THE EFFECTS OF LENTILS ON CALCIUM BALANCE IN HEALTHY MALES

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ABSTRACT

Nutritional recommendations encourage the Canadian population to increase its intake of complex carbohydrates with a resulting increase in dietary fibre. Diets high in insoluble fibre are thought to compromise calcium status by increasing faecal calcium excretion. Calcium status is of particular concern due to the risk of osteoporosis.

A six week study was conducted to determine the effect of Laird lentil fibre and starch on calcium balance. In a randomized experimental design, ten males (19-37 years) consumed a control diet for three weeks and the control diet plus 130 g lentils providing 13 g NSP; 80% insoluble, for three weeks. Seven-day food records were used to adjust the study diet to the subjects' usual calcium and energy intakes. Ultra-pure water was consumed throughout the study. Duplicate diets of food consumed were prepared. Radio-opaque markers were given and complete faecal collections were made. Twenty-four hour urine collections were made in weeks three and six. Tablets containing PABA were given to validate the completeness of urine collections.

Lentils increased faecal weight (control 130.6 $\pm$ 11.8 g/d (Mean $\pm$ SEM); lentil 189.6 $\pm$ 21.2 g/d (P =

0.027). Calcium balance was maintained during both the control (0.4 ± 0.8 mmol/d) and lentil periods (- 0.6 ± 0.9 mmol/d)(P = 0.503). Faecal calcium remained unchanged (28.5 ± 1.7 mmol/d; 29.7 ± 1.5 mmol/d)(P = 0.434). Urine calcium decreased from 5.43 ± 0.44 mmol/d to 4.53 ± 0.41 mmol/d (P = 0.0001). Possible mechanisms which may explain the change in renal calcium excretion are changes in dietary calcium, urinary sodium excretion, net acid excretion or renal potassium excretion. Dietary calcium did not significantly change (34.4 ± 1.8 mmol/d; 33.6 ± 1.9 mmol/d)(P = 0.095). Renal sodium excretion decreased (149 ± 4 mmol/d; 133 ± 3 mmol/d;)(P = 0.0003). Renal net acid excretion remained unchanged (49.2 ± 4.2 mmol/d; 52.3 ± 3.1 mmol/d)(P = 0.24). Renal potassium excretion increased (90.8 ± 4.7 mmol/d; 102.5 ± 3.1 mmol/d)(P = 0.015).

This study shows that adding Laird lentils to the diet does not adversely affect calcium status. The significant decrease in renal calcium excretion may be due to a combination effect from the changes in dietary phosphorus, renal sodium and potassium with the change in renal potassium excretion demonstrating the greatest effect. Also, the resistant starch present in Laird lentils may have had an effect on renal calcium excretion.

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NAE.....net acid excretion

PABA.....para-amino benzoic acid

PTH.....parathyroid hormone

SD.....standard deviation

SEM.....standard error measurement

TA.....titratable acidity

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1. REVIEW OF LITERATURE

1.1. Dietary Fibre

Dietary fibre has been historically defined as those food substances, particularly carbohydrate, which are resistant to digestion by the human digestive tract (Guthrie, 1989). This definition has posed certain problems. First, experimentally, it is difficult to determine which substances in foods and to what extent these substances are resistant to digestion. Second, no other nutrient is defined by factors of its digestion. In an attempt to clarify the definition, dietary fibre has been redefined as plant polysaccharides and lignin that resist hydrolysis by the human digestive enzymes (Trowell, Southgate, Wolever, Leeds, Gassul & Jenkins, 1976). This definition, however, still incorporates the physiological process of digestion. Dietary fibre should be defined and measured chemically as are other nutrients. There are a number of chemical methods currently in use that attempt to elucidate and measure the constituents of dietary fibre. For the purposes of

this manuscript, a chemical definition of dietary fibre derived from Englyst is used (Englyst, 1981). Englyst, who developed a method for dietary fibre analysis, defines dietary fibre as non-starch polysaccharide; this non-starch polysaccharide is further separated into soluble and insoluble fibre components. Insoluble fibre includes cellulose, some hemicelluloses and lignin; the latter, which is not a polysaccharide, is expressed separately. Soluble fibre includes pectins, gums and some hemicelluloses which are further described by their constituent sugars.

Dietary fibre is contained in plant foods only. Insoluble fibre is found primarily in cereal products, vegetables, and some legumes; an excellent source being wheat bran. Soluble fibre is found primarily in fruits, vegetables and some legumes.

There is current research that suggests that certain starches may act in the gut in ways similar to dietary fibre (Shetty & Kurpad, 1986; Macfarlane & Englyst, 1986). The term "resistant starch" is used to describe starch that is resistant to digestion by the human small intestine (Englyst & Cummings, 1990). Whether resistant starch should be included within the definition of dietary fibre is a matter of controversy (Englyst, Trowell, Southgate & Cummings, 1987).

1.1.1. Dietary Fibre and Health

A diet high in dietary fibre is thought to be protective in a number of diseases. The beneficial effects believed to be exerted by dietary fibre depends on the source or more specifically the composition of the fibre source. An increased consumption of insoluble fibre, an excellent source being wheat bran, has been found to increase stool bulk and lower intestinal transit time (Cummings, 1978; Cummings & Stephen, 1980). These effects on colonic function have been correlated with a decreased risk of diverticular disease (Painter & Burkitt, 1971). In addition, a diet low in dietary fibre has been found to directly correlate with the incidence of diverticular disease (Brodribb & Humphreys, 1976; Brodribb, 1980). An increased intake of soluble fibre such as from pectin has been shown to decrease serum cholesterol levels (Jenkins, Leeds, Newton, & Cummings, 1975; Hillman, Peters, Fisher, & Pomare, 1985). High serum cholesterol is known to be a major risk factor for coronary heart disease. Low fibre intakes are also associated with an increased risk of colonic cancer (Burkitt, 1971). Because of these perceived health benefits, current nutritional recommendations encourage the Canadian population to increase its intake of

complex carbohydrates with a resulting increase in dietary fibre (Nutrition Recommendations, 1990).

Dietary fibre, particularly the insoluble form, may not be without disadvantages. A possible negative effect of fibre is its effect on mineral balance. It has been known for some time that certain constituents in fibre, such as phytate, bind irreversibly to essential minerals and, therefore, could decrease their absorption from the gut (Spiller & Shipley, 1977). This decrease in absorption, if not compensated by a decrease in urinary excretion, may result in negative mineral balances. This is a matter of concern since a negative balance for calcium, for example, over an extended period of time will result in bone loss which may lead to osteoporosis.

1.1.2. Effect of Fibre Intake on Calcium Metabolism

The effect of dietary fibre intake on calcium metabolism has been investigated. Historical research by McCance and Widdowson (1942) found that an increase in fibre in the diet decreased intestinal absorption of calcium. Since that time, some research has been conducted on refined fibre sources. A diet supplemented with cellulose has been found to cause

negative calcium balances (Ismail-Beigi, Reinhold, Faraji & Abadi, 1977). Slavin and Marlett (1980) investigated the influence of purified cellulose on calcium balance and found a significant increase in faecal calcium but urinary calcium remained unchanged, with the calcium balances becoming more negative. The results of Godara, Kaur and Bhat (1981) show that the addition of cellulose to the diet significantly increased faecal calcium excretion but urine calcium was not measured. Other researchers found no change in faecal calcium output with the addition of soy polysaccharide to their experimental diet (Tsai, Mott, Owen, Bennick, Lo & Steinke, 1983).

More recently, Behall, Scholfield, Lee, Powell and Moser (1987) studied the effect of four different refined fibre sources and found that none of the fibres had a significant effect on calcium balance. Refined cellulose had no effect on faecal or urinary calcium excretion. The other refined fibres given, carboxymethylcellulose, karaya gum and locust bean gum, had varying effects on the route of calcium excretion, but it is difficult to make any conclusions from these findings since the calcium content of the fibre diets were all significantly different from the basal diet. Any effects seen may have been due to changes in calcium intake.

The nutritional significance of studies on cellulose and other refined fibre sources on calcium balance is questionable since human diets do not contain such refined fibre sources. In addition, it is possible that refined fibre may act very differently in the gut compared to fibre in its natural form.

A number of studies have been conducted on the effects of fibre from natural sources on calcium balance. Kelsay, Behall and Prather (1979) studied the effect of fibre from fruits and vegetables on calcium balance and found that calcium balance was significantly lower, faecal calcium was increased and urinary calcium was unchanged with the addition of fibre to the diet. Shah, Williams and Green (1980) found that feeding unprocessed bran decreased urinary calcium excretion in patients with hypercalciuria. Van Dokkum, Wesstra and Schippers (1982) concluded that faecal calcium increased with an increase in dietary fibre in the form of bran, but this increase paralleled the increase in calcium intake. Kelsay and Prather (1983) found that the calcium balances of their experimental subjects became negative when fruit fibre was added to the diet. The results of Schweizer, Bekhechi, Koellreutter, Reimann, Pometta and Bron (1983) showed higher faecal calcium excretions with the addition of soy bean fibre, but there was a concurrent

increase in calcium intake. The effect of a high fibre diet from a variety of food sources on calcium balance was assessed by Hallfrisch, Powell, Carafelli, Reiser and Prather (1987) and there were no significant differences between treatment and control. Kelsay, Prather, Clark and Canary (1988) found that the addition of fibre from fruits and vegetables did not change calcium balances but did increase faecal calcium excretion and decrease urinary calcium loss; however, calcium intakes were higher with the high fibre diet.

The review of the literature discussed above shows that conflicting data exist as to the effect of fibre on the route of calcium excretion and balance. The discrepancies in the literature may be due to a number of factors. First, none of the studies reviewed thus far utilizes a faecal marker that allowed for accurate assessment of faecal calcium excretion. An acceptable faecal marker should be unabsorbed, non-toxic, safe and easy to measure and not influence gastrointestinal metabolism (Whitby & Lang, 1960). Radio-opaque pellets satisfy these criteria but pigment markers, for example, do not. In addition, when the pellets are given continuously, they achieve a steady state (Cummings, Jenkins & Wiggins, 1976) and can be used to accurately correct for infrequency of defecation. Second, the studies contain varying amounts of dietary

fibre and perhaps, very high intakes of dietary fibre give an effect that is not seen with lower intakes. It is difficult to compare the effects of the fibre levels on calcium balance since different studies use different methods of fibre determination. Third, some of the studies failed to adequately control dietary intake, in particular, calcium intake and maintenance of macronutrient ratios with caloric adjustments.

Cummings, Hill, Jivraj, Houston, Branch, and Jenkins (1979) conducted a study on the effect of wheat bran on calcium balance which was well controlled. Adding bran, high in insoluble fibre, to the diet caused an impairment of calcium absorption in the gut, but urine calcium remained unchanged. Conversely, adding a soluble fibre source, pectin, to a controlled diet significantly changed the route of calcium excretion, increasing faecal calcium excretion and a decreasing urinary calcium excretion (Cummings, Southgate, Branch, Wiggins, Houston, Jenkins, Jivraj & Hill, 1979).

Andersson, Navert, Bingham, Englyst and Cummings (1983) found that bran, independent of its phytate content, had no effect on calcium balance or the pathway of excretion. Phytate, the storage form of phosphorus in plants, is commonly associated with fibre since it physically present with the insoluble fibre of

foods. The effect of phytate on calcium absorption is not a recent discovery since Bruce and Callow (1934) concluded that it is the phytate in bran that causes a decrease in intestinal absorption of calcium. Therefore, it appears that the insoluble fibre in the bran has no effect on calcium balance or the route of calcium excretion independent of phytate. Any fibre source containing phytate will be expected to decrease intestinal absorption of calcium, thereby increasing faecal calcium excretion.

It appears that the response observed to an increased fibre intake depends on the type of fibre consumed. Different fibre sources with their different ratios of soluble and insoluble fibre would be expected to have differing effects on mineral metabolism. Insoluble fibre is resistant to bacterial degradation in the colon while soluble fibre is partially or completely fermented in the colon by the microflora. Therefore, the addition of insoluble fibre to the diet would not be expected to have any effect on the metabolism of calcium in the body. Insoluble fibre may, however, decrease transit time to a point where passive calcium absorption may be limited, thus decreasing calcium absorption. Binding of calcium by insoluble fibre, thus increasing faecal calcium excretion appears unlikely. The fermentation of

soluble fibre gives rise to short chain fatty acids which are absorbed into the body (Ruppin, Bar-Meir, Soergel, Wood & Schmitt, 1980). Soluble fibre appears to have no effect on calcium balance, but may alter the route of excretion by increasing faecal calcium excretion and decreasing renal excretion. The mechanism of the effects of the products of soluble fibre fermentation on calcium metabolism is unknown.

A natural food fibre source containing phytate, soluble and insoluble fibre would be expected to exert a combination of these effects and the sum of or potential interaction of these effects on calcium balance is unknown and would need to be determined experimentally. A controlled study on the effects of resistant starch on calcium balance has not been conducted, therefore, the potential effects of resistant starch on calcium absorption and renal calcium excretion are unknown.

1.2. Calcium Metabolism

The average human body contains about 1000 g of calcium. Of this total, approximately 99 % of the calcium is contained within the skeleton in the form of hydroxyapatites (Vander, 1985). These calcium salts provide for skeletal structure, body support and a

calcium reservoir. The remainder of the body's calcium is found in the extracelluar fluid and soft tissues.

The calcium ion concentrations in the extracelluar fluid and cytosol are important to many biochemical processes and, therefore, it is crucial to life and health that these calcium compartments are maintained. Slight variations in extracelluar fluid concentrations can result in severe metabolic distress or death. Change in the calcium content of the skeleton becomes important when sufficient calcium is lost, decreasing bone mass and leading to skeletal weakness and possibly fracture. The regulation of calcium balance is, therefore, necessary to maintain health.

Bone metabolism is the vital regulatory mechanism of internal calcium homeostasis. Calcium homeostasis is regulated with the external environment primarily through the regulation of calcium absorption from the intestine and to a lesser extent by renal calcium excretion (Vander, 1985).

1.2.1. Bone Metabolism

Bone is a specialized connective tissue that has mechanical, protective and metabolic functions. The organic matrix of bone is formed from collagen fibres and a ground substance composed of glycoproteins and

proteoglycans (Baron, 1993). Calcium contained in the crystals of hydroxyapatite, $Ca_{10}(PO_4)_6(OH)_2$, are imbedded in and on the collagen fibres (Guyton, 1985).

The calcification of bone occurs by the following process (Guyton, 1985). Osteoblasts secrete collagen monomers and ground substance. The cartilage monomers polymerize and become osteoid allowing calcium salts to precipitate within the structure. The resulting products are the hydroxyapatite crystals which are formed when the amorphous precipitate becomes crystalline.

Bone remains active through the process of remodelling. Bone is continually being deposited by osteoblasts and reabsorbed by osteoclasts. Normally, bone deposition and reabsorption are in equilibrium. In the disease state of osteoporosis, decreased osteoblastic activity is seen (Guyton, 1985).

A portion of the skeletal calcium is easily available for the maintenance of plasma calcium. Parathyroid hormone functions to cause resorption of calcium salts when calcium ion concentration decreases. Calcitonin functions to decrease osteoclastic activity, increase osteoblastic activity and decrease the number of osteoclasts when calcium ion concentration becomes elevated (Guyton, 1985).

