily use of vitamin B_{12}, folic acid and viitamin B_6. An almost sevenfold increase in plasma homocysteine was observed in vitamin B_6 deficient rats given diet containing 0.6-1.85% methionine (154). Therefore, it seems clear that in vitamin B_6 deficiency, as well as in heterozygotes for cystathionine β-synthase deficiency, homocysteine metabolism is impaired.

Experimental evidence indicates that remethylation and transsulfuration pathways are coordinated; reduced activity in one pathway will lead to the more effective use of homocysteine by the second pathway (155, 156). Therefore, the impairment of only one homocysteine pathway should not lead to the accumulation of homocysteine, which is derived solely from methionine. The fact that it does accumulate, as it accumulated in the present study after niacin treatment, is explained by the fact where there is a defect in one pathway of homocysteine metabolism, this will lead to the impairment of the other. When the cellular SAM concentration is low, the synthesis of 5-methyltetrahydrofolate will proceed uninhibited, whereas cystathionine synthesis will be suppressed, resulting in the conservation of homocysteine for methionine synthesis. On the other hand, a high SAM concentration will inhibit 5-methyltetrahydrofolate synthesis accompanied by stimulation of the transsulfuration pathway because of increased

cystathionine synthesis. The direct effect of this coordination is the regulation of cellular SAM concentrations, but another important outcome is the maintenance of homocysteine concentrations compatible with the need for *de novo* methyl group. This hypothesis therefore predicts that homocysteine can accumulate when the cell is not able to coordinate between the two pathways (155). Impaired methylation will prevent induction of transsulfuration and prevent catabolism of excess homocysteine. Similarly, an impairment of the transsulfuration pathway will prevent the induction of remethylation and inhibit disposal of homocysteine through its conversion to methionine.

In a case study, a patient with partially defective SAM synthesis also had abnormal homocysteine conservation leading to excessive methionine synthesis and hypermethionemia (157). The authors predicted that high homocysteine resulted from a concentration of SAM insufficient to inhibit to methyltetrahydrofolate reductase activity and insufficient to stimulate cystathionine β-synthase to rid the cell of excess homocysteine through the transsulfuration pathway (157). When cystathionine β-synthase is completely inactive, the resulting homocysteine would initially induce accelerated de novo methionine synthesis until the concentration of methionine is increased enough to cause the accumulation of SAM. High concentrations of SAM will inhibit synthesis of 5-methyltetrahydrofolate and thus inhibit disposal of homocysteine through its conversion to methionine. Subsequently, SAM will be metabolized and its concentration will decrease, leading to renewed methionine synthesis from

homocysteine. Eventually, a state will be reached at which some homocysteine is converted to methionine, but the rest cannot be converted because of SAM's inhibitory effect on methyltetrahydrofolate reductase. This interpretation is consistent with the observation that patients with homocysteinemia due to homozygous cystathionine β-synthase also tend to have hypermethionemia (132). Decreased cystathionine β-synthase activity in vitamin B_6 deficient rats leads to the accumulation of homocysteine in tissue (158, 159). A marked increase of both free and protein-bound homocysteine has been observed in the rat within a few weeks of vitamin B_6 deficiency (160, 161). These high values of homocysteine were normalized within two days after supplementation with adequate amounts of vitamin B_6. Vitamin deficiency induced an even greater increase in free and protein-bound plasma homocysteine in pigs (162).

Apart from transsulfuration of methionine which is a major route of methionine degradation, existence of the transamination pathway in rats, sheep and humans has been observed (163-169). In this pathway methionine is transaminated to 4-methylthio-2-oxobutyrate. Oxidative decarboxylation of 4-methylthio-2-oxobutyratethe yeilds 3-methylthio propionate (163-166) and methanethiol (MT) is formed from 3-methyl thiopropionate (162, 167-169). However, it was demonstrated that quantitative significance of this pathway in methionine catabolism is only of minor importance in both normal subjects (170) and in patients with cystathionine β-synthase deficiency (170). This conclusion holds true even in complete cystathionine synthase deficiency, despite methionine levels higher than in the patient with methionine adenosyltransferase

deficiency. When intracellular accumulation occurs, the cell homocysteine export mechanism leads to the deposition of homocysteine into the blood and urine. This export maintains low intracelluar homocysteine concentrations and prevents toxicity to the cell but causes clinical homocysteinemia and homocystinuria.

