Evaluation of Urinary Creatinine as a Biochemical Index of Muscle Mass and Excretory Levels of Urea, Thiamine, Ascorbic Acid and Niacin for The Assessment of Nutritional Status amoung Adolescent Girls and Young College Women

By

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Introduction

CHAPTER I

INTRODUCTION

Measuring Muscle Mass was an important component of the nutritional assessment of an individual and 24-hour urinary excretion of creatinine can be considered as an index of muscle mass (Heymsfield *et al.*, 1983). Assuming that nearly all body creatine was within muscle and that muscle gives a constant creatinine output, urinary creatinine was expected to be proportional to muscle mass (Burger, 1949).

Muscle was roughly 80 per cent water and 20 per cent protein. Approximately muscle represented 4 to 10 Kg. of the body's total 10 to 12 Kg. of protein (Cohn *et al.*, 1981). Muscle mass and urinary creatinine were related due to the following reasons, namely,

Creatine was a nitrogenous organic compound which participated in cellular energy metabolism and was found primarily in muscle.

On a creatine free diet, the total body creatine pool and the average concentration of creatine per kilogram of muscle remained constant.

Creatine was converted non-enzymatically and irreversibly to creatine at a constant daily rate.

Creatinine once formed undergone renal excretion at a constant rate.

Bloch and Schoenheimer (1941) first proposed that creatine was derived from 3-aminoacids namely arginine, glycine and S-adenosyl methionine. The predominant bio-synthetic site in human was the kidney.

Bloch, Borsook and Dubnoff (1972) elucidated the first two bio-synthetic steps, the synthesis of guanido-acetate from glycine and argininine. The reaction was by the enzyme glycine aminotransferase which was found in human kidney, liver, pancreas, spleen, brain and mammary gland (Walker, 1966).

In the next reaction by the transfer of a methyl group from S-adenosyl methionine to guanidoacetate, creatine was formed (Walker, 1979). Synthesised creatine was released into the circulation, where the next step was active uptake against a concentration gradient, by muscle and other tissues (Haugland and Chang, 1975).

The conversion of creatine to creatinine was brought about purely on a physio-chemical basis. This irreversible reaction was now firmly established within muscle. Creatine existed in two forms, creatine and phosphocreatine. Creatine converted to creatinine at a rate of 1.1 per cent per day, while phosphocreatine conversion was 2.64 per cent per day. (Heymsfield *et al.*, 1983).

Once formed, creatinine diffused from the cell and ultimately appeared in the urine after glomerular filtration and to a small extent tubular excretion (Varley, 1980). Urinary creatinine level was directly proportional to the muscle mass of an individual. This was expressed as the creatinine coefficient, the amount of creatinine in milligrams excreted per 24 hour/kg of body weight. Creatinine coefficient varies from 18 - 32 in man and from 10 - 25 in women (Forbes et al., 1976).

There was a correlation between oxygen consumption and urinary output of creatinine (Zetelli et al., 1981). Creatinine excretion had been studied in a wide variety of stressful conditions and psychiatric illness. Scarimshaw et al (1966) concluded that creatinine output increases during stress. There was increased creatinine excretion during muscular dystropy (Barac-Nieto, 1984). Extremely strenous exercise increased urinary creatinine output by 5 - 10 per cent (Srivastava et al., 1957).

Urinary creatinine excretion was found to influenced to some extent by three dietary constituents namely protein, creatine and creatinine (Heymsfield et al., 1984).

Studies by Steven (1983) demonstrated a rise and fall in creatine and creatinine excretion during the menstrual cycle in women. The creatinine excretion was increased by 5 - 10 per cent late in the second half of the menstrual cycle and decreased in the days preceeding or during menstrual cycle.

In the adult, fat free body mass declined with increasing age and most of this loss in lean tissue had been attributed to the atropy of skeletal muscle (Srivastava *et al*., 1957).

The creatinine coefficient was low in obese and asthmatic persons and it was high in heavily muscled persons of average height. (Sauberlich *et al*., 1974). Creatinine height index was sensitive index of the body's nutritional status. There was a muscle wasting with retention of some subcutaneous fat during kwashiorkar and there was severe muscle wasting and subcutaneous fat during nutritional marasmas (Jelliffe, 1972).

Creatinine enabled the assessment of glomerular filtration and was used as a kidney function test in clinical biochemistry. (Varley, 1980).

Urea was the principle nitrogenous end product of amino acid metabolism (Lehninger, 1977). Excretion of urea was the direct function of total nitrogen intake, thus it was directly related with the quantity of protein consumed. The ratio of urea to creatinine in a random sample of urine reflected the amount of protein consumed. (Shankin *et al*., 1978). Since urea was the principal end product of protein metabolism in the human, the use of nitrogen to creatinine ratio and urea to creatinine ratio had been proposed for evaluating dietary protein intakes (Sauberlich, 1974). Under laboratory conditions, an increase or a decrease in the level of protein intake was paralleled by marked changes in the urinary urea/creatinine ratio (Simmons, 1972).

Urinary excretion of thiamine decreased proportionally with thiamine intake to a critical point after which further lowering of intake resulted in only minor and variable changes in urinary excretion (Pearson, 1967). Measuring 24-hour urinary thiamine had been useful in evaluating thiamine nutriture in man (FAO/WHO, 1967).

The functions of ascorbic acid included its role in the synthesis of collagen, wound healing, amino acid and carbohydrate metabolism, and for the synthesis of some hormones and iron (ICMR, 1981). Since urinary levels of ascorbic acid were probably prove to reflect immediate dietary intakes, measuring 24-hour urinary ascorbic acid had been useful in evaluating ascorbic acid nutriture in man (Ziporin et al., 1965).

The measurement of urinary excretion levels of N1-methyl nicotinamide had been commonly employed for evaluating niacin nutrition. Under normal conditions, adults excreted 20 - 30 per cent of their nicotinic acid as the N1-methylnicotinamide form and 40 - 60 per cent as the 2-pyridone (Lange and Jaarber, 1964).

Thus the nurinary excretion analysis for water soluble vitamins provided information pertaining to vitamin nutriture.

Thus, the study was aimed at estimating 24-hour urinary creatinine level as an index of muscle mass of adolescent girls and young women. Estimation of urinary urea, thiamine, niacin and ascorbic acid was done to assess the nutritional assessment of the same category. Ten adolescent girls of age ranging from 16 - 18 years, ten young college women of the under-graduate course of age ranging from 19 - 21 years and ten young women who were graduate students of age ranging from 20 - 23 years from Sri Avinashilingam Higher Secondary School and College were participated in the study. Their body height, weight, skinfold thickness, mid-arm circumference and also 24-hour urinary excretion of creatinine, urea, thiamine, niacin and ascorbic acid were analysed. Care was taken to collect urine samples only during non-menstruating days.

Creatinine excretion for early morning voiding, total six hours voiding, and 24-hour voiding urine samples were analysed, to evaluate the use of first two samples as compared to 24-hour excretion.

Review of Literature

CHAPTER II

REVIEW OF LITERATURE

Myers and Fine (1943) were the first to show that urinary creatinine output was directly proportional to the total body muscle mass. Krisho and Walker (1966) suggested that the higher creatinine coefficient (mg creatinine per kg body weight) was found in men as compared to women. The active uptake of creatine replaced about two per cent of the total amount of creatine in muscle each day (Walker, 1979). The rate of creatinine uptake was retarded by cooling and anaerobiosis and enhanced by insulin (Haugland and Chang, 1975).

About 98 per cent of the total creatine pool was with in muscle (Hunter, 1938). This creatine pool was slowly saturable (Fitch et al., 1968) and had a relatively slow daily turnover rate of about 1.5 to 2 per cent. Although the creatine concentration per kg and muscle varied from muscle to muscle, the average concentration was 3 to 5 g per kg of wet fat free tissue. The functions of muscle creatine were:

1) High energy intermediate in excitation - contraction coupling.

   Phospho creatine + ADP $\underset{2}{\overset{1}{\longleftrightarrow}}$ creatine + ATP

   Enzyme one was creatine phosphokinase and enzyme two was creatine phosphokinase.

2) Modulated glycolysis.

3) Increased intracellular flux of potassium and phosphorus.

4) Acted as a cofactor in muscle growth.

Other tissues (brain, kidney and liver) and fluids (blood and urine) contained measurable amounts of creatine.

Hamm and Mayer (1928) showed that creatine was converted to creatinine within muscle and was brought about purely on a physicochemical basis. Creatine dehydrated to creatinine at a rate of 1.1 per cent per day, while phospho creatine at the rate of 2.64 per cent per day.

Excretion of Creatinine in Urine

Once formed, creatinine diffuses from the cell and ultimately appears in the urine after glomerular filteration and to a small extent, tubular secretion (Bjornsson, 1979).