1.2.2. Calcium Absorption in the Gastrointestinal Tract

Calcium is absorbed through the intestinal mucosa via two mechanisms. Calcium is passively absorbed along its concentration gradient between the intestinal lumen and the blood. This route is paracellular and is unsaturable as are other passive routes of absorption. The proposed route is through leaky intercellular junctions of the intestinal epithelial cells. Calcium is also actively absorbed via a saturable, transcellular route. Calcitriol (1,25 dihydroxyvitamin D) stimulates active calcium absorption primarily in the duodenum and jejunum. Calcitriol causes the formation of a calcium-binding protein (CaBP) in the intestinal epithelial cells. The role of CaBP is uncertain although calcium transport in the duodenum varies directly with the concentration of CaBP (Bronner, Pansu & Stein, 1986). Movement of calcium through the intestinal epithelial cell is thought to be via facilitated diffusion. Calcitriol also causes the formation of a calcium-stimulated ATPase in the brush border of the cell and its function is uncertain (Bronner et al., 1986). Nellans (1990) has proposed that translocation of calcium across the plasma membrane of the enterocyte involves the

formation of vesicles to allow for transcytotic transport.

The dual absorptive process of calcium in the human intestine results in a curvilinear relationship between dietary calcium intake and net intestinal absorption (Heaney, Weaver & Fitzsimmons, 1990). Generally, the smaller the load of calcium, the greater the fractional absorption of calcium.

In addition to absorption, calcium is also secreted into the gut during the process of digestion making it necessary to differentiate amongst the terms used to describe absorption of calcium. *Fractional absorption* is the fraction of dietary calcium which is absorbed and *net fractional absorption* is fractional absorption less endogenous secretions. Nordin and Marshall (1988) define *true fractional absorption* as fractional absorption less endogenous secretions plus reabsorbed endogenous secretions. Finally, *apparent absorption* is a measurement of dietary calcium intake minus faecal calcium excretion.

1.2.3. Renal Handling of Calcium

Calcium in the plasma is regulated within narrow margin primarily though the action of parathyroid hormone. Calcium occurs is three forms in the plasma.

Forty percent is bound to plasma proteins, ten percent is non-ionized and an additional fifty percent of the plasma calcium is ionized (Guyton, 1985). It is the ionized calcium that is freely filterable in the nephron.

The normal fractional urinary excretion of calcium is less than 2% (Sutton & Dirks, 1986), thus the kidney must perform a great deal of reabsorption. The proximal tubule has a high permeability to calcium. This calcium reabsorption parallels sodium and water reabsorption and is concentration dependent. The route is paracellular via leaky epithelium. Under normal conditions, calcium does not appear to be regulated in this segment. Upwards to 70% of the calcium reabsorption in the kidney occurs by this passive process (Bronner, 1989). It is thought that the reabsorption in the thick portion of the ascending limb of the loop of Henle is also passive (Bronner, 1989).

In the distal convoluted tubule, the calcium concentration in the lumen is less than the plasma, the potential difference is lumen negative and the tubule is impermeable to diffusion of calcium (Sutton & Dirks, 1986). Calcium transport in the distal tubule must, therefore, be active. It is known that parathyroid hormone and cyclic AMP stimulate calcium absorption in the distal tubule segment (Sutton & Dirks, 1986). The

active, transcellular transport of calcium in the kidney is thought to occur in three steps. The first step is the entry of calcium into the renal tubule cell down its electrochemical gradient. This step is likely achieved through calcium channels (Bronner, 1989). The second step, the rate limiting step, is the movement of calcium through the cell. The cell cannot maintain the high rates of transport through the cell interior by unaided diffusion and at the same time maintain the intracellular concentration at its low level of 100 nmol. It has been suggested that calcium-binding protein (CaBP) functions as a "ferry" for calcium through the interior of the cell (Kretsinger et al, 1980). Parathyroid hormone may function by influencing this step (Bronner, 1989). Parathyroid hormone causes an increase in calcitriol production which in turn increases CaBP. Calcium transport in the duodenum varies directly with the concentration of CaBP and this is assumed to be similar in the distal tubule (Bronner, et al., 1986). Lysosomes may carry out some transport (Bronner, 1990). The third step is the extrusion of calcium through the basolateral membrane.

The known activity of Ca^{+2}ATPase present in the basolateral membrane of the distal tubule epithelial cells is sufficient to account for the calcium extrusion required (Bronner, 1990). The Na+/Ca+

exchanger may also be involved, but the contribution from this transporter has not been established. This transporter is known to function to maintain intracellular calcium concentration and has a regulatory role dealing with Na+ channel activity and intracellular pH (Bronner, 1990). While a great deal of knowledge concerning the mechanism of active transport in the kidney has been established, there is still much to be learned about how factors influence this system.

1.3. Dietary Factors Affecting Urinary Calcium Excretion

Urinary calcium excretion has a limited ability to compensate for changes in calcium intake. For instance, the kidney has the ability to decrease its active component of reabsorption, but a high enough intake can override this control through higher passive reabsorption (Bronner, 1990). Intake and excretion of nutrients other than calcium tend to have a greater influence on renal calcium excretion than does the intake of calcium (Sutton & Dirks, 1986). These effects may be unrelated to calcium homeostasis, instead being the result of a less than perfect evolution of the kidney. As Bronner (1989) states:

"In some cases there is evidence to suggest a primary defect in tubular reabsorption, to which dietary factors such as high salt or protein may contribute". A number of nutrients have been identified as affecting renal calcium excretion such as sodium, phosphate, protein and possibly potassium. Also, it is known that increased renal acid excretion affects renal calcium excretion (Lemann, Litzow & Lennon, 1967; Sutton, Wong & Dirks, 1979; Adams, Gray & Lemann, 1979). It is important to understand the effects of these nutrients since renal calcium losses in some cases may not be compensated for by increased intestinal absorption.

One might expect an increase in intestinal calcium absorption through the action of calcitriol as a result of renal losses. This has been found in response to an increased intake of sodium in humans (Breslau, McGuire, Zerwekh & Pak, 1982). This compensation may be less than expected in the elderly, groups with low calcium intakes and populations with higher requirements (Massey, 1993). The protective role by calcitriol has yet to be shown for the other nutrients that affect renal calcium excretion. The result of uncompensated renal losses will be a negative calcium balance and bone mineral loss.

Net acid excretion in humans rises in response to an increase in fixed acid production. Renal acid

excretion can be influenced by changes in the diet such as protein level (Lemann, Gray & Pleuss, 1989) and protein source. Animal protein sources have a greater content of the sulphur amino acids, cysteine and methionine. In the body, sulphur is oxidized to sulphate causing an increase in fixed acid load. Also, the overall acidity or alkalinity of the diet will influence renal acid excretion. Renal acid excretion is most often expressed as urinary net acid excretion which is measured as ammonium and titratable acid excretion minus bicarbonate excretion (Chan, 1972).

Early researchers found that base-forming diets decreased renal calcium excretion and acid-forming diets caused an increase in renal calcium excretion (Bogert & Kirkpatrick, 1922; Shohl & Sato, 1923; Farquharson, Salter, Tibbets & Aub, 1931). In a state of metabolic acidosis, hypercalciuria is present (Lemann et al., 1967). The administration of sodium bicarbonate in metabolic acidosis eliminated the calciuria immediately and the effect was maintained (Sutton et al., 1979). Breslau, Brinkley, Hill and Pak (1987) found that urinary calcium directly correlated with net acid excretion; calcium excretion increased with an increase in net acid excretion. Bicarbonate administration has been shown to cause a decrease in renal calcium excretion by augmenting renal

calcium absorption (Peraino & Suki, 1980).

It has been suggested that the cation administered with the anion may also affect renal calcium excretion. Sakhaee, Nicar, Hill and Pak (1983) found that the administration of potassium citrate was effective in decreasing renal calcium excretion but sodium citrate was not. Lemann and colleagues (1989) found that potassium bicarbonate improved calcium balance in healthy men while sodium bicarbonate did not. Research has confirmed that adding sodium chloride to experimental diets results in a significant increase in urinary calcium (Zarkadas, Gougeon-Reyburn, Marliss, Block, Alton-Mackey, 1989). Questions remain as to whether the effects seen in these studies are due to the calciuric effect of the sodium cation with or without an antagonistic effect by the anion, and if chloride or potassium have independent effects.

A number of studies have found that the administration of dietary phosphorus alters renal calcium excretion (Farquarson et al., 1931; Heyburn, Robertson & Peacock, 1982; Hegstead, Schuette, Zemel & Linkswiler, 1981). Heany and Recker (1982) conclude that a known increase in the intake of phosphorus causes a predictable decrease in urinary calcium, but phosphorus has no effect on calcium balance. Spencer, Kramer, Rubio and Osis (1986) conclude that a high

phosphorus intake decreases urinary calcium and this is the main effect of phosphorus on calcium metabolism. The mechanism by which dietary phosphorus affects renal calcium excretion is thought to be through mild secondary hyperparathyroidism (Calvo, 1993). This effect of phosphorus has been recognized as an important dietary consideration in that the RNI for phosphorus was increased to offset the calcium losses that result from the high protein intake of our Canadian diet (Nutrition Recommendations, 1990)

Table 1.1 gives a summary of the dietary factors that affect renal calcium excretion. Although the changes in urinary calcium brought on by these dietary factors are quite small, if they persist over long periods of time, the result may be significant bone loss.

Table 1.1. Dietary factors affecting renal calcium excretion.

Factor	Effect on Renal Calcium
Potassium	decrease
Net Acid Excretion	increase
Sodium	increase
Phosphorus	decrease

1.4. Dietary Factors Affecting Faecal Calcium Absorption.

As stated previously, there is a curvilinear relationship between calcium intake and calcium absorption from the gut. Generally, as calcium intake increases the amount of calcium absorbed by the gastrointestinal tract increases. In addition to the level of calcium intake, there are a number of other dietary factors that influence the amount of calcium absorbed from the gut. Dietary factors that are important to the present discussion include phosphorus, carbohydrate, fat and phytate.

Heaney and Recker (1982) found that a higher phosphorus intake was associated with increased intestinal secretion of calcium which, as stated above, was offset by a decrease in renal calcium excretion; the net result was no effect on calcium balance.

Carbohydrates are also thought to influence the absorption of calcium by the human gut. Sucrose loading has been shown to cause short-term calciuria attributed to an increase in intestinal absorption (Holl & Allen, 1987). Fat, if not completely absorbed can decrease calcium absorption from the gut through the formation of calcium soaps (Wardlaw, Insel &

Seyler, 1992).

Phytic acid forms complexes with calcium and other divalent cations. These mineral complexes are relatively insoluble and it is for this reason that phytic acid leads to a decrease in mineral bioavailability. Heaney, Weaver and Fitzsimmons (1991) compared the amount of calcium absorbed from soy beans with low and high phytate contents and found that the fractional calcium absorption was significantly lower with the high-phytate soy beans. These researchers conclude that plant fibre alone does not tend to reduce calcium absorption and that the phytate content of soy beans had a "definite and highly significant effect on calcium absorbability".

As stated above, insoluble fibre is believed to have no effect on intestinal calcium absorption (Andersson et al., 1983). Conversely, it has been found that soluble fibre functions to decrease the intestinal absorption of calcium (Cummings et al., 1979). The mechanism by which soluble fibre may decrease the absorption of calcium is not known.

Table 1.2. Dietary factors affecting calcium absorption by the gut.

Factor	Effect on Calcium Absorption
Phosphorus	decrease
Phytate	decrease
Insoluble Fibre	no effect
Soluble Fibre	decrease
Unabsorbed Fat	decrease

2. INTRODUCTION

Current nutritional recommendations encourage the Canadian population to increase its intake of complex carbohydrates (Nutrition Recommendations, 1990). This recommendation, if followed, will result in increased intakes of dietary fibre. High intakes of dietary fibre are beneficial for a number of reasons. Experiments have shown that certain fibre types, when added to the diet, cause an increase in faecal output thus decreasing the incidence of constipation (Cummings, 1978; Cummings & Stephen, 1980). Other fibre types have been found to lower serum cholesterol and serum glucose (Jenkins et al., 1975; Hillman et al, 1985). The effects of a fibre source on metabolism depend on its chemical constitution. Insoluble types, being resistant to microbial degradation in the human gut, exert their greatest effects on gastrointestinal function. Soluble types of fibre are degraded in the large intestine and, in ways not yet understood, the metabolites contribute to disease prevention (Stephen, 1990).

In addition to the effects of fibre on faecal output and blood cholesterol levels, some fibre types have been found to alter the route of mineral excretion (Andersson et al., 1983). High intakes of dietary fibre may compromise mineral status by increasing faecal excretion (Cummings et al., 1979). Calcium status of women is of particular concern due to the risk of osteoporosis. Osteoporosis is a condition of decreased bone mineral content and density which leads to fragile bones and breakage. For maintenance of bone density, it is necessary for calcium balance to be maintained, that is, calcium input must remain equal to output. A negative calcium balance can result from decreased intestinal absorption leading to increased faecal losses or from increased urinary losses providing there is a lack of compensation for these losses (Massey, 1993).

Many studies have been conducted on the effects of fibre on mineral balance. A number of these studies investigated the effects of isolated fibre portions on calcium balance (Ismail-Beigi et al., 1977; Slavin & Marlett, 1980; Godara et al., 1981). Individuals do not consume fibre in fractional portions. In the human diet, fibre comes from whole foods in a particular structure and with companion nutrients. More specifically, fibre in natural foods is invariably

accompanied by starch. As of the date of the commencement of the present study, no investigation on the effects of starch on mineral balance in humans had been conducted.

All types of mixed fibre sources are being recommended including pulses. One such pulse is lentils. As lentil protein is an excellent complement to cereal protein, it is suitable for inclusion into a vegetarian diet. Lentils are high in starch and fibre and low in fat. Lentils contain both soluble and insoluble fibre. In addition, lentils are low in anti-nutritional factors. The effects of lentils on calcium balance are unknown. It is for this reason that the present study was conducted.

The study had a number of objectives. The first objective of the study was to determine the effect of lentil fibre and starch on calcium balance. The second objective was to determine the effect of lentil fibre and starch on renal calcium excretion. The third objective was to identify possible mechanisms by which the lentil fibre and starch influence renal calcium excretion. This third objective would only be possible if lentils did in fact cause a significant change in renal calcium excretion as stated in the second objective. To my knowledge, there has not been an investigation of the effect of lentil fibre and starch

on calcium balance.

Lentils contain insoluble and soluble fibre, starch and phytate. The insoluble portion of the lentil fibre is predicted to cause no effect on mineral balance, although the phytate in lentils is predicted to cause an increase in faecal calcium excretion. But, it is predicted that the feeding of lentils will cause renal calcium excretion to decrease to maintain calcium balance. The effect of the soluble fibre is expected to be negligible since it makes up such a small percentage of the total dietary fibre found in lentils. The proposed mechanism is through a lower net acid excretion, a higher potassium intake or both.