The mechanism of interaction of niacin and vitamin B_6 is not known. Many studies in nondiabetic patients receiving niacin have demonstrated increases in plasma glucose concentrations (171-175). Studies have suggested that niacin induce hyperglycemia by stimulation of gluconeogenesis (176), development of insulin resistance (177) or interference with triglyceride synthesis leading to enhanced utilization of fatty acid at the expense of glucose, causing the liver to release glucose into the serum (178). Related to this is the observation that plasma PLP concentrations are depressed 20-30% within 1-2 hours following glucose load (179). More over, a significant proportion of people with either type I or type II diabetes mellitus have low plasma levels of B_6 vitamins (180-182). The mechanism by which PLP is lowered in the plasma is not known, but there are at least two possibilities. First, there may be decreased synthesis of PLP in the liver or inhibition of release from the liver. Secondly, there may be enhanced uptake of PLP into specific tissues. Data presented by Ink and co-workers (183) suppot the later mechanism. They examined the effect of glucose on the rate of uptake of PL by human erythrocytes and found that 10 mmol glucose/L enhances the uptake during 60 min of incubation. Moreover glucose metabolism as an energy subsrate in the red cell would provide adenosine triphosphate (ATP), required in the

pyridoxine kinase reaction. An increase in ATP results enhanced conversion of free forms of vitamin B_6 to their respective phosphoriated compounds. This vitamin is accumulated in the cells as a result of binding of PLP and PL to hemoglobin and metabolic trapping by phosphorylation of free forms of vitamin B_6.

If there is continued increase in glucose, and PLP is taken up into tissues, this could result in increased metabolism of PLP to PL and then conversion of PL to the metabolic end product 4-pyridoxic acid. In that case a gradual decrease in vitamin B_6 status may result. Increased PLP concentrations in the tissues as a result of the higher plasma glucose, may lead to a greater rate of turnover and increased degradation of PLP (and PL), eventually leading to a functional deficiency of vitamin B_6. Further niacin therapy has been shown to elevate plasma alkaline phosphatase activity. This elevated alkaline phosphatase activity may in part explain the decreased plasma PLP levels (184), because increased release of alkaline phosphatase activity has also been shown to be related to decreased plasma PLP concentrations (185).

4.1. CONCLUSIONS

The basal experimental diet used in this study contained niacin approximately 47 mg/kg diet. When the animals were fed this diet supplemented with either 400 or 4000 mg/kg diet, for 3 or 6 weeks, they exhibited decreased plasma and urinary levels of cysteine, while their homocysteine levels were markedly elevated. The later manifestation has

an important toxicological implecation. In recent years, there have been a number of studies suggesting that homocysteine can be potentially toxic to cells (186). Thus mild hyperhomocysteinemia has been linked to coronary heart disease (187-189) and cerebral (190, 191) as well as peripheral vascular diseases (192, 193). Elevated plasma homocysteine concentrations are involved in the pathogenesis of atherosclerosis (194). The mechanism by which homocysteine may promote atherogenesis include vascular endothelial injury (194), oxidative modification of LDL cholesterol (195) and enhanced binding of lipoprotein(a) to fibrin in atherosclerotic plaques (196). Homocysteine may also perturb vascular coagulation mechanisms and thus promote a thrombotic tendency (14).

In addition to homocysteinemia, mega dose level of niacin was found to decrease cysteine status in the body. This untoward effect of niacin appears to be caused by interfearing with vitamin B_6 as indicated by its reduced level in the plasma. This relationship between niacin and vitamin B_6 was further supported by the fact that the cysteine levels in both plasma and urine were reversed to their levels in control animals. It was noteworthy that concomittant administration of niacin and vitamin B_6 did not altered the hypolipidemic action of niacin. These results clearly suggest that a combination of these two vitamins may be better choice of therapy for lowering cholesterol status than niacin alone. Clinical trails are now wanted to determine if niacin therapy for the treatment of hypercholesterolemia will improve with the concurrent administration of niacin and vitamin B_6. However, it is essential that the