Conditions that modulate output of creatinine

Folin's Law (1904) stated that on a creatine free diet, the output of creatinine was constant from day to day for each individual. Some variability was however recognised in the healthy subject, more importantly some pathological states was able to alter normal creatinine output.

a) Normal day to day variability

Urinary excretion of an individual varied daily, the causes of which were largely unknown. Careful studies in variable subjects had shown a daily variation from 4 to 8 per cent in creatinine excretion, which can not be explained by minor variation in physical activity and diet (Cryer and Sode, 1976).

b) Exercise

Extremely strenuous exercise increased urinary output of creatinine by 5 to 10 per cent (Hobson, 1939) and (Srivastava, 1957). This effect was studied in normal subjects marching for 3 hour at two different speeds. The increase in daily creatinine output was about 10 per cent. The mechanism of these exercise induced charges in urinary creatinine output was unknown (Riley *et al*., 1981).

c) Emotional Stress

Creatinine excretion had been studied in a wide variety of stressful conditions and psychiatric illnesses (Scrimshaw *et al*., 1966). They concluded that the variability in creatinine output increased, during stress, but the underlying causes of this phenomenon were poorly understood.

d) Diet

Dietary protein was the main source of the aminoacid precursors of creatinine. The activity of the first enzyme in creatinine biosynthesis, transaminidase was influenced by dietary protein intake (Van Pilsum, 1957). For example Van Pilsum, fed rats of a protein free diet for 12 days and found on 85 per cent reduction in transamidinase activity. The level of protein intake per second appeared to have a small effect on urinary excretion of creatinine (Bleiler and Schedl, 1972). The large reduction in creatinine excretion from the basal diet occured because the men were ingesting a meat - free diet, although the total protein intake appears to have had some effect (Heymsfield et al., 1984).

Feeding the two dietary aminoacid precursors of creatinine, arginine and glycine, enhanced tranimidinase activity and was found to increase urinary excretion of guanidacetate. The activation of tranimidinase resulted in higher rate of creatinine production, as well as enlargement of creatine pool independant of change in muscle mass. The second dietery constituent that influence urinary creatinine excretion was preformed creatinine. The amount of dietary creatinine directly influenced the size of creatine pool which in turn was proportional to the output of creatinine in the urine (Bleiler et al., 1972). The average daily dietary intake of creatine and creatinine replaced about one-third to one-half of the daily urinary creatinine losses. The influence of dietary creatine on urinary creatinine excretion had been examined by a number of investigators. Switching to a meat - free protein source, Bleiler and Schedl (1972) showed a reduction in urinary creatinine. Crim et al., (1975) showed that nitrogen and potassium balance remained positive despite declining creatinine excretion. The third component of the diet that influensed creatinine excretion, mentioned above was creatinine. Dietary creatinine had undergone intestinal absorption and prompt renal excretion (Shaffer, 1908).

Menstrual Cycle

Studies by Smith (1942) demonstrated a rise and fall in creatine and creatinine excretion during the menstrual cycle, but changes were apparently unrelated to nitrogen levels. He showed that creatinine excretion increased by 5 to 10 per cent. Late in the second half of the menstrual cycle and then decreased in the days preceeding or during menstrual cycle.

Ageing

In the adult, fat free body mass declined with increasing age and most of this loss in lean tissue could be attributed to the atropy of skeletal muscle (Tomlinson, 1969). Creatinine excretion also declined with age and this presumably reflects the diminution in muscle mass. Dietary factors also accounted for some of the decrease in urinary creatinine with age, as the elderly tend to eat less meat (Bleiler and Schedl, 1972).

Infection, fever and trauma

Waterlow *et al* (1972) found increased creatinine turn over in rats suffering from lung abscesses. Frawley *et al* (1955) showed an increase in creatinine excretion but the daily output appeared to be independent of type, degree or location of injury. Schiller *et al* (1979) confirmed by demonstrating a rise in creatinine excretion with both fever and severe trauma.

Renal disease:

As glomerular filtration rate had fall in chronic renal failure, there was decrease in the excretion of creathinine. Goldman (1964) suggested that low output was due to either reduced creatinine production or an alternate excretory pathway. The studies of Mitch and Coworkers (1978) indicated metabolism rather than excretion of creatinine. These workers identified the two pathways of creatinine catabolism, a recycling of creatinine to creatine and intestinal degradation of creatinine to products other than creatine.

Starvation

Brief starvation was associated with an increase in gluconeogenesis suggesting increased Release of amino acid from muscle. Muscle release of the principle gluconeogenic amino acids were increased. The increased release for all amino acids averaged 69.4 per cent. This contrasted with the reduction in amino acid released characterising prolonged starvation (Pozefuky, 1976).

Urea

Urea was the principle nitrogenous end product of amino acid metabolism (Lehninger, 1977). Excretion of urea was the direct function of total nitrogen intake, thus it was directly related with the quantity of protein consumed (Varley, 1980). The ratio of urea to creatinine in a random sample of urine also reflected the amount of protein in the diet (Shenkin et al., 1978). Determination of urine urea excretion over 24-hour period was helpful in the management of cases of acute renal failure and in assessing the response of a renal transplant to dietary intake of protein (ibid). Since urea was the principal end product of protein metabolism in the human, the use of nitrogen to creatinine ratios and urea to creatinine ratios had been proposed for evaluating dietary protein intakes (Haddad, 1971).

Urinary thiamine, Ascorbic acid and Niacin thiamine

Urinary excretion of thiamine decreased proportionally with thiamine intake to a critical point after which further lowering of intake resulted only in minor and variable changes in urinary excretion. Tissue stores of thiamine was depleted with intake of the thiamine below the critical point and if continued resulted in symptoms of a deficiency (Oldham, 1962). When the daily intake of thiamine was reduced about 0.2 mg/1000 calories, urinary excretion was fall to 5.25 μg of thiamine per day. In cases of beriberi 24-hour urinary excretion of 0.15 μg of thiamine had been reported (Williams, 1961).

As a result of this observation measurement of the 24-hour urinary excretion of thiamine had been useful in evaluating thiamine nutriture (FAO/WHO, 1967). In controlled human studies a relationship was found to exist between thiamine intake and urinary excretion of thiamine and erythrocyte transketolase activities (Brin, 1967).

Thus, urinary excretion of thiamine was provided information pertaining to thiamine nutriture and was especially useful to confirm a diagnosis based on clinical symptoms. Such analysis will provide information also to thiamine intake levels, particularly with respect to immediate intakes.

Ascorbic acid:

Although most species of animals synthesised ascorbic acid man was unable to do so and needed to get his ascorbic acid therefore through diet. The function of ascorbic acid included its role in the synthesis of collagen, wound healing, amino acid and carbohydrate metabolism (Sauberlich, 1974). It also had an influence on the metabolism and synthesis of iron. Ingestion of very large amounts of ascorbic acid had beneficially influenced common cold (ICMR, 1981).

The urinary excretion of ascorbic acid had fall of rapidly during a depletion of this vitamin (Hodges et al., 1971). The average 24-hour excretion of ascorbic acid by the normal, well nourished adult had been reported to range from 0.27 mg (Telber et al., 1967) observed in children a urinary excretion of ascorbic acid from 35 - 54 mg/g of creatinine.

In scorbutic patients the urinary excretion of ascorbic acid was found to be essentially nil and hence had provided supportive diagnostic (Catalano, 1971).

Du Plessis (1967) was noted that sex approved to have no influence on the urinary excretion of ascorbi acid. Age did not influenced the urinary excretion of ascorbic acid. This would indicate that for children of various age an age adjusted sliding scale interpretive guide would be required to evaluate urinary ascorbic acid. Although, urinary ascorbic acid excretion rapidly declined to undetectable levels in ascorbic acid depletion, metabolite of ascorbic acid continue to be excreted. The metabolites included oxalic acid, ascorbate - 2 - sulfate and L. threonic acid (King *et al*., 1971) and (Baker *et al*., 1971). Thus, the urinary excretion analysis for water soluble vitamin had provided information pertaining to vitamin nutriture and was especially useful to confirm a diagnosis based on clinical symptoms (Jelliffe, 1974).

Niacin

In nutrition surveys, including those conducted by the Interdepartmental Committee on Nutrition for National Defense, the measurement of urinary excretion levels of N1- methylnicotinamide had been commonly employed for evaluating niacin nutrition (Sauberlich *et al*., 1974). The two major metabolites of niacin are N1-methylnicotinamide and N1-methyl - 2 - pyridone-5 carboxylamide (2 - pyridone). Under normal circumstances, adults excreted 20 to 30 per cent of their nicotinic acid as the N1-methylnicotinamide form and 40 to 60 per cent as the 2 - pyridone (Lange and Joubert, 1967). Thus, a ratio of

1.3 to 4 existed between 2 pyridone/N - methylnicotinamide excretion under normal conditions. (Holman and Lange, 1950).

When intakes of nicotinic acid were 8 to 10 mg and above/day, the excretion of nicotinic acid metabolite increased rapidly (Frazier *et al.*, 1965). N1 - methylnicotinamide excretion falled to a minimum at about the time of appearance of clinical symptoms of pellegra or niacin deficiency (Baker *et al.*, 1971). The excretion of 2 - pyridone was essentially absent for weeks before the clinical change were noted. Hence, the excretion ratio of these metabolites had been observed to be useful and probably the best criteria for evaluating niacin nutritional status under survey conditions (Duplesis, 1977).

Experimental Procedure

CHAPTER III

EXPERIMENTAL PROCEDURE

The study was aimed at assessing the muscle mass by estimating urinary creatinine level among young adolescent girls and college women. Further, an attempt was made to assess the nutritional status of the same group by the urinary excretion of urea, thiamine, ascorbic acid and niacin. Their body height, weight, skin fold thickness, arm circumference and also their urinary excretion of creatinine, urea, thiamine, ascorbic acid and niacin were studied.

1. Selection of the participants:

Thirty women students who were apparent in normal health of age 16 to 23 years studying in Sri Avinashilingam Higher Secondary School and College who were resided in the hostel were selected for the present investigation. They willingly cooperated in the study.