3. METHODS AND PROCEDURES

3.1. Experimental Design

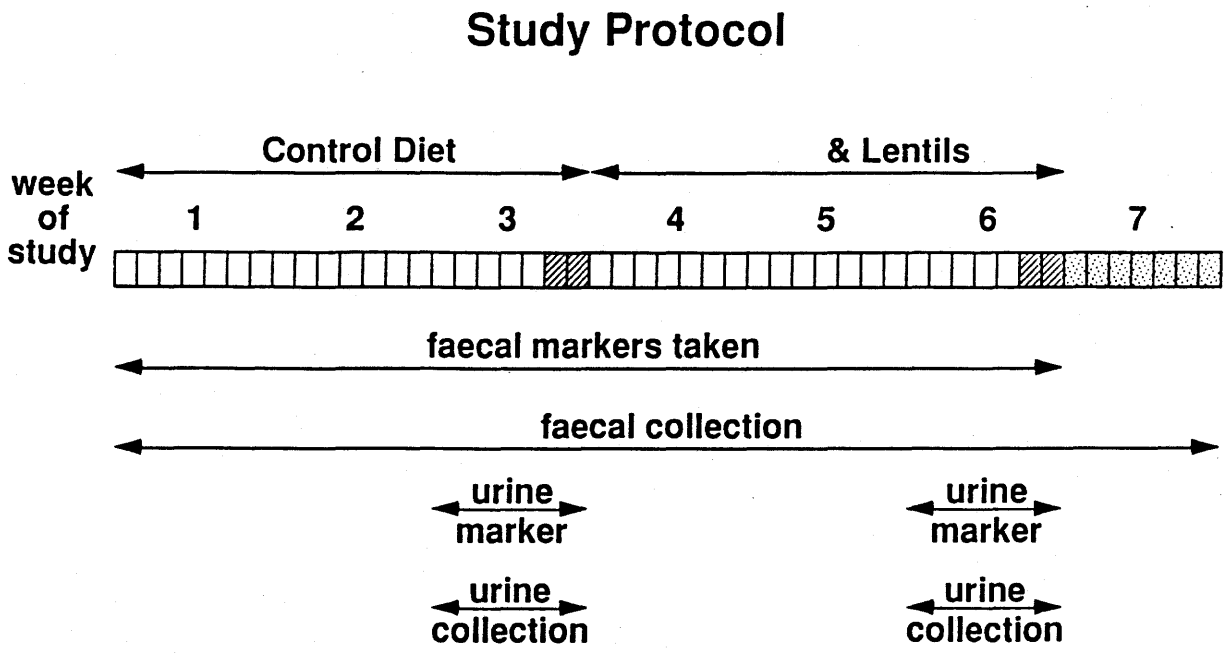
The six week study was divided into two, three week periods. In a random crossover design, the subjects were fed a control diet of typical North American foods for three weeks and the control diet plus 130 g of lentils for three weeks. The control diet was balanced for the protein provided by the lentils of the experimental period using soy protein isolate. The experimental period is referred to as the lentil period in the discussion that follows. **Table 3.1** gives the subject treatment order. Faecal markers were administered on a continuous basis throughout the study. Complete faecal collections were made throughout the six weeks and for an additional week following the study to allow for complete marker recovery. Once complete faecal collections were verified, samples from week three and six were used in analyses. Urine markers were given and twenty-four hour urine collections were made in weeks three and six. **Figure 3.1** gives a diagrammatic representation of

the study protocol. The study and all the procedures to be used were approved by the University Advisory Committee on Ethics in Human Experimentation (**Appendix 1**).

Table 3.1. Subject treatment order.

Subject	Treatment	
Number	Control	Lentil
27	wks 4-6	wks 1-3
30	wks 4-6	wks 1-3
38	wks 1-3	wks 4-6
39	wks 1-3	wks 4-6
40	wks 4-6	wks 1-3
47	wks 1-3	wks 4-6
48	wks 1-3	wks 4-6
49	wks 1-3	wks 4-6
50	wks 4-6	wks 1-3
51	wks 4-6	wks 1-3

Figure 3.1


Study Protocol
Control Diet
& Lentils
week of study
1
2
3
4
5
6
7
faecal markers taken
faecal collection
urine marker
urine marker
urine collection
urine collection

3.2. Subjects

Male volunteers were recruited by way of notices posted across the university campus. Female volunteers were excluded because of the possible confounding effect of the menstrual cycle on gastrointestinal function (Hinds, Stoney & Wald, 1989). Potential subjects completed a questionnaire to determine use of medications and medical problems. Only those men between the ages of 18-50 years of age who had no medical problems and used no medications were considered for study participation. Subject selection criteria also included a measurement of normal body weight. Height and weight were measured and Body Mass Index (BMI) was calculated. A BMI within the range of 20-27 (kg/m^2) was considered normal. Skin fold determinations were conducted on one individual with a body mass index in excess of 27 to determine whether the high figure was due to lean body mass, as in the case of an athlete, or due to an excess of adiposity. This individual was allowed to participate in the study, since his skin fold measurements ensured he did not have an excess of adiposity. Finally, the subjects must have been able to eat all the food on the proposed study menu. The characteristics of the subjects chosen to participate in the study are given in **Table 3.2.**

Subjects were weighed by study personnel at the beginning of the study and at one week intervals for the duration of the study.

Table 3.2. Characteristics of subjects.

Subject	Age(y)	Ht(m)	Wt(kg)	BMI(kg/m^2)
27	38	1.80	65	20
30	19	1.74	65	22
38	30	1.68	83	30
39	23	1.77	72	23
40	20	1.85	75	22
47	25	1.80	72	22
48	32	1.80	83	26
49	20	1.75	71	23
50	19	1.80	73	23
51	32	1.78	77	24
Mean	26	1.78	74	24
Range	(19-38)	(1.68-1.85)	(65-83)	(20-30)

Prior to the commencement of the study, the purpose and details of the study were explained to the subjects and each subject was required to read and sign

a consent form for participation in the human study (**Appendix 2**). All subjects were reimbursed for their participation in the study.

At the beginning of the study all subjects were given a study diary. In the diary, the subjects were instructed to record when faecal and urine markers were taken and when stool samples were passed. Problems with the food and any sickness, injury or unusual exercise were also to be reported. **Appendix 3** gives a sample page from a study diary.

3.3. Metabolic Diet

3.3.1. Dietary Assessment

Prior to the study, a seven-day weighed intake of each subject's usual diet was conducted to assess usual energy and nutrient intakes. PETRA^{R} (Portable Electronic Tape Recording Automated) scales were used by the subjects to weigh their food. NUTS^{R} Nutrient Assessment Program was used to determine usual nutrient intakes. The FIBREFIND program, developed in the Division of Nutrition and Dietetics, University of Saskatchewan, was used to determine usual intakes of non-starch polysaccharides (NSP).

3.3.2. Diet Formulation and Supplementation

The study consisted of three weeks of a control diet and three weeks of the control diet with the addition of Laird lentils. The lentils were incorporated to cakes, loaves and soups. The ratio of macronutrients (% energy) was maintained at 36% fat, 14% protein and 50% carbohydrate on both diet periods. In order to achieve this, the lentil starch of the lentil period was replaced with sucrose in the control period. Diet 7-up was given in the lentil period and regular 7-up was given in the control period. The protein content of the lentils was replaced with 30 g of soy protein isolate and 40 ml of milk in the control period. Soy protein isolate was chosen to replace the lentil protein since the amount and type of dietary protein may have an effect on the renal handling of calcium (Breslau et al., 1987; Whiting & McNally, 1989). The use of added salt was not permitted for the duration of the study due to the known calciuric effect of sodium (Zarkadas et al, 1989). Caffeine has been shown to increase urinary calcium excretion (Heany & Recker, 1982) thus, caffeine-containing foods and beverages were not included in the study diet except for instant coffee. The amount of instant coffee in grams per day remained constant throughout the study.

The level of calcium in the study diet was set to exceed each subject's usual calcium intake since it is generally accepted that it takes many weeks for the body to adjust to lower calcium intakes (Heany, Gallagher & Johnson, 1982). Any subject with a usual calcium intake above that of the study diet was given a daily tablet of $CaCO_3$ providing 500 mg of elemental calcium. Two subjects were given this supplemental calcium. The study diet either met or exceeded all current recommended nutrient intakes.

The diet was designed as a three day rotation. The basic control diet was given to all subjects and increments of certain foods to maintain the ratio of protein, fat and carbohydrate were added to the basic diet to adjust the energy level of the study diet to a subject's usual energy intake. Adjustments were made only within about $\pm$ two increments. During the first week of the study, energy adjustments of the diet were made to suit hunger/satiety needs as judged by the subjects. **Appendix 4** gives the calculated nutrient content of basic daily diet, lentil and control food products and foods used for the increments and the three day rotation.

3.3.3. Water

Due to the variable mineral content of the local water supply, all water used in the study was ultrapure (17 mohm). This water was produced by a Culligan reverse osmosis system followed by a Barnstead system of deionizing cartridges. The subjects used this water to drink and brush their teeth. Also, subjects were instructed to rinse their dishes and utensils with the water and to consume the resulting liquid.

3.3.4. Duplicate Diets

Duplicates of each subject's diet were made on days 16, 17 and 18 and on days 37, 38 and 39. Diets were homogenized using a Brinkman Polytron PT 6000 homogenizer. Hot ultrapure water was used to dissolve food components. Eight 30 mL samples of each diet on each day were taken and immediately frozen at -20°C.

3.4. Faecal Collection

Subjects collected all faeces into individual plastic bags. A toilet frame apparatus was provided to each subject to facilitate the faecal collections. Subjects were instructed to record the time of each

defecation in their study diaries. Once collected, faeces were placed with dry ice in thermos containers and later transferred by the subjects to a conventional freezer. Samples were individually weighed and then stored at -20° C until freeze-dried.

To ensure completeness of faecal collections, the subjects were given radio-opaque markers. Each day of the study, subjects were given three capsules containing ten markers each. The subjects were instructed to take a capsule at 8:00, 12:00 and 18:00. If they deviated from this schedule they were to record the times in their study diaries. Different shaped markers were used to differentiate between the faecal output of the control and lentil periods and for the first week of study to determine if subjects were making complete collections. All individual faecal samples were x-rayed at the Royal University Hospital's Department of Medical Imaging. The radiographs were used to determine the number of markers excreted. A photocopy of a faecal x-ray is given in **Appendix 5**. Periods with a marker recovery of less than 98.5% were considered incomplete. The markers were also used to correct for infrequency of stool output. **Appendix 6** gives the calculation for the marker correction factor which is used to adjust for infrequency of faecal output. Frozen samples were pooled by week and placed

in large polyethylene bags, and then freeze-dried in an Edwards Supermodulyo Freeze Drier for seven days until a stable weight was reached. After weighing, bags were sealed and the freeze-dried samples were crushed and transferred into air tight polyethylene containers.

Transit time is the length of time in hours that it takes ingested substances to be excreted in the faeces. Mean transit time is the average time it takes for 50% of ingested radio-opaque markers to be excreted. Mean transit time was calculated for the control and lentil periods using the continuous marker method of Cummings, Jenkins and Wiggins (1976) (**Appendix 6**).

3.5. Urine Collection and Marker Analysis

Subjects were required to make daily twenty-four urine collections during weeks three and six of the study. Upon rising on the first day of a urine collection, the subjects were instructed to discard their first morning urine. All urine passed throughout the day and night was collected into the one litre polypropylene bottles provided. The first morning urine of the following day was also added to the previous day's urine. Thimerosal was used as a preservative for the urine collections. Urine for each

twenty-four hour period was mixed, measured for volume and eight 30 mL aliquots were taken and frozen at -20°C.

To validate the completeness of the twenty-four hour urine collections, para-amino benzoic acid (PABA), in the form of PABACHECK^{R} tablets, was given as a marker. A dosage of 80 mg of PABA, three times a day, was given to the subjects during weeks three and six. Only urine collections with greater than 85% PABA recovery were considered to be complete. PABA has no known pharmacological effects and is completely absorbed by the human gastrointestinal tract. PABA, once absorbed, is converted within the body to p-aminohippuric acid and some glucuronide. It is the p-aminohippuric acid that is excreted in the urine; once hydrolysed, its concentration can be measured by colorimetry. Urinary measurement of PABA consists first of acid or alkali hydrolysis which releases the acetyl group from the compound. The free amine group can form diazonium salt. The colour reaction is outlined in Bratton and Marshall (1939). As stated previously, all intakes of drugs by the subjects were disallowed in the study. Finally, since PABA is light sensitive, subjects were advised to keep urine out of direct light.

As the determination of PABA consists of measuring

the content of an aromatic amine in the urine, it was necessary to eliminate the intake of any substances such as certain drugs which will interfere with the analysis (Bingham & Cummings, 1983). The analysis of PABA recovery in the urine was completed as follows. Urine samples were diluted to contain between 0.25 and 2.5 mg PABA. Three 10 mL aliquots were pipetted into glass boiling tubes with plastic lids. Standards containing 0.25, 0.75, 1.0, 1.5 and 2.5 mg PABA were prepared. Two mL of 5 mol/L sodium hydroxide was added to each tube. Hydrolysis was performed by placing tubes in a boiling water bath for two hours. Tubes were cooled and then 3 mL of 5 mol/L hydrochloric acid was then added to each tube. At two minute intervals, with thorough mixing, 1 mL sodium nitrite (1 g/L), then 1 mL ammonium sulphamate (5 g/L) and N - naphylethylene dihydrochloride (1 g/L) were added. One hour was allowed for maximum colour development. Absorbency was measured by spectrophotometer at 540 nm.

3.6. Mineral Determinations

The ashing procedure of Hill, Patterson, Veillon and Morris (1986) was used. Freeze-dried faecal and diet samples of approximately 1 g were placed into 16 mm by 100 mm borosilicate test tubes. Tubes were

covered with foil and placed in a muffle furnace. The temperature was raised to 400°C within 2-3 hours and held at this temperature for 48 hours. Tubes were cooled to room temperature and 0.20 mL deionized water and 0.20 mL concentrated nitric acid were added. Tubes were then placed in a Thermolyne Dri-Bath heating block and the temperature was raised to 90°C. Hydrogen peroxide (50% v/v) was added in 0.1 mL aliquots every 10 - 15 minute intervals until all black carbon particles were digested. Samples were evaporated to dryness and cooled. Two mL of deionized water and one mL of concentrated hydrochloric acid were added. Samples were reheated to dissolve residue. Samples were cooled and transferred to appropriate volumetric flasks (25 mL volumetric flasks were used for food samples and 100 mL volumetric flasks were used for faecal samples). Once made up to volume with ultrapure water, samples were transferred to polypropylene storage containers.

3.6.1. Calcium

Aliquots of urine were acidified with concentrated hydrochloric acid and centrifuged. Titrations were read for all samples in duplicate using an Corning 940 Calcium Analyzer. A standard of 2.5 mmol/l calcium is

used intermittently. The calcium analyzer works as follows. The dye calcein which forms a fluorescent complex with calcium is added to the samples. The fluorescence is eliminated by titrating with EGTA. The amount of EGTA used to squelch the fluorescence is converted to give the concentration of calcium present in the test solutions.

3.6.2. Sodium and Potassium

Flame photometry was used to measure sodium and potassium in diluted acidified urine samples (Corning Flame Photometer 410, Corning Medical and Scientific, Corning, NY). The electrons of sodium and potassium are excited when aspirated into a low temperature flame. A wavelength of visible light is emitted when the electrons return to ground state. The amount of visible light emitted is detected by the photodetector of the flame photometer and is proportional to the amount of ions in the sample (Tietz, 1987). All samples were completed in duplicate.

3.7. Net Acid Excretion

Net acid excretion (NAE) is defined as the sum of titratable acidity (TA) plus urinary ammonium (NH_4^+)

minus HCO_3. Net acid excretion was determined by the following procedure outlined by Chan, 1972: 5 mL aliquots of urine were pipetted into 25 mL containers and 5 mL 0.1 N hydrochloric acid was added. The containers were placed in a shallow boiling water bath for 10 minutes. The addition of acid converts the HCO_3 to CO_2 which is expelled in the boiling process. The tubes were cooled to 37°C. The solution was titrated back to pH 7.4 using 0.1 N sodium hydroxide. The volume of sodium hydroxide needed to titrate the sample back to 7.4 minus a titrated water blank gave the TA-HCO_3. Five mL of 8% (v/v) formaldehyde solution was added to the solution which served to release a hydrogen ion from the ammonium molecule thus, lowering the pH. Once again, the solution was titrated back to 7.4 using 0.1 N sodium hydroxide. The resulting volume of sodium hydroxide minus that of the blank gave the concentration of ammonium.

3.8. Dietary Fibre

Non-starch polysaccharides content of the lentils was analyzed by Englyst (Englyst, Wiggins & Cummings, 1982). The procedure is conducted in triplicate and to give total NSP and insoluble NSP. Soluble NSP is calculated by difference. The procedure involves a

series of enzymatic reactions to remove starch and then hydrolysis in sulphuric acid to produce monosaccharides. One of the three samples is rinsed in a phosphate buffer to remove soluble components. Following hydrolysis, and through a series of steps, monosaccharides are converted to their alditol acetates and concentrations of individual monosaccharides are determined by gas-liquid chromatography.

