# RELATIONSHIP OF NUTRIENT INTAKE, RENAL STONE AND DISTAL RENAL TUBULAR ACIDOSIS (dRTA) IN THE NORTH-EAST OF THAILAND

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**ชื่อวิทยานิพนธ์** ความสัมพันธ์ของสารอาหารที่ได้รับของคนในภาคตะวันออกเฉียงเหนือของประเทศไทย กับการเกิดโรคนิ่วในไต และโรคไตขับกรดไม่ได้

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## บทคัดย่อ

โรคนิ่วในไต จัดเป็นปัญหาที่พบได้ในคนผู้ใหญ่ในภาคตะวันออกเฉียงเหนือของประเทศไทย เช่นเดียวกับโรคไตขับกรดไม่ได้ที่เกิดขึ้นในท้องถิ่นเดียวกัน การศึกษาครั้งนี้จึงมีจุดประสงค์เพื่อศึกษาความสัมพันธ์ของการได้รับอาหารและสารอาหารในกลุ่มคนที่เป็นโรคนิ่วในไต โรคไตขับกรดไม่ได้ เปรียบเทียบกับคนที่ไม่ได้เป็นโรค การศึกษานี้ทำใน 2 ฤดู คือ ระหว่างฤดูฝนและฤดูร้อน โดยเก็บตัวอย่างอาหารเท่ากับปริมาณที่รับประทานจริงเป็นเวลา 3 วันติดต่อกัน จากกลุ่มคนตัวอย่างทั้ง 3 กลุ่มดังกล่าว ซึ่งเป็นประชากรของจังหวัดขอนแก่น รวม 44 คน (เพศชาย 21 คน เพศหญิง 23 คน) นำมาศึกษาโดยละเอียดถึงชนิดของอาหารและปริมาณสารอาหารที่รับประทาน ซึ่งศึกษาโดยการวิเคราะห์ทางเคมี

ในกลุ่มคนตัวอย่างที่ทำการศึกษาทั้งหมด พบว่ามีการบริโภคอาหารส่วนใหญ่ไม่แตกต่างกัน ได้รับกำลังงานเพียงพอ (2000-2600 กิโลแคลอรี่/วัน) แต่ได้รับจากสารอาหารหลักในปริมาณที่ไม่ได้สัดส่วนพอเหมาะ กล่าวคือได้รับสารอาหารคาร์โบไฮเดรทค่อนข้างสูง (400-540 กรัม/วัน) จากการบริโภคข้าวเหนียวในปริมาณมาก (น้ำหนักดิบ 770-950 กรัม/วัน) ได้รับไขมันน้อยมาก (13-18 กรัม/วัน) คิดเป็นค่าเฉลี่ยประมาณ 5-8 % ของกำลังงานที่ได้รับทั้งหมดเท่านั้น ได้รับ

สารอาหารโปรตีนในปริมาณที่เพียงพอ (60-72 กรัม/วัน) แต่ส่วนใหญ่ได้จากข้าวเหนียว ซึ่งมีกรดอะมิโนจำเป็นไม่ครบถ้วน เมื่อพิจารณาถึงสาเหตุสำคัญในการเกิดโรคนิ่วในไตในประเทศทางซีกโลกตะวันตก ประชากรในท้องถิ่นนี้มีการบริโภคสารในอาหารที่น่าจะเป็นผลดีในการป้องกันการเกิดโรคนิ่วในไต กล่าวคือบริโภคโปรตีนจากเนื้อสัตว์น้อย ได้รับไขมัน แร่ธาตุโซเดียม (730-850 มิลลิกรัม/วัน) และแคลเซียม (440-580 มิลลิกรัม/วัน) ในปริมาณน้อย และได้รับออกซาเลท (28-44 มิลลิกรัม/วัน) และกรดนิวคลีอิค (14-18 มิลลิกรัม/วัน) จากอาหารซึ่งเป็นสาเหตุสำคัญในการเกิดนิ่วในปริมาณที่น้อยมาก นอกจากนี้พบว่ามีการดื่มน้ำในปริมาณที่เพียงพอ (2700-4100 มิลลิลิตร/วัน) อย่างไรก็ตามโรคนิ่วในไตยังเป็นโรคที่พบได้ค่อนข้างแพร่หลายในคนภาคตะวันออกเฉียงเหนือ เมื่อดูปริมาณการบริโภคสารในอาหารที่จัดว่าเป็นสารป้องกันการเกิดของก้อนนิ่วในไตของกลุ่มคนตัวอย่างทุกกลุ่ม ร่วมไปกับผลการวิเคราะห์เลือดและปัสสาวะ 24 ชั่วโมง พบว่าคนกลุ่มนี้ได้รับฟอสฟอรัสเพียงพอ (680-770 มิลลิกรัม/วัน) แต่ส่วนใหญ่มาจากพืช (75-80 %) ซึ่งอาจดูดซึมได้ไม่ดี อันเป็นสาเหตุที่ทำให้พบฟอสเฟตในปัสสาวะต่ำ นอกจากนี้ยังพบว่าในปัสสาวะมีความเข้มข้นของซิเตรทต่ำอีกด้วย อธิบายได้ว่าอาจเป็นผลมาจากการได้รับแร่ธาตุโปแตสเซียมในอาหารต่ำ (1240-1470 มิลลิกรัม/วัน) เป็นเวลานานซึ่งอาจมีผลทำให้เกิดภาวะขาดโปแตสเซียมได้ เป็นสาเหตุทำให้พบซิเตรทขับออกมาในปัสสาวะต่ำ สมมติฐานนี้ยังต้องมีการศึกษาเพิ่มเติมอีก เนื่องจากทั้งฟอสฟอรัสและซิเตรทจัดเป็นสารสำคัญที่ช่วยป้องกันและขัดขวางการเกิดของก้อนนิ่วในไตได้ ดังนั้นการได้รับสารอาหารที่ไม่สมดุล ได้รับสารก่อนิ่วอย่างสม่ำเสมอแม้ว่าจะอยู่ในระดับต่ำ และการได้รับสารอาหารบางชนิดต่ำเป็นเวลาติดต่อกันนานๆ โดยเฉพาะสารยับยั้งต่อการเกิดก้อนนิ่วในไต อาจมีความสัมพันธ์กับการเกิดโรคนิ่วที่ไตได้ ข้อมูลที่ได้จากการศึกษานี้จัดได้ว่าเป็นข้อมูลเบื้องต้น ความสัมพันธ์ของอาหารต่อโรคนิ่วในไต โดยเฉพาะความสัมพันธ์ของการบริโภคข้าวเหนียวต่อโรคดังกล่าว ควรจะได้มีการศึกษาอย่างละเอียดต่อไป

**Thesis Title** Relationship of Nutrient Intakes, Renal Stone and Distal Renal Tubular Acidosis (dRTA) in the North-East of Thailand.

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## ABSTRACT

The attempt of this research was to study the food consumption pattern and nutrient intake in the subjects with renal stone and distal renal tubular acidosis (dRTA) compared to normal subjects. The relationship between the nutrient intake and the pathogenesis of the diseases was assessed.

The study was performed in three villages in Khonkhan province where high incidence of renal stone and dRTA were found. Daily foods consumed by forty-four subjects (21 males and 23 females) with renal stone, dRTA and healthy normal were collected for 3 consecutive days in rainy season and in summer. Multiple composite samples of individuals, 3-day duplicate meals, were prepared and chemically analysed for nutrient and some antinutrient composition.

The study showed that the nutrient and antinutrient consumptions of subjects with renal stone, dRTA and normal control did not differ significantly. The subjects had adequate intake of energy (2000-2600 Kcal/day), however it derived from an imba- lanced diet of high carbohydrate (400-450 mg/day) due to large amount (770-950 g/day) of glutinous rice consumption. Fat intake was quite low (13-18 g/day), contributing only 5-8% of total energy intake. This was mainly due to cooking methods which steaming, boiling, roasting, grilling and blanching were used. Adequate dietary protein (60-72 g/day) and phosphorus intakes (680-770 mg/day) were met but were mostly derived from plant origin which may affect their qualities and availabilies.

Low intake of animal protein, fat, calcium, sodium, oxalate and nucleic acids and high fluid intake were found among the subjects which should be beneficial for not having the risk factors in favouring stone formation. However, high incidence of renal stone and dRTA are still prevalence in this area. Blood and urine pictures of the subjects did not show typical pathogenesis of the diseases; calcium, magnesium, and phosphate in blood and calcium and uric acid in urine were within the normal ranges. However, low levels of urinary phosphate and citrate, the inhibitors for calcium stone formation, were found in all groups studied. Consumption of phosphorus mainly from plant origin may lower the availability and the amount of phosphate excreted in urine. Low potassium intake might lead to potassium deficiency and may have an important role in lowering urinary citrate excretion. From the findings, it could be suggested that consumption of imbalanced diets, regular consumption of

stone aggregators even at low concentration with inadequate quantity of stone forming inhibitors could be related to the incidence of renal stone and dRTA among the northeastern people of Thailand. The data of nutrient and antinutrient intakes obtained in this study provided basic information in understanding the ethiology of the renal stone disease. The relationship of glutinous rice consumption and the disease should be further investigated.

# TABLE OF CONTENTS

# LIST OF TABLES

# LIST OF FIGURES

page

## LIST OF ABBREVIATIONS

dRTA = distal renal tubular acidosis

g = gram

mg = milligram

n = number

y = year

d = day

kg = kilogram

cm = centimeter

$kg/m^2$ = $kilogram/meter^2$

BKK = Bangkok people

Vegetable (Mar) = Market vegetable

Vegetable (gat) = gathered vegetable

## LIST OF APPENDICES

page

# 1 INTRODUCTION

Renal stone is found commonly among population in the northeast of Thailand (1, 2). The study of renal stone in this area has been the field of interests, but little is known about their etiologies or risk factors. Results from the previous studies on the problem are as follows :

1. High prevalence of renal stone was documented in the northeast of Thailand especially in the villages with low socioeconomic status (3).

2. High prevalence of distal renal tubular acidosis (dRTA) (1-5%) was found in the same area as renal stones (4).

3. Hypokalemia was presented in all patient with complete dRTA (5).

4. A large number (30%) of the surveryed villagers (3007) in 5 villages in Khon Kaen province had very low urinary citrate excretion (6).

Most of renal stone formers were from the low-income, laboring rural class. This is in contrast to the western hemisphere where renal stone disease is found predominantly among the affluent (7, 8). Thus, the pathogenetic of renal stone disease in developing countries like Thailand may be dissimilar to that in the western world.

The impact of diet on the formation of renal stone has been recognized but the dietary pattern of the affected patients in the northeast of Thailand has never been studied. It was, therefore, the aim of this study to perform nutritional assessment of the patients with renal stone and dRTA compared to those without disease.

The results obtained would provide information to establish a guideline for the prevention and treatment of these diseases.

**The aim of the study**

1. to determine the nutrient and antinutrient intakes of normal and villagers with renal stone and dRTA in the northeast of Thailand, using chemical analysis of duplicate meal.

2. to evaluate the relationship between nutrient intake, renal stone, and distal renal tubular acidosis.

## 2 LITERATURE REVIEW

Robertson and Peacock explained urinary stone disease as being due to periods of excessive crystalluria which may be so great or persistent that crystal aggregated and become lodged at some narrow section of the urinary tract and there formed the nucleus of the stone (9). Stone disease has been one of the common afflictions of mankind since antiquity. Prior to the industrial revolution, the bladder was the most common site of stone disease in the urinary system. It was until the early 1900s, when there was an increase in industrialisation, an increased occurrence of renal stone and ureteral calculi in Europe, the British Isles, and the United states was observed (10). This phenomenon seemed to be parallel with the economical status of the population, thus for urinary stone was found gradually vanished.

By 1950, investigators began to report some significant physiological observations that were associated with the production of urinary culculi which included the importance of diet consumed. Anderson (11) presented a multifaceted theory of epidemiology of urinary calculi. The incidence of upper urinary tract calculi varied greatly with age, anatomical site, geographical distributions and there were unexplained increases during different periods of history. At least two separate epidemiological factors were involved in the genesis of urinary calculi. The first one was intrinsic factor, which was related to inherited biochemical or anatomical make up of individuals. The second was extrinsic or environmental factors. These included climate, water available for drinking, the presence or

absence of trace elements in foodstuffs and drinking water, and occupations of the patients.

It was found that a significant risk to stone formation was related to certain nutritional factors, such as inadequate fluid intake and excessive ingestion of foods rich in oxalate, calcium, sodium or animal protein (12). Many dietary elements have been cited as contributing factor in stone formation. The results of their studies were reviewed and presented in the particular topics as follows.

## 2.1 General overview of nutritional influences on stone formation

There were many types of stone and great variations of the incidence of each type were reported. Calcium oxalate stones accounted for 50 to 80 % of all renal stones. Approximately 20, 10 and 5 % of the other types of stone were calcium phosphate, mixed (uric acid, cystine and xanthine) and oxalate stone respectively (13, 14). The dietary elements that have been identified as contributors to stone formation are calcium, oxalate, purine, sodium, dietary acid ash, animal protein, and etc.

### 2.1.1 Dietary calcium as a risk factor

The association between calcium excretion and stone formation has long been recognized. High prevalence of hypercalciuria was found in stone forming patients (15, 16). Though hypercalciuria encountered in stone formation is not a homogenous entity, without individualized disease responsible for secondary hypercalciuria, increased intestinal

calcium is found in the majority of patients (17). However, decreased calcium intake can also result in enhanced oxalate absorption and excretion which can have an important effect on urinary saturation of calcium oxalate (18, 19).

#### 2.1.1.1 Pathogenetic role of dietary calcium in stone formation

A high calcium intake could stimulate calcium stones formation by raising urinary calcium excretion and the saturation of stone-forming calcium salts. There are basically three forms of hypercalciuria, not all of which can be significantly altered by dietary modifications of calcium. The first one is resorptive hypercalciuria which occurs primarily in those patients with hyperparathyroidism. The second type of hypercalciuria is renal hypercalciuria, and it often persists in periods of restriction of dietary calcium. Because enhanced calcium excretion is maintained in the presence of dietary calcium restriction, low-calcium diets can be detrimental to these patients. The disorder is usually treated instead with thiazide. The third and most common type encountered is absorptive hypercalciuria. It is characterized by normocalcemia and urinary calcium excretion exceeding 200-350 mg in 24 hours, depending on the dietary calcium intake.

A reduction in dietary intake of calcium results in decreased urinary excretion of calcium, in both normocalciuric and hypercalciuric patients. Peacock, et al (18) examined nine male stone formers with persisted hypercalciuria (defined as excretion of calcium

in urine greater than 300 mg per day) and nine male control subjects. Urine samples collected after fasting for 10 hours demonstrated a significantly greater excretion of calcium in stone-formers as compared to control subjects, although the excretion of calcium in stone-formers was lower than when they had been on a random calcium diet (20). Urinary calcium excretion in stone formers, after fasting more than six hours decreased but after a 1 g calcium load was administered orally, the urinary calcium in patients with stones rose significantly more than in the control subjects.

#### 2.1.1.2 Beneficial value of dietary calcium restriction

Bataille P, et al (21) studied the effect of dietary calcium restriction on renal excretion of calcium and on the risk of stone formation in ambulatory patients, using a controlled diet containing 400 mg calcium per day. Twenty women and thirty men, with an average age of 30 years, served as a control group for the 18 women and 30 men (average age, 40 years) with idiopathic calcium urolithiasis. Subjects were maintained on their regular diet for one week, while two 24-hour urine collections were analyzed. They then ingested a diet contain 400 mg calcium per day for four days, after which another 24 hour urine was analyzed. After dietary restriction, daily urinary calcium excretion decreased, an average of 75 mg was found in the control subjects, while in patients with absorptive hypercalciuria (with urinary calcium exceeding 380 mg/day in men and 280 mg/day in women on a free diet) an average declined level of 240 mg was found.

In 1976, Ettinger (22) demonstrated an ability of reduced calcium intake to lower calcium excretion. Controlled studies were conducted in assessing the value of restricting calcium intake as a form of treatment in patients with high calcium oxalate excretion. Urinary calcium was determined when patients were maintained on a 600 mg calcium per day diet. Hypercalciuria was defined as urinary calcium exceeding 300 mg/day in men and 250 mg/day in women. All subjects had normal serum calcium and uric acid. The subject were divided into three therapeutic groups : the first group received phosphate therapy alone; the second was given a combination of phosphate therapy and dietary calcium restriction, and the third was placed on dietary calcium restriction with no medication. Dietary calcium was reduced in groups II and groups III from 600 to 300 mg per day. There was a 25 % reduction in urinary calcium excretion in 38 % of the subjects in group I, 25 % in group II, and 38 % in group III. There was also a decrease in the stone passing rate in all three groups. This study was one of the first controlled trials demonstrating the effectiveness of dietary modifications without other treatments for recurrent calcium oxalate urolithiasis.

### 2.1.2 Dietary oxalate as a risk factor

The study on the influence of urinary oxalate on the stone-forming process was started following the multiple observations that calcium oxalate stone-formers sometimes have no detectable abnormalities in calcium metabolism (23, 24). Another important aspect of the work being carried out in oxalate metabolism relates to

the more recent devolopment of realiable assays to urinary oxalate. Robertson and Achieles have demonstrated that small increases in urinary oxalate concentration resulted in supersaturation of urine with calcium oxalate (25, 26).

Oxalate is filtered by the glomerulus, reabsorbed in proximal tubule and finally secreted at a more distal site. The origin of urinary oxalate is comprised of endogenous sources (60 %), dietary sources (10 %), and metabolism of ascorbic acid (25-30 %) (27). Although dietary sources constitute the smallest component, excess dietary oxalate content occasionally is found in patients with nephrolithiasis (28). Major common dietary sources of oxalic acid are dark green leafy vegetables, tea, cocoa, nuts, and pepper.

#### 2.1.2.1 Pathogenetic role of dietary oxalate in stone formation

As previously noted, dietary oxalate has a significant effect on urinary oxalate excretion. Even small increments in oxalate excretion might result in quite important effects on urinary supersaturation of calcium oxalate. In this manner dietary oxalate can contribute toward formation of calcium oxalate stones. Therefore, to evaluate patients with calcium oxalate stone, 24 hour oxalate excretion as well as the dietary histories that may reveal excessive intake of the product should be obtained. Although overall consumption and 24 hour excretion of oxalate may be normal, periodic exaggerated urinary excretion of oxalate can follow periods of high dietary intake. This information can only be realized with a careful dietary history.

### 2.1.2.2 Control of intestinal oxalate absorption

Oxalate is a compound commonly found in plant food such as green leafy vegetables. Most of which is excreted unchanged by the body. In human, half of the ingested oxalate is destroyed by intestinal bacteria, 25 % is excreted unchanged in the feces, while 25 % appears in the urine (29). Pinto and Patenain (30) found that hyperabsorption of oxalate seemed to be one of the mechanigus responsible for hyperoxaluria in susceptible individuals. The calcium in the small intestine can complex with oxalate, thus preventing the oxalate from being absorbed. Therefore, urinary oxalate could be decreased either by decreasing dietary oxalate or by increasing dietary calcium.

Zarembski and Hodgkinson examined some factors influencing the urinary excretion of oxalic acid in man (31). Fifty-six normal healthy adults were employed as controls for 13 patients with renal calculi and idiopathic hypercalciuria. All subjects were maintained on a metabolic ward with controlled dietary regimens. The amounts of calcium and oxalate were not specified. In all cases, excretion of oxalate was higher on high oxalate diet than on low oxalate diet. This demonstrated the effect of dietary oxalate on urinary oxalate. Furthermore, the control subjects experienced an average of 3.4 % increase in urinary oxalate, while in the stone formers the urinary oxalate increased by 10.3 %, suggesting an abnormality in oxalate absorption in the latter group.

Marangella, et al (32) performed an experiment to determine what effect calcium had on oxalate absorption. Seventeen healthy

volunteers (13 men and 4 women) served as controls for 63 patients (44 men and 19 women) with a history of calcium - containing renal stones. Patients with resorptive hypercalciuria or elevated serum calcium were excluded from the study. Urine samples, 24 hour, were collected when the subjects were on a free diet at home and when they were maintained on a controlled diet containing 850 mg calcium and 80 mg oxalate per day, and again when they were kept on a controlled diet containing only 150 mg calcium and 80 mg oxalate/day. Dietary oxalate was labelled with $^{14}$C-oxalate in order to measure intestinal oxalate absorption. The results showed that the decrease in calcium excretion was associated with a significant increase of $^{14}$C-labeled oxalate absorption. On the 850 mg Ca/day diet, these subjects excreted an average of 314 mg calcium and 46.6 mg oxalate/day, significantly elevated from the average of normocalciuric patients. This difference was accounted for an increased absorption of calcium, which left a smaller amount of free calcium in gut to bind with oxalate, thus leaving a larger amount of free oxalate for absorption.