2. Anthropometric Data:

Data on body height, weight, mid arm circumference and skinfold thickness of thirty participants were collected.

a) Height measurements

A vertical measuring scale fixed to a wall was employed. After removing the foot wear, the subjects were allowed to stand on a flat floor by the scale with feet parallel and with heads, buttocks, shoulders and backs of head touching the upright. The head was held comfortably erect, with the lower border of the orbit in the same horizontal plane in a natural manner, the arms were hanging at the sides in a natural manner. The head piece, a wooden block was gently lowered, crushing the hair and making contact with the top of the head. The measuring scale was capable of measuring to an accuracy of 0.5 cm.

b) Weight measurement:

For measuring weights of the participants platform beam balance was used. Weighing was not done after a full meal. Theoretically the bladder was emptied prior to measurement. The subjects were allowed to stand on the centre of the platform without touching anything else. The weights of the participants were entered in kilograms.

c) Measurement of Skinfold thickness:

The skin was carefully selected, half-way down the arm, between the tip of the acromion process of the scapula and the olecranon process of the Ulna. The measurement was made with the armhanging relaxed at the side. The skin fold parallel to the long axis was picked up between the thumb and fore-finger of the left hand, clean away from the underlying muscle and measured at this point. The readings were entered in millimetres.

d) Measurement of mid arm circumference:

The mid arm circumference was measured to the nearest 0.1 cm with a fibre glass tape, which was placed gently, but firmly round the limb to avoid compression of the soft tissues. The left arm was measured while hanging freely, at its mid point, which was selected in the same way as for the triceps skin-fold.

Bio-chemical analysis of Urine:

All the estimations were done in duplicates.

a) Estimation of Creatinine in Urine: Creatinine in urine was estimated by alkaline picrate method (Jaffe's reaction), as described in Annexure I.

b) Estimation of Urea in Urine: Urinary urea was estimated by DAM-TSC method (Varley, 1980) as described in Annexure II.

c) Estimation of Thiamine in Urine: Urinary Thiamine was estimated by Thiochrome method by Johnson et al 1945 (Varley, 1980) as described in Annexure III.

d) Estimation of ascorbic acid in Urine: Urinary ascorbic acid was estimated by 2, 4 - Dinitro phenyl hydrazine method by Roe and Kuether, 1961 (Varley, 1980) as described in Annexure IV.

e) <u>Estimation of N1-Methyl Nicotinamide in Urine</u>: Urinary N1-Methyl Nicotinamide was estimated by a method given by Johnson *et al* 1945 (Varley, 1980) as described in Annexure V.

Statistical Analysis

't' tests were conducted wherever necessary to check if the results were significant using the formula.

$$t = \frac{\bar{X}_1 - \bar{X}_2}{S} \times \frac{n_1 \, n_2}{n_1 + n_2}$$

$\bar{X}_1$ = mean of the 1st sample

$\bar{X}_2$ = mean of the 2nd sample

S = Combined SD

n_1 and n_2 = No. of observations of the First and Second samples

$$S = \frac{(X_1 - \bar{X}_1)^2 + (X_2 - \bar{X}_2)^2}{n_1 + n_2 - 2}$$

Results and Discussion

CHAPTER IV

RESULTS AND DISCUSSIONS

Body muscle mass of selected adolescent girls and young college women students were assessed by evaluating 24-hour urinary excretion of creatinine. Furthermore, an assessment of the nutritional status of the same group was made by measuring their height, weight, mid arm circumference and skinfold thickness. Twenty four hour urinary excretory levels of urea, thiamine, ascorbic acid and niacin of the same group of women were used for evaluation of their nutritional status. The participants were selected from Sri Avinashilingam Higher Secondary School and College. The target participants of the study belonged to three different age groups. They were adolescent girls of age 16 - 18 years, young undergraduate college women of age ranging from 19 to 20years and post-graduate women of age 21 to 23 years. The results of the above study is discussed in this chapter.

1. <u>The Body weight and height of the participants</u>

Table I shows the mean body weight and height of the women participants in the study.

TABLE I

Mean body weight and height of the participants

The target participants	Age (years)	Weight (Kg) Mean ± SD	Height (cm) Mean ± SD
Adolescent girls (School)	16 - 18	40.5 ± 4.3	142 ± 6.7
Undergraduate women (college)	19 - 20	45.5 ± 2.8	148 ± 3.2
Post-graduate Women (college)	21 - 23	48.0 ± 3.6	151.5 ± 6.3

Size of each age group was ten.

According to Gopalan and Vijayaraghavan (1971), the weight of the adolescent girls of age 16 to 18 years in India ranged from 41.1 to 42.4 Kg. The mean weight of the adolescent girls in the present study was 40.5 ± 4.3 which was similar to the values given by Gopalan and Vijayaraghavan (1971). The mean height of adolescent girls in the present study was 142 +_ 6.7 cm. The mean height and weight of the undergraduate college women in the present study was 148 ± 3.8 cm and 45.5 ± 2.8 Kg. The mean height and weight of the post-graduate college women was found to be 155 ± 6.3 cm and 48 ± 3.57 Kg. These values are compared with the values reported for their counterparts in India.

Table II shows the mean weights and heights of the participants compared with those reported in this study.

TABLE II

<u>Mean weights and heights of the participants compared with those reported in literature</u>

Reference	Age (years)	Height (cm)	Weight (Kg)
Present Study	16 - 18	142.0	40.5
a) Adolescent girls	19 - 20	148.0	45.5
b) Undergraduate women	21 - 23	151.5	48.0
c) Post-graduate women			
Girija Bai (1972)	20.3	158.0	47.7
Banerjee (1961)	19.0	156.6	46.0
Banerjee (1962)	18.2	156.6	46.5
Goyal and Clerke (1960)	20.8	155.7	49.0
Nirmala <u>et al</u> (1974)	18.6	152.8	41.5
Nutrition Atlas of India (1971)	18.6	156.4	49.9

The height and weight of the present study was similar to other data reported in India. There was no significant difference in the mean heights and weights of three age groups namely adolescent girls, young college women and the post-graduate women.

2. **24-hour urinary volume of the participants:**

Table III shows the mean 24-hour urine volume voided by the participants.

TABLE III

24-hour urinary volume of the participants

The target participants	Age (years)	Volume of Urine ml/day Mean ± SD	Groups Compared	't' Value
Adolescent girls (a)	16 - 18	990 ± 227	a and b	0.1584 NS
Undergraduate women (b)	19 - 20	1007 ± 230	b and c	0.3867 NS
Post-graduate women (c)	21 - 23	1049 ± 231	a and c	0.5467 NS

NS - Not significant.

Size of each age group was ten.

The mean volume of urine voided by the adolescent girls was 990 ± 227 ml. Undergraduate women voided 1007 ± 230 ml per day and it was 1049 + 231 ml by the post-graduate women for a period of 24-hour. The daily output of urine on an average diet and normal fluid intake was between 1200 to 1500 ml, which may vary widely both physiologically and pathologically (Varley, 1980). Only one day urine sample was collected in this study.

3. 24-hour urinary excretion of creatinine by the target women participants

Table IV shows the mean 24-hour creatinine excretion by the target women of this study.

TABLE IV

Mean 24-hour urinary creatinine excretion of the participants

The target participants	Age (years)	Excretion of creatinine mg/day Mean ± SD	Groups Compared	't' value
Adolescent girls	16 - 18	634 ± 110	a and b	3.990**
Undergraduate women	19 - 20	805 ± 67	b and c	3.368**
Post-graudate women	21 - 23	933 ± 93	a and c	6.232**

** Significant at one per cent level.

Varley (1980) had reported that healthy women eating normal foods are in a steady state of urinary creatinine excretion. The creatinine excretion of women ranged between 1.0 to 2.0 g per day according to Varley (1980). The lower range of excretion indicated that the participants were shorter in height and smaller in weight. Upper range indicated that they are taller and heavier. Girija Bai (1972) had indicated that the mean creatinine excretion of young Coimbatore women ranged between 659 to 1033 mg per day. Brin (1973) had reported in his study the value of young women ranged between 482 to 1113 mg per day.

Table V shows the mean creatinine excretion of the participants according to their body weights.

TABLE V

Mean creatinine excretion based on the body weight of the participants

Weight (Kg)	Number of participants	Creatinine excretion (mg/day) Mean ± SD	Groups Compared	't' Value
Less than 40 (a)	7	590.4 ± 80	a and b	3.404**
40 to 45 (b)	9	733.4 ± 76	b and c	4.256**
Above 45 (c)	14	906.7 ± 92		

** - Significant at one per cent level.

The participants whose weight was less than 40 Kg. showed the mean creatinine excretion of 590.4 ± 80 mg the participants of weight 40 to 45 Kg. had mean creatine excretion of 733.4 ± 76 mg. The mean creatinine excretion of 906.7 ± 92 mg was found in the participants of weight above 45 Kg. There was significant difference in creatinine excretion of the participants of weight above 45 Kg. than the other two groups. Creatinine excretion of participants who weighed 40 to 45 Kg was significantly greater than that of the individual whose body weight was less than 40 Kg. This shows that the creatinine excretion increases with increase in body weight in terms of lean body mass.

Table VI shows the mean creatine excretion based on their body heights.