3.9. Phosphorus and Phytate

The phosphorus and phytate content of the lentils and the soy protein isolate was analyzed in the Department of Crop Science, College of Agriculture, University of Saskatchewan. The method used is outlined in Harland and Oberleas (1986).

3.10. Statistical Analysis

Results in the text are stated as mean $\pm$ standard error measurement (SEM). Tables give both standard error measurement and standard deviation (SD). Since order of treatment was randomized and the subjects served as their own controls, results were analyzed for significance using paired t-tests. A probability of < 0.05 was used to indicate significance. ANOVA tests

were also carried out to determine if the order of treatment had any effect.

4. RESULTS

4.1. Exclusion of LEN038

Subject LEN038 made only one complete twenty-four hour urine collection, using the criteria of greater that 85% PABA recovery, in each of weeks 3 and 6. Two complete collections out of a possible fourteen was not considered adequate for the purpose of balance calculations. In addition, LEN038's faecal output throughout the study was extremely large and it was suggested that this was an indication of malabsorption (Bingham, S., personal communication; **Appendix 7**). Based on these factors, the data collected on LEN038 was excluded from the following analyses.

4.2. Subject Body Weights

Table 4.1 gives the weekly body weights of the subjects and the net change for each individual. There was no significant difference between the subjects' initial and final body weights.

Table 4.1. Subject body weights (kg).

	Weeks							
Subject	0	1	2	3	4	5	6	Net
27	66.1	65.5	65.5	65.5	65.5	65.9	65.0	-1.1
30	70.9	70.5	70.7	70.9	70.5	70.5	70.9	0
39	71.8	70.9	69.8	69.1	69.1	70.2	70.0	-1.8
40	81.6	80.5	80.5	81.6	80.0	81.1	81.4	-0.2
47	73.4	70.9	70.0	69.3	69.1	68.9	68.8	-4.6
48	86.1	85.9	84.5	85.0	83.6	83.9	83.2	-2.9
49	71.8	72.5	72.3	72.5	72.3	72.7	73.2	+1.4
50	78.9	79.5	80.0	80.5	81.4	82.7	81.8	+2.9
51	79.8	79.1	80.0	80.2	78.9	78.6	80.0	+0.2
Mean	75.6	75.0	74.8	75.0	74.5	74.9	74.9	-0.7
P = 0.392								

4.3. Subjects' Usual Diet

The calculated nutrient content of the subjects' usual diet assessed by 7-day weighed intakes is given in **Table 4.2.** Fat, protein and carbohydrate are expressed as percent of energy.

Table 4.2. Usual daily nutrient intakes.

Subject	Energy kJ	Fat %	Protein %	CHO %	NSP g
27	12850	36	16	47	17.7
30	12470	49	13	38	14.9
39	14760	27	13	61	35.6
40	13670	33	11	56	14.4
47	12740	26	14	59	18.9
48	13690	42	15	41	15.7
49	14220	33	12	54	16.5
50	15760	41	12	48	17.3
51	12450	38	13	46	19.2
Mean	13620	36	13	50	18.9
SD	1130	7	2	8	6.5

4.4. Duplicate Diets

The analyzed calcium, potassium and sodium content of the duplicate diets are given in **Tables 4.3**, **4.4** and **4.5**. Both three and seven day means are given for each subject. The three day mean is an arithmetic average of each day in the three day rotation. The seven day

mean is an adjustment of the three day rotation to an estimated calculation of the entire seven days used for balance study calculations using the following calculation: 3 x day 1, 2 x day 2 and 2 x day 3. No significant difference was found between the calcium content of the lentil diet as compared with the control diet ($P = 0.095$). The sodium content of the lentil diet was found to be significantly less than that of the control diet ($P = 0.004$). The potassium content of the lentil diet was found to be significantly greater than that of the control diet ($P = 0.0001$).

Table 4.6 compares the usual energy intakes and metabolic diet energy intakes of the subjects. The caloric level of the study diet was not significantly different than the subjects' usual diet ($P = 0.330$).

Table 4.7 gives the subjects' usual calcium intakes and the calculated and analyzed calcium content of the study diet. There was no significant difference between the subjects' usual calcium intakes and the calcium content of the metabolic diet ($P = 0.861$). It appears that the NUTS Nutrient Assessment Program consistently overestimated the calcium provided in the diet, reported as "calculated", as compared to the analyzed values ($P = 0.0001$).

Table 4.3. Analyzed calcium content of duplicate diets (mmol/d)

Subject	27	30	39	40	47	48	49	50	51
Control									
	28.6	30.7	32.2	29.9	42.8	29.7	31.8	42.6	28.6
	31.3	33.3	37.9	33.4	47.5	35.1	37.9	45.6	33.9
	29.6	31.7	32.7	29.2	41.1	30.4	34.7	42.8	30.1
3d* Mean	29.8	31.9	34.3	30.8	43.8	31.7	34.8	43.7	30.9
7d# Mean	29.7	31.7	34.0	30.7	43.7	31.4	34.4	43.5	30.5
Group mean = 34.4				SD = 5.4				SEM = 1.8	
Lentil									
	28.3	27.9	35.2	26.4	40.1	28.5	30.1	43.1	30.2
	33.9	35.4	35.1	33.8	45.6	33.3	34.6	48.6	34.9
	26.3	28.9	31.8	28.4	43.2	29.5	29.7	42.1	28.8
3d* Mean	29.5	30.7	34.0	29.5	43.0	30.4	31.5	44.6	31.3
7d# Mean	29.3	30.3	34.2	29.1	42.6	30.2	31.3	44.4	31.1
Group mean = 33.6				SD = 5.8				SEM = 1.9	

* Arithmetic average for a 3 day rotation.

\# Calculated average for 7 days.

Table 4.4. Analyzed sodium content of duplicate diets (mmol/d).

Subject	27	30	39	40	47	48	49	50	51
Control									
	151	157	172	176	166	152	160	182	172
	157	161	180	171	163	165	174	162	161
	148	152	144	151	142	135	138	154	149
3d* Mean	152	157	165	166	157	151	157	166	161
7d# Mean	152	157	166	167	158	151	158	168	162
Group mean = 160				SD = 6.3			SEM = 2.1		
Lentil									
	155	158	174	158	155	150	162	158	153
	152	151	160	147	160	147	153	150	150
	115	139	150	132	150	143	143	135	124
3d* Mean	141	149	161	146	155	147	153	148	142
7d# Mean	143	151	163	147	155	147	154	149	144
Group mean = 150				SD = 6.3			SEM = 2.1		

* Arithmetic average for a 3 day rotation.

\# Calculated average for 7 days.

Table 4.5. Analyzed potassium content of duplicate diets (mmol/d).

Subject	27	30	39	40	47	48	49	50	51
Control									
	97	94	108	91	104	97	100	96	85
	90	83	122	90	117	108	115	92	96
	90	97	110	93	105	101	110	106	102
3d* Mean	92	91	113	91	109	102	108	98	94
7d# Mean	93	92	113	91	108	101	107	98	93
Group mean = 100				SD = 8.1			SEM = 2.7		
Lentil									
	119	111	139	111	117	111	112	118	113
	131	131	122	122	121	113	114	135	127
	112	110	142	112	140	130	125	119	112
3d* Mean	121	117	134	115	126	118	117	124	117
7d# Mean	120	116	135	114	125	117	116	123	117
Group mean = 120				SD = 6.6			SEM = 2.2		

* Arithmetic average for a 3 day rotation.

\# Calculated average for 7 days.

Table 4.6. Comparison of reported usual energy intakes to metabolic diet energy intake (kJ/d).

Subject	Usual Energy*	Diet Energy
27	12850	12410
30	12470	13250
39	14760	15760
40	13670	13250
47	12740	14090
48	13690	13250
49	14220	14923
50	15760	14923
51	12450	13250
Mean	13620	13901
SD	1130	1090
SEM	380	363
P = 0.330		

* Reported from 7-day weighed intake.

Table 4.7. Reported usual calcium intake and metabolic diet calcium (mmol/d).

Subject	Usual Ca Calculated	Diet Ca Calculated	Diet Ca Analyzed
27	25.0	34.0	29.7
30	34.7	35.0	31.3
39	34.4	37.0	34.1
40	29.4	35.0	30.2
47	46.8	46.5*	43.4
48	31.5	35.0	31.1
49	25.7	36.0	33.1
50	44.6	49.5*	44.1
51	34.0	34.0	31.1
Mean	34.0	38.0	34.2
SD	7.5	5.8	5.6
SEM	2.5	1.9	1.9
		P = 0.014	P = 0.861

* Subject given daily supplement providing 500 mg calcium.

The phosphorus and phytic acid contents of the Laird lentils and soy protein isolate are given in **Table 4.8**. The soy protein isolate contained more phosphorus and phytic acid on a dry weight basis than the lentils. The total phosphorus per day coming from dietary intakes of 130 g of lentils was 12.9 mmol/d. This compares to only 8.4 mmol/d from the soy protein isolate. Other food items were added to the control diet to balance the protein, fat and carbohydrate of the lentils. Milk was one of these items, and 40 mL of 2% milk was added to the control diet each day. Based on the average phosphorus content of cows milk being 0.910 g/ 1000 ml (Beal, 1986), an additional 1.2 mmol/d phosphorus was provided by the control diet. The difference in phosphorus content between the diets, therefore, was about 3.3 mmol/d.

The lentils contained 440 mg/d phytic acid and the soy protein isolate contained 420 mg/d phytic acid. Thus, there was virtually no difference in phytate content between the control and the treatment diets.

The content of non-starch polysaccharides in Laird lentils is given in **Table 4.9**. As 130 g of lentils were given and the lentils are 93 % dry matter, this resulted in an additional 12.2 g of NSP in the treatment diet.

Table 4.8. Phosphorus and phytate content of lentils and soy protein isolate (g/100 g ± SD dry weight).

Total Phosphorus	
Lentil	0.33 ± 0.01
Soy Protein Isolate	0.85 ± 0.01
Phytic Acid	
Lentil	0.37 ± 0.01
Soy Protein Isolate	1.41 ± 0.01

Table 4.9. Non-starch polysaccharides (NSP) in Laird lentils (g/100g dry matter)

Soluble NSP	2.0
Insoluble NSP	8.1
Total NSP	10.1

4.5. Colonic Function

4.5.1 Marker Recovery

Table 4.10 shows the subjects' faecal marker recoveries. Subject 48 reported that he missed taking one capsule containing ten markers, therefore, his

total of markers taken was 620 compared to 630 for the other subjects. All subjects exceeded the 97% recovery level, therefore, all subjects were judged to have complete faecal collections.

Table 4.10. Faecal marker recoveries.

Subject	Weeks 1-3	Weeks 4-6
	%	%
27	100.0	100.0
30	99.8	99.7
39	100.0	100.0
40	99.2	100.0
47	100.0	99.8
48	99.7	99.4
49	99.7	100.0
50	100.0	98.4
51	99.8	100.0

Mean Marker Recovery = 99.8 %

4.5.2 Faecal Weights

Table 4.11 gives the marker corrected faecal weights in g/d for the individual subjects. There was a significant increase in faecal weight by 59 g/d with

the addition of lentils to the diet (P = 0.027). An analysis of variance was conducted, but no order effect was found.

Table 4.11. Marker corrected faecal weight (g/d)

Subject	Control	Lentil
27	90.7	135.0
30	92.2	156.9
39	145.0	180.8
40	120.6	204.2
47	170.0	140.1
48	167.2	202.0
49	178.4	346.5
50	95.3	163.9
51	116.0	176.9
Mean	130.6	189.6
SD	35.4	63.6
SEM	11.8	21.2
		P = 0.027

4.5.3 Transit Time

Table 4.12 gives the mean transit times for the individual subjects. No difference was found between transit time in the control compared to the treatment period.

Table 4.12. Mean transit time (hours).

Subject	Control	Lentil
27	91	60
30	42	39
39	38	41
40	38	40
47	47	58
48	26	34
49	28	20
50	55	46
51	45	47
Mean	46	43
SD	19	12
SEM	6	4

P = 0.523

4.5.4. Faecal Calcium Excretion.

The data on faecal calcium excretion is given in **Table 4.13**. The mean faecal calcium excretions for the control period, 28.5 $\pm$ 1.7 mmol/d, and the lentil period, 29.7 $\pm$ 1.5 mmol/d, were not significantly different ($P = 0.434$). There was a significant correlation between faecal calcium and dietary calcium intake ($y = .98 x + 5.57$; $r = 0.831$; $P = 0.0001$). In addition, there was a significant positive correlation between urinary calcium excretion and apparent calcium absorption ($y = 0.08 x + -2.00$; $r = 0.533$; $P = 0.023$).

4.6. Renal Function

4.6.1. Marker Recovery

Urine collections with PABA recoveries of greater than 85% were considered to be complete. **Appendix 8** gives the PABA recoveries. Complete collections were then matched by day of the diet. For instance, if a subject had two complete day 1, one day 2 and no day 3 collections in the control period, corresponding collections in the lentil period for this subject would be used in the calculations. The number of collections used in the urinary mineral excretion calculations are given in brackets in **Table 4.14**.

Table 4.13. Faecal calcium excretion (mmol/d).

Subject	Control	Lentil
27	29.4	25.8
30	25.4	26.3
39	25.3	30.7
40	23.8	26.7
47	39.3	31.4
48	27.2	28.3
49	26.0	31.4
50	34.4	39.7
51	25.5	27.1
Mean	28.5	29.7
SD	5.1	4.4
SEM	1.7	1.5
		P = 0.434

4.6.2. Mineral Excretion

Average urinary excretions of calcium, sodium and potassium for individual subjects are given in **Table 4.14**. A significant decrease in urinary calcium excretion was found in the lentil period as compared to

the control period (P = 0.0001). Sodium excretion in the lentil period was significantly less than in the control period (P = 0.003). Finally, the potassium excretion in the lentil period was significantly higher than that of the control period (P = 0.015).

4.6.3. Renal Net Acid Excretion

Data for urinary titratable acid, ammonium and net acid excretion are given in **Table 4.15**. No significant differences were found for titratable acid, ammonium or net acid excretion between the control and the lentil periods.

4.6.4. Calcium Balance

Balance was determined by summing faecal and urinary calcium and comparing the total to the seven day average of dietary calcium. No significant difference was found between the balance results of the control period as compared to the lentil period. If an adjustment of 1.6 mmol/d is made for dermal losses (Charles, Eriksen, Hasling, Sondergard & Mosekilde, 1991), balances become more negative but there is still no significant difference between the balances. Calcium balance data is given in **Table 4.16**.

Table 4.14. Urinary mineral excretion (mmol/d).

Subject		Control			Lentil		
	#*	Ca	Na	K	Ca	Na	K
27	(6)	3.61	148	95.9	2.78	138	107.8
30	(4)	6.49	128	79.7	4.49	129	100.4
39	(5)	7.06	159	105.3	6.02	123	107.3
40	(3)	5.58	161	98.0	4.98	138	108.9
47	(2)	7.39	154	117.1	6.63	140	108.4
48	(4)	4.30	156	81.4	3.27	133	112.2
49	(4)	5.19	150	76.1	4.35	125	91.9
50	(5)	5.19	153	86.2	4.28	146	101.5
51	(6)	4.07	131	77.9	4.00	122	84.1
Mean		5.43	149	90.8	4.53	133	102.5
SD		1.33	12	14.1	1.22	8	9.2
SEM		0.44	4	4.7	0.41	3	3.1

Ca	Na	K
P = 0.0001	P = 0.003	P = 0.015

* # - Represents the number of complete urine samples used to calculate the mean mineral excretions.

Table 4.15. Urinary titratable acid (TA), ammonium (NH_4^+) and net acid excretion (NAE)(mmol/d).