Research on the effect of calcium and oxalate ingestion on calcium and oxalate appearance in the urine has repeatedly demonstrated. The following results were obtained :

(1) a reduction of dietary calcium decrease urinary calcium;

(2) a reduction of dietary oxalate decreases urinary oxalate;

(3) decreasing dietary calcium, or increasing absorption of calcium, as in absorptive hypercalciuria; causes a rise in urinary oxalate; and

(4) increasing dietary calcium decreases urinary oxalate (13, 21, 24, 33)

Thus, a diet restricted in calcium is beneficial only in patients with absorptive hypercalciuria. Dietary calcium restriction alone does not decrease the probability of stone formation because of the increased oxalate excretion which offsets the benefit of lowered urinary calcium (34). A simultaneous oxalate restriction is suggested whenever any patient with calcium stones (with/without absorptive hypercalciuria) is placed on calcium-restricted diets (21).

### 2.1.2.3 Beneficial Value of dietary oxalate restriction

As shown before, calcium content in the diet influences oxalate absorption and subsequent excretion. Dietary oxalate can also have a significant influence on the content of oxalate in the urine. A high oxalate consumption will result in a rise in oxalate excretion and conversely limiting oxalate intake will reduce urinary oxalate excretion.

A defect in cellular transport of oxalate may be presented among patients with calcium oxalate kidney stone. Baggio et al, measured transmembrane oxalate flux in red blood cell of 98 patients with idiopathic urolithiasis and 25 nonstone-forming control subjects, and found the values to be higher in the patients (35). The mean oxalate exchange rate, derived by an equation using .measurement of $^{14}C$-label oxalate and time, was 0.31 unit in the control subjects and 0.95 unit in stone formers. The levels above the upper limit of normal was found in 78 out of 98 patients. Patients with

hyperparathyroidism and primary hyperoxaluria had values in the normal range.

### 2.1.3 Dietary purines as a risk factor

Uric acid is a metabolic breakdown product of purines, derived for the most part from endogenous biosynthesis, and to a lesser extent from ingested proformed purines. The hyperuricosuria which results from excessive purine intake has been demonstrated to have a significant role in the formation of calcium oxalate stone (36, 37).

#### 2.1.3.1 Dependence of urinary uric acid excretion on dietary purine intake

Sources of urinary uric acid include dietary purines and endogenous production. In the majority of hyperuricosuric calcium oxalate stone-formers, excess intake of dietary purine is the main cause of hyperuricosuria. Dietary sources containing high purines content are organ meats, seafood, and legumes. Most hyperuricosuric patients excreted normal levels of uric acid when placed on purine restricted diets, and the levels excreted were comparable with that of the normal nonstone-forming patients (38).

#### 2.1.3.2 Pathogenetic role of hyperuricosuria i stone formation

Urinary uric acid and urate salts have a dual effect on calcium oxalate nucleation and subsequent crystal growth. These effects

relate to epitaxy or heterogeneous nucleation in which the uric acid or urate salt crystals induce calcium oxalate crystallization. Pak et al (39) presented the pathogenic importance of hyperuricosuria in the formation of calcium-containing renal stones. In 11 male patients with hyperuricosuric calcium urolithiasis, renal excretion of uric acid was altered with low-purine diets, allopurinol therapy, and oral purine loads. As renal excretion of uric acid increased, urine specimens become more supersaturated with respect to monosodium urate, which was found to facilitate spontaneous nucleation of calcium oxalate.

### 2.1.4 Dietary acid ash content as a risk factor

A high acid ash intake results from consumption a diet rich in animal proteins (meat, poultry, and fish). The acid load from such a diet lowers urinary pH and citrate and increases urinary calcium (40, 41, 42), A consumption of a diet rich in animal proteins also increases urinary uric acid and exaggerates the potential of stone-forming. The effect of such a diet on urinary oxalate has been studied but found to be variable.

#### 2.1.4.1 Dependence of citrate excretion on dietary acid ash content

Although it is well established that hypocitraturia is associated with systemic acidosis, potassium depletion, starvation urinary tract infection, and renal tubular acidosis but the actual cause of the reduced citrate excretion in patients with hypocitraturia

and calcium oxalate urolithiasis is still unknown. The acid ash load associated with excessive intake of animal proteins results in diminished citrate excretion and reduced urinary pH. Both factors associate with an increase risk of calcium stone formation.

### 2.1.4.2 Other factors influencing of citrate excretion

Citrate excretion by the kidney is strongly influenced by the acid-base status. Serum bicarbonate is the essential factor influencing renal citrate excretion. After ingestion, all of the absorbed citrate is converted to bicarbonate which contributes excessive base to the kidney. This in turn causes the enhanced in citrate excretion. It is, therefore, understandable that factors other than excessive dietary intake of acid-ash can also significantly influence citrate excretion. Distal renal tubular acidosis and chronic dirrheal states with associated acidosis will result in a reduction in urinary citrate. Urinary tract infections have also been implicated in reducing urinary citrate probably secondary to the degradation of molecule by bacterial enzymes (43).

### 2.1.4.3 Role of citrate in preventing stone formation

Citrate is a potent chelator of calcium; thus, its presence in tubular fluid reduces the concentration of ionized calcium and subsequently decreases the risk of calcium salt crystallization in the

tubular fluid and urine (44). Urinary saturation of calcium salts (calcium oxalate and calcium phosphate) is reduced because of the formation of these citrate-calcium complexes. Nucleation, agglomeration, and crystal growth of calcium salts are also directy impaired by citrate.

### 2.1.5. Dietary sodium as a risk factor

Modlin studied the 24-hour excretion of sodium and calcium in stone-forming and non-stone forming white patients and non-stone-forming Bantu patients (Bantus rarely form stones) (45, 46). Modlin believed that a high urinary sodium : calcium ratio conferred protection against stone disease. The results of the study showed that the ratio was higher in the white stone-formers than that in the nonstone-formers and was lowest in the Bantu population. However, Robertson et al (47) suggested the more important factor of the calcium excretion and not its relationship to urinary sodium. Subsequent report have shown that a low dietary intake of sodium was associated with a reduced incidence of calcium stone formation (48). Recently, a study on this aspect demonstrated no difference in sodium excretion between stone formers and non stone formers (49).

#### 2.1.5.1 Pathogenetic role of dietary sodium in stone formation

Studies in stone formers have demonstrated that a high sodium intake was associated with increase urinary calcium excretion by expanding the extracellular fluid volume and there by inhibiting renal

tubular calcium reabsorption. Studies have shown that a 100 meq rise in dietary sodium resulted in a 25-30 mg increase in urinary calcium excretion (51, 51). The enhanced excretion of sodium can also cause sodium urate-induced crystallization of calcium salts which may be an important factor in stone formation (37, 52). Moreover, there was a preliminary evidence suggesting that a high sodium intake reduces the renal excretion of citrate (53).

### 2.1.6. Fluid intake as a risk factor

A low urinary output increases urinary saturation with respect to stone forming salts by raising the urinary concentration of constituent ions. Low daily urinary volume was a very important factor in predisponsing patients to stone formation. Additionally Blacklock noted that the incidence of urinary calculi in sailors decreased by 86 percent by increasing daily urinary volumes from 800 to 1200 ml (54). The type of fluid consumed can also have a significant effect on stone-forming propensity by altering excretion of specific ions or altering urinary pH. Although a high fluid intake will reduce the concentration of urinary inhibitor, there has been no evidence to suggest any loss of inhibitor activity (55).

### 2.1.6.1 Pathogenetic role of reduce fluid intake on stone formation

A lower urinary output increases urinary saturation will respect to stone forming salts by raising the urinary concentration of constituent ions (56). Pak et al (57), presented the increase intake of distilled water by 0.5, 1.0, and 1.5 liters per day in four patients with nephrolithiasis while on a constant metabolic diet. This urinary dilution significantly reduced the urinary concentration product ratio (state of saturation) of calcium oxalate and other crystal product. However, a theoritical cautionary note that urine dilution could reduce activity coefficients of urinary stone-forming constituents and thus increase ion collisions which could potentially contribute to crystal formation. Fluid diuresis facilitated transit of crystalline product through the nephron, results in reduction of crystals to be in contact with all surfaces. Moreover, the components of urine that may crystallize are also diluted by increasing urinary volume. It can be concluded therefore that the dilution effects associated with an increased urine production probably outweigh any changes in activity coefficients and therefore is effective in preventing stone formation.

## 2.2 Dietary assessment

Evaluation of dietary intake of individual subject is the most difficult nutritional assessment. It is desirable to assess person's food intake without influencing changes in food habits. In order to get the best estimation of intake, assessment of a subject's habitual food consumption should be determined along with the recent dietary habits.

### 2.2.1 Methods for assessing dietary intake

2.2.1.1 24-Hour recall. The most popular and easiest method for obtaining an idea of a person's dietary intake is the 24-hour recall. The individual completes a questionnaire or is interviewed by a dietitian/nutritionist or whoever experience in dietary interviewing. The subject is asked to recall what and how much he or she had eaten within the last 24 hours, or the previous day. In surveys of population intakes, this method has been found to be a fairly good tool. However, there are significant sources of error such as (1) the person may not be able to accurately recall the amount of food eaten (2) the previous day's intake may be atypical intake; and (3) the person may not be telling the truth, one of which may be embarrassment.

2.2.1.2 Food frequency questionnaire. To help overcome some of the weaknesses inherrent in the 24-hour recall method, a food frequency questionnaire may also be completed. Using this tool, the

health professional can collect information on how many times per day, week or month the individual eats particular foods. This information can aid in validating the accuracy of 24-hour recall food consumption pattern.

2.2.1.3 Dietary history. The dietary history is more complete than either the 24-hour recall or food frequency questionnaire, although it usually includes errors from both of these sources. It contain additional information about economics, physical activity, homelife and meal patterns, dietary or nutritional problems, etc. Remember that dietary habits are personal and an individual may be unwilling to talk about them, especially if he or she perceives the interviewer as being judgemental. It is necessary to be as objective as possible during interviewing in order to gain a complete and accurate insight into a person's eating pattern.

2.2.1.4 Food diary or record. This method involves more time, understanding, and motivation on the part of the patient or client. The subject is asked to write down everything he and she eats or drinks for a certain time period. Three days, particularly two week days and one weekend day, have been found to be a representative time period for most people. The length of time that the food diary must be carried out in order to get an accurately reflect of usual nutrient intake depends on whether the person had a regular food pattern. A daily food pattern requires fewer days of recording than a random, haphazard eating style. The nutrient contribution for each food is calculated using national food composition table. The total

daily intake is divided by the number of days to give an average daily intake. The health practitioner can gain information about lifestyle, companions, and meal eating atmosphere by asking the person to noted the time, place and people with whom he or she eats. This is a very good way to get to know a person's lifestyle, since food habits are an intimate part of the lifestyle.

2.2.1.5 Observation of food Intake. Observation of food intake is the most accuratly method of dietary intake assessment using food composition tables, but also the most time consuming, expensive and difficult. Observation must be non-intrusive and is most easily done when the person's meals are provided for him, as in the case of a hospitalized person, nursing home resident or child at a boarding school. It requires knowing the amount and kind of food presented to the person and a record of the amount actually eaten. The ultimate in a controlled situation is that in a metabolic unit when a weighed amount of food is presented, the amount of uneaten food is re-weighed, and the differrence is recorded as the amount eaten.

2.2.1.6 Household food consumption. This method involves visiting a household periodically and recording the amounts and types of food purchased for household and the disappearance of that food. The food unaccounted for is assumed to be that consumed by the family. It is most commonly used in the large population serveys and is not a good evaluation of individual intake, because of food wastage and lack of a record of individual household members consumption. However, in attempting to gain insight into nutrition situation in the community, this information can be very valuable.

2.2.1.7 Duplicated meal analysis. Precision of food sample collection is an important for quantitative data. In a duplicated meal analysis, the food comsumption involved each subject keeping a weighed collection inventory 3-5 days (do not only estimate by eyes). They also kept their diet and duplicate portion was chemically analysed using suitability of method to measure dietary intake by stuff of food laboratory.

There are major limitations to these method of assessing intake. Errors in duplicated meal analysis may be more serious. First, subject will often admit to a change in their eating pattern, and secondly the sampling error increased with the heterogeneity of the food. Disadvantages of the methods was high price for field survey costs and analytical costs per subject.

### 2.2.2 Problems in estimating dietary intakes

There are two basic factors in obtaining information which can provide a true reflection of food intake (58).

(1) method chosen

(2) period of assessment

The method chosen must provide an accurate measure of each subject's usual intake of specific nutrients. Todd and associates conducted a study on food intake measurements using 18 male graduate students (aged 22 to 31) (59). The methods used were (1) diary record of estimated portion sized of food eaten, (2) weighed food intake recorded on tape, and (3) a 24-hour dietary recall obtained by advanced dietetics students. The study lasted for 30 days - six

periods of five days each. The subjects were divided into two groups, following alternate schedules of the methods. In the first method, food was weighed by the investigator and assistant in the dining hall. As a control to determine the accuracy of subjects' records, waste was also recorded and weighed. For the second method, small scales were given to each subject to use at home. The 24-hour recall taken for the third method, was performed on a recorded day. The results were analysed separately from the weighed and estimated record intake for energy and protein intake. Both recorded methods were combined to determine a 30-day mean. The results showed no significant difference in the 15-day mean energy and protein intake reported by the taped and written recording methods. A single 24-hour recall, however, was not as accurate estimate of individual intake as recorded in a diary for that day. A one-day diet record also did not accurately represent the overall 30-day mean.

Young and associates compared the 24-hour recall to the diet history method and the seven-day food record (60). Groups interviewed were : 28 pregnant women in Massachusette, 51 seventh and eighth graders in New York, and 87 high school and college students in Rhode Island. The 24-hour recall was the first information obtained, then the history. Later, after individual and group instruction, seven-day dietary records were kept. The 24-hour recall was generally not found to give the same estimate of intake for an individual as the seven-day record and cannot be substituted for the seven day record with any assurance of obtaining the same picture of nutrient intake. The diet history was compared to the seven-day record in another study by Young and associates (61). Dietary record were selected and standardised

and used on groups in six different Northeastern states : 63 subjects in Maine, 49 in Massachusetts, 129 in New Jersey, 68 in West Virginia, 164 in New York, and 77 in Rhode Island. For the diet history method, the interviewer first obtained the usual dietary pattern, the variations in usual intake, along with food not eaten, when and where meals were eaten, and with whom. Usual dietary pattern was then cross-checked by a checklist. Glasses, dishes, and spoons were used to estimate portion size. Seven-day records had heading of "time of day", "food", and "amount". Instructions for keeping records were given by the interviewer. Foods were estimated for energy, protein, calcium, iron, phosphorus, vitamin A, C, thiamin, riboflavin, and niacin intakes. The results showed that the history did not give the same estimate of intakes for an individual as the seven-day record. The history generally gave higher estimates for all nutrients.

# 3 MATERIALS AND METHODS

This study was carried out at three villages : Ban Bungchim, Ban Pachad, Ban Nonelan, situate in amphur Mueng, Khonkaen province, Thailand. The dietary assessment was performed in two seasons, during June-July (rainy season) in 1989 and March (summer) in 1990.

## 3.1 Subjects

The study are performed in 44 villagers which can be categorized into three groups. Group I, normal control, consisted of 6 males and 8 females. They were proved to be healthy on the basis of clinical assessment with no history of renal stone, normal plain Kidney urinary bladder radiographly and normal urine analysis. Group II, 8 males and 7 females, was confirmed to have renal stone by radiographical examination and was referred as renal stone group. Group III, 7 males and 8 females had acidification defect of kidney as assessed by $NH_4Cl$ loading test and was referred as dRTA (5). Patients with urinary tract infection, systemic diseases and cases with serum creatinine over 2.0 mg/dl were not included in this study.

## 3.2 Dietary assessment

### 3.2.1 Food sample collection and preparation.

The dietary intake of each subject was studied for 4 consecutive days in each seasons (Figure 1). On the first three days, amount of foods consumed by the subjects, categorized according to food groups (i.e. cereal, fish, meat, egg, vegetables, fruits, water and beverage), were assessed by 24-hour weighing method. Daily food consumed by individual subjects over the first three-consecutive days as recorded were prepared and cooked by individual subject on the forth days. They were collected and kept frozen for chemical analysis. Foods consumed in each day were separately homogenized. A multiple composite sample of a 3-day duplicate meal was prepared by pooling 15 to 30 % of the homogenized daily food samples and blended with equal proportion of glutinous rice consumed in 3 days. The multiple composite samples of foods consumed in each season were prepared separately and the composition were chemically analysed.

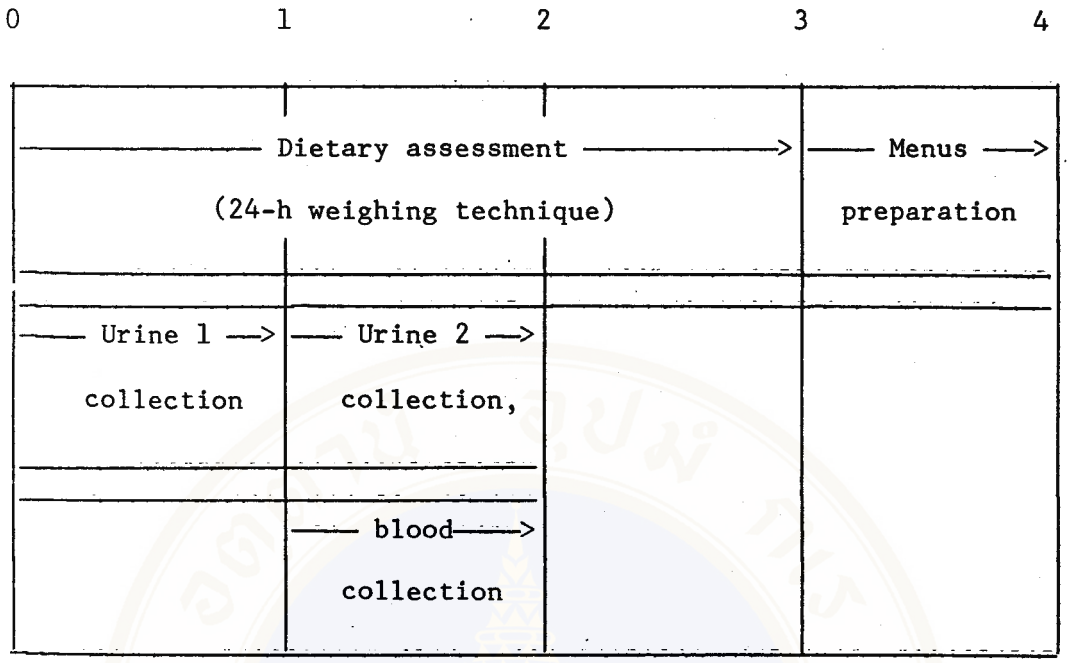


**Figure 1** : Experimental design for each study period

3.2.2 Drinking water collection.

The total volume (ml) of drinking water in each day in raing season and summer was recorded. Each type of drinking water was collected in the cleaned bottle (100 ml) and kept at $4^{o}C$ until analysis. Macro element in drinking water was analysed separately following the methods as shown in Appendix (1-4).

3.2.3 Chemical analysis

3.2.3.1 Determination of proximate composition.

The main component in food samples were chemically analysed using standard methods as follows.

| Nutrients | Method/Principle used | Appendix £ |
|---|---|---|
| Protein | Kjeldhal method | 5 |
| Lipid | Acid digestion and solvent extraction using soxhlet apparatus | 6 |
| Dietary fibre | Enzymetic-gravimetric method | 7 |
| Moisture | Drying method at 100±5° C | 8 |
| Ash | Drying method at 450° C | 9 |
| Carbohydrate | Calculation by subtracting the percent of protein, fat moisture, ash and dietary fibre from 100 percent. | |
| Energy | Calculation by using factor of 4, 4 and 9 Kcal/g for energy derived from protein, carbohydrate and fat respectively. | |

The results were expressed as weight of nutrient intake per day.