TABLE VI

Mean creatinine excretion based on the body height of the participants

Height (cm)	Number of participants	Creatine excretion (mg/day) Mean ± SD	Groups compared	't' Value
Less than 140 (a)	3	765 ± 167	a and b	0.1941 NS
140 - 150 (b)	14	805 ± 73	b and c	0.1627 NS
Above 150 (c)	12	861 ± 87	a and c	1.284 NS

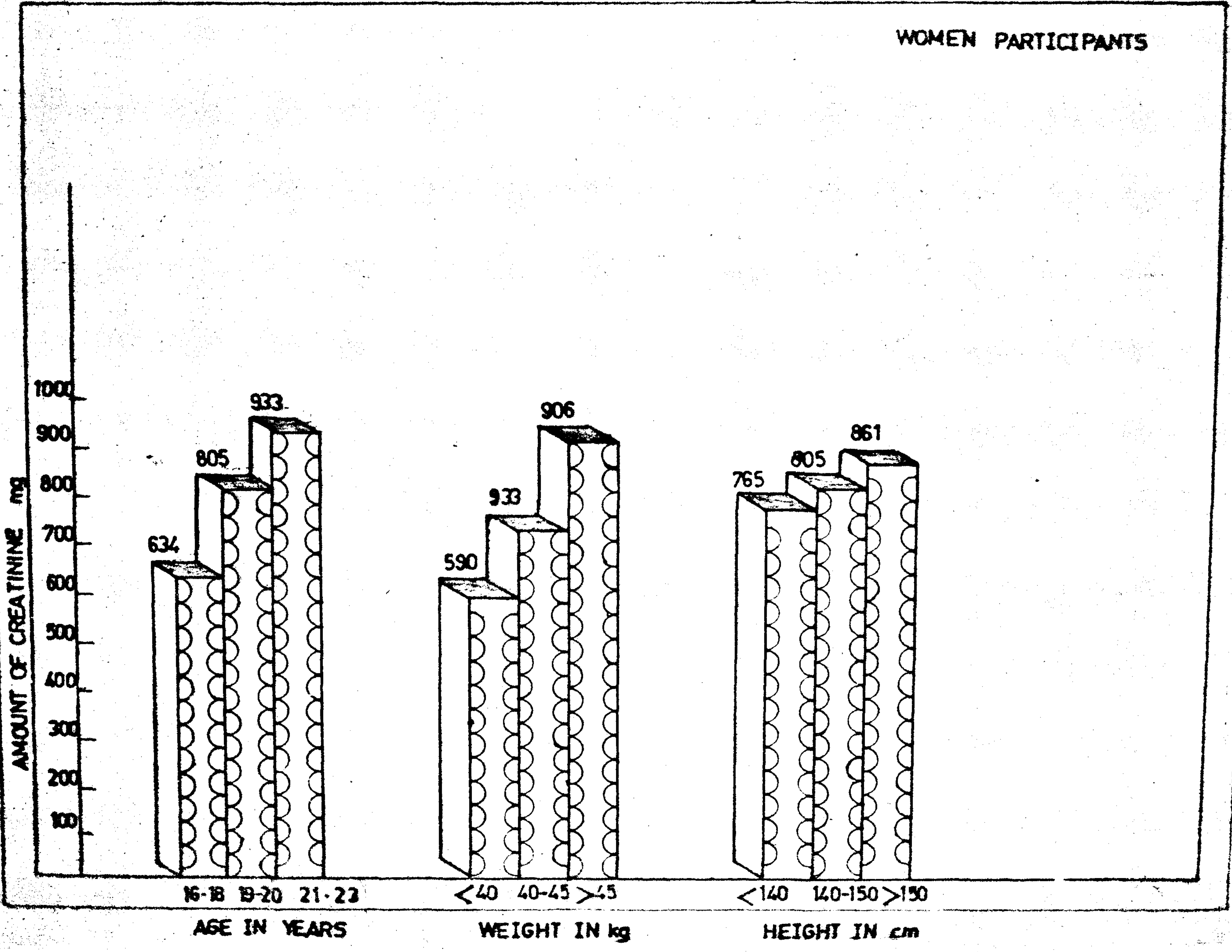
NS - Not significant.

Young women of height less than 140 cm had mean creatinine excretion of 765 ± 167 mg per day, those of height 140 to 150 cm showed the mean creatinine excretion of 805 ± 128 mg and those whose height was above 150 cm, it was 861 ± 87 mg per day.

Figure I shows the mean 24-hour creatinine excretion level based on age, body height and weight of the target women participants.

FIGURE-I

MEAN 24-HOUR URINARY CREATININE LEVEL ACCORDING TO AGE, WEIGHT AND HEIGHT OF THE TARGET



While the mean values showed a tendency to increase with increase in height, none of these groups were statistically significant. Thus body weight but not body height correlates with creatinine excretion.

Table VII shows the mean creatinine coefficient of the target women participants.

TABLE VII

Mean creatinine coefficient of the women participants in the study

The target participants	Age (years)	Creatinine coefficient (mg/day/Kg of Body weight) Mean ± SD	Groups compared	't' Value
Adolescent girls (a)	16 - 18	15.58 ± 1.28	a and b	4.444**
Undergraduate women (b)	19 - 20	17.70 ± 0.64	b and c	4.750**
Post-graduate women (c)	21 - 23	19.60 ± 1.05	a and c	8.040**

** - Significant at one per cent level.

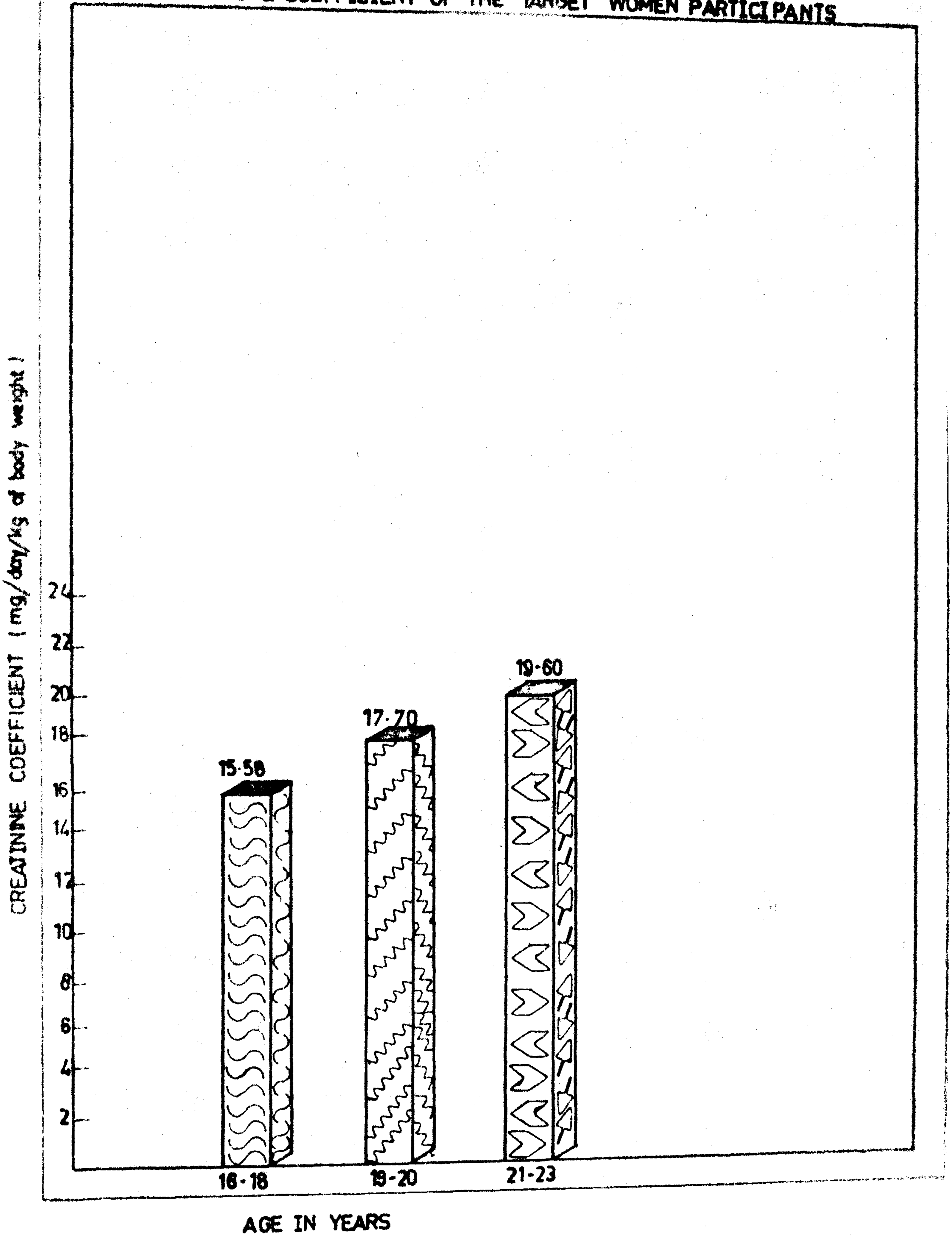
The size of each age group was ten

Figure II shows the mean creatinine coefficient of the target women participants diagrammatically.

Urinary creatinine level can be expressed as creatinine coefficient (milligram of creatinine per day per kilogram of body weight). According to Varley (1980), the normal

FIGURE-II

MEAN CREATININE COEFFICIENT OF THE TARGET WOMEN PARTICIPANTS



creatinine coefficient for women was 15 to 21. According to Forbes (1976), the creatinine coefficient for women was found to be 10 to 25. In the present study the creatinine coefficient of women participants ranged between 13.4 to 21..

Mean creatinine coefficient of the target compared with those in literature are presented in Table VIII.

TABLE VIII

Mean creatinine coefficient of the target groups compared with those reported in literature

Sample size	Reference	Creatinine coefficient
	Present study	
10	a) Adolescent girls (16 - 18 years)	15.58
10	b) Undergraduate women (19 - 20 years)	17.70
10	c) Post-graduate women (21 - 23 years)	19.60
6	Girija Bai (1972)	17.8
6	Nirmala *et al* (1962)	21.8
6	Maragatham (1968)	8 - 29
6	Geetha (1971)	8.81 - 19.23
104	Ritchy *et al* (1973)	18.7

Thus, the creatinine coefficient obtained for the participants was found to be the same range as those reported in the literature for young women. Creatinine coefficient was used as an index of muscle mass (Heymsfield *et al*., 1983).