Subject	Control			Lentil		
	TA	NAE	NH_4^+	TA	NAE	NH_4^+
27	-2.6	27.5	30.1	2.6	34.0	31.4
30	2.1	38.8	36.7	3.2	47.6	44.4
39	11.6	47.4	35.8	17.6	56.7	39.1
40	8.5	48.8	40.3	8.7	47.6	38.9
47	02.1	37.4	35.3	6.2	47.4	41.2
48	13.0	59.3	46.3	28.3	62.8	34.5
49	18.3	61.3	43.0	36.4	65.0	28.6
50	16.2	59.9	43.7	10.6	55.2	44.6
51	16.7	62.6	45.9	5.8	54.0	48.2
Mean	9.5	49.2	39.7	13.3	52.3	39.0
SD	7.5	12.6	5.5	11.9	9.4	6.5
SEM	2.5	4.2	1.8	4.0	3.1	2.2
TA	NAE		NH_4^+			
P = 0.257	P = 0.204		P = 0.791			

Table 4.16. Calcium balance (mmol/d).

Control Subject	Faecal	Urinary	Total	Dietary	Balance
27	29.4	3.61	33.0	29.7	- 3.3
30	25.4	6.49	31.9	31.7	- 0.2
39	25.3	7.06	32.4	34.0	+ 1.6
40	23.8	5.58	29.4	30.7	+ 1.3
47	39.3	7.39	46.7	43.7	- 3.3
48	27.2	4.30	31.5	31.4	- 0.1
49	26.0	5.19	31.2	34.4	+ 3.2
50	34.4	5.19	39.6	43.5	+ 3.9
51	25.5	4.07	29.6	30.5	+ 0.9
Mean					+0.4
SD					2.5
SEM					0.8

Table 4.16 (continued)

Lentil Subject	Faecal	Urinary	Total	Dietary	Balance
27	25.8	2.78	28.6	29.3	+ 0.7
30	26.3	4.49	30.8	30.3	- 0.5
39	30.7	6.02	36.7	34.2	- 2.5
40	26.7	4.98	31.7	29.1	- 2.6
47	31.4	6.63	38.0	42.6	+ 4.6
48	28.3	3.27	31.6	30.2	- 1.4
49	31.4	4.35	35.8	31.3	- 4.5
50	39.7	4.28	44.0	44.4	+ 0.4
51	27.1	4.00	31.1	31.1	0.0
Mean					-0.6
SD					2.6
SEM					0.9
					P = 0.503

5. DISCUSSION

The addition, daily, of 130 g of lentils, containing 13 g of dietary fibre, significantly increased faecal weight, therefore, this amount of lentils can be considered an effective fibre source. The excellent compliance of the subjects supports the conclusion that the amount of lentils fed was well tolerated, thus, this is considered a practical way to increase the intake of complex carbohydrates and fibre.

The study conducted was sufficiently controlled and of adequate length to make substantiated conclusions regarding calcium balance. The administration of 130 g of lentils to a controlled diet had no significant effect on calcium balance by three weeks of feeding. This result is consistent with the findings of other investigators who have found no change in calcium balance with dietary fibre administration. For example, Spencer, Norris, Derler and Osis (1991) found that oat bran had no significant effect on calcium balance. But, as the researchers state: "the lack of effect of the intake of the oat

bran muffins on mineral metabolism in humans in the present study may be due to the fairly high content of soluble fibre in the oat bran". A better comparison would be to the study conducted by Wisker, Nagel, Tanudjaja and Feldheim (1991), who found that barley fibre concentrate exerted no negative effect on calcium balances when compared to the low fibre diet given. Barley fibre contains a greater percentage of insoluble fibre than oat bran, therefore, is more comparable to lentils.

Although no significant difference was found between the balance figures of the control compared to that of the lentil period, it must be emphasized that total output as calculated in the present study excludes any calculation of dermal losses of calcium. Charles, Eriksen, Hasling, Sondergard and Mosekilde (1991) estimate dermal losses in normal individuals to be 1.6 mmol/d. Incorporating this estimation of the dermal losses into the present study, the mean balance for the control period decreases from + 0.4 mmol/d to - 1.2 mmol/d and the mean balance for the lentil period increases in negativity from - 0.6 to - 2.2 mmol/d. The inclusion of dermal losses makes the average balance of the control period negative and that of the lentil diet more negative.

The negative calcium balances observed in this

study are of some concern. The present study was conducted on healthy young men and it is difficult to extrapolate to women. If it is assumed that young healthy women have similar results, it is likely that post-menopausal women may experience more negative balances due to their decreased ability to absorb calcium from the intestine (Riggs & Melton, 1986). If the post-menopausal women were to exhibit similar results, the calcium losses from such a diet could result in significant bone loss since a loss of only 1.0 mmol/day can explain the 1% to 1.5% loss in skeletal mass observed each year in postmenopausal women (Heany and Recker, 1982).

No significant change in faecal calcium excretion was found with the feeding of lentils. It was hypothesized that the only effect of insoluble fibre on calcium metabolism would be through a decrease in transit time. No significant change in mean transit time occurred, therefore, faecal calcium excretion would not be expected to change. The results support this hypothesis. In addition, it was proposed that the phytate contained in lentils would increase faecal calcium excretion. The lentils fed per day contained 440 mg phytic acid. Whether or not this level of phytate would result in an increase in faecal calcium cannot be determined from the results of this study

since the soy protein isolate, fed in the control period to balance the protein of the lentil period, contained 420 mg phytic acid. Thus, the lentil diet differed from the control by only 20 mg of phytate. Contrary to the present findings, Wisker and coworkers (1991) found faecal calcium excretion to be lower with the low-phytate barley fibre diet than the low fibre control. The low-phytate barley fibre diet differed from the control by 10 mg of phytate. The results of their study, however, are complicated by differing protein levels.

The negative calcium balances, including dermal losses, of both the control and the lentil periods may be explained by the level of phytate in both experimental periods. The total phytate in the control diet is not known, but the foods given were very low or deficient of phytate. It can be assumed that the most of the phytate in the study diets came from the lentils and the soy bean isolate. A phytate level of about 400 mg/d is a typical dietary level of phytate in the North American diet (Murphy & Calloway, 1986) and would not be expected to have any detrimental effects on calcium balance.

Faecal calcium is known to vary with dietary calcium intake (Heaney et al., 1990). The dietary calcium level fed in the control and the lentil periods

were not significantly different, thus one would not expect faecal calcium to differ between the study periods. The results of the study support this expectation. Faecal calcium, however, did correlate with dietary calcium intake ($r = 0.831$; $P = 0.0001$), that is, as dietary calcium increased faecal calcium increased. Since the relationship between dietary and faecal calcium approaches linearity, one can conclude that at the high dietary calcium levels fed, increases in absorption are through the passive mechanism.

The control and the lentil diet differed by 3.3 mmol/d of phosphorus. This figure is based on the analysis of the lentils and soy protein isolate for total phosphorus and an estimation for the additional milk, as the diets were identical in all other respects. It can be concluded that this amount of phosphorus has no effect on faecal calcium excretion. In the study conducted by Spencer and coworkers (1991), the oat bran period exceeded the control period by almost 25.8 mmol/d phosphorus and no change was seen in faecal calcium excretion. It should be noted that the amount of phosphorus associated with phytic acid in oat bran is relatively low (Loas, Palamidis & Markakis, 1976).

The lentils caused a significant decrease in urinary calcium excretion. This finding was not

expected from a fibre source so high in insoluble fibre. This result is particularly interesting since the decrease in renal calcium excretion does not appear to be compensating for increased faecal losses. Spencer and coworkers (1991) found a decrease in urinary calcium with the feeding of oat bran, but oat bran is a fibre source high in soluble fibre unlike lentils, which are high in insoluble fibre.

A number of factors may have contributed to the decrease in renal calcium excretion during the lentil period, including calcium intake. Since calcium intake did not change, it would not have had any effect on renal calcium excretion. The factors to be discussed, net acid excretion, phosphate, sodium and potassium, are known to influence urinary calcium excretion.

One possible mechanism for the alteration in renal calcium excretion by lentils is through a change in net acid excretion (NAE), but no significant change in NAE was found. At first consideration, it is not surprising that there was no change in net acid excretion since the dietary sulphur content should have been similar since the lentil protein was replaced by soy protein isolate, both of which are legume protein sources. Lentils contain about 1.8 g of sulphur amino acids per 100 g of protein (Bhatty, 1988) compared to soy beans which contain about 3.0 g/ 100 g (Pennington,

1994). If an animal protein such as egg white had been used in the control period to replace the lentil protein, a higher NAE would have been expected in the control as compared to the lentil period. Egg white contains approximately 6.1 g of sulphur amino acids per 100 g of protein (Pennington, 1994). The lower NAE in the lentil period would have resulted in a further decrease in renal calcium excretion. Net acid excretion can also be affected by the overall acid load of the diet. Lentils are actually considered to be a food with an excess acidity (Krause & Mahan, 1979), but this acidity was not reflected by an increase in NAE.

Renal calcium excretion is known to change with increases of dietary phosphorus (Spencer et al., 1986). Further, with a known change in dietary phosphorus, it is possible to predict the change in urinary calcium excretion (Heany & Recker, 1982). The predicted change in urinary calcium is 0.43 mmol/d with a 18 mmol/d increase in phosphorus. In the present study, there was a 3.3 mmol/d increase in dietary phosphorus. This would result in a 0.075 mmol/d decrease in renal calcium excretion. The change observed in renal calcium excretion was 0.90 mmol/d, therefore, the effect, if any, of phosphorus on renal calcium excretion was very small (8%) compared to the overall change in renal calcium excretion. The proposed

mechanism for the effect of dietary phosphorus on renal calcium excretion is likely "a phosphate-induced PTH-mediated increase in the recovery of Ca from the filtered urine" (Draper, Piche & Gibson, 1991).

Another way by which lentils may have caused the observed decrease in renal calcium excretion is through reduced renal sodium excretion. The diet in the lentil period contained an average of 150 mmol/d of sodium compared to the diet of the control which contained 160 mmol/d of sodium. The sodium excretion in the urine reflected this difference in dietary sodium. The average sodium excretion in the control period exceeded the average sodium excretion in the lentil period by 16 mmol/d. Bleich & Moore (1979) state that "some data suggest that urinary calcium increases about 0.6 mmol (25 mg) per 100 meq (2300 mg) increment in urinary sodium". Nordin, Need, Morris and Horowitz (1993) state that sodium and calcium excretion in the kidney are interrelated depending on relative doses. These researchers estimate that when sodium is the determining factor in the interaction, each 100 mmol of sodium causes the removal of about 1.0 mmol of calcium from the urine. In the present study, a difference of 16 mmol/d of sodium excretion in the urine would result in a change in renal calcium excretion of about 0.16 mmol/d. This effect would account for a small

percentage (18%) of the observed change in renal calcium excretion. It is important to consider that this sodium-dependent calcium loss is thought not to be a transitory occurrence, but may continue indefinitely leading to continued calcium loss. Therefore, any diet lower in sodium could be considered beneficial in terms of maintenance of bone density.

The alternate hypothesized mechanism by which urinary calcium excretion was decreased with the feeding of lentils was through an increased intake of potassium. The diet of the lentil and the control period differed by 20 mmol/d potassium, with the lentil diet having the excess. Urinary potassium excretion reflected this difference in the diet. The average renal potassium excretion in the lentil period was 102.5 mmol/d compared to 90.8 mmol/d of the control period; with the difference being 11.7 mmol/d. Lemann, Pleuss and Gray (1993) describe an inverse relationship between urinary calcium and potassium and propose a formula for the relationship. The change in urinary calcium (mmol/d) appears to be about -0.015 times the change in urinary potassium (mmol/d). If this relationship is true, the change in potassium of the present study would result in a 0.17 mmol decrease in urinary calcium excretion. This would account for about 19% of the observed decrease in renal calcium

excretion. It has been suggested that potassium enhances the renal tubular calcium reabsorption, but this proposed mechanism has not been confirmed. (Lemann et al., 1993).

Table 5.1. Summary of factors reducing renal calcium excretion.

Factor	Change in Factor	Predicted Decrease in Renal Calcium Excretion
Dietary P	+ 100 mmol	- 2.4 mmol[a]
Renal Na	- 100 mmol	- 1.0 mmol[b]
Renal K	+ 100 mmol	- 1.5 mmol[c]

[a] Spencer et al. (1986)

[b] Nordin et al. (1993)

[c] Lemann et al. (1993)

If one is to sum the effects discussed thus far, about half of the observed decrease in renal calcium excretion has been explained. There still remains over 50% of the renal calcium excretion that has yet to be explained.

Research has shown that resistant starch is degraded in the large intestine in much the same way as soluble fibre (Stephen, 1990). It is likely that some of the starch in lentils is resistant to digestion in the small intestine and enters the colon to be fermented. Thus, it is possible that the resistant starch in lentils acted in a similar way as soluble fibre would, particularly causing the decrease in urinary calcium excretion.

It is likely that in the lentil period, renal calcium excretion also decreased by other mechanisms not considered thus far. Further investigation into the effects of lentil starch and fibre would be necessary to identify these mechanisms.

Subjects exhibited some change in body weight. Change in body weight ranged from + 2.9 to - 4.6 kg with the average change being - 0.7 kg. The study was conducted during spring and the weather changed considerably over the duration of the study. It is likely that as the weather warmed, some subjects became more active. For instance, subject 47, who lost the

most weight, was a long distance runner and began to increase his activity level as the study progressed. This increase in activity may explain his weight loss.

The radio-opaque faecal marker recoveries ensured that complete faecal collections were made. Not all subjects made complete urine collections, but the use of the PABA urine marker allowed any incomplete collections to be identified and omitted from the analysis.

Although the study was well controlled using urine and faecal markers, compliance to the diet was further ensured. Sodium is virtually completely absorbed by the gastrointestinal tract leaving only about one mmol to be excreted in the faeces each day (Guyton, 1985), therefore, urine sodium should closely reflect dietary sodium intake. Renal sodium excretion as a percentage of dietary sodium intake averaged 91%. Greater than 98% of sodium intake is expected when there is no sweating or diarrhoea (Luft, 1990). The weather during the study, however, was considerably warmer than the subjects were accustomed to, so allowances for significant sweating must be considered.

The present study had some limitations. First, the study was conducted on young healthy males. Calcium balance is most important to those individuals who are at greatest risk of developing osteoporosis.

The elderly and postmenopausal women are at greatest risk of developing osteoporosis. Second, there may have been a problem with the levels of calcium administered in the experimental diet. Although it was necessary to adjust the calcium intake to the subjects' usual calcium intake, all subjects had relatively high calcium intakes. It is advantageous to study calcium absorption and excretion at intakes that are marginal. Third, in nutritional research there is always the problem concerning the appropriateness of the control diet. In the present study, soy protein isolate was used in the control diet to replace the protein of the lentils. It was chosen to control for sulphur amino acids, but unintentionally controlled for phytate.

In conclusion, lentils are a good source of insoluble fibre and are effective in increasing faecal bulk. Compared to an experimental control diet that is similar to a typical Canadian diet, the addition of a considerable amount of lentils to the diet does not appear to impair calcium absorption or calcium balance. Lentils have a significant effect on renal calcium excretion. Decreased renal sodium excretion, increased renal potassium excretion and increased phosphorus intake are believed to have caused some of the observed change in renal calcium excretion. The fermentation of lentil starch, through some unknown mechanism, is

speculated to have caused some of the change in renal calcium excretion.

Further study is recommended in the following areas. Since postmenopausal women are at greatest risk of developing osteoporosis, a balance study involving this portion of the population would supply useful data. In addition, this study should involve individuals with marginal calcium intakes. It would be useful to study the effects of lentils using animal protein in the control diet to replace the protein of the lentils since Canadians are still thought to consume an excess of animal protein. Finally, although there is some research on the dietary factors that influence renal calcium excretion, further studies are needed to outline the exact physiological mechanisms by which sodium, potassium, phosphorus and starch influence renal calcium excretion.