3.2.3.2 Determination of macro-elements in food sample.

Macro-elements i.e. calcium, phosphorus, magnesium, sodium, and potassium were analysed from multiple composite samples and drinking water. After dry ashing, sodium and potassium was determined by flame emission photometry (Appendix 1) and phosphorus by gravimetic method (Appendix 2). Calcium (Appendix 3) and magnesium in ash prepared by dry ashing (Appendix 4) were measured by flame atomic absorption spectrophotometry using air-acetylene flame. Operational parameters, such as lamp current and wavelength were those recommended by the Varian Techtron (Model AA-6). Concentrations of the elements were calculated from the standard curve.

3.2.3.3 Determination of oxalic acid and nucleic acid in food sample.

Oxalic acid and nucleic acid are present as antinutrients in foods. Drying of multiple composition sample at 50-60 c overnight was conducted prior to oxalic acid and nucleic acid determination. The oxalic acid by was analysed by calcium oxalate precipitation method (Appendix 10) and nucleic acid colorimetric method (Appendix 11).

## 3.3 Blood and Urine analysis

Ten milliliters of blood sample were collected on the second day of each study period and serum was seperated. They were kept frozen until analysis. The nonprotein nitrogen in serum, i.e.

creatinine, blood urea nitrogen, uric acid, was analysed, and the serum protein i.e. albumin, globulin, alkaline phosphatase as well as blood electrolytes were analysed by staff of Renal Unit, Department of Medicine, Siriraj hospital.

On the first and second day during each study period, urine sample were collected using toluene as a preservative and the total volume of urine was measured. Thirty milliliters of urine sample was devided into 2 parts, the first ten milliliters was determined for citratelyase and the other portion (20 ml) was analysed for creatinine, urea nitrogen, and urine electrolytes using the same methods as serum and by the same group of staff at Siriraj Hospital.

### 3.4 Statistical analysis

All data in this study were presented as mean±standard diviation. Comparisons of nutrient and antinutrient intakes among the studied subjects were made using one-way analysis of variance. Correlation between variables, within particular groups, was analysed by linear regression analysis.

# 4 RESULTS

## 4.1. Demogrophy of subjects

The subjects involved were 21 males and 23 females. Mean values of age, weight, height and body mass index (BMI) of the subjects were ranging from 41-45 years, 55-57 Kg, 152-157 cm, and 22-23 $Kg/m^2$ respectively (Table 1). There were no significant differences in these parameters among the normal, renal stone and dRTA groups.

**Table 1** Demography of the subjects

| Subjects | No. (n) | Age (yr) | Weight (Kg) | Height (cm) | BMI ($Kg/m^2$) |
|---|---|---|---|---|---|
| Normal | 14 | 42.5±3.5 | 57.7±4.7 | 157.1±6.0 | 23.5±2.6 |
| Renal stone | 15 | 41.5±9.0 | 56.6±8.2 | 156.0±7.2 | 23.2±2.4 |
| d RTA | 15 | 45.5±12.1 | 55.2±6.2 | 152.3±7.2 | 22.4±2.5 |

Values are mean±SD.

## 4.2 Food consumption pattern

Table 2 shows the average amounts (g per day) of each type of foods consumed by each group of subjects during rainy season and summer. Glutinous rice is the staple food of the people in this area. The amount of cereal consumed was not significantly different among the three studied groups, ranging from 770 to 950 g per person per day. During both seasons no significant difference was found in the amounts consumed of fish egg and other animal protein source (major : chicken (flesh and organ), beef, pork, dried squid; minor : frog, bull-frog, lizard, insects ant-egg, young frog) consumed, ranging from 26 to 38, 17 to 30 and 44 to 67 g per person per day respectively. Vegetables consumed were classified into two types - **market vegetables** (vegetables that were purchased from the local markets) which included kale, chinese cabbage, long bean, cucumber, egg plant, tomato, chili and **gathered vegetables** (vegetables that were gathered daily by the subjects from their backyard, garden or rice fields) which included some local vegetables such as mushroom, bamboo shoot, leadtree leaves, pak-tiew, pak kra-jiew, pak mek. There was no significant difference in the mean daily intake of market vegetables among these groups of subjects during both seasons. In rainy season, the amount of vegetables consumed was 120-150 g per person per day compared to 130-175 g per person per day in summer. For gathered vegetables, the average amounts consumed by normal, renal stone and d-RTA groups were 75, 50 and 87 g per person per day respectively in rainy season and 45, 21 and 48 g per person per day in summer. As shown in Figure 2, higher amounts of gathered vegetables were consumed by most of the

**TABLE 2** AVERAGE AMOUNT OF DAILY FOOD CONSUMED BY NORMAL, RENAL STONE AND dRTA SUBJECTS DURING RAINY SEASON AND SUMMER

| Food | Normal | | Renal stone | | dRTA | | BKK |
|---|---|---|---|---|---|---|---|
| | Rainy | Summer | Rainy | Summer | Rainy | Summer | |
| Cereal (raw) | 866±234 | 770±245 | 854±271 | 784±271 | 947±325 | 912±330 | 220±50 |
| Fish | 31±22 | 31±22 | 38±31 | 33±24 | 30±15 | 26±24 | 39±44 |
| Egg | 17±10 | 28±17 | 30±22 | 25±16 | 20±8 | 26±11 | 29±26 |
| Meat | 45±32 | 67±35 | 44±32 | 54±27 | 44±30 | 47±33 | 106±65 |
| Vegetable(M) | 150±98 | 128±58 | 120±94 | 174±69 | 136±76 | 149±109 | 90±44 |
| Vegetable(g) | 75±45 | 45±37 | 50±41 | 21±28 | 87±50[a] | 48±44 | - |
| Fruit | 101±80 | 74±70 | 75±62 | 88±93 | 89±130 | 40±31 | 298±204 |

Values are mean±SD.

a. significcant difference between renal stone and dRTA ($P<0.05$).

subjects in rainy season than in summer and the amount consumed by the dRTA group was found significantly higher ($p<0.005$) than those consumed by the renal stone group. (Table 2). Although variable amounts of the vegetables were consumed during summer by the subjects in different groups, no significant difference on amount intakes were found.

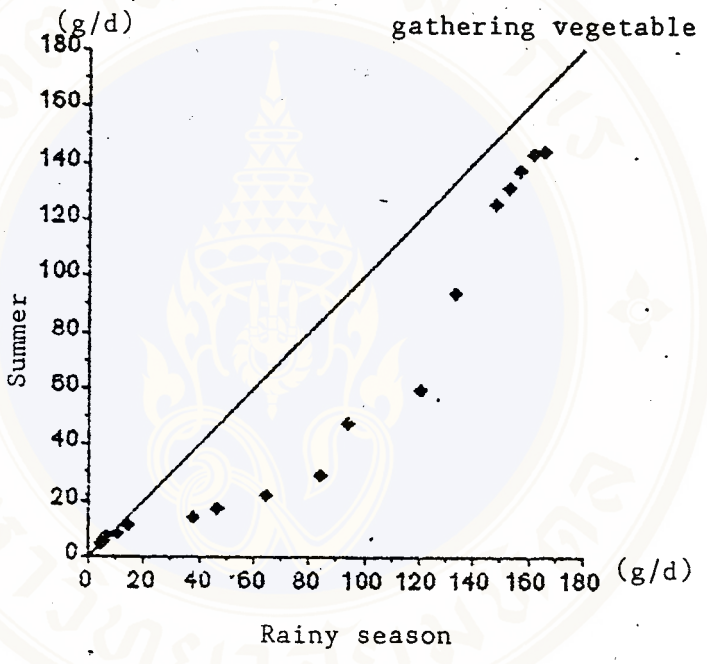


Fig 2. Consumption of gathered vegetables by villagers in rainy season and summer.

Generally above foods were mainly prepared by steaming (glutinous rice), boiling, roasting, grilling and blanching. Frying, dipped in batter or deep-fried were not commonly applied for cooking. Some food items i.e. fermented fish - pla-ra, pla-som, pla-joa - were eaten raw and most of vegetables were eaten fresh.

A wide range of amount of fruits consumed by the studied group were observed in both rainy season and summer. The average ranges were 75-101 and 40-88 g/person/day in rainy season and summer respectively. Fruits commonly consumed were banana, mango, water-melon, orange and guava. According to Figure 3, it was noticed that about 5 % of the total subjects consumed more fruits in rainy season, (more than 275 g/day) than in summer.

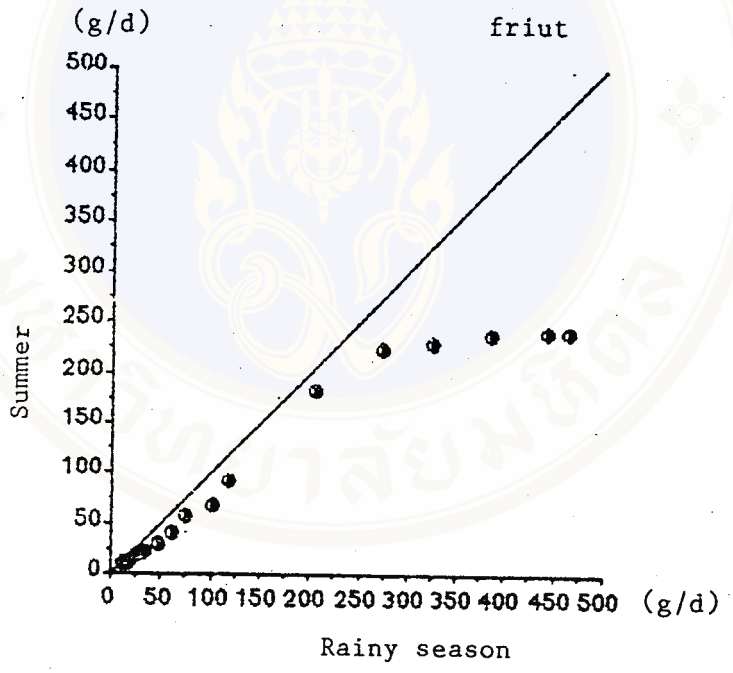


Fig3 . Consumption of fruit by villagers in rainy season and summer.

## 4.3 Nutrient intake

The nutrient and antinutrient intakes of the subjects in each group during rainy season and summer are shown in Table 3. The table also includes data of Bangkok inhabitants which obtained from the study of Chitchumroonchokchai in 1987 (62). Comparation of the data from this study to the data of Bangkok will be discussed. The data of nutrient intakes of the renal stone and d RTA patients were compared with those of normal subjects using pair t-test. The distribution of data on nutrient and antinutrient intakes among each group, during rainy season and summer, are shown in Figure 4 to Figure 15. Individual data of nutrient intake are shown in Appendix 12-29.

### 4.3.1 Energy intake

The mean daily energy intake of the subjects was ranging from 2289 to 2587 kcal/person/day in rainy season and 2035 to 2323 kcal/person/day in summer. The data of individuals are presented in (Appendix 15-20). The average daily energy intake of the subjects obtained in this study was slightly lower than the Thai RDA (1989) of this age group (2500 kcal/day). Nevertheless it was noticed that the mean daily energy intake of all groups studied was slightly higher in rainy season than in summer but no significant different in the value was observed.

**TABLE 3** DAILY DIETARY INTAKE OF VILLAGERS IN KHONKAEN PROVINCE

| Diet | Normal | | Renal Stone | | dRTA | | |
|---|---|---|---|---|---|---|---|
| | Rainy | Summer | Rainy | Summer | Rainy | Summer | BKK |
| Energy (Kcal) | 2289±500 | 2035±662 | 2438±810 | 2136±663 | 2587±746 | 2323±818 | 1866±153 |
| Protein (g) | 66.4±18.0 | 59.7±20.9 | 67.7±21.0 | 64.8±18.6 | 71.9±21.9 | 67.0±23.4 | 63.5±6.0 |
| Fat (g) | 13.4±5.5 | 17.7±9.5 | 15.9±6.9 | 16.7±8.1 | 15.9±6.3 | 15.8±7.8 | 67.0±6.5 |
| Carbohydrate (g) | 473.8±103.7 | 409.3±131.7 | 506.1±174.9 | 435.9±142.9 | 539.3±164.8 | 478.2±175.5 | 252.2±22.5 |
| Dietary fiber (g) | 33.2±21.6 | 15.9±6.2 | 16.4±8.2[a] | 16.5±7.0 | 21.2±8.8[b] | 14.8±6.4 | 35.5±3.5 |
| Calcium (mg) | 470±188 | 500±262 | 554±334 | 442±246 | 490±299 | 577±398 | 456±241 |
| Phosphorus (mg) | 741±45 | 710±287 | 771±300 | 678±270 | 767±257 | 752±280 | 1386±345 |
| Magnesium (mg) | 181±45 | 175±70 | 204±72 | 173±74 | 232±65[b] | 175±70 | 268±105 |
| Sodium (mg) | 752±208 | 782±306 | 800±363 | 731±288 | 852±336 | 844±427 | 3713±1336 |
| Potassium (mg) | 1348±384 | 1242±416 | 1479±632 | 1250±567 | 1468±515 | 1342±613 | 1824±846 |
| Oxalic acid (mg) | 28±13 | 28±9 | 42±22[a] | 44±14[aa] | 33±18 | 36±11 | - |
| Nucleic acid (mg) | 14±5 | 14±5 | 18±7 | 14±4 | 14±7 | 15±6 | - |

Values are mean±SD.

a significcant difference between normal and renal stone in rainy season ($p<0.05$).

aa significcant difference between normal and renal stone in summer ($p<0.05$).

b significcant difference between normal and dRTA ($p<0.05$).

The data of energy intake for male and female subjects in all studied groups during rainy season and summer was presented separately in Figure 4 along with that of Bangkok inhabitants. The mean daily energy intake of the male and female total subjects were 2800-3000 and 1700-1900 kcal/ person/day respectively, compared to 2300 and 1400 kcal/person /day of male and female Bangkok people, respectively.

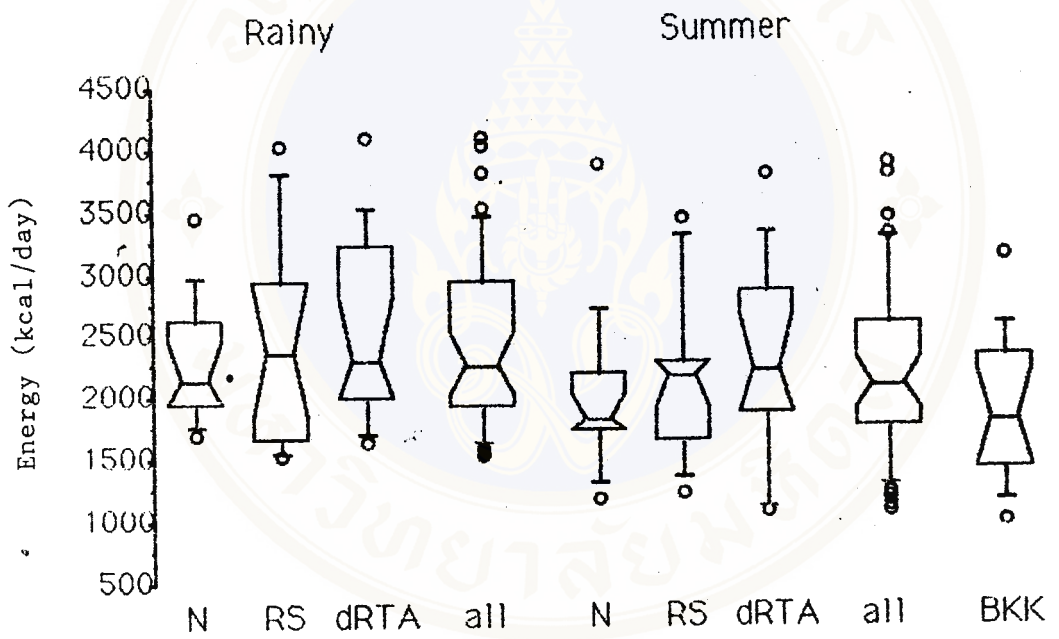


**Fig 4.** Energy intake of villagers during rainy season and summer

N : normal, RS : renal stone

dRTA : distal renal tubular acidosis

BKK : Bangkok

The energy distribution (Table 4) in the diets derived from protein, lipid and carbohydrate for normal, renal stone and d-RTA was in the same range of 11-12, 5-8 and 80-84 % respectively. The corresponding values for the diet of Bangkok residence were 14, 32 and 54 %.

**TABLE 4** ENERGY DISTRIBUTION (%) OF NUTRIENT INTAKES OF VILLAGERS

| Nutrient | Normal | | Renal stone | | dRTA | | BKK |
|---|---|---|---|---|---|---|---|
| | Rainy | Summer | Rainy | Summer | Rainy | Summer | |
| Protein | 11.6±1.8 | 11.7±1.7 | 11.2±1.5 | 12.2±1.4 | 11.1±1.6 | 11.7±1.9 | 14 |
| Fat | 5.3±1.9 | 7.7±3.1 | 5.9±2.0 | 7.1±2.2 | 5.8±2.6 | 6.4±2.6 | 32 |
| Carbohydrate | 83.1±2.9 | 80.6±4.3 | 82.8±2.6 | 80.8±2.6 | 83.1±3.7 | 82.0±4.3 | 54 |

Values are mean±SD.

4.3.2 Protein intake

The means of daily protein intake of the subjects in each group during rainy season and summer were in the same ranges, 66-72 and 60-67 g/person/day respectively. Figure 5 shows the distribution of protein intake levels among three groups of subjects during the two seasons. These levels are higher than the recommended level of protein intake (about 1 g/kg/day) and comparable well with that of Bangkok subjects. They contributed about 11-12 % of total energy in the daily diet.

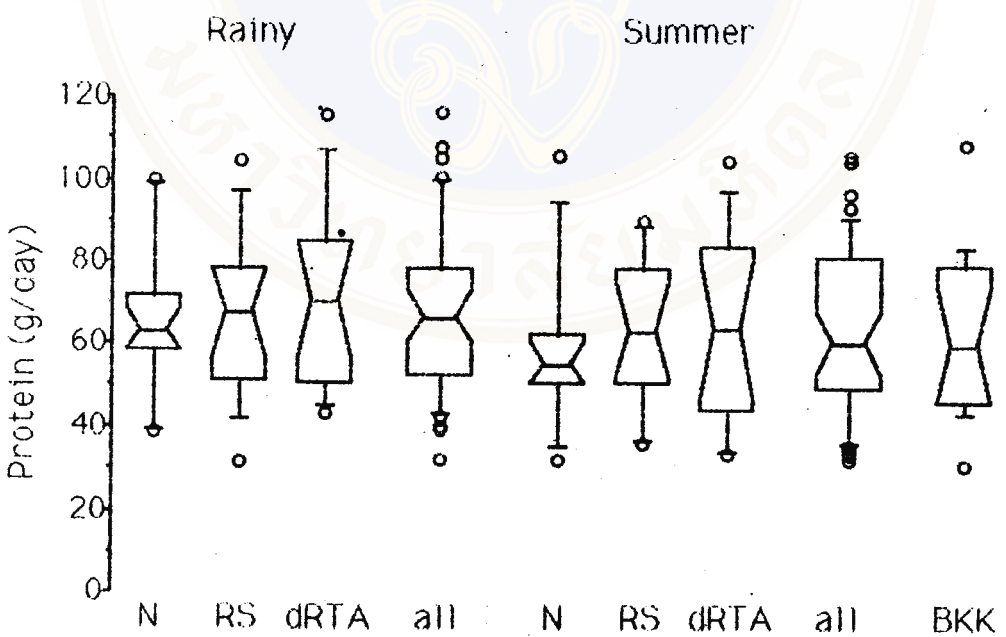


**Fig 5.** Protein intake of villagers during rainy season and summer.

4.3.3 Lipid intake

On average, the subjects consumed 13-17 g of lipid per person per day (Fiqure 6). There were no significant differences of the intake levels of subjects in different groups and in different seasons. The energy contributed from lipid in these diets was only 5-8 % of total energy which was much lower than that of Bangkok subjects, (more than 30 % distribution). The lipid intake of these subjects was about 1/6 of the level recommended by the RDA.