From the creatinine coefficient measured for the participants, it indicated that the muscle mass of the post-graduate women (21 to 23 years) may be greater than the muscle mass of undergraduate women (19 to 20 years) and adolescent school girls (17 to 19 years) and the difference was significant at one per cent level. The muscle mass of the undergraduate women based on creatinine coefficient may be greater than the adolescent school girls and there was significant difference at one per cent level.

Table IX shows the mean creatinine height index of the women participants.

TABLE IX

Mean creatinine height index of the women participants

The target participants	Age (years)	Creatinine height Index (mg/cm) Mean $\pm$ SD	Groups compared	't' Value
Adolescent girls (a)	16 - 18	4.7 $\pm$ 1.0	a and b	0.1931^{NS}
Undergraduate women (b)	19 - 20	5.4 $\pm$ 0.4	b and c	0.0270^{NS}
Post-graduate women (c)	21 - 23	5.6 $\pm$ 1.9	a and c	0.1217^{NS}

NS - Not significant.

Size of each age group was ten.

According to Sauberlich (1974), the relationship of milligrams of creatinine excreted per unit time per centimeter of body height had been useful in evaluating protein nutritional status and the value above 0.9 was found to be in normal protein nutritional status. The value obtained in the present study was found to be above 0.9 which indicated the normal protein nutritional status of the participants. From the mean creatinine height index value for the above three age groups, it was found out that the difference in mean creatinine height index between the three groups was not significant.

4. Comparison of creatinine content of early morning, six hour and 24-hour sample voided by the target women

Table X presents the mean volume of urine of early morning, 6-hour and 24-hour samples.

TABLE X

Mean volume of urine of early morning, 6-hour and 24-hour samples

Sample type	Number studied	Volume of urine per sample (ml) Mean ± SD
Early morning	8	221 ± 29
6-hour sample	8	201 ± 36
24-hour sample	10	990 ± 227

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LIBRARY
COIMBATORE-641 043.

The mean volume of early morning voided urine was 221 ± 29 ml. The mean volume of 6-hour voided urine sample was 201 ± 36 ml and the mean volume of 24-hour voided sample was 990 ± 227 ml. Not only the volume of urine but also the composition of urine determines the creatinine content of urine.

Table XI shows the creatinine content of early morning, 6-hour and 24-hour urine samples.

TABLE XI

Creatinine content of early morning, six hour and 24-hour urine sample voided by the target women

Type of sample	Number of sample	Amount of creatinine (mg/sample) Mean ± SD
Early morning sample (a)	8	161.0 ± 33.0
Six-hour sample (b)	8	134.0 ± 21.5
24-hour sample (c)	10	640.0 ± 71.0

The mean amount of creatinine present in early morning sample was 161.3 ± 33.18 mg, the mean amount of creatinine present in 6-hour urine sample and 24-hour urine sample was 134 ± and 640 ± 71.

Using 6-hour urinary content of creatinine, 24-hour urinary creatinine level was computed. This was done in order to find out whether 6-hour urine sample can be used instead of 24-hour urine sample to measure creatinine co-efficient (mg/day/kg of body weight), which is an index of evaluating muscle mass. This study was mainly aimed at reducing the rate of 24-hour urinary collection.

Table XII shows the comparison of creatinine coefficient values obtained from actual 24-hour urine sample and computed 24-hour excretion using 6-hour sample collection.

TABLE XII

Comparison of creatinine coefficient values obtained from actual 24-hour urine samples and computed 24-hour excretion using 6-hour sample collection

Type of Sample	Age in years	Creatinine coefficient (mg/day/kg of body weight) Mean ± SD	Groups compared	't' value
Actual 24-hour urine sample (a)	16 - 18	15.6 ± 1.28	a and b	3.276**
24-hour excretion computed from 6-hour collection (b)	16 - 18	13.2 ± 1.65		

** Significant at one per cent level.

Creatinine coefficient of the actual 24-hour urine sample was 15.6 ± 1.28. But in computed 24-hour urine excretion using 6-hour sample collection was 13.2 ± 1.65. There was a significant lesser creatinine coefficient in the computed 24-hoururine sample. Hence it indicated that 6-hour urine sample even though easy to collect is not dependable to obtain creatinine coefficient and only 24-hour urine should be collected.

5. Measurement of arm circumference, and skinfold thickness

Table XIII represents the arm circumference of the women participants.

TABLE XIII

The mean arm circumference of the women participants

The target Participants	Age (years)	Arm circumference (cm) Mean ± S.D.	Groups compared	't' value
Adolescent girls (a)	16 - 18	18.5 ± 0.78	a and b	1.612 NS
Undergraduate women (b)	19 - 20	19.5 ± 0.96	b and c	1.958 NS
Post-graduate women (c)	21 - 23	20.5 ± 1.00	c and a	4.342**

** - Significant at one per cent level.

NS - Not significant

Size of each age group was ten.

Allowed to separate or centrifuged. Transferred 1 ml of the supernatant butanol layer to a cuvette and read in the fluorimeter at 460 nm using an excitation wavelength of 360 nm. Readings were taken not earlier than 5 min. shaking with the sodium hydroxide. For the working standard a concentration of 0.1 mg/litre was convenient. No blank correction was required so long as the urine concentration was below 15 mg/litre.

Creatinine coefficient of the actual 24-hour urine sample was 15.6 ± 1.28. But in computed 24-hour urine excretion using 6-hour sample collection was 13.2 ± 1.65. There was a significant lesser creatinine coefficient in the computed 24-hoururine sample. Hence it indicated that 6-hour urine sample even though easy to collect is not dependable to obtain creatinine coefficient and only 24-hour urine should be collected.

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Undergraduate women (b)	19 - 20	19.5 ± 0.96	b and c	1.958 NS
Post-graduate women (c)	21 - 23	20.5 ± 1.00	c and a	4.342**

** - Significant at one per cent level.

NS - Not significant

Size of each age group was ten.

According to Hertzberg *et al* (1963), the arm circumference of adult women was 22.8 cm. According to Nutrition Atlas of India it was 24.8 cm. In the present study the mean arm circumference of adolescent girls was 18.5 ± 0.78, for undergraduate women the mean arm circumference was 19.5 ± 0.96 and for post-graduate women 20.5 ± 1.0.

Table XIV shows the skinfold thickness of the women participants.

TABLE XIV

Mean skinfold thickness of the participants

The target participants	Age (years)	Skinfold thickness (mm) Mean ± S.D.
Adolescent girls	16 - 18	15.4 ± 0.74
Undergraduate women	19 - 20	16.0 ± 0.36
Post-graduate women	21 - 23	17.0 ± 0.20

According to Jelliffee (1974), the mean triceps skinfold thickness of the adult women was 16.5 mm. According to Nutrition Journal of India (1971) the triceps skinfold of adult women was 14.4 mm. In the present study the mean skinfold thickness of the adolescent girls, undergradute women and post-graduate women was found to be 15.4 ± 0.74 mm, 16 ± 0.36 mm and 17 ± 0.2 mm. Thus the triceps skinfold thickness of the participants was similar to the values given by above references. The mean skinfold thickness of post-graduate women was significantly greater than the other two age group of women.

Table XV represents the mean muscle arm circumference of the participants.

TABLE XV

The mean muscle arm circumference of the participants

The target participants	Age (years)	Mean Muscle arm circumference (cms) Mean ± S.D.	Groups compared	't' value
Adolescent girls (a)	16 - 18	18.00 ± 0.770	a and b	2.55 NS
Undergraduate women (b)	19 - 20	19.00 ± 0.954	b and c	2.042 NS
Post-graduate women (c)	21 - 23	20.00 ± 1.062	c and a	4.5 **

** - Significant at one per cent level

NS - Not significant

According to Hertzberg et al (1963) the normal muscle circumference for adult women was 20.9. In the present study the muscle arm circumference of the participants ranged from 16.5 to 21.98. By anthropometric measurement also the muscle mass of post-graduate women was found to be greater than the undergraduate college women and adolescent girls and muscle mass of undergraduate college women may be greater than the adolescent school girls.

6. Urinary excretion of urea

Table XVI shows the mean urea excretion of the target women participants.

TABLE XVI

Mean 24-hour urinary urea excretion of the target women participants

The target participants	Age (years)	Urea excretion (g/day) Mean ± SD	Groups compared	't' value
Adolescent girls (a)	16 - 18	9.54 ± 0.58	a and b	0.166 NS
Undergraduate women (b)	19 - 20	9.13 ± 0.87	b and c	1.225 NS
Post-graduate women (c)	21 - 23	9.64 ± 0.88	c and a	0.285 NS

NS - Not significant

Size of each sample was ten.

The daily urea excretion reflected the dietary protein intake in adult women. According to Haman, (1974), the daily urea excretion of adult women was 12.8 to 25.7, Nirmala *et al* (1968) reported as 5.25 to 12.29 of urea per day for post adolescent women students. Kamalanathan *et al* (1965) reported as 8.29 - 11.13 of urea for women per day. According to Tietz (1976) the daily urea excretion per day was 12 to 20 g. In the present study the daily urea excretion of the adolescent girls, young undergraduate women and post-graduate women was found to be 9.54 ± 0.58 g per day, 9.13 ± 0.87 g per day and 9.64 ± 0.88 g per day. From the mean excretion of urea, it was indicated that the participants are in normal protein nutritional status. There was no significant difference in urinary urea excretion between the three groups.