6. References

Adams, N.D., Gray, R.W. & Lemann, J.Jr. (1979). The calciuria of increased fixed acid production in humans: Evidence against a role for parathyroid hormone and 1,25(OH)2-vitamin D. Calcified Tissue International, 28, 233-238.

Andersson, H, Navert, B, Bingham, S.A., Englyst, H.N., Cummings, J.H. (1983). The effects of breads containing similar amounts of phytate but different amounts of wheat bran on calcium, zinc and iron balance in man. British Journal of Nutrition, 50, 503-510.

Baron, R. (1993). Anatomy and ultrastructure of bone. In M.J. Favus (Ed.), Primer on the Metabolic Bone Diseases and Disorders of Mineral Metabolism (pp. 3-9). New York, NY: Raven Press, Ltd.

Beal V.A. (1986) Nutrition through the Life Span (p. 192). New York: Macmillian Publishing Company.

Behall, K.M., Scholfield, DJ, Lee, K, Powell, AS, Moser, P.B. (1987). Mineral balance in adult men: Effect of four refined fibres. American Journal of Clinical Nutrition, 46, 307-314.

Bhatty, R.S. (1988). Composition and quality of Lentil (*Lens culinaris* Medik): A review. Canadian Institute of Food Science and Technology, 21, 144-160.

Bingham, S, & Cummings, J.H. (1983). The use of 4-aminobenzoic acid as a marker to validate the completeness of 24 hr urine collections in man. Clinical Science, 64, 629-635.

Bogert, L.J. & Kirkpatrick, E.E. (1922). Studies in inorganic metabolism: III The effects of acid-forming and base-forming diets upon calcium metabolism. Journal of Biological Chemistry, 54, 375-386.

Branch, W.J., Cummings, J.H. (1978). Comparison of radio-opaque pellets and chromium sesquioxide as inert markers in studies requiring accurate faecal collections. Gut, 19, 371-376.

Bratton, A.C. & Marshall, E.K. (1939). A new coupling component for sulphanilamide determination. Journal of Biological Chemistry, 128, 537-541.

Breslau, N.A., Brinkley, L. Hill, K.D., & Pak, C.Y.K. (1987). Relationship of animal protein-rich diet to kidney stone formation and calcium metabolism. Journal of Clinical Endocrinology and Metabolism, 66, 140-46.

Breslau, N.A., McGuire, J.L., Zerwekh, J.E. & Pak, C.Y.C. (1982) The role of dietary sodium on renal excretion and intestinal absorption of calcium on vitamin D metabolism. Journal of Clinical Endocrinology and Metabolism, 55, 369-373.

Brodribb, J.M. (1980). Dietary fibre in diverticular disease of the colon. In G.A. Spiller and R.M. Kay (Eds.), Medical Aspects of Dietary Fibre, (pp. 43-66). New York: Plenum Press.

Brodribb, J.M. & Humphreys, D.M. (1976) Diverticular disease: Three studies: Part 1: Relation to other disorders and fibre intake. British Medical Journal, 1, 424-430.

Bronner, F. (1989). Renal calcium transport: Mechanisms and regulation - an overview. American Journal of Physiology, 257, 707-711.

Bronner, F. (1990). Intestinal calcium transport: The cellular pathway. Mineral and Electrolyte Metabolism, 16, 94-100.

Bronner, F., Pansu, D. & Stein, W.D. (1986). An analysis of intestinal calcium transport across the rat intestine. American Journal of Physiology, 250, G561-G569.

Burkitt, D.P. (1971). Epidemiology of cancer of the colon and rectum. Cancer, 28, 3.

Calvo, M.S. (1993). Dietary phosphorus, calcium metabolism and bone. Journal of Nutrition, 123, 1627-1633.

Chan, J.C.M. (1972). The rapid determination of urinary titratable acid and ammonium and evaluation of freezing as a method of preservation. Clinical Biochemistry, 5, 94-98.

Charles, P., Eriksen, E.F., Hasling, C., Sondergard, K. & Mosekilde, L. (1991). Dermal, intestinal, and renal obligatory losses of calcium: Relation to skeletal calcium loss. American Journal of Clinical Nutrition, 54, 266S-273S.

Cummings, J.H. (1978). Diet and transit through the gut. Journal of Plant Foods, 3, 83-95.

Cummings, J.H., Hill M.J., Jivraj, T, Houston, H, Branch, W.J., Jenkins, D.J.A. (1979). The effect of meat protein and dietary fibre on colonic function and metabolism 1. Changes in bowel habit, bile acid excretion, and calcium absorption. American Journal Clinical Nutrition, 32, 2086-2093.

Cummings, J.H., Jenkins, D.J.A. & Wiggins, H.S. (1976). Measurement of the mean transit time of dietary residue through the gut. Gut, 17, 210-19.

Cummings, J.H., Southgate, D.A.T., Branch, W.J. Wiggins, H.S., Houston, H., Jenkins, D.J.A., Jivraj, T. & Hill, M.J. (1979). The digestion of pectin in the human gut and its effect on calcium absorption and large bowel function. British Journal of Nutrition, 41, 477-485.

Cummings, J.H. & Stephen, A.M. (1980). the role of dietary fibre in the human colon. Canadian Medical Association Journal, 123, 1109-1114.

Draper, H.H., Piche, L.A, & Gibson, R.S. (1991). Effects of a high protein intake from common foods on calcium metabolism in a cohort of postmenopausal women. Nutrition Research, 11, 273-281.

Englyst, H. (1981) In James W. P. T. & Theander, O. (ed.), The Analysis of Dietary Fibre in Food. New York, New York: Dekker.

Englyst, H.N. & Cummings, J.H. (1990). Dietary fibre and starch: Definition, classification and measurement. In Leeds, A.R. (ed), Dietary Fibre Perspectives - Reviews and Bibliography, vol 2, (p. 20) London: John Libby Pr.

Englyst, H.N., Trowell, H., Southgate, D.A.T. & Cummings, J.H. (1987). Dietary fibre and resistant starch. American Journal Clinical Nutrition, 46, 873-874.

Englyst, H.N., Wiggins, H.S. & Cummings, J.H. (1982). Determination of the non-starch polysaccharides in plant foods by gas liquid chromatography of constituent sugars as alditol acetates. Analyst, 107, 307-318.

Farquharson, R.F., Salter, W.T. & Aub, J.C. (1931). Studies of calcium and phosphorus metabolism. 13. The effect of ingestion of phosphates on the excretion of calcium. Journal of Clinical Investigation, 10, 251-269.

Godara, R., Kaur, A.P. & Bhat, C.M. (1981). Effects of cellulose incorporation in a low fiber diet on faecal excretion and serum levels of calcium, phosphorus, and iron in adolescent girls. American Journal Clinical Nutrition, 34, 1083-1086.

Guthrie, H.A. (Ed.) (1989). Introductory Nutrition (7 ed.) (p. 79). St. Louis, Missouri: Time Mirror/Mosby College Publishing.

Guyton, A. (Ed.) (1985). Textbook of Medical Physiology (7 ed.). Toronto: W.B. Saunders Company.

Hallfrisch, J., Powell, A., Carafelli, C. Reiser, S. & Prather, E.S. (1987). Mineral Balances of men and women consuming high fibre diets with complex or simple carbohydrate. Journal Nutrition, 117, 48-55.

Harland, B.F. & Oberleas, D. (1986). Anion-exchange method for the determination of phytate in foods:collaborative study. Journal of the Association of Official Analytical Chemistry, 69, 667.

Heaney, R.P., Gallagher, J.H. & Johnson, C.C.Jr., Neer, R., Parfitt, A.M. & Whedon, G.D. (1982). Calcium nutrition and bone health in the elders. American Journal of Clinical Nutrition, 36, 986-1013.

Heaney, R.P., Recker, R.R. (1982). Effects of nitrogen, phosphorus and caffeine on calcium balance in women. Journal of Laboratory Clinical Medicine, 99, 46-55.

Heaney, R.P., Weaver, C.M. & Fitzsimmons, M.L. (1990). Influence of calcium load on absorptive fraction. Journal of Bone and Mineral Research, 5, 1135-1138.

Heaney, R.P., Weaver, C.M., & Fitzsimmons, M.L. (1991). Soybean phytate content: Effect on calcium absorption. American Journal of Clinical Nutrition, 53, 745-747.

Hegstead, M.S., Schuette, M., Zemel, M.B. & Linkswiler, H.M. (1981). Urinary Ca balance in young men as affected by level of protein and phosphorus intake. Journal of Nutrition, 111, 553-562.

Heyburn, P.J. Robertson, W.G. & Peacock, M. (1982). Phosphate treatment of recurrent calcium stone disease. Nephron, 32, 314-319.

Hill, A.D., Patterson, K.Y., Veillon, C. & Morris, E.R. (1986). Digestion of biological materials for mineral analyses using a combination of wet and dry ashing. Analytical Chemistry, 58, 2340-2342.

Hillman, C. Peters, S.G., Fisher, C.A. & Pomare, E.W. (1985). The effects of the fiber components pectin, cellulose and lignin on serum cholesterol levels. American Journal Clinical Nutrition, 42, 207-213.

Hinds, J.P., Stoney, B. & Wald, A. (1989). Does gender or the menstrual cycle affect colonic transit? American Journal of Gastroenterology, 27, 123-126.

Holl, M.G. & Allen, L.H. (1987). Sucrose ingestion, insulin response and mineral metabolism in humans. Journal of Nutrition, 117, 1129-1233.

Ismail-Beigi, F., Reinhold, J.G. Faraji, B. & Abadi, P. (1977). Effects of cellulose added to diets of low and high fiber content upon the metabolism of calcium, magnesium, zinc and phosphorus by man. Journal of Nutrition, 107, 510-518.

Jenkins, J.A., Leeds, A.R., Newton, C., Cummings, J.H. (1975). Effect of pectin, guar gum and wheat fibre on serum cholesterol. Lancet, , 1116-1117.

Kelsay, J.L., Behall, K.M. & Prather, E.S. (1979). Effect of fiber from fruits and vegetables on metabolic responses of human subjects II. Calcium, magnesium, iron, and silicon balances. American Journal Clinical Nutrition, 32, 1876-1880.

Kelsay, J.L. & Prather, E.S. (1983). Mineral balances of human subjects consuming spinach in a low-fiber diet and in a diet containing fruits and vegetables. American Journal of Clinical Nutrition, 38, 12-19.

Kelsay, J.L., Prather, E.S., Clark, W.M. & Canary, J.J. (1988). Mineral balances of men fed a diet containing fiber in fruits and vegetables and oxalic acid in spinach for six weeks. Journal of Nutrition, 118, 1199-1204.

Krause, M.V. & Mahan, L.K. (1979). Food, Nutrition and Diet Therapy. (p. 901) Philidelphia, PA: W.B. Saunders Company.

Lemann, J. Jr., Adams, N.D. & Gray, W. (1979). Urinary calcium excretion in human beings. The New England Journal of Medicine, 301, 535-541.

Lemann, J. Jr., Gray, R.W. & Pleuss, J.A. (1989). Potassium bicarbonate, but not sodium bicarbonate, reduces urinary calcium excretion and improves calcium balance in healthy men. Kidney International, 35, 688-695.

Lemann, J.Jr., Litzow, J.R. & Lennon, E.J. (1967). Studies of the mechanism by which chronic acidosis augments urinary calcium excretion in man. Journal of Clinical Investigation, 46, 1318-1328.

Lemann, J. Jr., Pleuss, J.A. & Gray, R.W. (1993). Potassium causes calcium retention in healthy adults. Journal of Nutrition, 123, 1623-1626.

Loas, G.M., Palamidis, N. & Markakis, P. (1976). The phytic acid-total phosphorus relationship in barley, oats, soybeans and wheat. Cereal Chemistry, 53, 867-871.

Luft, F. (1990). Sodium, chloride, and potassium. In M. Brown (Eds.), Present Knowledge in Nutrition (pp. 23-240). Washington: International Life Sciences Institute-Nutrition Foundation.

McCance, R.A. & Widdowson, E.M. (1942). Mineral metabolism of healthy adults on white and brown bread dietaries. Journal of Physiology, 101, 44-85.

Macfarlane, G.T. & Englyst, H.N. (1986). Starch utilization by the human large intestinal microflora. Journal of Applied Bacteriology, 60, 195-201.

Massey, L.K. (1993). Dietary factors influencing calcium and bone metabolism: Introduction. Journal of Nutrition, 123, 1609-1610.

Murphy, S.P. & Calloway, D.H. (1986). Nutrient intakes of women in NHANES II, emphasizing trace minerals, fiber, and phytate. Journal of the American Dietetic Association, 86, 1366-1371.

Nellans, H.N. (1990). Intestinal calcium absorption: Interplay of paracellular and cellular pathways. Mineral and Electrolyte Metabolism, 16, 101-108.

Nordin, B.E.C. & Marshall, D.H. (1988). Dietary requirements for calcium. In B.E.C. Nordin (Ed.), Calcium in Human Biology (pp. 447-471). New York: Springer-Verlag.

Nordin, B.E.C., Need, A.G. Morris, H.A. & Horowitz, M. (1993). The nature and significance of the relationship between urinary sodium and urinary calcium in women. Journal of Nutrition, 123, 1615-1622.

Painter , N.S. & Burkitt, D.P. (1971). Diverticular disease of the colon: A deficiency of western civilization. British Medical Journal, 2, 450.

Pennington, J.A.T. (Ed.)(1994). Bowes & Church's Food Values of Portions Commonly Used.. Philidelphia: J.B. Lippincott Company.

Peraino, R.A. & Suki, W.N. (1980). Urine HCO_3- augments renal Ca^{+2} absorption independent of systematic acid-base changes. American Journal of Physiology, 238, F394-F398.

Raman, A.& Chong, Y.K. (1974). Determination of calcium in serum and urine with an automatic calcium titrator. Clinical Biochemistry, 7, 106-111.

Riggs, B.L. & Melton, L.J. III. (1986). Involutional osteoporosis. New England Journal of Medicine, 306, 446-450.

Ruppin, H., Bar-Meir, S., Soergel, K.H., Wood, C.M. & Schmitt, M.G.Jr. (1980). Gastroenterology, 78, 1500-1507.

Sakhaee, K. Nicar, M. Hill, K. & Pak, C.Y.C. (1983). Contrasting effects of potassium citrate and sodium citrate therapies on urinary chemistries and crystallization of stone-forming salts. Kidney International, 24, 348-352.

Scientific Review Committee (1990). Nutrition Recommendations. Ottawa: Minister of Supply and Services Canada.

Schweizer, T.F., Bekhechi, A.R. Koellreutter, B., Reimann, S., Pometta, D. & Bron, B.A. (1983). Metabolic effects of dietary fiber from dehulled soybeans in humans. American Journal Clinical Nutrition, 38, 1-11.

Shah, P.J.R., Williams, G. & Green, N.A. (1980). Idiopathic hypercalciuria: Its control with unprocessed bran. British Journal of Urology, 52, 426-429.

Shetty, P.S. & Kurpad, A.V. (1986). Increasing starch intake in the human diet increases faecal bulking. American Journal Clinical Nutrition, 43, 210-212.

Shohl, A.T. & Sato, A. (1923). Acid-base metabolism II. Mineral metabolism. Journal of Biological Chemistry, LVIII, 257-266.

Slavin, J.L., Marlett, J.A. (1980). Influence of refined cellulose on human bowel function and calcium and magnesium balance. Nutrition, 33, 1932-1939.

Spencer, H., Kramer, L., Rubio, N. & Osis. (1986). The effect of phosphorus on endogenous faecal calcium excretion in man. American Journal Clinical Nutrition, 43, 844-851.