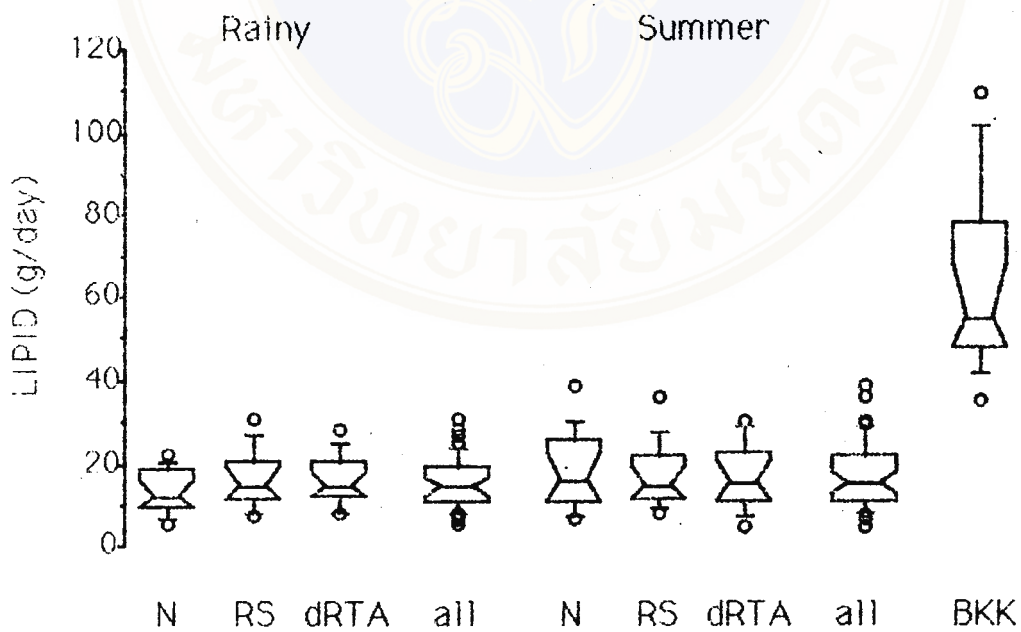


**Fig 6.** Lipid intake of villagers during rainy season and summer.

4.3.4 Carbohydrate Intake

The mean daily carbohydrate intake among the subjects was 473-539 g per day in rainy season and 409-478 g per day in summer (Figure 7). There was no significant difference in the amount of carbohydrate intake among the studied groups. The subjects consumed more carbohydrate in rainy season than in summer but no significant difference in the mean intake of carbohydrate was observed. Carbohydrate was found to be the main source of energy among the studied subjects, contributed more than 80% of total energy. In both seasons, male subjects have carbohydrate intake (>550 g/day) more than females (>350 g/d) and the average level of intake, >470 g/day were higher than that of Bangkok subjects (252 g/d).

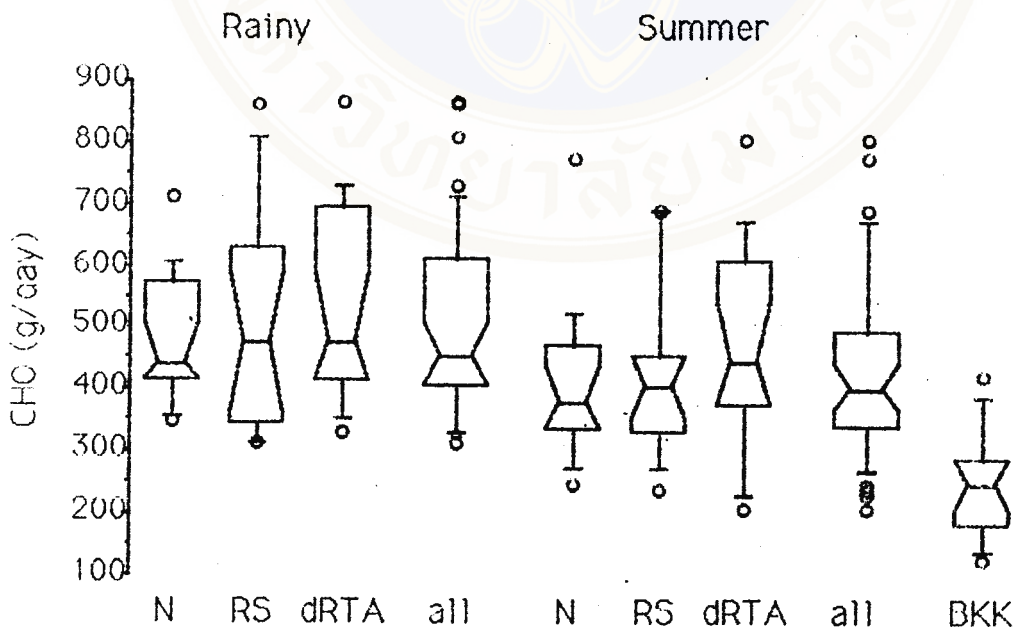


**Fig 7.** Carbohydrate intake of villagers during rainy season and summer.

4.3.5 Dietary Fiber Intake

As shown in Table 3, the average dietary fiber intake of normal control, renal stone and dRTA groups (Figure 8) in rainy season were significantly different, they were 33, 16, and 21 g/day respectively. The mean dietary fiber intake in summer ranged from 15 to 16 g per day. The amount of dietary fiber consumed by the subjects in two seasons except normal subjects in rainy season, were lower than the level recommended by RDA (30 g/d).

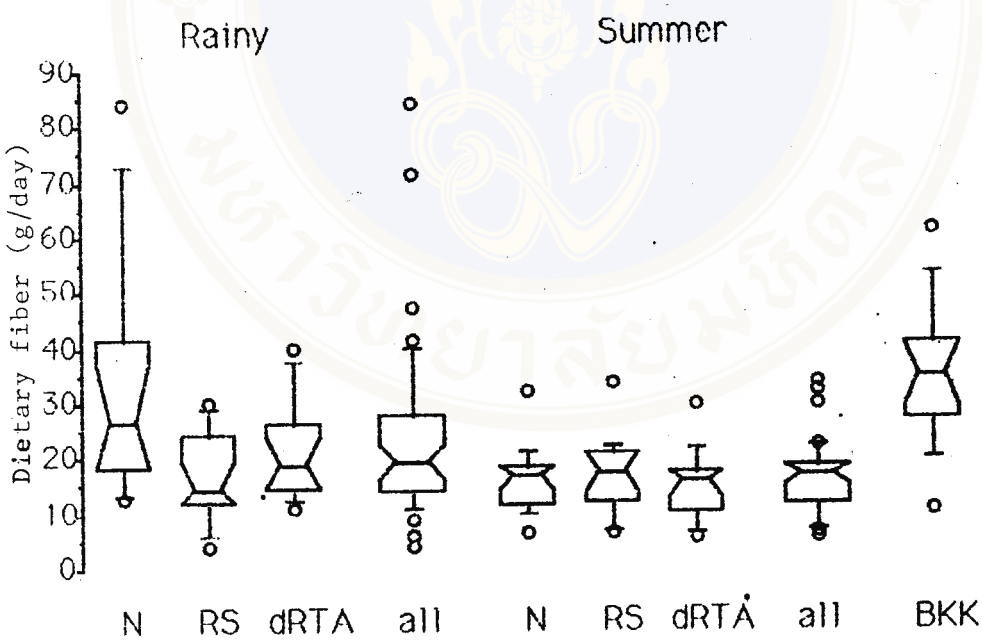


Fig 8. Dietary fiber intake of villagers during rainy season and summer.

4.3.6 Macro element

The level of macro element intake of the subjects was obtained from the amounts in foods and drinking water. Data on the average daily intake of macro-elements : calcium, phosphorus, magnesium, sodium, and potassium of the subjects in two seasons are shown in Table 3.

4.3.6.1 Calcium intake

The average calcium intake of normal, renal stone, and dRTA subjects in rainy season were 470, 554, and 490 mg per day and those in summer were 500, 442, and 577 mg per day respectively. These levels were about 55-70 percent of the level recommended (800 mg per day) for Thai adults (RDA 1989). There was no statistically different in the means of daily calcium intakes of all groups in both seasons (Figure 9).

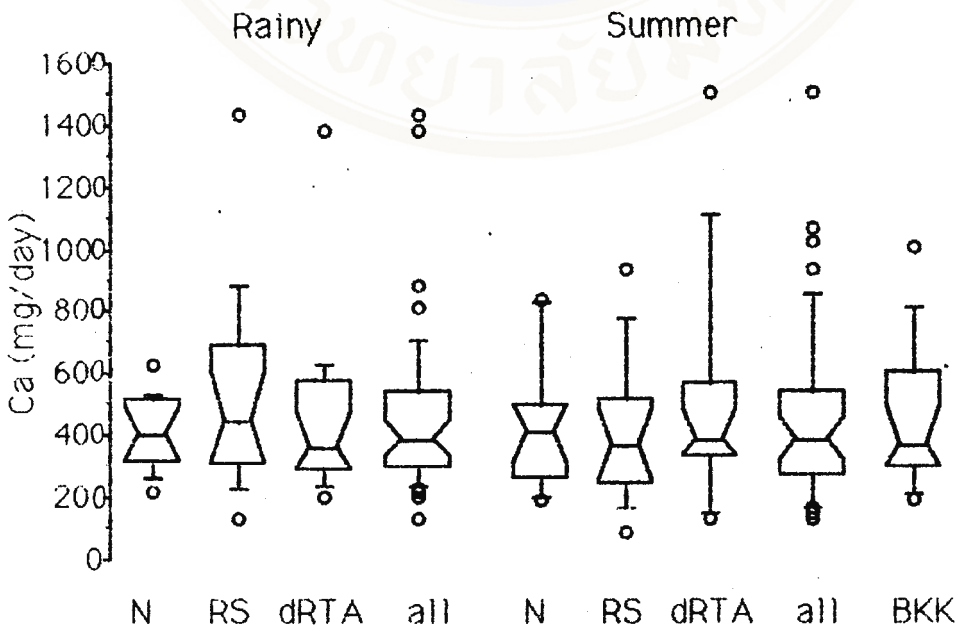


Fig 9. Calcium intake of villagers during rainy season and summer.

4.3.6.2 Phosphorus intake

The average phosphorus intakes of normal, renal stone, and dRTA were 741, 771, 767 mg per day in rainy season and 710, 678, 752 mg per day in summer respectively (Figure 10). No significant difference was found in mean phosphorus intake by different groups in both seasons. The amounts of daily phosphorus intakes was within the recommended level of 800 mg per day for Thai adults (RDA 1989).

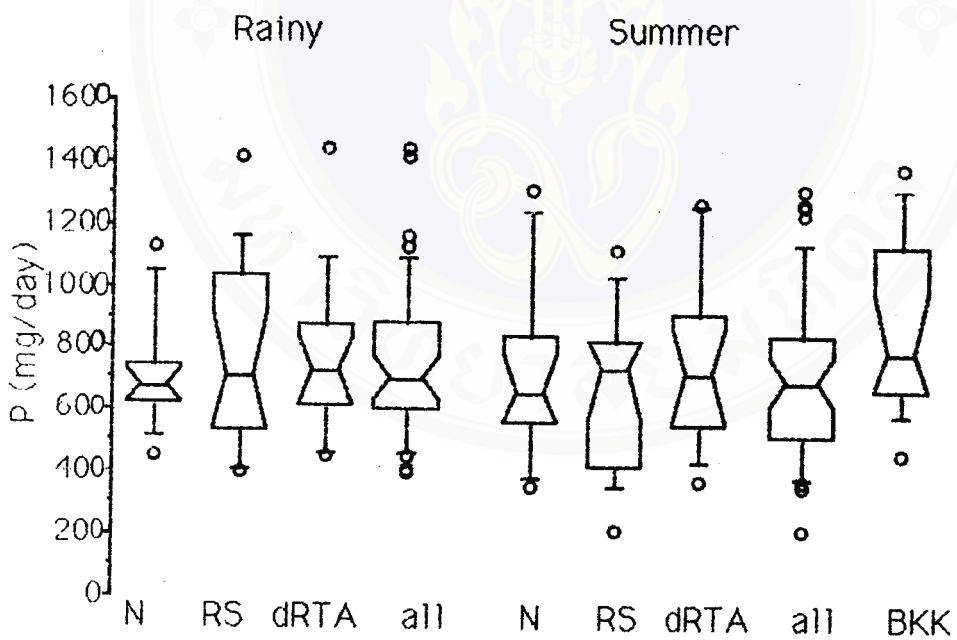


Fig 10. Phosphorus intake of villagers during rainy season and summer.

4.3.6.3 Magnesium intake

In rainy season, the average magnesium intake of the renal stone, dRTA and normal subjects was 204, 252 and 181 mg/d respectively. A significant difference in the daily magnesium intake ($p<0.05$) was found when compare the intake of normal group to that of dRTA group. On average, magnesium intake in summer was about 175 mg per day which is about a half of recommended level (RDA; 350 mg of Mg per day) (Figure 11).

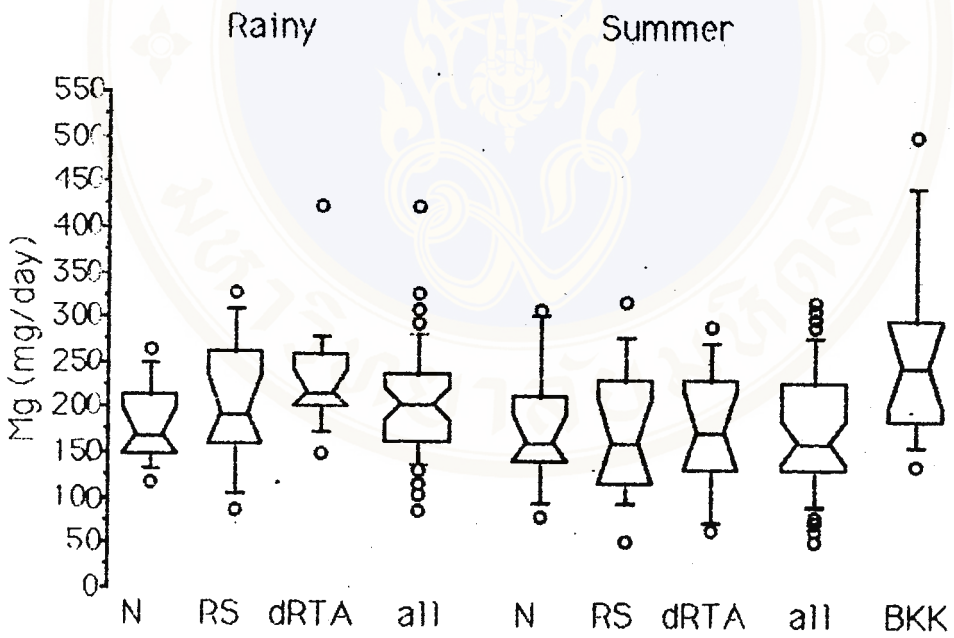


**Fig 11.** Magnesium intake of villagers during rainy season and summer.

4.3.6.4 Sodium intake

As shown in Table 3, the average intakes of sodium in normal, renal stone, and dRTA groups were 752, 800, 852 mg per day in rainy season and 782, 731, 844 mg per day in summer. The subjects consumed sodium less than the recommended level of the RDA (2500 mg/d) (Figure 12).

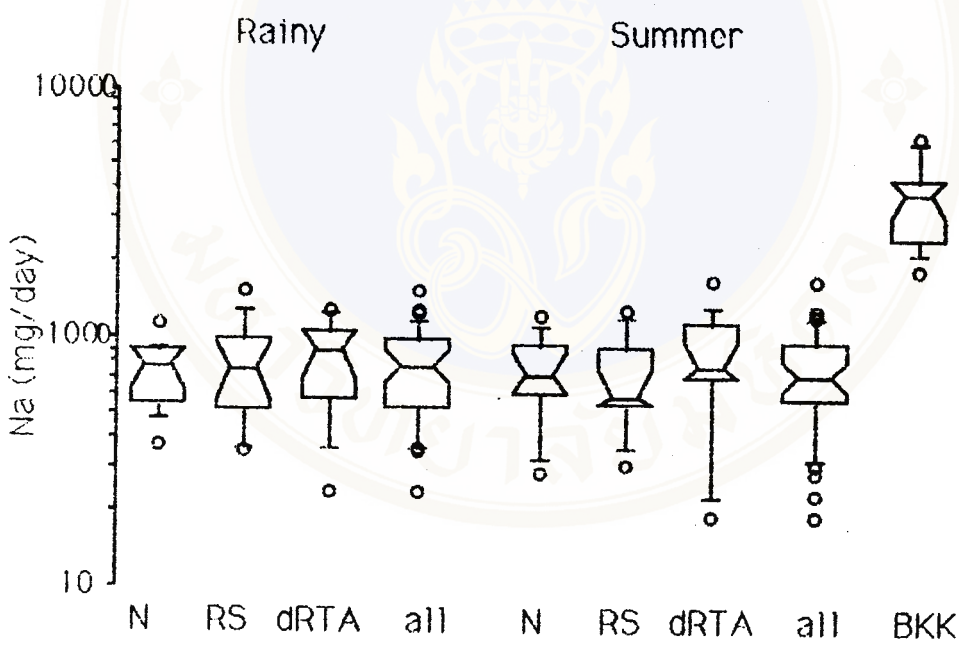


**Fig 12.** Sodium intake of villagers during rainy season and summer.

5.3.6.5 Potassium intake

The average potassium intake of all groups ranging from 1350 to 1480 mg/d in rainy season and 1240 to 1340 mg/d in summer. No significant differences of potassium intakes among the three groups of studied subjects were found and no significant variation of potassium intake during the two seasons (Figure 13).

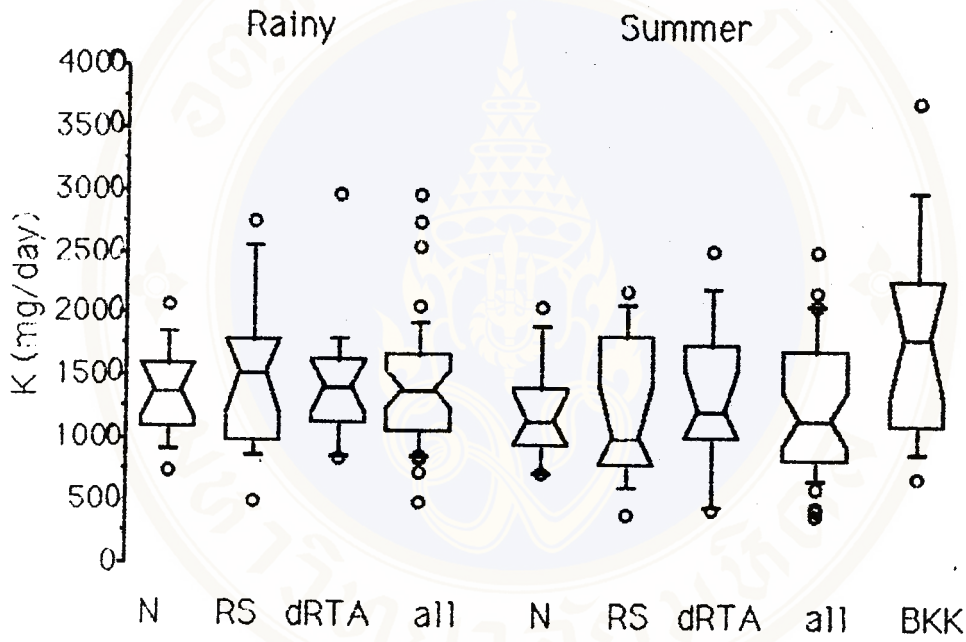


**Fig 13.** Potassium intake of villagers during rainy season and summer.

Considering the levels of macro-element intakes in Table 3, the studied subjects consumed about the same level of calcium but less amounts of phosphorus, magnesium and potassium than the levels consumed by the Bangkok subjects. The amount of sodium intake of Bangkok people was about 4-5 times more than that consumed by the studied subjects.

## 4.4 Antinutrient intakes

### 4.4.1 Oxalic acid

The means of oxalic acid intake were 28, 43 and 35 mg/day in normal, renal stone and dRTA respectively and no variation of intakes in each group during rainy and summer season was observed. Oxalic acid intake by the renal stone group was found the highest, 42 mg/day in rainy season and 44 mg/d in summer. They were significantly different ($p<0.05$) from the levels consumed by the normal subjects but no significant differences when compared to the dRTA (Figure 14).