7. Urinary excretion of Thiamine

Mean thiamine excretion of the participants are given in Table XVII.

TABLE XVII

Mean thiamine excretion of the participants

The target participants	No. studied	Age (years)	Thiamine excretion (ug per day) Mean ± S.D.	Groups compared	't' value
Adolescent girls (a)	10	16 - 18	191.20 ± 23.12	a and b	0.6350[NS]
Undergraduate women (b)	10	19 - 20	196.25 ± 10.18	b and c	0.7049[NS]
Post-graduate women (c)	10	21 - 23	191.83 ± 15.59		

NS - Not significant

Size of each sample was ten.

Daily thiamine excretion ranged from 50 u to 500 u mg. (Varley, 1980). Daily thiamine excretion of the participants was found to be 148 to 220 ug. Thus the thiamine excretion obtained in the present study was similar with the values given by Varley. Since the urinary excretion analysis of thiamine provided information pertaining to thiamine nutriture and participants excreted normal thiamine level it indicated the normal thiamine intake of the participants. There was no significant difference in thiamine excretion between the participants.

Table XVIII shows the excretion of thiamine related to creatinine excretion.

TABLE XVIII

Mean thiamine excretion of thiamine related to creatinine excretion

The target group participants	Age (years)	Thiamine excretion per gram of creatinine (ug/g) Mean ± S.D.	Groups compared	't' value
6				
Adolescent (a) girls	16 - 18	321.99 ± 54.98	a and b	3.956**
Undergraduate women (b)	19 - 20	238.47 ± 31.47	b and c	2.263*
Post-graduate women (c)	21 - 23	207.59 ± 26.16		

** - Significant at one per cent level.

* - Significant at five per cent level.

Size of each sample was ten.

A correlation of urinary excretion of thiamine per gram of creatinine had been useful for assessing thiamine nutritional status, (Pearson, 1966) and the value of thiamine per gram of creatinine greater than 100 was accepted value (low risk) for adults. According to Pearson (1962) the thiamine excretion is greater than 150 mg per day was normal for adult women. According to FAO/WHO, the normal daily thiamine excretion for adults ranged between 50 to 400 micro gram. Participants namely adolescent girls, undergraduate women and post-graduate women had thiamine creatinine ratio above 100 which indicated that the participants belonged to 'acceptable' group according to Pearson which also indicated the normal thiamine nutritional status of the participants.

8. Urinary excretion of ascorbic acid

Table XIX shows mean ascorbic acid excretion of the target women participants.

Figure III shows the mean 24-hour urinary excretion ascorbic acid of the participants diagramatically.

TABLE XIX

Mean Ascorbic acid excretion of the participants

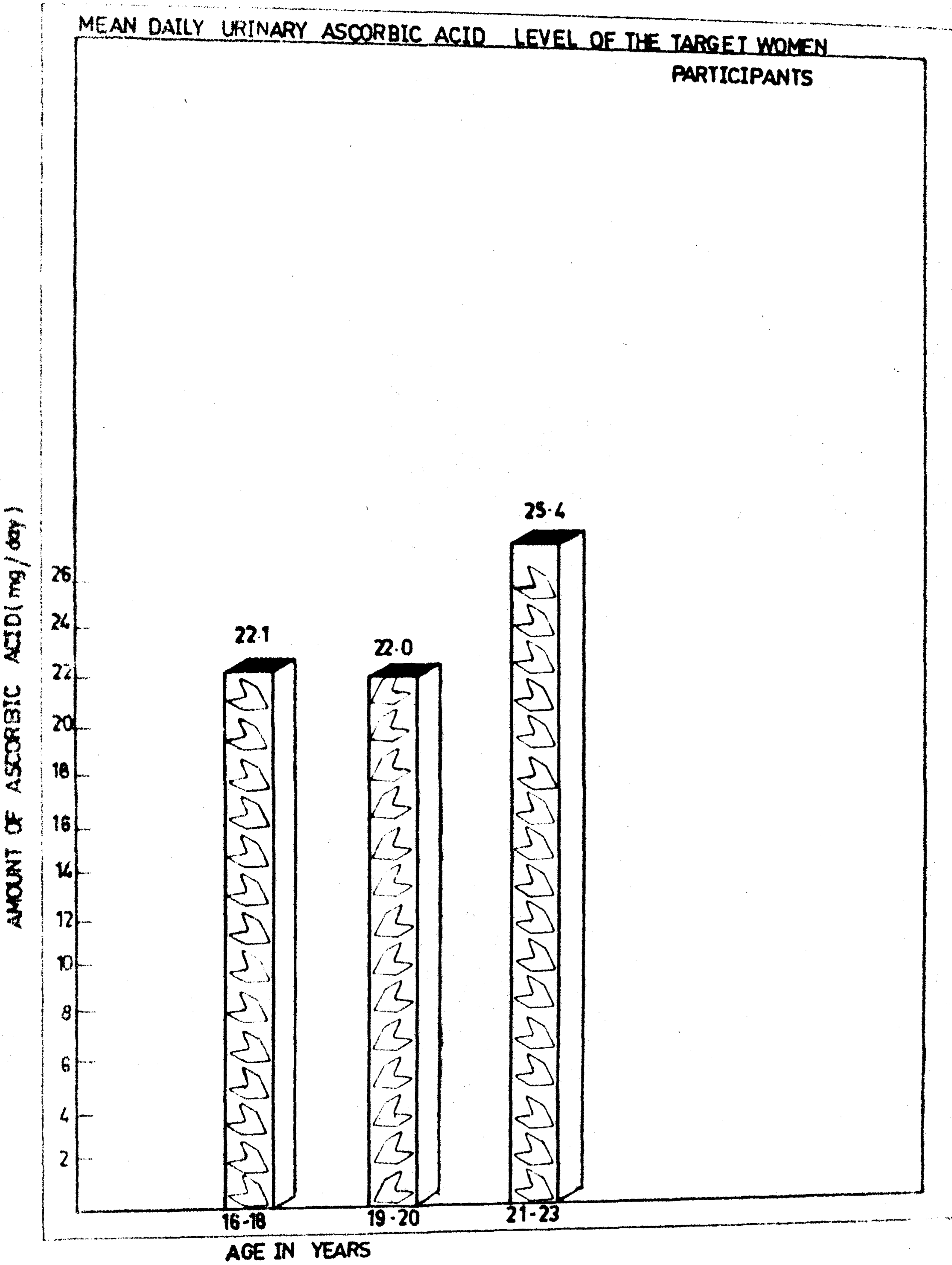
The target participants	Age (years)	Excretion of ascorbic acid in (mg/day) Mean ± S.D.	Groups compared	't' value
Adolescent girls (a)	16 - 18	22.15 ± 3.24	a and b	0.169^{NS}
Undergraduate women (b)	19 - 20	22.00 ± 1.41	b and c	4.268**
Post-Graduate women (c)	21- 23	25.40 ± 2.48	a and c	2.561*

NS - Not significant

** - Significant at one per cent level

* - Significant at five per cent level

FIGURE-III


MEAN DAILY URINARY ASCORBIC ACID LEVEL OF THE TARGET WOMEN PARTICIPANTS
AMOUNT OF ASCORBIC ACID (mg/day)
26
24
22
20
18
16
14
12
10
8
6
4
2
22.1
22.0
25.4
16-18
19-20
21-23
AGE IN YEARS

The daily output of ascorbic acid was found to be 20 - 30 mg (Varley, 1980). The daily urinary excretion of ascorbic acid reported by Rodervele *et al* (1965) was 8 to 27 mg.

According to Tolber *et al* (1964), the average 24-hour excretion of ascorbic acid was reported as 8 to 27 mg. The mean ascorbic acid excretion ranged between 9 to 34 mg per day (Mármala *et al*., 1979). According to $U_p$john (1973), the adult excreted 30 - 60 mg of ascorbic acid per day.

The daily output of ascorbic acid of the participants was 19.9 to 30 which agreed with the normal value given by above reports. According to Brin (1974), the well nourished adults excreted 8 to 27 mg per 24-hour with which also the ascorbic excretion of participants agreed, since the urinary levels of ascorbic acid reflected immediate dietary intakes, it indicated the normal ascorbic acid intake of the participants. There was no significant difference in ascorbic acid excretion between adolescent girls and under-graduate students.

9. Urinary excretion of Niacin

Table XX represents the mean niacin excretion of the target women participants.

Figure IV shows the mean 24-hour urinary niacin excretion of the target women participants diagrammatically.

FIGURE IV

MEAN 24 HOUR URINARY NIACIN EXCRETION OF THE TARGET WOMEN PARTICIPANTS.