Spencer, H., Norris, C., Derler, J. & Osis, D. (1991). Effect of oat bran muffins on calcium absorption and calcium, phosphorus, magnesium and zinc balance in men. Journal of Nutrition, 121, 1976-1983.

Spiller, G.A. & Shipley, E.A. (1977). Perspectives in dietary fibre in human nutrition. World Review of Nutrition and Diet, 27, 105.

Stephen, A. (1990). Starch in human nutrition. NIN Review No. 15, 5, 1-4.

Sutton, R.A.L., Wong, N.L.M. & Dirks, J.H. (1979). Effects of metabolic acidosis and alkalosis on sodium and calcium transport in the dog kidney. Kidney International, 15, 520-533.

Sutton, R.A.L. & Dirks, J.H. (1986). Calcium and magnesium: Renal handling and disorders of metabolism, In Breener, B.M. & Rector, F.C. (eds.), The Kidney, Third Edition, Volume 1, Philidelphia, PA: W.D. Saunders Company.

Tietz, N. (1987). Fundamentals of Clinical Chemistry, Philidelphia: W.B. Saunders Company.

Trowell, H.D., Southgate, A.T., T.M.S. Wolever, T.M.S., Leeds, A.R., Gassel, M.A. & Jenkins, D.J.A. (1976). Dietary fibre redefined. Lancet, 1, 967.

Tsai, A.C., Mott, E.L., Owen, G.M., Bennick, M.R., Lo, G.S. & Steinke, F.H. (1983). Effects of soy polysaccharide on gastrointestinal functions, nutrient balance, steroid excretions, glucose tolerance, serum lipids, and other parameters in humans. American Journal of Clinical Nutrition, 38, 504-511.

Van Dokkum, W., Wesstra, A. & Schippers, F.A. (1982). Physiological effects of fibre-rich bread. 1. The effect of dietary fibre from bread on mineral balance of young men. British Journal of Nutrition, 47, 451-460.

Vander, A.J. (1985). Regulation of calcium balance and extracelluar concentration in Renal Physiology, section 10, New York, NY: McGraw Hill.

Wardlaw, G.M., Insel, P.M. & Seyler, M.F. (1992). Contemporary Nutrition: Issues and Insights, St. Louis, MO.: Mosby-Year Book, Inc.

Wisker, E., Nagel, R., Taudjaja, T.K. & Feldheim, W. (1991). Calcium, Magnesium, zinc, and iron balances in young women: Effects of a low-phytate barley fibre concentrate. American Journal of Clinical Nutrition, 54, 553-559.

Whitby, L.G. & Lang, D. (1960). Experience with the chromic oxide method of faecal marking in metabolic balance investigations on humans. Journal of Clinical Investigation, 39, 854-863.

Whiting, S.J. & McNally, M.E. (1989). Calciuric effect of diets high in soy, beef or lactalbumin protein in the rat. Nutrition Reports International, 40, 199-205.

Zarkadas, M., Gougeon-Reyburn, R., Marliss, E.B., Block, E. & Alton-Mackey, M. (1989). Sodium chloride supplementation and urinary calcium excretion in postmenopausal women. American Journal of Clinical Nutrition, 50, 1088-94.

Appendix 1. Ethics Committee Approval Form

UNIVERSITY ADVISORY COMMITTEE ON ETHICS IN HUMAN EXPERIMENTATION

me and E.C. File #: A.M. Stephen 90-08 February 20, 1990

ur project entitled: The effect of lentils on gastrointestinal function, lipid metabolism and mineral balance in human subjects.

s been approved by the Committee.

Therefore you are free to proceed with the project subject to the llowing conditions:

Approved.

Please submit the revisions requested above to the Director of Research rvices, Room 50, Murray Building.

Any significant changes of your protocol should be reported to the rector of Research Services for Committee consideration in advance of its plementation.

ncerely,

. E.A. McKenna, Chairman
iversity Advisory Committee on
hics in Human Experimentation

Appendix 2. Consent Form

University of Saskatchewan

DIVISION OF NUTRITION AND DIETETICS
College of Pharmacy

CONSENT FOR PARTICIPATION IN A HUMAN STUDY

I, _____

the undersigned, agree to participate in a study entitled "The effect of lentils on gastrointestinal function, lipid metabolism and mineral metabolism in healthy human subjects," under the supervision of Dr. A. M. Stephen at the University of Saskatchewan.

The study covers a period of 42 days (divided into two three week periods) during which time:

1. I will eat a controlled dietary intake which will be prepared in the Division of Nutrition and Dietetics and provided to me everyday. I must not eat any food other than that which is given to me. The amount of food will be that which will maintain my weight, it will be a typical Canadian intake and will provide me with sufficient levels of all essential nutrients.

2. I will take radioopaque pellets, 30 each day (as 3 capsules each containing 10 pellets) throughout the 42 day period to allow the researchers to measure intestinal transit time and to correct for the infrequency of bowel movements, when they analyze compounds in stools.

3. For 21 days of the 42, I will consume 120-130 g per day of dry lentils which will be incorporated in some of the foods in my diet. (This amount of dietary fibre on top of my intake on the control period will be equivalent to the recommendation for the amount of fibre Canadians should be consuming each day.)

4. I will have 20 mL of venous blood drawn on the first day of the study and on 2 days at the end of each 3 week period, i.e. 5 times in total.

5. I will make stool collections throughout the 6 weeks of the study to allow the investigators to measure faecal output and transit time and for one week after the diet period is over to ensure the complete collection of markers.

6. Although it is desirable that the 3 week periods follow right after one another, it is possible that some time be left between the 2 periods if I find this more convenient.

7. I will make 24h urine collections throughout weeks 3 and 6 of the study to allow the investigators to measure urine minerals.

8. I will take Pabacheck tablets (p-amino benzoic acid), 3 tablets per day each containing PABA 80 mg throughout weeks 3 and 6 to allow researchers to measure completeness of urine collection.

I have been informed that there are no known harmful effects of taking this amount of dietary fibre and that the researcher has administered similar doses to volunteers in the past.

I have been informed that there are no known harmful effects of taking radioopaque pellets or PABACHECK markers and that these are used routinely in human nutritional and gastrointestinal experiments.

I understand that I may experience some slight bruising at the site of needle insertion when blood is drawn, but that any further complications from blood taking are extremely rare.

I understand that it is hoped to obtain information about the effect of lentils on gastrointestinal function, serum cholesterol and mineral metabolism. My participation will result in no direct benefit to me.

I consent to participate in this study knowing that:

1. if I notice any irregularities or have any abdominal pain, or am required to take medications during the period of the study, I will inform the investigators.

2. if there are any problems or concerns, I can contact Dr. Stephen at any time at 966-5847 (work) or 373-5640 (home).

3. I am free to withdraw from the study at any time.

I have read the above and fully understand its contents which have been explained to me by _____.

_____ (participant)

_____ (witness)

_____ (date)

_____ (investigator)

Appendix 3. Diary Instructions

STUDY DIARY AND CHECKLIST

In this diary, there is a space allocated for each day you are taking part. For each day, there is achecklist for each procedure you have to complete. There is also space for any notes you want to make.

Tick off each procedure when you have done it.

Make sure that times for stool samples are marked a.m. or p.m. or use the 24 hour clock.

NOTES: We are interested in knowing:

1. any problems you had with the food
2. any medications you took and why. Include all details of amount and how often you took them.
3. anything unusual you noticed about your bowel movements
4. any unusual exercise you took part in - heavy exertion, competition etc.
5. any special events you attended or visits to the doctor or dentist
6. any sickness or injury - cold, flu, toothache, sprained ankle etc.

DAY ☐ DATE: _____

MARKERS TAKEN: 8 A.M. ☐ 12 NOON ☐ 6 P.M. ☐

STOOL SAMPLE: ☐ TIME: _____ _____ _____

NOTES:

DAY ☐ DATE: _____

MARKERS TAKEN: 8 A.M. ☐ 12 NOON ☐ 6 P.M. ☐

STOOL SAMPLE: ☐ TIME: _____ _____ _____

NOTES:

Appendix 4. Calculated Nutrient Content of Metabolic Diet

LENTIL STUDY - METABOLIC DIET

i) Basic Daily

FOOD ITEM	Wt g	ENERGY kcal	kJ	Protein g	CHO g	Fat g	Starch g	Sugars g	DIETARY FIBRE g NSP	Ca	Zn
Orange Juice	500	224	936	3.8	53.2	---	---	53.2	---	48	1.0
Sugar	20	77	322	---	19.9	---	---	19.9	---	--	—
White Bread	50	108	454	3.8	23.4	---	21.9	1.5	0.84	43	0.85
Butter	20	143	596	---	---	15.7	---	---	---	5	0.03
Cheese Cheddar	40	160	669	9.6	---	13.2	---	---	---	289	1.60
Iceberg Lettuce	40	6	22	0.4	---	---	---	---	0.36	27	0.08
Cucumber	20	3	11	0.1	0.5	---	---	0.5	0.10	3	0.02
Canned Beets	20	6	25	0.4	1.4	---	---	1.4	0.50	3	0.02
Salad Dressing	8	53	222	---	---	6.0	---	---	---		
Potato Cooked	80	70	293	1.2	15.9	---	15.6	0.3	1.02	4	0.16
Apple (no skin)	80	37	155	0.3	9.5	---	0.1	9.5	1.28	6	0.08
Ice Cream(10% BF)	50	101	424	2.1	12.1	5.7	1.1	11.0	---	66	0.20
Cornflakes	20	74	308	1.1	16.8	---	15.5	1.4	0.18	---	0.06
Milk 2%	500	248	1037	17.4	23.2	9.7	---	23.2	---	609	1.75
TOTAL BASIC		1310	5476	40.2	175.9	50.3	54.2	121.9	4.28	1103	5.91

ii) Day 1

FOOD ITEM	Wt g	ENERGY kcal	kJ	Protein g	CHO g	Fat g	Starch g	Sugars g	DIETARY FIBRE g NSP	Ca	Zn
Apricots (canned)	120	100	417	0.4	25.9	---	---	25.9	1.56	11	0.12
Pork	30	73	305	7.7	---	4.5	---	---	---	2	1.05
Broccoli	50	14	60	1.4	2.8	---	0.2	2.6	1.35	57	0.20
Carrots (frozen)	50	18	75	0.6	4.2	---	0.1	4.1	1.25	14	0.15
TOTAL		205	857	10.1	32.9	4.5	0.3	32.6	4.16	84	1.52
+ Lentil Product		1270		46.9	192.4	36.4			16.01	154	5.35
+ Basic Daily		1310		40.2	175.9	50.3			4.28	1103	5.91
Day 1 Total		2785		97.2	401.2	91.2			24.45	1341	12.78
				14.0%	57.6%	29.5%					

iii) Day 2

FOOD ITEM	Wt g	ENERGY kcal	kJ	Protein g	CHO g	Fat g	Starch g	Sugars g	DIETARY FIBRE g NSP	Ca	Zn
Fruit Cocktail	100	72	301	0.4	19.0	---	---	19.0	1.50	6	---
Chicken Breast	35	58	242	11.0	---	1.2	---	---	---	5	0.35
Green Beans	60	16	67	0.8	3.8	---	---	3.8	1.38	27	0.18
Coleslaw	69	---	---	---	---	---	---	---	---	---	---
Cabbage	30	8	33	---	1.6	---	0.1	1.6	0.87	14	0.12
Carrot	15	6	25	0.2	1.6	---	0.1	1.6	0.36	4	0.06
Mayonnaise	6	43	180	---	---	4.7	---	---	---	---	0.02
TOTAL		203	849	12.4	26.0	5.9	0.2	26.0	4.11	56	0.73
+ Lentil Products		1237		44.3	177.8	36.3			15.59	161	6.32
+ Basic Daily		1310		40.2	175.9	50.3			4.28	1103	5.91
Day 2 Total		2750		96.9	379.7	92.5			23.98	1320	12.96
				14.0%	55.2%	30.3%					

iv) Day 3

FOOD ITEM	Wt g	ENERGY kcal	kJ	Protein g	CHO g	Fat g	Starch g	Sugars g	DIETARY FIBRE g NSP	Ca	Zn
Peaches (canned) with syrup)	140	104	447	0.5	28.0	---	---	---	1.40	4	---
Beef	30	55	656	8.2	---	3.1	---	---	---	3	1.65
Mushrooms (canned)	40	9	51	0.7	1.9	---	---	2.4	.44	4	0.04
Cauliflower	80	15	88	---	6.7	---	---	6.7	1.28	13	0.16
TOTAL		183	765	9.4	36.6	3.1	---		3.12	24	1.85
+ Lentil Products		1282		41.1	185.8	41.6			16.65	166	5.81
+ Basic Daily		1310		40.2	175.9	50.3			4.28	1103	5.91
Day 3 Total		2775		90.8	398.3	95.0			24.05	1293	13.57
				13.1%	57.4%	30.8%					

Day 1 Loaf (Lentil Burgers #4)

FOOD ITEM	Wt g	ENERGY kcal	kJ	Protein g	CHO g	Fat g	Starch g	Sugars g	DIETARY FIBRE g NSP	Ca	Zn
Lentils	500	530	---	37.9	97.2	2.4	---	---	17.50	126	5.95
Breadcrumbs (white)	40	108	---	28.8	20.0	---	---	---	0.60	34	0.32
Oatmeal (dry)	30	115	---	4.9	20.2	2.1	---	---	2.13	15	0.90
Onion (raw)	60	20	---	0.8	4.2	---	---	---	---	15	0.06
Fat (Butter)	50	350	---	---	---	39.3	---	---	---	12	0.08
Egg	50	79	330	6.0	---	6.0	---	---	---	28	0.75
TOTAL		1202		78.4	141.6	44.8			21.43	230	8.06
PORTION	/5	240		15.7	28.3	10.0			4.29	46	1.61

Day 1 Cake (Lentil Banana Loaf)

		ENERGY							DIETARY FIBRE g		
FOOD ITEM	Wt g	kcal	kJ	Protein g	CHO g	Fat g	Starch g	Sugars g	NSP	Ca	Zn
Fat (Butter)	100	700		---	---	78.6	---	---	---	22	0.15
White Sugar	200	744		---	194	---	---	194	---	---	---
Egg	100	168	660	12.0	---	---	---	---	---	56	1.50
Lentils	400	425	---	30.3	77.7	1.9	---	---	14.00	100	4.76
Flour (White)	280	1019		29.5	212.6	2.1	208.5	4.0	8.6	44	1.96
Milk (2%)	70	35		2.4	3.3	1.4			---	85	0.24
Banana	225	207		2.0	53.3	---			2.48	14	
TOTAL		3298		76.2	540.9	84.0			25.08	321	8.61
PORTION	/4	824		19.0	135.2	21.0			6.27	80	2.15
CAKE & LOAF		1064		34.7	163.5	31.0			10.56	126	3.76

Day 1 Soup (Lentil Soup #1)

FOOD ITEM	Wt g	ENERGY kcal	kJ	Protein g	CHO g	Fat g	Starch g	Sugars g	DIETARY FIBRE g NSP	Ca	Zn
Lentils	200	608		47.6	106.4	2.0	101.6	4.8	18.20	78	6.2
Carrots	25	11		---	2.4	---			0.60	7	0.10
Turnip	50	14		0.4	3.3	---			1.99	15	---
Onion	50	17		0.7	3.5	---			1.00	12	0.05
Margarine	25	175		---	---	19.7	---	---	---	---	---
TOTAL		825		48.7	21.7	115.6			21.79	112	6.35
PORTION	/4	206		12.2	5.4	28.9			5.45	28	1.59
+ Day 2 Cake and Loaf		1064		34.7	163.5	31.0			10.56	126	3.76
Total Lentil Products		1270		46.9	192.4	36.4			16.01	154	5.35