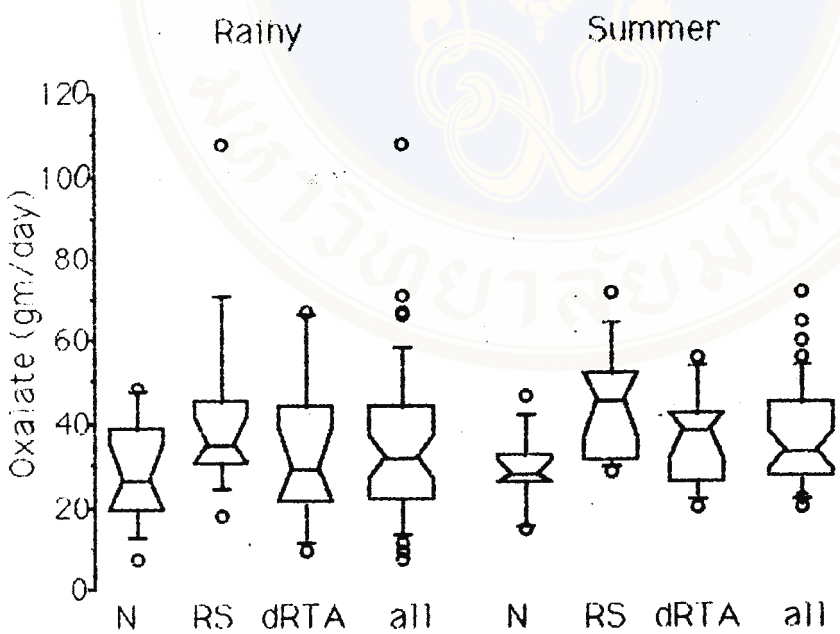


Fig 14. Oxalic acid intake of villagers during rainy season and summer.

### 4.4.2 Nucleic acid

The means intake of nucleic acid of normal, renal stone, and dRTA were 14, 18, 14 mg/day in rainy season and there were 14, 14, 18, mg/day in summer. The average of daily nucleic acid intake of cases was similar and no significant difference in means was found during the two seasons (Figure 15).

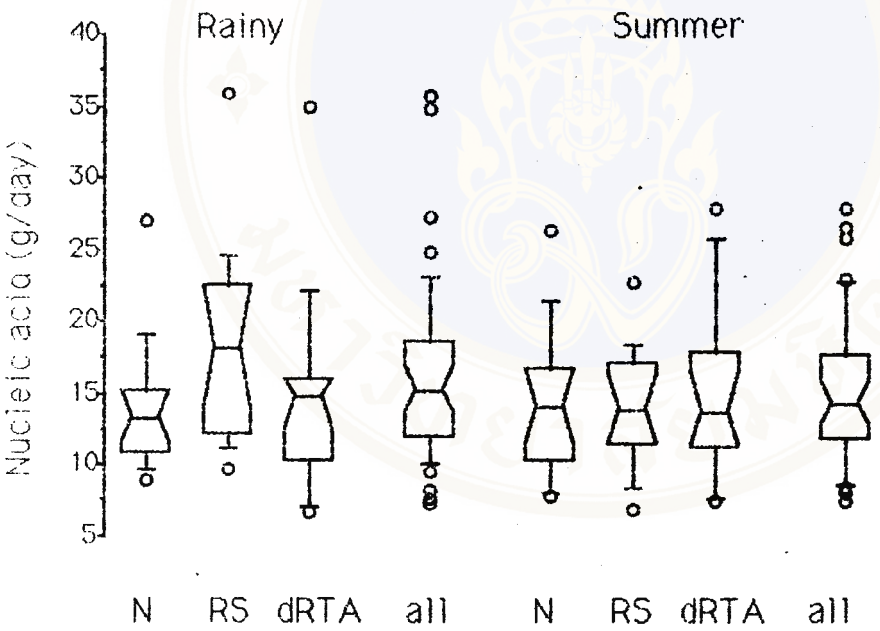


Fig 15. Nucleic acid intake of villagers during rainy season and summer.

## 4.5 Fluid intake

Average daily intake of drinking water in three studied groups in rainy season and summer was presented in Table 5. The water intake in rainy season among the normal, renal stone and dRTA groups were not significantly different, they were 2960, 3020 and 3718 ml respectively. In summer, water intake of dRTA group (4125 ml) was significantly higher than those consumed by the normal group (2760 ml) but not significantly different from the renal stone group (3706 ml).

## 4.6 Urine excretion

Each group excreted about the same volume of 24-h urine, 1224 and 1240 ml in normal, 1707 and 1708 ml in renal stone and 2167 and 2191 ml in dRTA group during the studied periods in rainy season and summer respectively (Table 5). There was significant difference ($P < 0.05$) in the urine volume excreted by dRTA compared to normal group in both seasons but not to renal stone group.

**Table 5** AVERAGE VOLUME OF DRINKING WATER AND 24-hr URINE EXCRETED BY VILLAGERS

| Volume (ml) | Normal | | Renal stone | | dRTA | |
|---|---|---|---|---|---|---|
| | Rainy | summer | Rainy | Summer | rainy | summer |
| Drinking Water | 2950±567 | 2671±641 | 3028±1053 | 3306±1252 | 3718±1470 | 4125±1609[aa] |
| 24-h Urine | 1224±632 | 1240±730 | 1707±901 | 1708±629 | 2167±1473[a] | 2191±1365[aa] |

Values are mean±SD.

a significant difference between normal and dRTA in rainy season ($p<0.05$).

aa significant difference between normal and dRTA in summer ($p<0.05$).

TABLE 6 URINARY COMPOSITION OF VILLAGERS IN KHONKAEN PROVINCE

| URINE | Normal value | Normal | | Renal-Stone | | dRTA | |
|---|---|---|---|---|---|---|---|
| | | Rainy | Summer | Rainy | Summer | Rainy | Summer |
| Citrate (mM/d) | 0.64-3.61 | 0.56±0.36 | 0.46±0.24 | 0.47±0.30 | 0.39±0.48 | 0.35±0.28 | 0.18±0.15 |
| Creatinine (g/d) | 0.8-1.8 | 0.9±0.4 | 1.2±0.8 | 0.9±0.3 | 1.4±1.1 | 0.9±0.4 | 1.7±0.9 |
| Urea N. (g/d) | 6.0-17.0 | 24.7±5.44 | 15.1±1.0 | 14.4±5.1 | 15.2±12.1 | 14.7±6.0 | 18.3±12.1 |
| Uric acid (mg/d) | 250-750 | 273±128 | 331±193 | 401±213 | 437±166$^{aa}$ | 486±231$^{b}$ | 594±340$^{bb}$ |
| Ca (mg/dl) | 100-240 | 95±42 | 110±67 | 150±81$^{a}$ | 149±104 | 150±65$^{b}$ | 144±100 |
| $PO_4$ (mg/d) | 900-1300 | 363±150 | 327±171 | 394±169 | 452±396 | 361±146 | 567±410 |
| Mg (mEq/d) | 0.3-4.3 | 3.4±1.4 | 3.6±2.4 | 4.7±2.9 | 4.1±1.5 | 5.1±3.2 | 5.3±3.6 |
| Na (mEq/d) | 43-217 | 89±52 | 110±90 | 107±60 | 76±42 | 81±38 | 139±88$^{cc}$ |
| K (mEq/d) | 26-133 | 18±9 | 20±14 | 22±11 | 19±14 | 16±7 | 26±16 |
| Cl (mEq/d) | 170-254 | 98±51 | 112±90 | 126±60 | 82±53 | 100±40 | 149±90 $^{cc}$ |
| $HCO_3$ (mM/d) | varies | 12±7 | 9±6 | 14±8 | 12±5 | 16±11 | 17±9$^{bb}$ |

Values are mean±SD.

a significcant difference between normal and renal stone in rainy ($p<0.05$).

aa significcant difference between normal and renal stone in summer ($p<0.05$).

b significcant difference between normal and dRTA in rainy ($p<0.05$).

bb significcant difference between normal and dRTA in summer ($p<0.05$).

cc significcant difference between renal stone and dRTA in summer ($p<0.05$).

TABLE 7 BLOOD CHEMISTRY PROFILES OF VILLAGERS IN KHONKAEN PROVINCE

| SERUM | Normal value | Normal | | Renal-stone | | dRTA | |
|---|---|---|---|---|---|---|---|
| | | Rainy | Summer | Rainy | Summer | Rainy | Summer |
| Albumin (g/dl) | 3.5-5.0 | 4.6±0.3 | 3.9±0.1 | 4.7±0.3 | 4.0±0.2 | 4.5±0.4 | 4.0±0.2 |
| Globulin (g/dl) | 2.5-3.2 | 3.0±0.3 | 3.7±0.3 | 3.2±0.2[a] | 3.8±0.3 | 3.1±0.4 | 3.8±0.4 |
| Alk. phosphatase(u/l) | 9.0-35.0 | 24.5±5.8 | 24.3±8.9 | 28.9±9.8 | 25.9±11.9 | 26.0±6.5 | 24.1±6.0 |
| Creatinine (mg/dl) | 0.6-1.4 | 1.0±0.1 | 1.1±0.2 | 1.4±0.6[a] | 1.3±0.3[aa] | 1.5±0.7[b] | 1.6±0.8[bb] |
| BUN (mg/dl) | 8.0-20.0 | 13.1±2.8 | 11.7±3.5 | 16.6±6.2[a] | 13.1±2.2 | 15.5±10.8 | 15.6±9.1 |
| Uric acid (mg/dl) | 3.0-7.8 | 4.2±0.8 | 4.1±0.7 | 5.0±1.1[a] | 4.6±1.1 | 5.0±1.1[b] | 4.5±.9 |
| Ca (mg/dl) | 8.5-10.5 | 9.3±0.6 | 9.2±0.2 | 9.9±0.6[a] | 9.2±0.5 | 9.7±0.7 | 9.1±0.3 |
| $PO_4$ (mg/dl) | 2.5-4.8 | 3.8±0.9 | 3.6±0.6 | 4.2±0.9 | 3.5±0.5 | 3.9±0.8 | 3.7±0.6 |
| Mg (mM/L) | 0.7-1.3 | 1.4±0.3 | 1.2±0.1 | 1.6±0.3 | 1.1±0.1[bb] | 1.4±0.3 | 1.2±0.1 |
| Na (mM/L) | 135.0-145.0 | 144.1±7.8 | 148.2±1.7 | 141.6±4.3 | 145.6±5.4 | 141.0±4.6 | 147.5±2.5 |
| K (mM/L) | 3.5-5.0 | 3.8±0.5 | 3.6±0.2 | 3.8±0.6 | 3.5±0.4 | 3.4±0.4[b,c] | 3.2±0.4[bb,cc] |
| Cl (mM/L) | 95.0-105.0 | 102.7±4.7 | 100.8±2.8 | 100.8±4.0 | 99.7±5.9 | 99.5±3.2[b] | 99.9±3.5 |
| $HCO_3$ (mM/L) | 25.0-30.0 | 26.2±4.8 | 25.9±1.2 | 28.2±4.8 | 24.6±2.4 | 25.9±3.8 | 25.7±2.0 |

Values are mean±SD.

a significiant difference between normal and renal stone in rainy season ($p<0.05$).

aa significiant difference between normal and renal stone in summer ($p<0.05$).

b significiant difference between normal and dRTA in rainy season ($p<0.05$).

bb significiant difference between normal and dRTA in summer ($p<0.05$).

c significiant difference between renal stone and dRTA in rainy season ($p<0.05$).

cc significiant difference between renal stone and dRTA in summer ($p<0.05$).

# 5 DISCUSSION

The impact of dietary factors on the formation of renal stone has been recognized for many years (63). Since dietary pattern of the Thai population in the northeast where renal stone was prevalent is remarkably different from other parts of the country, we therefore study the dietary composition of the villagers in this area to investigate its role in the pathogenesis of renal stone disease and dRTA.

Form this study, it was found that the main source of energy in the diets of the studied subjects was derived from glutinous rice. The amount consumed was two to three times more than the rice consumed by BKK people. However, the energy intake of the subjects was not much higher than that of BKK (2800-3000 and 1700-1800 Kcal/d in males and females respectively compared to 2300 and 1400 Kcal/d of BKK subjects). This is because BKK people consumed more caloric intake from lipid (30-35% of total energy intake) than the studied subjects (5-8% of total energy intake). The average lipid intake of BKK people was 52-82 g per day (Chitchumroonchokchai 1987) (62) compared to 13-16 g per day of the subjects in this study.

Fish and egg are major animal protein sources among the subjects. It was also observed that grilled chicken organ was often eaten. However, these subjects consumed large amount of glutinous rice contributed about 50-60 g protein per day (more than 80% of total protein intake). Glutinous rice is, therefore, the major protein

source among the studied subjects. It must be aware that eventhough protein intake of the subjects was found adequate (60-72 g per day) and higher than the Thai recommended level (Thai RDA 1989), the majority composed of incomplete essential amino acid mainly from glutinous rice.

Subjects in the studied area consumed lipid about 20-25% of the amount consumed by the people in Bangkok. It was mainly due to the fact that the subjects usually cooked their diets without using oil. In addition, not large amount of animal protein (about 2/3 of the amount consumed by Bangkok inhabitants) which contributed invisible fat was eaten.

It is clearly demonstrated that the average amount of daily dietary fiber intake (Table 3) varied with the amount of vegetables and fruits consumed (Table 2). In rainy season, normal subjects consumed the highest amount of vegetables and fruits which resulted in the highest dietary fiber intake in this group (about 30% higher than that of the dRTA and about 50% higher than the others). However, these amounts were slightly lower than those of BKK inhabitants and comparable well with the recommended level by Harmuth-Hoene (64). If there is any inhibitory effect of dietary fiber on the absorption of minerals which in turn reduce the mineral excretion in urine, it should affect mostly in the normal group than the others. The result of urine analysis in Table 6 shows the lowest excretion of urinary calcium and magnesium in normal group compared to the others.

As for the role of dietary minerals, in this study, hypercalcemia, hypomagnesemia, hypophosphatemia and hyperuricemia were not found among the subjects of renal stone and dRTA.

One of the pathogenetic factors contributed to renal stone formation is urinary calcium excretion, the aggregator of stone formation. Hypercalciuria will eventually lead to calcium stone formation, however this was not the case in this study.

A high dietary phosphate intake was reported to have benificial effect in decreasing urinary calcium excretion when dietary protein was increased (65). In addition, high dietary protein from animal source was reported to increase the urinary excretion of pyrophosphate, an important inhibitor of calcium - phosphate crystal formation. This effect is complex and involves both direct stimulation of distal nephron calcium reabsorption (66). and decreased bone resorption (67). In this study, the main dietary phosphorus was derived from glutinous rice (75-80% of total intake), and the rest was from animal protein. Could it be that the low concentration of urine phosphate found among all groups of the subjects in this study was due to the decreased absorption of phosphorus from plant origin which in turn lower the inhibitory factors of calcium-crystal formation. This speculation however need to be further investigated.

Magnesium deficiency was shown to cause hypercalciuria in rats resulted in renal parenchymal calcification with calcium deposition in the tubular cells and tubular lumen (68,69). In this study, low

magnesium intake was found in all groups of subjects including the normal. However, the level of calcium in urine of all the subjects was not increased. Although the levels of urinary calcium excretion were in the normal ranges, it was noticed that in rainy season the levels in renal stone and dRTA were significantly higher than that of the normal. Due to the wide variation of the data of these two groups and the small numbers of subjects in each particular groups, the significance of this difference in pathogenesis of stone formation should be taken with cautions. Johansson et al (70) also found no significant differences of serum and urine calcium and magnesium values in recurrent stone formers compared to normal controls matched for sex and age. but reported low magnesium/calcium ratio. In this study, no significant difference in the ratio between the three groups in the two seasons was found. Therefore, it is unlikely that calcium and magnesium would play a major role in pathogenesis of renal stone in this region.

Potassium intake of the subjects was low, about one half of the recommended level (1240-1470 vs 2500 mg per day) but no significant difference was found among the normal, stone formers and dRTA. Concentration of serum potassium was in the normal range except lower in the villagers with renal acidification defect (dRTA) than in those without (3.2-3.4 vs 3.6-3.8 mM/L, $p<0.05$). The villagers excreted much less potassium in their urine than did people in Bangkok, 16-26 vs 36.5 mM/day (71). These findings are in accordance with the previous report of studies performed in the population of the same area by Nilwarangkur, S. et al (3). Potassium is one of the

most abundant intracellular cations and is involved in many vital cellular functions. Potassium deficiency is one of the well-known cause of hypocitraturia. The finding of low urinary citrate excretion among the subjects (0.18-0.56 mM/d) may will be from potassium deficiency. Since citrate is one of the potent inhibitors of stone formations, low urinary citrate could promote nephrocalcinosis and nephrolithiasis. Thus hypocitraturia in the northeast may be one of the pathogenic factors of renal stone. If there was the case, low dietary potassium may contribute significantly to the pathogenesis of renal stone formation.

Previous studies reported that many of local vegetables in northeast Thailand contain high oxalic acid content (72). Duplicate meals analysis showed that the daily oxalic intake of all groups studied was less than 50 mg per day which was classified as low-oxalic acid diet (73). However, different levels of oxalic acid intake among these groups were observed, the highest was found in renal stone group (42-44 mg per day) followed by the dRTA (33-36 mg per day). In addition, urinary oxalate is derived from metabolic pathways, with approximately 40% derived from ascorbic acid and the remainder from the conversion of glycolate, glycine, hydroxyproline and alpha-hydroxy-beta-ketoadipate (74). For ascorbic acid, the metabolism of ascorbate to oxalate is saturated, and thus an increase in dietary intake does not necessarily result in arise in urinary oxalate excretion. As noted earlier in this study, people in the studied area consumed large amount of glutinous rice, about three to four times more than ordinary rice consumed by Bangkok people.

Eventhough glycine content in glutinous rice and ordinary rice are similar (256 vs 238 mg/100 g), large amount of glycine (>2000 mg/day) from glutinous rice (770-950 g (uncooked)/day) consumed by people in the north-east of Thailand may contribute high oxalate in urine. Unfortunately, urinary oxalate has not yet sucessfully measured in this study, the relationship of dietary oxalate and its precursor intake which related to the formation of renal stone cannot be therefore evaluated.

The nucleic acid content in food relates directly to its purine content. Due to some difficulties in determination of purine in this study the analysis of nucleic acid was therefore undertaken. Hyperuricosuria was not found in all studied subjects. It might be therefore concluded in this study that nucleic acid was not a major causing factor in developing of renal stone among the studied subjects.

As for the role of fluid intake, a high fluid intake is the only nutritional modification that is universally agreed to be useful in all forms of nephrolithiasis (75). It increases urine output which in turn dilutes urinary concentration of constituent ions and lower the saturation of stone forming salts. For normal activity, drinking of approximately 3 liters of fluid daily was recommended to achieve a minimum urine output of 2 liters per day. (76). In the studied villages, subjects drunk up adequate amount of water as recommended during rainy season and summer. However, only dRTA subjects whose daily water intake was approximately 4 liters excreted

24-h urine of more than 2 liters. The others excreted lower volume of 24-h urine, 1.3-1.7 liters per day and no significant difference in the urine volume were found during rainy season and summer. Since this study was performed in the northeast which is the temperate area of Thailand and the subjects are mainly farmers, the considerable amount of the fluid loss was probably due to sweating. The higher volume of 24-h urine found in the dRTA might be due to their higher water intake or their pathological condition of loosing capacity to concentrate urine. The consistent lower volume of urine output found in normal than in renal stone in both seasons might indicate similarity of the tendency for stone formation in the normal group compared to the stone former. Therefore, there should be some inhibitory factors in urine of the normal subjects that can inhibit stone formation which might be absent or present in an inadequate amount.

Refering to the available data of calcium and uric acid in urine which are accounted as urinary aggregators for stone formation, these values were still within the normal range and not expected to be the important factor of renal stone formation in this study. In addition, although low level of urinary citrate, the inhibitors for calcium-oxalate crystal forming, was found in all groups studied, the citrate concentration in the urine of normal subjects were slightly higher than the others. For phosphate, its role depends on the form of phosphate in urine, pyrophosphate inhibit calcium-phosphorus crystal formation while orthophosphate reduces urinary saturation of calcium oxalate. In this study lower level than normal range of

phosphate in urine was found in all groups studied and no data on the form of phosphate was available.