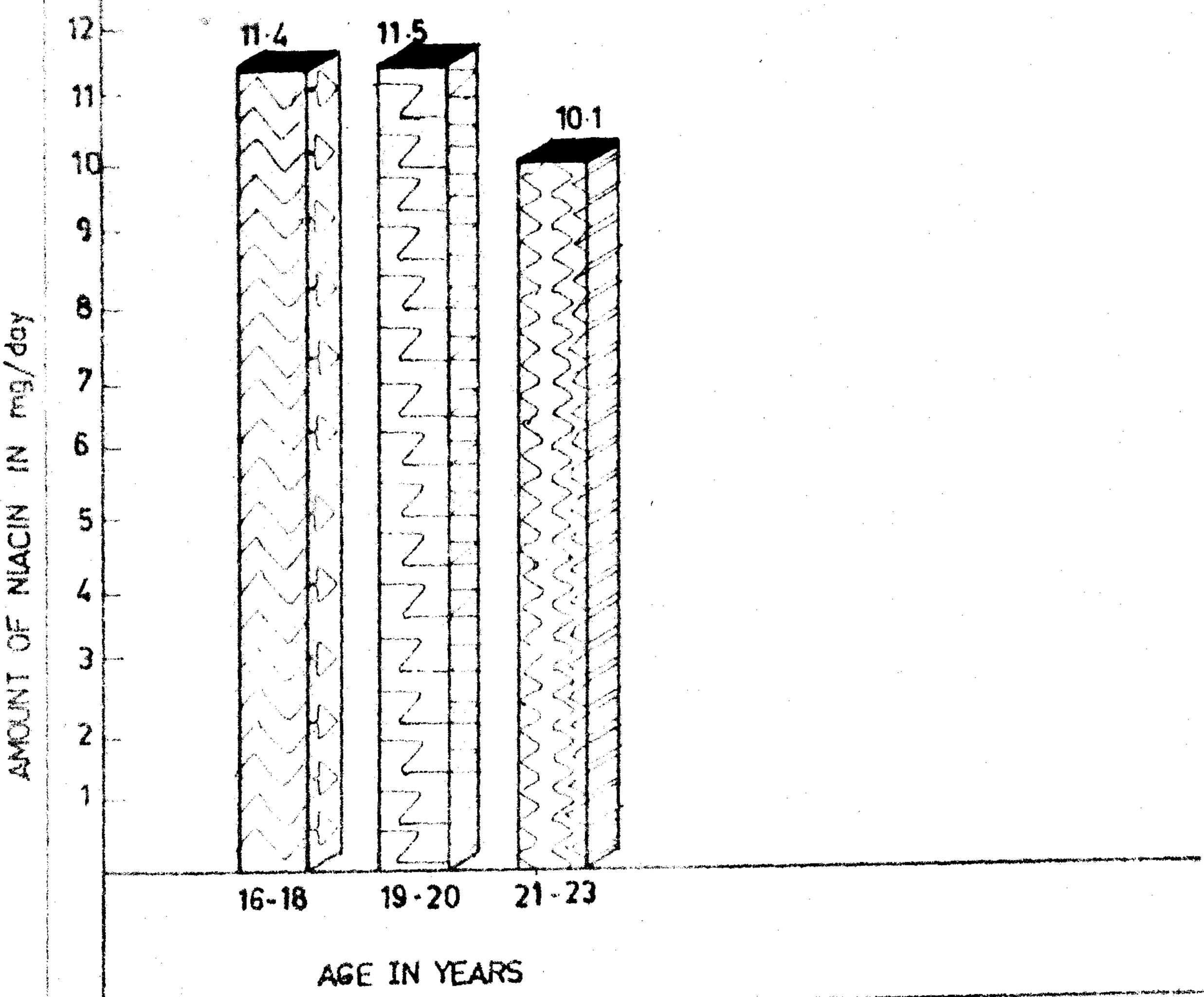


TABLE XX

Mean Niacin excretion of the target women participants

The target participants	Age (years)	Niacin excretion (mg/day) Mean ± S.D.	Groups compared	't' value
Adolescent girls (a)	16 - 18	11.40 ± 3.37	a and b	0.8974 NS
Undergraduate women (b)	19 - 20	11.54 ± 1.66	b and c	1.045 NS
Post-graduate women (c)	21 - 23	10.137 ± 0.70	a and c	1.101 NS

NS - Not significant

Size of each sample = 10.

Under normal circumstances, adults excrete 20 to 30 per cent of their nicotinic acid as the N'- Methyl Nicotinamide form, with adequate nicotinic acid the daily excretion of N'- methyl nicotinamide was found to be 3 to 17 mg, the average being 7 mg (Varley, 1980).

According to ICMR (1963) DATA, daily urinary excretion of N'- methyl nicotinamide less than 0.8 milligram was considered as deficiency state, 0.8 to 2.36 mg was considered to be in low niacin nutritional status and 2.4 to 6.36 mg was considered to be in acceptable niacin nutritional status and greater than 6.5 mg was considered to be in high niacin nutritional status. The daily excretion of N'- methyl nicotinamide of the participants in the present study was 7.21 to 16.25 mg which agreed with the normal value given. Urinary excretory level of niacin was an index of niacin nutriture (Sauberlich, 1974). From the mean value of N'- methyl nicotinamide excretion, it was found out that the participants are in High niacin nutritional status.

According to Duplessie (1967) the daily excretion of niacin varied between 8 mg to 15 mg per day. Since the daily niacin excretion of participants was normal it indicated the normal intake of niacin by the participants. There was no significant difference in N'- methyl nicotinamide excretion between undergraduate and post-graduate women and also between adolescent girls and post-graduate women.

Summary and Conclusion

CHAPTER V

SUMMARY AND CONCLUSION

This study namely "Evaluation of Urinary Creatinine as a Bio-chemical index of muscle mass and excretory levels of urea, thiamine, ascorbic acid and niacin for the assessment of Nutritional status among adolescent girls and young women" was aimed to assess the body muscle mass of the target young women, and assessment of their nutritional status by evaluating their 24-hour urinary excretion of urea, thiamine, ascorbic acid and niacin.

Thirty participants of three different age groups were selected from Sri Avinashilingam Home Science College and Higher Secondary School. They were adolescent girls of age 16 - 18 years, young undergraduate students of age 19 - 20 years and post-graduate women of age 21 - 23 years.

An Evaluation of creatinine content of early morning sample and six-hour sample was made. From the creatinine content of 6-hour sample, 24-hour urinary creatinine level was computed to find out whether 6-hour sample can be used instead of 24-hour urinary collection for clinical purpose. The results of the above study is summarised below:

Measurement of height and weight of the participants showed that the mean weight and height of the target adolescent girls of age 16 - 18 years was 40.5 ± 4.3 Kg and 142 ± 6.7 cm. The mean weight and height of the undergraduate women of age 19 - 20 years was 45.5 ± 2.8 kg and 148 ± 3.2 cm. The mean weight and height of the post-graduate women was 48.0 ± 3.6 kg and 151.5 ± 6.3 cm which was similar with the values given by Girijabai (1972), Banerjee (1962) and Goyal and Clerke (1966).

The muscle circumference of the participants was measured from mid arm circumference and triceps skinfold thickness of the participants. The mean arm circumference of the adolescent girls was 18 ± 0.7 cm, the mean arm circumference of the undergraduate young women and post-graduate women were 19 ± 0.95 cm and 20 ± 1.06 cm.

The volume of 24-hour urine was measured accurately.
The mea
The mean volume of urine voided by adolescent girls, undergraduate women and post-graduate women were found to be 990 ± 227 ml, 1007 ± 230 ml and 1049 ± 231 ml.

24-hour urinary excretion of creatinine by the participants was used as an Index of body muscle mass. The mean 24-hour urinary creatinine exeretion of the adolescent girls was found to be 634 ± 110 mg and the mean urinary creatinine excretion of undergraduate women and post-graduate women were found to be 805 ± 7 mg and 933 ± 93 mg. The mean creatinine excretion of post-graduate women was significantly greater than the adolescent girls and undergraduate college women and the mean urinary creatinine level of undergraduate women was significantly greater than the adolescent girls. According to Girijabai (1972) the mean creatinine excretion of young Coimbatore women ranged between 659 to 1033 mg per day. According to Brin (1973) it was 890 to 1720 mg per day.

Creatinine coefficient (mg/day/kg of body weight) was calculated to assess the muscle mass of the participants. The mean creatinine coefficient of the adolescent girls, young undergraduate women and post-graduate women were found to be 15.6 ± 1.3, 17.7 ± 0.64, and 19.6 ± 1.05, which showed that the muscle mass of selected post-graduate women seem to be greater than the muscle mass of selected undergraduate women and adolescent girls. The muscle mass of selected undergraduate women seem to be greater than the adolescent girls. The creatinine coefficient obtained in the present study was found to be similar with the values given by Girijabai (1972), Nirmala et al (1962), Maragatham (1968) and Korambelkar (1950).

Creatinine excretion of the participants based on their weight was also measured. Participants of weight below 40 kg had mean urinary creatinine level as 590 ± 80 mg per day, participants of weight ranging 40 - 50 kg showed the mean creatinine excretion as 733.4 ± 76 mg and it was 906.7 ± 92 mg per day by the participants of weight above 50 kg. From the mean creatinine level it was found out that the creatinine excretion increases with increase in weight.

Creatinine excretion of the participants based on their body height was also measured. The participants of height lesser than 140 cm showed mean creatinine excretion of 7.65 ± 167 mg per day. The participants of height 140 - 150 cm and participants of height above 150 cm showed the mean creatinine excretion of 805 ± 73 mg and 861 ± 87 mg per day.

According to Sauberlich (1974) the creatinine height index (milligram of creatinine excreted per unit time per centimeter of body height) value above 0.9 was found to be in normal protein nutritional status. The mean creatinine height index of adolescent girls, undergraduate college women and post-graduate women was found to be 4.72 ± 1.04, 5.40 ± 0.4 and 5.57 ± 1.9 mg/cm which showed that the participants have normal protein nutritional status.