Day 2 Loa

FOOD ITEM	Wt g	ENERGY kcal	kJ	Protein g	CHO g	Fat g	Starch g	Sugars g	DIETARY FIBRE g NSP	Ca	Zn
Lentils	500	530		37.9	97.2	2.4			17.50	126	5.95
Cheese Cheddar	130	523		31.7	---	43.3			---	135	5.20
Onion (raw)	40	13		0.5	2.8	---	---		0.80	10	0.04
Egg	50	79	330	6.0	---	6.0	---	---	---	28	0.75
Breadcrumbs (white)	50	135		3.6	25.0	---			0.75	43	0.40
Carrots	80	34		---	7.8	---			1.92	21	0.32
TOTAL		1314		79.7	132.8	51.7			20.97	363	12.66
PORTION	/5	263		15.9	26.6	10.3			4.19	73	2.53

Day 2 Cake (Nut Bread)

FOOD ITEM	Wt g	ENERGY kcal	kJ	Protein g	CHO g	Fat g	Starch g	Sugars g	DIETARY FIBRE g NSP	Ca	Zn
Lentils	400	425		30.3	77.7	1.9			14.0	100	4.76
Butter	100	700		---	---	78.6	---	---	---	22	0.15
Sugar White	200	744		---	194	---	---	194	---	---	---
Eggs	100	168	660	12.0	---	---	---	---	---	56	1.50
Flour White	250	910		26.3	189.8	1.9	182.6	3.6	7.7	39	1.75
TOTAL		2947		68.6	461.5	82.4			21.70	217	8.16
PORTION	/4	737		17.2	115.4	20.6			5.42	54	2.04
CAKE & LOAF		1000		31.7	142.0	30.9			9.61	127	4.57

Day 2 Soup (Lentil Soup #3)

FOOD ITEM	Wt g	ENERGY kcal	kJ	Protein g	CHO g	Fat g	Starch g	Sugars g	DIETARY FIBRE g NSP	Ca	Zn
Lentils	200	608		47.6	106.4	2.0	101.6	4.8	18.20	78	6.2
Onion	100	34		1.4	7.0	---			2.00	24	0.10
Potato	100	86		1.5	20.0	---			1.30	8	0.30
Margarine	25	175		---	---	19.7	---	---	---	---	---
TOTAL		947		50.5	143.0	21.7			23.90	138	7.00
PORTION	/4	237		12.6	35.8	5.4			5.98	34	1.75
+ Day 1 Cake and Loaf		1000		31.7	142.0	30.9			9.61	127	4.57
Total Lentil Products		1237		44.3	177.8	36.3			15.59	161	6.32

Day 3 Loaf (Bean Loaf #2)

FOOD ITEM	Wt g	ENERGY kcal	kJ	Protein g	CHO g	Fat g	Starch g	Sugars g	DIETARY FIBRE g NSP	Ca	Zn
Lentils	500	530		37.9	97.2	2.4			17.50	126	5.95
Potatoes	225	193		3.3	45.0	---			2.92	18	0.68
Tomato Soup (Canned cond.)	200	140		3.1	27.9	3.1			---	20	0.60
Egg	50	79	330	6.0	---	---	---	---	---	28	0.75
Rolled Oats (Quick Cook)	80	307		13.1	53.9	5.6			5.68	40	2.40
Onion	170	57		2.3	11.9	---			3.40	42	0.17
Fat (Butter)	30	210		---	---	23.7			---	7	0.04
TOTAL		1516		65.7	235.9	40.8			29.50	281	10.59
PORTION	/5	303		13.1	47.2	8.2			5.90	56	2.12

Day 3 Cake (Carrot-Lentil Muffins)

FOOD ITEM	Wt g	ENERGY kcal	kJ	Protein g	CHO g	Fat g	Starch g	Sugars g	DIETARY FIBRE g NSP	Ca	Zn
Lentils	400	425	---	30.3	77.7	1.9			14.00	100	4.76
Eggs	100	168	660	12.0	---	---	---	---	---	56	1.50
White Sugar	220	818		---	213	---	---	213	---	---	---
Flour (White)	150	546		15.8	113.9	1.1	111.7	2.1	4.6	24	1.05
Carrot	120	51		---	11.7	---			2.88	32	0.48
Oil (corn)	120	1061		---	120	---	---	---	---	---	---
TOTAL		3069		58.1	416.3	123			21.48	212	7.79
PORTION	/4	767		14.5	104.1	30.8			5.37	53	1.95
CAKE & LOAF		1070		27.6	151.3	39.0			11.91	108	4.07

Day 3 (Lentil Soup #2)

FOOD ITEM	Wt g	ENERGY kcal	kJ	Protein g	CHO g	Fat g	Starch g	Sugars g	DIETARY FIBRE g NSP	Ca	Zn
Lentils	200	608		47.6	106.4	2.0	101.6	4.8	18.2	78	6.2
Onion	100	34		1.4	7.0	---			2.0	24	0.1
Potato	100	86		1.5	20.0	---			1.31	8	0.3
Milk (2%)	100	62		3.5	4.7	1.9			---	122	0.35
Margarine	8	56		---	---	6.3	---	---	---	---	---
TOTAL		846		54.0	138.1	10.2			21.51	232	6.95
PORTION	/4	212		13.5	34.5	2.6			5.38	58	1.74
+ Day 3 Cake and Loaf		1070		27.6	151.3	39.0			11.27	108	4.07
Total Lentil Products		1282		41.1	185.8	41.1			16.65	166	5.81

Appendix 5. Faecal X-ray

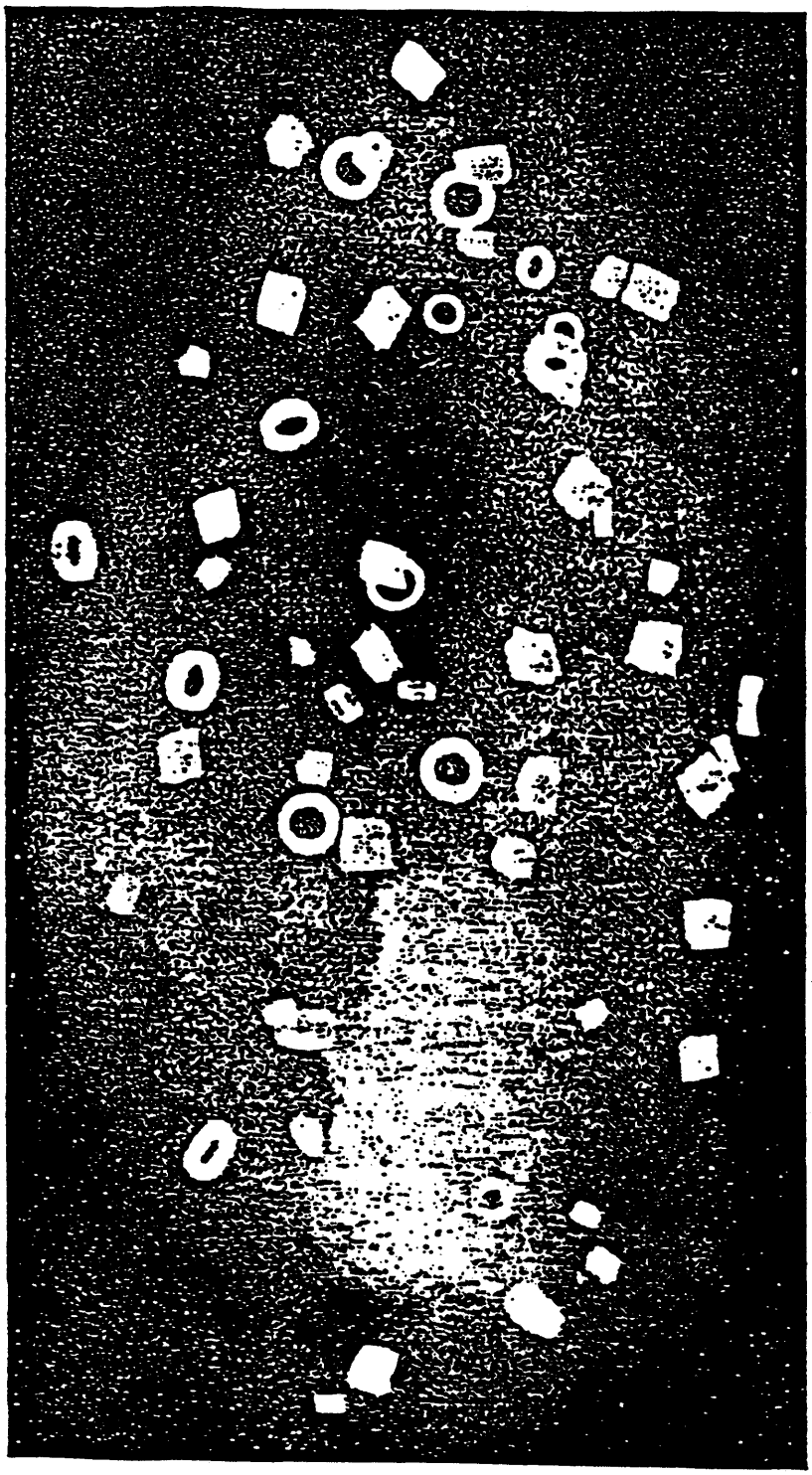


Fig. 1 *Radiograph of a stool containing all four types of marker. These are evenly distributed and clearly distinguishable in the stool.*

Appendix 6. Mean Transit Time

Mean transit time

Mean transit time (MTT) is calculated according to the continuous marker method of Cummings et al. (29). Briefly, this method measures turnover time, taking into account the times and numbers of markers taken in during each day and the times and numbers of markers excreted.

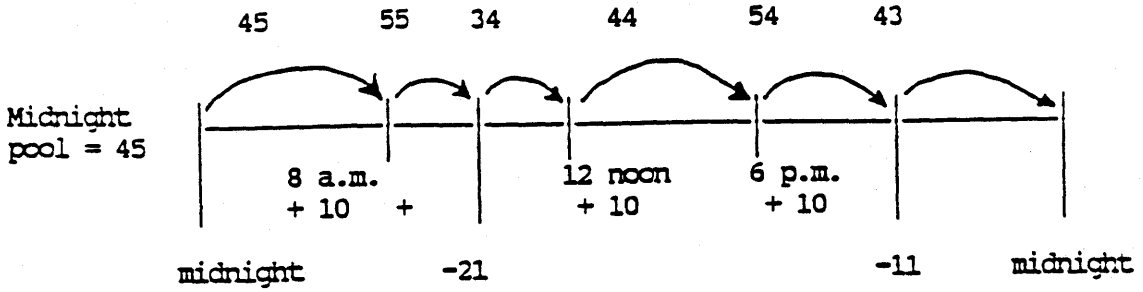
$$\text{MTT} = \text{Turnover time} = \frac{\text{Mean Marker Pool Size}}{\text{Number of markers ingested daily}} \times 24 \text{ (h)}$$

Mean Marker Pool (MMP) = Sum of products of number of markers and the period of time during which that number of markers are present in the gut.

The total number of periods is determined by the frequency with which the number of markers present changes either by ingestion or defecation and includes the final period from the last change to midnight.(29)

A typical day might be:

8:00 a.m.	10 markers in
10:30 a.m.	faecal sample - 21 markers out
12:00 noon	10 markers in
6:00 p.m.	10 markers in
9:30 p.m.	faecal sample - 11 markers out



$$\text{MTT} = \frac{(45 \times 8)+(55 \times 1.5)+(34 \times 1.5)+(44 \times 6)+(54 \times 2.5)+(43 \times 1.5)+(32 \times 2.5)}{30}$$

$$= \frac{360 + 82.5 + 51 + 264 + 135 + 64.5 + 80}{30}$$

$$= \frac{1037}{30}$$

$$= 34.6 \text{ h}$$

Markers come in different shapes (see Appendix C) and can therefore be changed when periods change or at any other time. Markers can be changed after one week into the study, which allows the investigators to determine if the subjects are collecting their faecal samples correctly, since in one week they should excrete 210 markers (intake = 30/d x 7 days).

At the end of the study, the total number of markers excreted is calculated for: i) the third week of each 3 week period, ii) for each total 3 week period, and iii) for the entire study.

i) The excretion in the third week of each 3 week period is used to correct the mean daily faecal weight of the third week for the infrequency of bowel movements.(28) Since 210 markers are taken in a week, in an ideal situation, 210 markers should be excreted. However, because bowel movements occur infrequently, somewhat less or more than 210 markers may be excreted. Faecal outputs are therefore corrected using a marker correction factor:

$$\text{Marker Correction Factor (MCF)} = \frac{210}{\text{Number of markers excreted in weeks}}$$

$$\text{Marker Corrected Faecal Weight (MCFW)} = \text{Mean daily faecal weight} \times \text{MCF}$$

ii) The total number of markers excreted in each three week period were used to estimate total recovery. In addition, mean transit time calculations assume a 100% recovery of markers. Therefore the difference in markers between the actual excretion and 100% is added back to the stools excreted, distributed according to marker excretion.

iii) The total number of markers excreted in the total study gives the marker recovery for that subject and allows the investigators to be certain that the subject collected all stool samples. Only subjects with total marker recoveries greater than 98.5% are used in faecal analysis. The faecal collection data from one subject is shown in Appendix D.

At the end of the study, all stool samples collected were checked against samples as noted in the subjects' diaries. When all samples had markers counted and all marker recoveries have been calculated and checked, stools from the third week of each period are removed from their individual bags, combined in large bags, weighed and freeze-dried in an Edwards Supermodulyo Freeze Dryer for one week. The dried stools were removed after this time, weighed, sealed, crushed with a rolling pin and stored for later analysis.

Appendix 7. Letter of Correspondence

UNIVERSITY OF CAMBRIDGE AND MEDICAL RESEARCH COUNCIL
MRC DUNN NUTRITION UNIT

Please reply to:

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MRC LABORATORIES
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Cable address: CAMNUT, Cambridge

SAB/mpr

11th September 1990.

Dr. Alison M. Stephen,
College of Pharmacy,
Division of Nutrition & Dietetics,
University of Saskatchewan,
Saskatoon,
S7N OWO,
Canada.

Dear Alison,

Thank you for your letter.

It sounds like your subject has something wrong with his small gut which is highly likely to affect his PABA absorption. I did look at PABA excretion in some gastroenterology subjects, but deliberately excluded those with possible absorption problems - such as short bowel. So I think you are wise to exclude that subject, but sorry that I don't have anything to send you specifically. I have looked at renal failure and PABA recovery and it is probable that recovery is reduced in patients with plasma creatinine in excess of the normal values. This paper is currently doing the rounds, but I enclose a copy for your interest.

Glad to hear things are going very well.

Best wishes,

Yours sincerely,

Dr. S. Bingham

Encl.

Appendix 8. PABA Recoveries

Completeness of urine collections - % PABA recovered

	Day of Study														
Subject	15	16	17	18	19	20	21	36	37	38	39	40	41	42	% Incomp.
LEN 27	82	85	93	94	89	95	88	89	90	89	88	85	96	92	7.1
LEN 30	78	78	98	81	91	87	91	89	88	86	80	77	85	58	35.1
LEN 38	77	78	80	84	83	94	80	68	86	87	79	65	81	70	78.6
LEN 39	120	42	88	93	92	105	89	82	79	91	86	85	88	74	14.3
LEN 40	80	86	80	71	85	95	85	46	86	88	91	84	94	87	28.6
LEN 47	79	75	97	98	69	83	48	100	69	73	94	93	85	92	50.0
LEN 48	80	90	93	65	100	114	89	87	150	79	85	73	93	107	28.6
LEN 49	75	80	78	86	83	108	88	91	93	42	80	75	92	85	50.0
LEN 50	82	73	96	93	92	93	98	85	87	100	86	108	98	90	7.1
LEN 51	81	86	87	99	84	119	98	100	89	98	113	74	87	94	21.4

Recovery < 85% is considered incomplete. Total number of incomplete collections was 48 out of 140 (34.3%).