The results of the study suggested that low urinary inhibitors for stone formation might play an important role in the pathogenesis of renal stone in the northeast. However, it remains to be investigated why stone farmation occurred only in a subgroup of the population.

# 6 CONCLUSION

The attempt of this research was to study the food consumption pattern and nutrient intake, by duplicate meal analysis, in the population of north east Thailand where high incidence of renal stone and dRTA were found. The relationship between the nutrient intake and the pathogenesis of the diseases was assessed.

The results of the study showed that stone formers do not differ significantly from normal and dRTA in their consumption of either nutrients and antinutrients. There are many dietary intakes that beneficial to the people in the studied area in terms of not having the risk factors in favouring stone formation. Low intakes of animal protein, fat, sodium, calcium, oxalate and nucleic acid and high intake of fluid found among the studied population are the main related beneficial factors. However, high incidence of renal stone and dRTA are still prevalence in this area.

According to the fact that dietary pattern of the people in this area is quite typical and substantially differ from those of Bangkok people and other parts of Thailand, it may play an important role in increasing risk of the diseases among the people in the northeast region.

From the results obtained in this study, it can be proposed that the following factors may relate to the high incidence of the diseases in the population in the studied area.

(1) Consumption of large amount of glutinous rice may provide high concentration of carbohydrate, glycine or other amino acids which are precursor sources of endogenous oxalate.

(2) Low potassium intake might lead to potassium deficiency and may have an important role in the pathogenesis of renal stone and dRTA. This hypothesis, however needs to be further investigated.

(3) Main dietary phosphorus of the people in the studied area derived from plant origin which may not available for absorption. This in turn lower the amount of phosphate especially pyrophosphate (inhibitor of calcium crystal formation) excreted in urine found in this study.

(4) Having regular foods with crystal aggregators i.e. nucleic acid, oxalate, even at low amounts together with foods low in crystal forming inhibitory factors could be an important risk condition for forming of renal calculi.

It can be therefore concluded that there are some relationship between the nutrient intake of the people in the north-east of Thailand and the pathogenesis of the renal stone and dRTA.

# 7 SUMMARY

At present, information on nutrient intake of patients with upper urinary tract stone is still not available. The impact of diet on the formation of renal stone has been recognized, especially for the people in the northeast of Thailand. The aim of this study, therefore, to assess the nutrient intake of subjects with renal stone and distal renal tubular acidosis (dRTA) compared to those without diseases. The study was carried out in three villages, Ban Bungchim, Ban Pachad, Ban Nonelan, situate in amphur Mueng, Khonkaen province, Thailand. Daily food consumed by forty-four villagers, (21 males and 23 femeals) which can be categorized into three groups : the normal, renal stone, and dRTA, were collected for 3-consecutive days in rainy season and summer. Multiple composite samples of individual subjects, 3-day duplicated meal, and drinking water were chemically analysed. The relationship between nutrient and antinutrient intakes and information of blood and urine pictures among these groups was statistically evaluated.

The results showed that the subjects had adequate intake of energy (2000-2600 kcal/day), however it derived from an imbalanced diet of high carbohydrate (400-450 g/day) due to large amount (770-950 g/day) of glutinous rice, the staple carbohydrate food which contributes most of energy intake, was consumed. Lipid intake was quite low, contributed only 5-8% of total energy intake. This was mainly due to the way of cooking foods which involved mainly steaming, boiling, roasting, grilling and blanching. Adequate dietary protein

and phosphorus intakes derived from plant origin which may not be available for absorption. Fish is an animal protein source and amount intake was high in rainy season than in summer. Dietary fiber intake of normal group (33 g/day) in the rainy season was significantly higher than those of dRTA (21 g/day) and renal stone (16 g/day) groups. This is in accordance with the observation that higher amounts of gathered vegetables were consumed by most groups of subjects in rainy season than in summer, and the sums of average vegetables and fruits consumed by normal was more than renal stone and dRTA groups. Calcium, sodium and potassium intake were found below the recommended levels and no significant differences were observed among the three studied groups. Low potassium intake might lead to potassium deficiency and may have an important role in lowering urinary citrate excretion. Magnesium intake of all groups was lower than the requirement recommended by the Thai RDA but that of dRTA (252 mg/day) was significantly higher ($p<0.05$) than that of normal group (181 mg/day). In both seasons, oxalic acid intake by renal stone group was the highest, 40 mg/day, among the three studied groups. The level was significantly higher ($p<0.05$) than that of normal group. Nucleic acid intake of subjects was similar (about 20 mg/day) and no significant difference in means was found during the two seasons. The study showed that stone formation do not differ significantly from renal stone and their controls in their consumption of either nutrients and antinutrients. However, the dietary pattern of the population in this area is differ from those of Bangkok people and other parts of Thailand. Blood and urine composition were not found the pathogenesis of the hypercalcimia,

hypomagnesemia, hypophosphatemia, and hyperuricosuria among the subjects of renal stone and dRTA. It is interesting that the stone aggregators such as calcium and uric acid related the stone formation were still within the normal range. But low levels of urinary citrate and phosphate, the inhibitors for calcium stone formation, were found in all groups of studied. From the findings, it could be suggested that consumption of imbalanced diets, regular consumption of stone aggregators even at low concentration with inadequate quantity of stone forming inhibitors could be related to the incidence of renal stone and dRTA among the northeastern people of Thailand. The data of nutrient and antinutrient intakes obtained in this study provided basic information in understanding the ethiology of the renal stone disease. The relationship of glutinous rice consumption and the disease should be further investigated.

# 8 SUGGESTIONS FOR FUTURE RESEARCH

1. Relationshrip between carbohydrate and vegetable protein in Vake from glutinous rice on the urinary oxalate excretion.
2. Determination of urinary oxalic acid and pyrophosphate in renal stone patients.
3. Role of increasing of lipid intake, on the decreasing of glutinous rice consumption and renal stone formation.
4. Dietary fiber and mineral availability subjects with calcium stone forming.
5. The relationship of trace element in the diet of normal and renal diseases.
6. High fluid intake and inhibitor activities of stone formation in normal and nephrolithiasis subjects.

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## Appendix 1 : Sodium, Potassium (Na, K)

Sodium is the major cation in extracellular fluids whereas potassium is the chief intracellular cation in human and animals. Most vegetable foods contain little sodium, while animal foods have considerable amounts. Dry ashing methods may be used for sample preparation without losses. In this study, sodium and potassium were measured by using flame emission photometry.

### Reagents and Procedure

Standard sodium and potassium chloride containing 100 mM/L were purchased from the Beckman company. The sodium and potassium content of diluted acid sample were measured using flame emission photometer and compared with standards.

Recommended instrument setting (Rence : Food analysis by atomic absorption spectroscopy, 1973)

| | |
|---|---|
| Wavelength | 766.5 nm |
| Spectral band pass | 0.1 nm |
| Fuel/Support | propane/air |

The concentration of Na and K in terms of mM/L was then recorded and then calculated as followed.

$$\text{Na or K (mg/100 g)} = \frac{\text{reading of unknow} \times \text{M.W.} \times 100}{100 \times \text{weight of sample (g)}}$$

molecular weight of Na = 22.98977

molecular weight of K = 39.0983

In the air propane flame, sodium imigation is negligible and no chemical interference have been reported. For potassium, it is partially ionized in the air-acetylene flame. To suppress ionization, cesium nitrate or chloride solution is added to give a final concentration of 1000 ug/ml cesium in all solutions including the blank. In the air-propane flame ionization is negligible but interference from perchlorates is expected.

## Appendix 2 : Phosphorus

(Gravimetric Method)

### Principle

The procedure is suitable for any sample containing interfering elements such as iron, magnesium etc. Precipitating reagent used in this determination is ammonium molybdate in the presence of nitric acid. When the reagent is mixed with a solution of orthophosphate, a yellow precipitate of ammonium phosphomolybdate is formed, having a composition approach :

$$(NH_4)_3\ PO_4 \cdot\ 12MoO_3 \text{ or } (NH_4)_3\ (PO_4 \cdot\ 12MoO_3) \cdot\ 12H_2O$$

This compound should be called ammonium molybdophosphate and the formula is :

$$(NH_4)_3\ [P\ (Mo_{12}O_{40})] \cdot\ 12H_2O$$

Diammonium salt is the other possible form to occur as follow :

$$(NH_4)_2\ HPO \cdot\ 12MoO_3 \cdot \text{ aqeous}$$

which will be converted to the triammonium salt after washing with ammonium nitrate. The exact composition of the yellow precipitate depends on the composition of the molybdate reagent, nitric acid and ammonium nitrate, etc.

### Reagents

1. Ammonium molybdate solutions.

**Solution A** : dissolve 100 g of ammonium molybdate in a mixture of 400 ml of distilled water and 80 ml of concentrated ammonium hydroxide.

**Solution B** : dilute 400 ml of concentrated nitric acid with 600 ml of distilled water. Immediately before use, slowly pour a suitable volume of solution A into twice its volume of solution B.

2. Ammonium nitrate (5%) solution
3. Ethanol solution (95%)
4. Diethylether (anhydrous)
5. Glass filter crucible No. 4

**Procedure**

1. Dissolve ash in a porcelain crucible with 10 ml of 4 N nitric acid, filter into 100 ml volumetric flask using 541 filter paper.

2. Wash the residue in the crucible several times with distilled water and dilute to the mark.

3. Transfer quantitatively 20 ml of the solution into a 250 ml beaker.

4. Add 10 ml of conc. nitric acid and dilute the solution to 50 ml with distilled water.

5. Boil the solution on bunsen burner for exactly 3 min.

6. Add 50 ml of molybdate solution, let stand, every 5 min, swirl thoroughly for 30 min.

**Calculation**

$$\text{mg \% p} = \frac{Y \times (b-a) \times 0.015287 \times 100 \times 1000)}{\text{weight of sample (g)}}$$

(used for ash determinaton)

Y = dilution of sample.

Note : 1. If b-a is more than 1.5 g, dilute the sample and reanalysed.

2. Clean glassfilter crucible with distilled water, then 4 N NaOH, distilled water, 4 N nitric acid, distilled water, 70% ethanol, and ether, respectively.

## Appendix 3 : Calcium

Most foods contain considerable quantities of calcium, levels up to 100 ug/g for meat and about 1000 ug/ml for milk are typical (Underwood, 1971). At higher levels, titration with EDTA or CDTA is often used for analysis, but at lower levels atomic absorption is usually preferred. Wet or dry ashing methods are suitable for sample pretreatment.

**Reagents**

1. Standard calcium solution 100 mg% stock standard calcium solution was purchased from Merck company. The 5 mg% of intermediate calcium standard was also prepared by diluting 10 ml of stock standard to 200 ml with deionized distilled water. Working standard ranging form 0.25 to 1.25 mg/100 ml were prepared with 10% 4N nitric acid and 20% 5% lanthanum solution.

2. 5% stock lanthanum solution 58.659 of lanthanum oxide (calcium free was g of lanthanum oxide (Calcium free) was dissolved in 250 ml of concentrated hydrochloric acid. The solution was stirred until clear and then diluted to 1000 ml with deionized distilled water.

3. 4N nitric acid

4. Blank; deionized distilled water containing 10% 4N nitric acid (V/V) and 20% of 5% lanthanum solution (V/V) was used for zero setting.

**Procedure**

The sample filtrate (Portion I) obtained (see section III) was diluted appropriately with 10% 4N nitric acid and 20% (V/V) of 5% lanthanum solution in a volumetric flask and finally the calcium concentration in filtrate was adjusted with deionized distilled water until the desired concentration was within the range of working standard. Calcium determination was conducted using atomic absorption spectrophotemetry (Vaian Techtron AA-6). Instrument settings were as follows:

| | |
|---|---|
| Flame | air/acetylene |
| Lamps current | 3 mA |
| Spectral bandless | 0.5 nm |
| Wavelength | 422.7 nm |

Flame Stoichimetry reducing: red cone 1-1.5 cm high. The standard curves and the corresponding absorbance of both calcium standard concentrations and of sample were plotted. The slope was also determined. From the latter value we can find the quantity of calcium content in food sample which is generally expressed in mg/100 g.

Chemical interferences in air acetylene flame are pronounced and have been well documented. Apart frrom a 5-10% enhancement caused by the alkali metals (Na, K, Li) due to suppression of ionization all other interferences such as aluminium phosphate sulfate etc can be eliminative by lanthanum addition (10,000 ug/ml).

## Appendix 4 : Magnesium

Magnesium is present in all foods and is essential to nutrition; like potassium, it is a major intracellular cation. The concen tration is typically 10-1000 ug/g; high levels are found in leafy vegetables and lower levels in meat (Underwood 1971). Dry and wet ashing (Gorsuch 1970) may be used for sample preparation. Flame determination for magnesium is very sensitive and present no difficulty.

**Reagents**

1. Stock standard; 1000 ppm (1 mg/ml) of stock magnesium nitrate solution.

2. Intermediate standard; 10 ml of 1000 ppm stock magnesium standard was diluted with deionzed distilled water to 100 ml and then 10 ml of the later standard was further diluted to 100 ml of which this final concentration was 10 ppm.

3. Working standard; the series of working magnesium standard were prepared from the intermediate standard (10 ppm) by diluting with deionized distilled water accompanying with added 10% of 4 N nitric acid to make up the desired volume the final concentration of working standard was ranged form 0.1 to 1.0 ppm.

4. 4 N nitric acid

5. Blank; blank solution was prepared by making dilution of 10% of 4N nitric acid (V/V) in volumetric flash with deionized distilled water up to 100 ml.

**Procedure**

Magnesium content in sample filtrate from wet-ashing was determined by flame atomic absorption spectrophotometer in normal working conditions:

| | |
|---|---|
| Flame | air/acetylene |
| Lamp current | 3 mA |
| Spectral band pass | 0.5 |
| Wavelength | 285.2 nm |
| Flame stoichometry | oxidizing |

Magnesium concnetration of food sample was calculated form the formula:

$$\text{Mg (mg/100 g)} = \frac{\text{OD of sample x dilution x 100 x 100}}{\text{slope x weight of sample (g) x 1000}}$$

wheras; OD = absorbance

Slope = the value that obtained form the elaborated graph resulted from the various concentrations of working magnesium standard along the x-axis vs. the corresponding OD along th y-axis.

The detection limit by flame atomic is 0.0002 ug/ml in airacetylene and the most common interferences can be overcome by the addition of known excess of a releasing agent such as strontium (1000-5000 ug/ml) or lanthanum (10.000 ug/ml). In the carbon rod atomizer, phosphates affect the result by effecting the atomization kinetics. Samples should be prepared with excess phosphate and compared with similar standards.

## Appendix 5 : Crude Protein

### Macro-Kjeldahl Method

**Principle**

The foodstuff is oxidised by heating with concentrated sulphuric acid in the presence of catalyst. Sodium or potassium sulphate is frequently added to raise the boiling point of sulphuric acid. In this digestion process, nitrogen in the sample is converted to ammonium sulphate. After making alkali with concentrated sodium hydroxide, the ammonia is distilled and trapped with a known amount of standard acid. The unreacted acid is then determined by titration with standard alkali solution. The amount of acid used indicates the ammonia liberated from the foodstuff. Nitrogen content is hence determined. To obtain protein content, the amount of the nitrogen in the sample is multiplied by the converting factor, 6.25 being usually used since most protein contain 16% nitrogen.

**Reagents**

1. Concentrated sulfuric acid.
2. Catalyst : sodium sulfate + copper (II) sulfate pentahydrate + selenium (IV) dioxide (96 + 3.5 + 0.5 w/w).
3. 50% Sodium hydroxide.
4. 0.1 N sodium hydroxide (standardized with potassium acid pthalate)
5. Indicator solution : 0.1% methyl red (in 95% ethanol).
6. 0.1 N hydrochloric acid (add 0.1% methyl red 7 ml/1000 ml).

7. 30% Hydrogen peroxide.

**Procedure**

1. Weigh 0.5-5 g of sample and transfer to 750 ml kjeldahl digestion flask.

2. Add about 17 g of catalyst mix and 5 glass beads.

3. Add 25 ml of conc. sulfuric acid and 5 ml of hydrogen peroxide, then place on a preheated digester.

4. Digest of additional 30 min after the solution becomes clear or until oxidation is complete.

5. Cool, then add 300 ml of distilled water.

6. Place 500 ml erlenmeyer flask containing 50 ml of 0.1 N hydrochloric acid of the cork with the tip of the condenser extending below the surface of the acid solution.

7. Add 100 ml of 50% sodium hydroxide to the digested produce and immediately connect to the condensing unit. The ammonia distillate is led through the splash head and condensed into a flask containing acid solution.

8. Collect about 150 ml of distillate, then let the tip of the condenser above the solution and collect another 50 ml of distillate.

9. Titrate with standardized 0.1 N sodium hydroxide until the first appearance of yellow.

10. Carry out blank determination following exactly the same method as the sample.

**Calculation**

$$N\ (g\ \%) = \frac{\text{titer (blank-sample)} \times N \text{ of NaOH} \times 14.007 \times 100}{\text{weight of sample} \times 100}$$

$$\text{protein (g \%)} = \%\ N \times \text{appropriate converting factor}$$

## Appendix 6 : Crude Fat

### Continuous Extraction Method

**Principle**

The foodstuff is hydrolysed by diluted acid prior to continuous extraction with petroleum ether using Soxhlet apparatus. The residue in the extraction flask after solvent removal represents the fat content of the sample.

**Reagent**

4 N hydrochloric acid

Petroleum ether (B.P. 35-60°C).

**Procedure**

1. Weigh 5 g of sample into an erlenmeyer flask.
2. Add 50 ml of 4 N hydrochloric acid, then mix.
3. Connect to an air condenser and reflux with gentle boiling for 1 hr.
4. Filter and wash with hot water until the filtrate becomes free of acid (using pH papers).
5. Dry the filter paper containing digested sample in an oven at 60°C for 4-6 hr. Then transfer it into an extraction thimble.
6. Add 130 ml of petroleum ether into a preweighed round flat bottom flask, then connect to a soxhlet extractor. Extract the sample for 5-8 hr.

7. Disconnect the flask and evaporate off the petroleum ether on a steam bath in a fume hood. (the petroleum ether is evaporated using soxhlet apparates, redistilled and resused).

8. Dry the flask in an oven at 60°C until constant weight is obtained.

**Calculation**

$$\% \text{ crude fat} = \frac{\text{average weight of fat}}{\text{weight of sample}} \times 100$$

## Appendix 7 : Total Dietary Fibre

### Enzymatic-Gravimetric Method

**Principle**

Dietary fibre is usually defined as the sum of undigestible polysaccharide and lignin. The dietary fibre polysaccharide include celluloses, hemicelluloses, pectins, mucillages and gums which have quite variable compositions.

Dried sample, fat extraction with petroleum ether, 40-60°C B.P, 2-3 times is necessary if contains >5% fat, is gelatinized and hydrolyped with Termanyl (heat stable -amylase). The resistant starch is treated with dimethyl sulphoxide (DMSO) and -amyloglucosidase (AMG). Soluble dietary fibre is collected by precipitation in 95% alcohol and then completely separated together with insoluble dietary fibre by centrifugation. Total residue is filtered and weighed after drying. Protein and ash in the residue are measured and subtracted from the total residue, then the total dietary fibre content of the food sample is obtained.

**Apparattus** : - 50 ml test tube (or 50 ml screw-capped tube)

- Water baths a) boiling b) constant temperature adjustable to 60°C, with multistation magnetic stirrer to provide constant agitation of digested solution during enzymatic hydrolysis.
- Centrifuge

- Fritted crucible - porosity No.2 with celite as filter aid.
- Gooch crucible with glass/orcelain filter and acid washed sand as filter aid.
- Hot air oven - temperature 100±°C
- Desiccator
- Muffle furnace
- Magnetic bar 12 mm. length,
- Magnetic stirrer

Reagents :
- 0.1M Tris-aleate bffer pH 6.7 in $2.5 \times 10^{-3}$ M $CaCl_2$

  Weigh 6.057 g of Tris hydroxymethy laminomethane and 0.13873 g $CaCl_2$ anhydrous into a500 ml beaker, dissolve in deionised water(450 ml wasused), transfer to a 500 vol. flask and the volume isthenadjusted.
- Termamyl (heat-stable -amylase) -No. 120L, Novo Industri A/S, Copenhengen, Denmark. Store in refrigerator.
- 95% ethanol (v/v)
- -Amyloglucosidase
- Dimethyl sulphoxide
- Dimethyl sulphoxide (DMSO)
- 0.1M acetate buffer pH 4.6

**Calculation**

$$\text{Total dietary fibre} = \text{residue (unk-residue (bl)-[(prot.<unk>-prot.<bl>) + (ash <unk>-ash <bl>)] x 100}}{\text{weight of sample}}$$

## Appendix 8 : Moisture

### Air-oven Method

**Principle**

The direct heating or drying method is also called. A well ground sample is dried in an oven (usually at 100±5°C) until constant weight. The weight loss due to heating is the moisture content of the sample. Acid washed sand is used to mixed with the wet sample prior to drying in order to increase the surface area for rapid and complete evaporation of water from the wet sample.