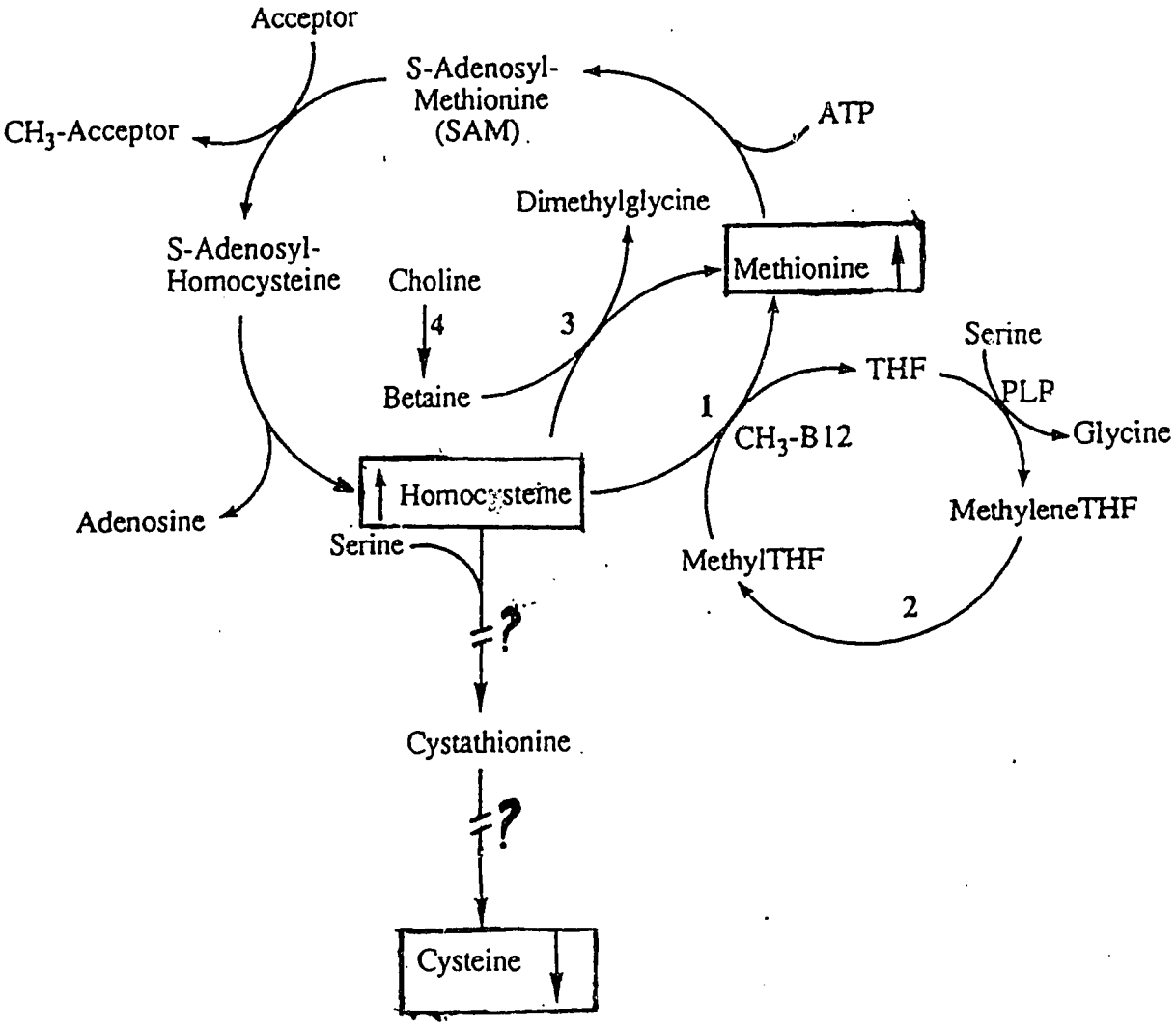


Figure 4.1 Interruption of methionine metabolism following niacin (large doses) treatment.

optimal niacin to vitamin B_6 ratio should be established before clinical trails are initiated.

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