**Procedure**

1. Weigh approximately 20 g of acid washed sand into a porcelain dish containing a small glass stirring rod. Dry in hot oven at 105°C for 30 min.

2. Remove the sand dish and allow it to cool in a desiccator. Weigh (=a g) and add approximately 5 g of sample. Reweigh (=b g).

3. Add sufficient distilled water to disperse the sample thoroughly while warming on a hot water bath.

4. Evaporate off the water as much as possible on the water bath. The sample + sand should be frequently mixed until it is near the completion of evaporation.

5. Transfer the sample dish to an oven, dry the sample at 100±5°C for 2 hr.

6. Remove the sample dish and cool it in a desiccator and weigh (=c g).

7. Return the dish to the dry until a constant weight is obtained. Reweigh every 30 min. The difference should not be more than 1-3 mg when the residual solids is 2-5 g.

**Calculation**

$$\% \text{ moisture} = \frac{b-c}{b-a} \times 100 \text{ (w/w)}$$

$$\% \text{ total solid} = 100 - \% \text{ moisture (w/w)}$$

## Appendex 9 : Ash

### Principle

The ash content is determined by incenerating a known quantity of foodstuff, previously dried, in a muffle furnace at 450° C, until constant weight is obtained.

The ash obtained is not necessarily the same composition as the mineral matter present in the original food as there may be losses due to volatilization of some interaction between constituents. The amount of ash in each sample may be used as an indicator of food quality. Higher figures suggest the possibility of an adulteration.

In addition to the determination of ash, mineral content can be obtained. The residue (the ash) is allowed to dissolve in acid solution, then subjected to anlysis of each element with appropriate methods.

### Procedure

1. Dry porcelain (or platinum, see note 1 below) crucible in the furnace at 450° C for 15 min.

2. Cool the crucible in a desiccator for 20 min. Weigh with accuracy of 1 mg (a g).

3. Weigh exactly 2-10 g of homogeneous or 5-20 ml of liquid sample into the weighed crucible.

4. Evaporation of sample on hot water bath until dry is necessary if the sample is liquid.

5. Heat the dried sample over a low flame of bunsen burner or on an electric hot plate until char. This is to burn away some of the organic matter.

6. Incinerate the charred sample in a furnace at 450° C until complete ashing is obtained (white or light gray ash). Remove the crucible and cool in a desiccator (see note 2 below).

7. Weigh with anaccuracy of 1 mg (b g).

8. If the ashing is not complete, carefully add 2 ml of 50 % nitric acid. Evaporate off the acid and re-ignite the sample and re-incinerated.

**Calculation**

$$\text{Ash} = \frac{b-a \times 100}{\text{weight or volume of sample}} \text{ \% w/w or \% w/v}$$

**Note**

Calcium, iron, magnesium, and phosphorus can be determined from the ash obtained. A platinum crucible is suitable for ashing sample subjected to sodium and potassium analyses.

## Appendix 10 : Oxalic acid

Determination of oxalic acid by calcium oxalate precipitation method.

**Reagent**

**1. Acctate buffer solution (pH 4.5)**

Dissolve 2.5 g anhydrous $CaCl_2$ in 50 mll acctic acid (1+1) and add to a 50 ml solution containing $CH_3COONa.\ 3H_2O$ 33 g.

**2. 6 N HCl**

**3. Tungstophosphoric acid**

Dissolve 2.5 g $Na_2WO_4\ 2H_2O$ in a mixlure of 4 ml $H_3PO_4$ and 50 ml $H_2O$, and dilute to 100 ml with $H_2O$

**4. Washed liguid**

Dilute 12.5 ml acctic acid to 250 ml with Dissolve calcium oxalate powder in the dilated acctic $H_2O$. Repeat addition of calcium oxalate and shaking until calcium oxalate solution is saturated. Coo to $4^{o}$ C and store in a refrigerator. Just before use, filter the amount needed. Keep cold during filtration and used.

5. **Calcium standard solution** (see appendix of calcium determination)

**Procedure**

Accurately weigh sample 3 g (dried weight) into 400-ml beaker. Add $H_2O$ to 150 ml, then add 27-28 ml 6N HCl, and boil 15 min. Cool, transfer quantity to a 250-ml volumetric flask, dilute to the volume with $H_2O$, mix, and let stand overnight. Mix, and filter through fast quantity paper, diseanding firs 100 ml filtrate. Pipet 25 ml filtrate into a 50-ml centrifuge tube, add 5.0 ml tungstophosphoric acid reagent, mix, and let stand >5 hr. Centrifuge at $4^{\circ}$ C for 10 minutes. Pipet 20 ml of the supernatant into a 50-ml centrifuge tube and adjust pH of the solution to 4-4.5 (using pH meter) by Add 5 ml of acctate buffer solution, and stir using magnetic stirrer. Rinse magnetic bar into centrifuge tube with small stream of $H_2O$ and let stand overnight. Centrifuge for 15 min at 1700 rpm. Decant supernate with one smooth continuons inversion of centrifuge tube. Hold tube upside down and let remaining supernate drip completely onto clean filter paper. Do not disturb the calcium oxalate precipitation. Wash precipitation by completely breaking the precipitate into fine suspension with fine jet stream of 20 ml filtered cold washed lignid. Repeat centrifugation and decanting steps, make sure that the precipitate is drained completely. Diseard paper. Dissolve the precipitate 5 ml $H_2SO_4$ (1+9). Quantity transfer entire contents of centrifuge tube into a 10-ml volumetric flask with $H_2SO_4$ (1+9) and dilute to the volume with same solution. Pipet 2 ml the diluted solution 50-ml volumetric flask containing 10 ml lanthanum solution. Dilute to the volume with $H_2O$

and analyse for calcium using atomic absorption spectrophotometer as describe in appendix 3 (calcium determination).

**Calculation**

$$\text{mg \% oxalic acid} = \frac{\text{OD of sample} \times K \times \text{dilution factor} \times 2.246}{\text{weight of sample (g)}}$$

where; 2.246 = to convert mg Ca to mg oxalic acid

## Appendix 11 : Nucleic acid

**Reagents**

1. Stock standard DNA - (400 ug/ml)
2. Stock standard RNA - (2 mg/ml)
3. 1 N $HClO_4$
4. 0.5 N $HClO_4$
5. 0.25 N $HClO_4$
6. 5 mM NaOH
7. Diphenylamine solution (solution A, 20 : solution B, 0.1)

**Solution A**

dissolve 1.5 g diphenylamine with 100 ml of glacial acetic acid and add 1.5 ml of $H_2SO_4$, keep in brown bottle

**Solution B**

dilute 1.6 g of Acetaldehyde with 100 ml of distilled water

8. Orcinol reagent (Solution A, 4 : solution B, 1)

**Solution A**

dissolve 0.45 g of $FeCl_3$ $6H_2O$ with 500 ml of conc. HCl, store in $0^{\circ}C$

**Solution B**

dissolve 1 g orcinol with 100 ml of distilled water, store in $0^{\circ}C$

**Standard solution**

- **Stock DNA solution** (400 ug/ml)

dissolve 0.04 g DNA with 5 mM NaOH and adjust to 100 ml (store $4^{\circ}C$, 6 months)

**Working standard solution**

Dilute aliquots of stock DNA, with 0.5 N $HClO_4$ to obtain > 5 standard solution within range of determination, 12.5-200 ug/ml, in final solution

- **Stock RNA solution** (2 mg/ml)

dissolve 0.2 g RNA in water and dilute to 100 ml

**Warking standard solution**

Dilute aliquots of series of stock RNA, with water to obtain 5 standard solution within range of determination, 0.0625-1 mg/ml, in final solution.

**Calculation**

Nucleic acid (mg/100 g) =

$$\frac{(\text{Reading OD of DNA} \times \text{dilution} \times 100)}{\text{Weight of sample (g)} \times 1000 \times 1000} + \frac{(\text{Reading OD of RNA} \times \text{dilution} \times 100)}{\text{Weight of sample (g)} \times 100}$$

## Appendix 12

**DEMOGRAPHY OF NORMAL VILLAGERS.**

| Subject code | Sex | Age (y) | Weight (Kg) | Height (cm) | Body mass index ($Kg/m^2$) |
|---|---|---|---|---|---|
| 1. B9 | Female | 43 | 58.0 | 156.2 | 23.8 |
| 2. B82 | Female | 35 | 55.0 | 148.6 | 24.9 |
| 3. B102 | Male | 38 | 65.5 | 166.4 | 23.6 |
| 4. B556 | Male | 39 | 53.5 | 160.0 | 20.9 |
| 5. P42 | Female | 42 | 64.0 | 149.1 | 28.8 |
| 6. P57 | Female | 44 | 55.5 | 160.7 | 21.7 |
| 7. P176 | Female | 40 | 57.5 | 153.0 | 24.6 |
| 8. P414 | Male | 45 | 54.0 | 157.0 | 21.9 |
| 9. P426 | Male | 42 | 54.0 | 160.8 | 20.9 |
| 10. L62 | Female | 47 | 54.0 | 148.2 | 24.6 |
| 11. L68 | Female | 44 | 67.5 | 154.3 | 28.3 |
| 12. L139 | Male | 48 | 60.0 | 164.5 | 22.2 |
| 13. L140 | Female | 45 | 54.0 | 155.9 | 22.2 |
| 14. L192 | Male | 43 | 55.5 | 164.6 | 20.5 |
| mean±SD | | 42.5±3.5 | 57.7±4.7 | 157.1±6.0 | 23.5±2.6 |

## Appendix 13

**DEMOGRAPHY OF RENAL STONE VILLAGERS.**

| Subject code | Sex | Age (y) | Weight (Kg) | Height (cm) | Body mass index ($Kg/m^2$) |
|---|---|---|---|---|---|
| 1. B226 | Male | 33 | 61.0 | 166.0 | 22.1 |
| 2. B236 | Male | 52 | 52.0 | 152.6 | 22.3 |
| 3. B337 | Female | 42 | 60.0 | 157.9 | 24.1 |
| 4. B361 | Male | 35 | 67.5 | 163.2 | 25.3 |
| 5. P63 | Female | 43 | 42.0 | 140.8 | 21.2 |
| 6. P110 | Female | 29 | 55.0 | 149.9 | 24.5 |
| 7. P193 | Male | 39 | 64.0 | 161.5 | 24.5 |
| 8. P315 | Female | 39 | 39.6 | 148.6 | 17.9 |
| 9. P418 | Female | 50 | 57.5 | 152.9 | 24.6 |
| 10. L226 | Female | 51 | 68.0 | 154.9 | 28.3 |
| 11. L259 | Female | 53 | 56.0 | 151.4 | 24.4 |
| 12. L259 | Female | 51 | 60.0 | 158.9 | 23.8 |
| 13. L404 | Male | 45 | 60.0 | 167.5 | 21.4 |
| 14. L405 | Male | 36 | 57.5 | 161.0 | 22.2 |
| 15. L489 | Male | 24 | 49.0 | 153.4 | 20.9 |
| mean±SD | | 41.5±9.0 | 56.6±8.2 | 156.0±7.2 | 23.2±2.4 |

## Appendix 14

**DEMOGRAPHY OF dRTA VILLAGERS.**

| Subject code | Sex | Age (y) | Weight (Kg) | Height (cm) | Body mass index ($Kg/m^2$) |
|---|---|---|---|---|---|
| 1. B56 | Female | 68 | 64.5 | 152.4 | 27.8 |
| 2. B70 | Male | 26 | 53.0 | 161.6 | 22.3 |
| 3. B160 | Male | 41 | 54.0 | 159.7 | 21.2 |
| 4. B208 | Female | 39 | 47.5 | 160.9 | 18.3 |
| 5. B262 | Male | 54 | 58.5 | 163.4 | 21.9 |
| 6. B357 | Female | 62 | 51.0 | 143.4 | 24.8 |
| 7. B410 | Female | 41 | 60.0 | 156.0 | 24.6 |
| 8. P303 | Female | 63 | 51.0 | 146.6 | 23.7 |
| 9. L13 | Male | 51 | 58.0 | 164.7 | 21.4 |
| 10. L150 | Male | 53 | 51.0 | 164.7 | 18.8 |
| 11. L191 | Female | 33 | 47.0 | 145.5 | 22.2 |
| 12. L228 | Male | 34 | 65.0 | 159.8 | 25.4 |
| 13. L297 | Female | 64 | 49.0 | 153.7 | 20.7 |
| 14. L299 | Male | 41 | 54.5 | 160.3 | 21.2 |
| 15. L300 | Male | 46 | 52.0 | 157.7 | 20.9 |
| 16. L474 | Male | 32 | 66.5 | 166.6 | 24.0 |
| mean$\pm$SD | | 45.5$\pm$12.1 | 55.2$\pm$6.2 | 157.3$\pm$7.2 | 22.4$\pm$2.5 |

## Appendix 15

**DAILY MAJOR NUTRIENT INTAKES OF NORMAL VILLAGERS IN RAINY SEASON**

| Subject | Energy (Kcal) | Protein (g/day) | Fat (g/day) | Carbohydrate (g/day) | Dietary fibre (g/day) |
|---|---|---|---|---|---|
| 1. B9 | 1800.76 | 63.19 | 16.44 | 350.01 | 38.24 |
| 2. B42 | 2194.61 | 63.69 | 10.93 | 460.37 | 47.19 |
| 3. B57 | 1910.88 | 51.15 | 6.73 | 411.43 | 27.43 |
| 4. B82 | 2145.64 | 66.59 | 19.81 | 425.25 | 41.45 |
| 5. B102 | 3443.03 | 99.97 | 22.36 | 710.48 | 83.86 |
| 6. B176 | 2143.70 | 64.52 | 8.53 | 425.21 | 26.05 |
| 7. B556 | 2860.93 | 77.92 | 18.08 | 596.63 | 71.41 |
| 8. P414 | 2082.61 | 72.65 | 8.26 | 429.42 | 23.42 |
| 9. P426 | 2920.48 | 101.10 | 17.89 | 588.77 | 27.89 |
| 10. L62 | 1781.86 | 40.76 | 5.47 | 392.40 | 17.77 |
| 11. L68 | 2146.05 | 59.33 | 11.05 | 452.32 | 19.30 |
| 12. L139 | 2288.68 | 67.82 | 19.89 | 459.60 | 12.95 |
| 13. L140 | 1712.32 | 40.22 | 12.09 | 360.66 | 15.12 |
| 14. L192 | 2612.62 | 60.13 | 9.91 | 570.73 | 13.28 |
| mean±SD | 2289±500 | 66.4±18.0 | 13.4±5.5 | 473.8±103.7 | 33.2±21.6 |

## Appendix 16

**DAILY MAJOR NUTRIENT INTAKES OF RENAL STONE VILLAGERS IN RAINY SEASON**

| Subject | Energy (Kcal) | Protein (g/day) | Fat (g/day) | Carbohydrate (g/day) | Dietary fibre (g/day) |
|---|---|---|---|---|---|
| 1. B56 | 1581.46 | 33.05 | 14.66 | 329.33 | 5.93 |
| 2. B70 | 4026.18 | 105.47 | 17.23 | 862.31 | 14.05 |
| 3. B160 | 3282.84 | 98.51 | 30.71 | 653.10 | 24.56 |
| 4. B208 | 1555.55 | 55.50 | 7.74 | 315.97 | 10.96 |
| 5. B262 | 2661.24 | 70.04 | 18.71 | 553.17 | 8.99 |
| 6. B357 | 1585.89 | 43.54 | 11.92 | 326.11 | 3.74 |
| 7. B410 | 2485.46 | 68.87 | 21.62 | 503.85 | 13.45 |
| 8. P303 | 1525.67 | 47.48 | 9.32 | 312.97 | 13.22 |
| 9. L13 | 2349.18 | 74.30 | 26.71 | 452.95 | 16.11 |
| 10. L91 | 2268.20 | 64.59 | 12.67 | 473.95 | 30.13 |
| 11. L150 | 1761.77 | 51.31 | 7.45 | 372.37 | 15.99 |
| 12. L228 | 3800.15 | 94.76 | 20.70 | 808.70 | 23.05 |
| 13. L297 | 2089.03 | 54.11 | 11.04 | 443.31 | 24.96 |
| 14. L300 | 2585.99 | 81.17 | 17.50 | 525.95 | 29.24 |
| 15. L474 | 3009.03 | 72.14 | 9.91 | 657.82 | 12.14 |
| mean±SD | 2438±810 | 67.7±21.0 | 15.9±6.9 | 506.1±174.9 | 16.4±8.2 |

## Appendix 17

**DAILY MAJOR NUTRIENT INTAKES OF dRTA VILLAGERS IN RAINY SEASON**

| Subject | Energy (Kcal) | Protein (g/day) | Fat (g/day) | Carbohydrate (g/day) | Dietary fibre (g/day) |
|---|---|---|---|---|---|
| 1. B193 | 3217.92 | 80.09 | 8.33 | 705.65 | 26.43 |
| 2. B226 | 3373.92 | 87.02 | 12.25 | 728.90 | 17.57 |
| 3. B236 | 3516.31 | 116.85 | 23.72 | 708.86 | 37.39 |
| 4. B337 | 1640.93 | 46.83 | 14.30 | 331.23 | 13.50 |
| 5. B361 | 2244.09 | 71.52 | 28.16 | 426.14 | 22.53 |
| 6. P63 | 1701.63 | 44.26 | 11.96 | 354.25 | 12.41 |
| 7. P110 | 2184.51 | 74.17 | 18.32 | 430.74 | 28.28 |
| 8. P315 | 1901.44 | 50.33 | 7.88 | 407.30 | 15.29 |
| 9. P393 | 1879.88 | 54.75 | 14.08 | 383.54 | 16.08 |
| 10. P418 | 2292.67 | 73.59 | 24.74 | 443.91 | 18.64 |
| 11. L226 | 2166.38 | 48.55 | 8.03 | 474.98 | 13.19 |
| 12. L259 | 2940.79 | 68.48 | 17.20 | 628.02 | 11.00 |
| 13. L404 | 4086.96 | 108.53 | 20.88 | 866.23 | 25.95 |
| 14. L405 | 3165.87 | 87.88 | 16.94 | 665.47 | 19.66 |
| 15. L489 | 2498.73 | 65.26 | 11.18 | 534.27 | 39.56 |
| mean±SD | 2587±746 | 71.9±21.9 | 15.9±6.3 | 539.3±164.0 | 21.2±8.8 |

## Appendix 18

**DAILY MAJOR NUTRIENT INTAKE OF NORMAL VILLAGERS IN SUMMER**

| Subject | Energy (Kcal) | Protein (g/day) | Fat (g/day) | Carbohydrate (g/day) | Dietary fibre (g/day) |
|---|---|---|---|---|---|
| 1. B9 | 1683.39 | 56.93 | 27.66 | 301.68 | 15.81 |
| 2. B82 | 2180.77 | 56.95 | 16.12 | 451.97 | 16.45 |
| 3. B102 | 3872.21 | 106.48 | 38.41 | 775.15 | 20.07 |
| 4. B556 | 2569.28 | 81.13 | 29.22 | 495.49 | 18.15 |
| 5. P42 | 2087.34 | 55.72 | 11.05 | 441.25 | 18.08 |
| 6. P57 | 1315.15 | 36.06 | 7.60 | 275.63 | 10.49 |
| 7. P176 | 2180.50 | 53.11 | 10.02 | 467.47 | 10.96 |
| 8. P414 | 1728.60 | 53.52 | 18.45 | 337.12 | 17.74 |
| 9. P426 | 2563.65 | 93.60 | 25.49 | 489.96 | 31.91 |
| 10. L62 | 1709.07 | 40.28 | 15.44 | 352.25 | 6.16 |
| 11. L68 | 1828.82 | 50.60 | 9.75 | 384.67 | 17.08 |
| 12. L139 | 1813.17 | 55.59 | 10.68 | 373.67 | 10.77 |
| 13. L140 | 1185.91 | 32.72 | 6.68 | 248.75 | 10.99 |
| 14. L192 | 1777.31 | 62.70 | 21.43 | 333.41 | 18.53 |
| mean±SD | 2035±662 | 59.7±20.9 | 17.7±9.5 | 409.3±131.7 | 15.9±6.2 |

## Appendix 19

**DAILY MAJOR NUTRIENT INTAKE OF RENAL STONE VILLAGERS IN SUMMER**

| Subject | Energy (Kcal) | Protein (g/day) | Fat (g/day) | Carbohydrate (g/day) | Dietary fibre (g/day) |
|---|---|---|---|---|---|
| 1. B70 | 3438.76 | 88.54 | 36.32 | 688.43 | 22.29 |
| 2. B160 | 2168.22 | 77.83 | 27.29 | 402.82 | 17.44 |
| 3. B208 | 1821.22 | 53.94 | 9.53 | 379.92 | 6.71 |
| 4. B262 | 2192.15 | 60.80 | 22.83 | 435.87 | 17.24 |
| 5. B357 | 1217.75 | 36.64 | 12.19 | 204.37 | 6.20 |
| 6. B410 | 1883.90 | 58.89 | 7.67 | 394.73 | 17.30 |
| 7. B566 | 1481.50 | 41.31 | 10.83 | 304.70 | 11.56 |
| 8. P303 | 1345.93 | 37.49 | 10.00 | 276.49 | 9.03 |
| 9. L13 | 2206.44 | 63.86 | 14.67 | 454.74 | 18.78 |
| 10. L150 | - | - | - | - | - |
| 11. L191 | 2232.17 | 72.66 | 21.65 | 436.67 | 16.59 |
| 12. L228 | 2943.65 | 89.11 | 20.74 | 600.14 | 33.27 |
| 13. L297 | 1538.14 | 48.99 | 11.18 | 310.39 | 10.94 |
| 14. L299 | 2294.71 | 79.04 | 18.95 | 452.00 | 16.70 |
| 15. L300 | 1989.71 | 72.91 | 8.94 | 404.40 | 21.11 |
| 16. L474 | 3289.31 | 90.43 | 18.18 | 690.99 | 22.34 |
| mean±SD | 2136±663 | 64.8±18.6 | 16.7±8.1 | 435.9±142.9 | 16.5±7.0 |

## Appendix 20

**DAILY MAJOR NUTRIENT INTAKE OF dRTA VILLAGERS IN SUMMER**

| Subject | Energy (Kcal) | Protein (g/day) | Fat (g/day) | Carbohydrate (g/day) | Dietary fibre (g/day) |
|---|---|---|---|---|---|
| 1. B193 | 2821.47 | 64.62 | 15.90 | 604.97 | 7.82 |
| 2. B226 | 3775.82 | 104.77 | 14.77 | 805.95 | 16.56 |
| 3. B236 | 3278.99 | 97.20 | 28.56 | 658.29 | 17.18 |
| 4. B337 | 1969.88 | 63.56 | 22.01 | 379.39 | 15.16 |
| 5. B361 | 2829.74 | 83.72 | 14.62 | 590.82 | 17.02 |
| 6. P63 | 1113.30 | 34.20 | 4.90 | 233.10 | 5.62 |
| 7. P110 | 2082.68 | 81.47 | 30.21 | 371.23 | 16.47 |
| 8. P315 | - | - | - | - | - |
| 9. P393 | 1070.10 | 37.88 | 9.77 | 207.66 | 6.60 |
| 10. P418 | 1879.83 | 61.02 | 12.08 | 381.76 | 20.82 |
| 11. L226 | 1550.79 | 35.05 | 7.76 | 335.19 | 9.12 |
| 12. L259 | 1833.17 | 44.09 | 8.52 | 395.03 | 12.09 |
| 13. L404 | 3122.00 | 89.21 | 22.36 | 640.98 | 29.36 |
| 14. L405 | 2848.01 | 82.04 | 15.01 | 596.19 | 19.29 |
| 15. L489 | 2354.03 | 59.58 | 15.32 | 494.46 | 13.36 |
| mean±SD | 2323±818 | 67.0±23.4 | 15.8±7.6 | 478.2±175.5 | 14.8±6.4 |

## Appendix 21

**DAILY MACRO ELEMENT INTAKE OF NORMAL VILLAGERS IN RAINY SEASON**

| Subject | Calcium (mg/day) | Phosphorus (mg/day) | Magnesium (mg/day) | Sodium (mg/day) | Potassium (mg/day) |
|---|---|---|---|---|---|
| 1. B9 | 331.15 | 677.48 | 218.46 | 901.81 | 1842.22 |
| 2. B82 | 294.56 | 650.15 | 168.33 | 524.13 | 1504.38 |
| 3. B102 | 668.79 | 1123.81 | 264.48 | 892.24 | 2074.31 |
| 4. B556 | 470.10 | 873.21 | 214.07 | 766.53 | 1657.74 |
| 5. P42 | 371.06 | 739.74 | 139.52 | 544.01 | 951.02 |
| 6. P57 | 439.18 | 589.71 | 116.22 | 375.33 | 744.00 |
| 7. P176 | 490.22 | 697.13 | 211.69 | 915.52 | 1587.86 |
| 8. P414 | 557.73 | 640.43 | 133.24 | 766.16 | 1386.02 |
| 9. P426 | 548.09 | 1038.70 | 247.86 | 783.85 | 1362.01 |
| 10. L62 | 254.13 | 454.31 | 143.59 | 487.69 | 1062.08 |
| 11. L68 | 412.04 | 607.83 | 179.60 | 1143.68 | 1591.65 |
| 12. L139 | 548.30 | 724.68 | 170.39 | 788.99 | 1104.43 |
| 13. L140 | 305.06 | 517.86 | 152.85 | 674.60 | 937.42 |
| 14. L192 | 349.01 | 661.74 | 149.58 | 707.62 | 1067.42 |
| mean±SD | 470±186 | 714±185 | 181±45 | 752±208 | 1348±384 |

## Appendix 22

**DAILY MACRO ELEMENT INTAKE OF RENAL STONE VILLAGERS IN RAINY SEASON**

| Subject | Calcium (mg/day) | Phosphorus (mg/day) | Magnesium (mg/day) | Sodium (mg/day) | Potassium (mg/day) |
|---|---|---|---|---|---|
| 1. B56 | 350.59 | 411.09 | 106.06 | 335.58 | 871.55 |
| 2. B70 | 1485.92 | 1417.94 | 267.22 | 988.44 | 1726.46 |
| 3. B160 | 824.80 | 1165.13 | 308.32 | 964.86 | 2760.82 |
| 4. B208 | 255.14 | 501.62 | 172.18 | 752.15 | 1543.32 |
| 5. B262 | 371.10 | 658.33 | 177.06 | 630.18 | 1164.63 |
| 6. B357 | 301.72 | 398.17 | 86.74 | 364.03 | 513.01 |
| 7. B410 | 458.12 | 700.20 | 192.38 | 791.11 | 1535.02 |
| 8. P303 | 308.01 | 497.23 | 147.98 | 381.38 | 949.12 |
| 9. L13 | 170.00 | 708.70 | 163.85 | 735.57 | 1149.24 |
| 10. L150 | 696.30 | 704.79 | 154.79 | 579.44 | 908.27 |
| 11. L191 | 890.92 | 1055.48 | 293.53 | 1343.27 | 1800.46 |
| 12. L228 | 509.76 | 1014.44 | 326.44 | 1571.68 | 2568.22 |
| 13. L297 | 425.30 | 718.66 | 225.08 | 861.63 | 1929.09 |
| 14. L300 | 718.50 | 1034.40 | 240.13 | 1182.80 | 1734.77 |
| 15. L474 | 544.69 | 592.23 | 205.28 | 494.62 | 1026.06 |
| mean±SD | 554±334 | 771±300 | 204±72 | 800±363 | 1479±632 |

## Appendix 23

**DAILY MACRO ELEMENT INTAKE OF dRTA VILLAGERS IN RAINY SEASON**

| Subject | Calcium (mg/day) | Phosphorus (mg/day) | Magnesium (mg/day) | Sodium (mg/day) | Potassium (mg/day) |
|---|---|---|---|---|---|
| 1. B63 | 290.97 | 465.18 | 208.66 | 367.06 | 958.89 |
| 2. B193 | 402.14 | 773.85 | 211.46 | 1043.71 | 1479.53 |
| 3. B226 | 591.47 | 846.25 | 266.44 | 1309.37 | 1804.62 |
| 4. B236 | 369.16 | 886.09 | 216.05 | 1062.76 | 1632.99 |
| 5. B337 | 278.07 | 450.92 | 237.33 | 240.02 | 859.12 |
| 6. B361 | 389.69 | 676.04 | 196.20 | 1194.10 | 1462.99 |
| 7. P110 | 268.16 | 769.68 | 175.03 | 731.32 | 1418.25 |
| 8. P315 | 226.19 | 533.45 | 280.65 | 452.60 | 874.77 |
| 9. P393 | 303.04 | 593.01 | 151.65 | 461.68 | 1061.00 |
| 10. P418 | 419.64 | 939.67 | 237.22 | 1095.47 | 1645.27 |
| 11. L226 | 678.52 | 687.50 | 217.58 | 893.42 | 1367.24 |
| 12. L259 | 628.04 | 721.36 | 180.20 | 689.06 | 1312.06 |
| 13. L404 | 1429.54 | 1442.49 | 424.58 | 1282.55 | 2967.83 |
| 14. L405 | 666.39 | 1090.50 | 280.30 | 854.07 | 1782.41 |
| 15. L489 | 416.00 | 628.05 | 202.45 | 1021.37 | 1394.29 |
| mean±SD | 490±299 | 767±257 | 232±65 | 852±336 | 1468±515 |

## Appendix 24

**DAILY MACRO ELEMENT INTAKE OF NORMAL VILLAGERS IN SUMMER**

| Subject | Calcium (mg/day) | Phosphorus (mg/day) | Magnesium (mg/day) | Sodium (mg/day) | Potassium (mg/day) |
|---|---|---|---|---|---|
| 1. B9 | 456.10 | 756.61 | 186.21 | 716.35 | 1429.08 |
| 2. B82 | 250.11 | 551.11 | 151.89 | 627.48 | 1129.58 |
| 3. B102 | 861.88 | 1230.38 | 301.50 | 1237.73 | 1908.61 |
| 4. B556 | 522.40 | 870.71 | 214.71 | 1107.10 | 1698.82 |
| 5. P42 | 501.87 | 758.00 | 174.39 | 750.13 | 1226.88 |
| 6. P57 | 211.16 | 402.05 | 99.14 | 337.41 | 733.54 |
| 7. P176 | 390.19 | 655.70 | 150.32 | 630.69 | 1083.55 |
| 8. P414 | 273.33 | 659.22 | 144.76 | 899.22 | 1299.01 |
| 9. P426 | 853.00 | 1313.51 | 310.83 | 1107.37 | 2075.13 |
| 10. L62 | 317.18 | 388.74 | 81.38 | 476.98 | 740.87 |
| 11. L68 | 517.86 | 551.48 | 231.76 | 952.65 | 1194.03 |
| 12. L139 | 355.20 | 603.63 | 136.79 | 591.45 | 932.87 |
| 13. L140 | 270.45 | 360.68 | 99.27 | 304.90 | 794.04 |
| 14. L192 | 606.48 | 842.41 | 170.11 | 719.74 | 1236.98 |
| mean±SD | 500±262 | 710±287 | 175±70 | 782±306 | 1249±416 |

## Appendix 25

**DAILY MACRO ELEMENT INTAKE OF RENAL STONE VILLAGERS IN SUMMER**

| Subject | Calcium (mg/day) | Phosphorus (mg/day) | Magnesium (mg/day) | Sodium (mg/day) | Potassium (mg/day) |
|---|---|---|---|---|---|
| 1. B56 | 178.18 | 356.39 | 99.16 | 366.52 | 730.43 |
| 2. B70 | 387.91 | 1001.08 | 278.44 | 974.16 | 1572.24 |
| 3. B160 | 372.51 | 835.46 | 194.18 | 945.98 | 1930.92 |
| 4. B208 | 196.54 | 466.86 | 97.28 | 491.65 | 1020.74 |
| 5. B262 | 490.70 | 630.53 | 182.86 | 838.65 | 1438.66 |
| 6. B357 | 104.48 | 217.04 | 52.72 | 325.44 | 408.29 |
| 7. B410 | 362.13 | 644.68 | 162.28 | 801.02 | 1185.15 |
| 8. P303 | 251.57 | 391.431 | 128.44 | 355.68 | 955.30 |
| 9. L13 | 567.20 | 734.70 | 148.53 | 561.42 | 786.21 |
| 10. L150 | - | - | - | - | - |
| 11. L191 | 458.78 | 795.94 | 246.64 | 873.79 | 2092.25 |
| 12. L228 | 867.26 | 1031.89 | 317.93 | 1241.92 | 2108.97 |
| 13. L297 | 285.47 | 382.84 | 109.75 | 558.78 | 635.14 |
| 14. L299 | 615.45 | 786.59 | 183.13 | 609.10 | 874.35 |
| 15. L300 | 518.08 | 785.31 | 142.49 | 593.62 | 818.00 |
| 16. L474 | 980.59 | 1118.07 | 245.57 | 1253.86 | 2197.27 |
| mean±SD | 442500±246 | 678±270 | 173±74 | 731±288 | 1250±597 |

## Appendix 26

**DAILY MACRO ELEMENT INTAKE OF dRTA VILLAGERS IN SUMMER**

| Subject | Calcium (mg/day) | Phosphorus (mg/day) | Magnesium (mg/day) | Sodium (mg/day) | Potassium (mg/day) |
|---|---|---|---|---|---|
| 1. B63 | 156.42 | 374.68 | 65.24 | 194.02 | 476.39 |
| 2. B193 | 395.45 | 607.69 | 128.16 | 676.32 | 997.98 |
| 3. B226 | 587.69 | 1000.77 | 272.63 | 1145.61 | 2197.77 |
| 4. B236 | 1533.83 | 1266.64 | 202.45 | 1321.75 | 1307.28 |
| 5. B337 | 372.74 | 576.22 | 155.04 | 774.78 | 1179.99 |
| 6. B361 | 1076.10 | 793.54 | 243.26 | 1150.33 | 1743.09 |
| 7. P110 | 429.83 | 913.61 | 200.90 | 1132.63 | 1795.34 |
| 8. P315 | - | - | - | - | - |
| 9. P393 | 182.60 | 493.24 | 75.54 | 240.22 | 469.33 |
| 10. P418 | 551.05 | 737.78 | 231.87 | 737.61 | 1778.86 |
| 11. L226 | 303.35 | 441.28 | 99.11 | 355.41 | 762.52 |
| 12. L259 | 367.40 | 692.16 | 159.90 | 690.70 | 1196.72 |
| 13. L404 | 303.35 | 1261.22 | 290.13 | 1721.85 | 2544.96 |
| 14. L405 | 639.90 | 817.73 | 189.15 | 895.42 | 1322.95 |
| 15. L489 | 367.40 | 545.55 | 140.24 | 785.10 | 1055.76 |
| mean±SD | 577±398 | 752±280 | 175±70 | 844±427 | 1342±613 |

## Appendix 27

**DAILY OXALIC ACID AND NUCLEIC ACID INTAKE OF NORMAL VILLAGERS IN RAINY SEASON AND SUMMER**

| Subject | Oxalic acid (mg) | | Nucleic acid (mg) | |
|---|---|---|---|---|
| | rainy | summer | rainy | summer |
| 1. B9 | 20.88 | 15.17 | 10.03 | 14.65 |
| 2. B82 | 26.88 | 25.57 | 10.67 | 12.54 |
| 3. B102 | 47.51 | 41.17 | 27.05 | 26.32 |
| 4. B556 | 48.63 | 26.32 | 13.96 | 16.60 |
| 5. P42 | 38.76 | 32.01 | 17.03 | 14.62 |
| 6. P57 | 33.86 | 14.47 | 8.98 | 8.11 |
| 7. P176 | 40.83 | 24.60 | 12.05 | 18.03 |
| 8. P414 | 35.75 | 32.61 | 14.50 | 10.04 |
| 9. P426 | 25.80 | 46.31 | 18.23 | 20.87 |
| 10. L62 | 6.89 | 30.48 | 14.94 | 13.32 |
| 11. L68 | 12.95 | 28.87 | 15.08 | 13.61 |
| 12. L139 | 12.90 | 26.42 | 12.77 | 14.71 |
| 13. L140 | 21.39 | 29.82 | 9.79 | 7.81 |
| 14. L192 | 18.23 | 21.73 | 11.74 | 9.62 |
| mean±SD | 33±22 | 16±6 | 14±5 | 14±5 |

## Appendix 28

**DAILY OXALIC ACID AND NUCLEIC ACID INTAKE OF RENAL STONE VILLAGERS IN RAINY SEASON AND SUMMER**

| Subject | Oxalic acid (mg) | | Nucleic acid (mg) | |
|---|---|---|---|---|
| | rainy | summer | rainy | summer |
| 1. B56 | 34.61 | 29.18 | 11.15 | 6.87 |
| 2. B70 | 32.07 | 71.70 | 24.59 | 22.70 |
| 3. B160 | 57.47 | 49.28 | 22.40 | 13.99 |
| 4. B208 | 30.91 | 39.68 | 11.64 | 15.03 |
| 5. B262 | 70.81 | 52.87 | 22.46 | 17.06 |
| 6. B357 | 38.37 | 28.32 | 9.67 | 8.47 |
| 7. B410 | 29.36 | 45.32 | 19.81 | 13.83 |
| 8. P303 | 43.77 | 29.18 | 12.32 | 11.16 |
| 9. L13 | 24.35 | 47.01 | 16.90 | 11.04 |
| 10. L150 | 17.88 | - | 11.76 | - |
| 11. L191 | 34.53 | 45.13 | 15.22 | 12.57 |
| 12. L228 | 44.15 | 63.95 | 35.79 | 17.27 |
| 13. L297 | 26.17 | 32.68 | 18.30 | 12.98 |
| 14. L299 | - | 38.52 | - | 18.38 |
| 15. L300 | 46.03 | 29.45 | 18.19 | 11.17 |
| 16. L474 | 107.39 | 59.64 | 22..79 | 17.11 |
| mean±SD | 42±22 | 44±14 | 18±7 | 14±4 |

## Appendix 29

**DAILY OXALIC ACID AND NUCLEIC ACID INTAKE OF dRTA VILLAGERS IN RAINY SEASON AND SUMMER**

| Subject | Oxalic acid (mg) | | Nucleic acid (mg) | |
|---|---|---|---|---|
| | Rainy | Summer | Rainy | Summer |
| 1. B63 | 22.89 | 24.91 | 12.64 | 7.43 |
| 2. B193 | 53.42 | 37.80 | 22.17 | 17.74 |
| 3. B226 | 17.39 | 41.58 | 14.94 | 27.74 |
| 4. B236 | 29.04 | 55.69 | 15.54 | 25.49 |
| 5. B337 | 8.88 | 24.79 | 10.30 | 10.11 |
| 6. B361 | 10.94 | 37.69 | 11.64 | 13.27 |
| 7. P110 | 26.02 | 42.17 | 7.08 | 17.64 |
| 8. P315 | 20.17 | - | 7.75 | - |
| 9. P393 | 28.93 | 19.65 | 6.65 | 7.75 |
| 10. P418 | 20.91 | 37.85 | 9.92 | 11.10 |
| 11. L226 | 44.65 | 21.82 | 16.11 | 12.25 |
| 12. L259 | 42.46 | 23.25 | 14.86 | 14.19 |
| 13. L404 | 66.66 | 53.29 | 34.83 | 22.39 |
| 14. L405 | 66.29 | 43.73 | 16.04 | 15.99 |
| 15. L489 | 42.62 | 38.17 | 15.46 | 12.37 |
| mean±SD | 33±18 | 36±11 | 14±7 | 15±6 |