Mean urinary volume and creatinine content of early morning, 6-hour and 24-hour urine samples were measured. The mean volume of early morning sample of adolescent girls were found to be 221 ± 29 ml; the mean volume of 6-hour voided urine sample was 201 ± 36 ml and the mean volume of 24-hour voided urine sample was 990 ± 227 for adolescent girls. Creatinine content of early morning and 6-hour sample was found to be 161.0 ± 33 mg and 134 ± 22 mg and the mean creatinine content of 24-hour sample was found to be 590 ± 80 mg. Using the amount of creatinine present in

6-hour sample, creatinine coefficient was computed and it was found to be 13.2 ± 1.65 for adolescent girls, were as the creatinine coefficient found out from the actual 24 hours urinary creatinine excretion for adolescent girls was found to be 15.6 ± 1.28. This indicated that it is better to evaluate the creatinine coefficient from 24-hour urinary excretion of creatinine.

Mean urea excretion of the target participants was measured to evaluate the protein nutritional status of the participants. The mean daily urea excretion of the adolescent girls, young undergraduate women and post-graduate women were found to be 9.54 ± 0.58 g, 9.13 ± 0.87 g and 9.64 ± 0.88 g. According to Tietz (1976) the normal urinary urea of women per day was 12 - 20 g. Since the participants showed a daily urinary excretion within this limit it indicated the normal protein nutritional status of the participants.

According to Pearson (1962), the thiamine excretion per day ranged between 50 - 500 µg. The mean daily thiamine excretion of the adolescent girls, undergraduate women and post-graduate women were found to be 191 ± 23, 196 ± 10 and 192 ± 15 µg respectively. Since the urinary excretion of thiamine indicates the thiamine nutriture, the normal thiamine excretion by the participants indicated the normal thiamine nutritional status of the same. Mean thiamine creatinine ratio the target participants also indicated the normal thiamine nutritional status of the participants.

The mean daily Urinary excretion of ascorbic acid by the adolescent girls, undergraduate women and post-graduate women were found to be 22 ± 3.24 mg, 22 ± 1.40 mg and 25.4 ± 2.5 mg which was similar to the values reported by Roderude et al (1965) (8 - 27 mg). Tolber et al (1974) (8 - 27 mg) and Nirmala et al (1979) (9 - 34 mg) per day. This indicates the normal ascorbic acid nutritional status of the target women. Mean daily niacin excretion of the participants namely adolescent girls, undergraduate women and post-graduate women were found to be 11.4 ± 3.4 mg, 11.5 ± 1.7 mg and 10.1 ± 0.7 mg, which was similar to the values reported by Varley (1980) and according to ICMR (1963) the participants were in high niacin nutritional status.

From the results obtained it can be concluded that 24-hour urinary creatinine output was more for post-graduate women. From creatinine coefficient obtained for the participants, an evaluation of muscle mass was made which indicated that the muscle mass of the selected group of post-graduate women was found to be greater than the adolescent girls and undergraduate women. From the anthropometric measurements and by evaluating 24-hour urinary excretion of urea, thiamine, ascorbic acid and niacin all the participants were shown to be in normal and acceptable nutritional status.

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Annexures

ANNEXURE I

ESTIMATION OF CREATININE IN URINE (ALKALINE PICRATE METHOD) (VARLEY, 1980)

Principle:

This method makes use of Jaffe's reaction, the production of Mahogany red colour with an alkaline picrate solution. The intensity of the red colour developed is compared in a colorimeter against a reagent blank at 500mμ.

Reagents:

1. Picric acid - 0.04 M Solution (9.16 g of crystalline picric acid is dissolved in 1000 ml of water.
2. 0.075 N Sodium hydroxide.
3. Stock standard creatinine solution. Dissolved 100 mg of creatinine in N/10 hydrochloric acid and made up to 100 ml with the same.
4. Working standard solution: Diluted 1.0 ml of the stock solution to 50.0 ml with water. This contains 20 μg of creatinine per ml.

<u>Procedure:</u>

Tubes containing 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 ml of the standard solution corresponding to the γ values of 10 - 60 were taken. The volume was made up to 3.0 ml with water in all the tubes. 3.0 ml of water was taken as blank.

1.0 ml of urine was diluted to 100 ml with water. 3.0 ml of the diluted urine was taken for the experiment. To all the tubes added 1.0 ml of picric acid and then 1.0 ml of 0.75 N Sodium hydroxide. Allowed to stand for 20 minutes for the colour to develop. The tubes were shaken well and the colour developed was read in a colorimeter at 500 mμ against a reagent blank.

A standard graph was drawn by plotting concentration of creatinine on X-axis and colorimeter reading on Y-axis.

ANNEXURE II

ESTIMATION OF UREA
(DAM-TSC METHOD) (VARLEY, 1980)

Principle:

Urea reacted with Diacetyl monoxime in the presense of thiosemicarboside to form a red coloured product which was measured colorimetrically.

Reagents:

Acid Reagent:

Water - 100 ml, Phosphoric acid - 20 ml

Concentrated sulphuric acid - 8.0 ml, 5% ferric chloride - 1.0 ml.

Colour Reagent:

Acid Reagent - 30 ml, Water - 20 ml.

Diacetyl monoxime (DAM) 2.5% - 1.0 ml.

Thiosemicarboside (TSC) 0.25% - 0.25 ml.

Colour Reagent should be prepared just before use. This solution is not stable more than one hour.

Stock Standard:

Dissolved 100 mg urea in 100 ml of distilled water. (This solution is prepared in a standard solution of benzoic acid for long use).

Working Standard:

2.0 ml of the Stock standard was diluted to 100 ml with water. 1.0 ml of this solution contains 20 µg of Urea.

Procedure:

Into a series of test tubes added 0.5 - 2.5 ml of the working standard solution corresponding to ug values of 10 - 50 respectively. 1.0 ml of urine was diluted to 100 ml with water. 0.2 ml of the diluted urine was taken for the experiment. Made up to 3.0 ml with water in all the tubes, and then added 5.0 ml of the colour reagent to all the tubes. Mixed well, stoppered and heated in a vigorously boiling water bath for 20 minutes. Along with this a blank was also prepared. Removed the tubes, cooled and read against the reagent blank at 540 mµ in a colorimeter.

A standard graph was drawn by plotting the concentration of urea on X-axis and colorimeter reading on Y-axis.

ANNEXURE III

ASCORBIC ESTIMATION IN URINE
(2, 4 DINITROPHENYL HYDROZINE METHOD BY ROE AND KWETHER, 1961)

Principle:

Ascorbic acid was oxidised to dehydro ascorbic acid by bromine water. The excess of bromine is aerated and dehydro ascorbic acid was treated with 2, 4 - dinitrophenyl hydrozine and then with 85% Sulphuric acid to produce a red colour which was measured colorimetrically.

Reagents:

1) Standard Ascorbic acid: 100 mg of ascorbic acid in 100 ml of 4% oxalic acid. 10.0 ml of this was diluted to 100 ml with 4% oxalic acid.

2) 2% Solution of 2, 4 - dinitrophenyl hydrazine: 2 g of 2, 4 - dinitrophynyl hydrazine was dissolved in 9 N sulphuric acid and made up to 100 ml with the same.

3) 10% Thiourea Solution: 10 g of thiourea was dissolved in 100 ml of water.

4) 85% Sulphuric acid.

Procedure:

10.0 ml of the working standard was taken in a clean dry conical flask and a few drops of bromine water was added till the solution was yellow. Excess of bromine was aerated and the solution was made up to 50 ml. This 50 ml contained one mg of ascorbic acid and hence 1.0 ml of this solution was equivalent to 20 µg of ascorbic acid. In a series of clean tube known volume of solution 0.5 - 2.5 ml were taken. The concentration of each tube was noted and 1.0 ml of 2, 4 - dinitrophenyl hydrazine was added.

Took 10.0 ml of the urine in a clean conical flask, added a few drops of bromine water, aerated the excess of bromine and made up to 25.0 ml. In clean dry tubes pipetted out 2.0 ml of the solution. To each added a drop of thiourea solution and 1.0 ml of 2, 4 - dinitrophenyl hydrazine, and made up all the solution in the test tube to 6.0 ml with 4% oxalic acid. Incubated for 3 hours at 37° C. Removed and cooled in ice. Then added 4.0 ml of 85% sulphuric acid to each of the tubes and read against a reagent blank at 540 mµ. Calculated the amount of ascorbic acid by comparison with standard curve.

ANNEXURE IV

ESTIMATION OF THIAMINE BY THIOCHROME REACTION (JOHNSON et al., 1945) (VARLEY, 1980)

Thiamine has been determined in urine by oxidizing it to thiochrome by alkaline ferricyanide. This tricyclic derivative is then extracted into butanol and measured fluorimetrically.

Reagents:

1) Activated Decalso or permutit: About 200 mg were shaken with water in a 10 ml measuring cylinder all the particles should settle rapidly to the bottom in not more than 2 mins. To obtain a suitable product, suspended material which will pass a 100 mesh sieve in acetic acid (10 ml/100 ml of water) in large cylinders. Separated the granules which settled down to the bottom in 2 mins. from the lighter particles, by decanting. Boiled these three times with dilute acetic acid with settling and decenting between fresh addition of acetic acid. Washed the product with distilled water and dried at 110° C.

2) Potassium chloride solution: Approximately 250 g/litre

3) Sodium hydroxide solution 150 g/litre

4) Potassium ferricyanide solution: 2.5 g/litre prepared freshly.

5) Isobutanol or n-butanol

6) Acetic acid (10 ml/litre in water)

7) Stock standard hydrochloride solution 40 mg/litre in water.

8) Working standard 2 mg/litre. Diluted 5.0 ml stock standard to 100 ml with water and added 400 mg oxalic acid.

Technique:

Collected urine in glass vessels and stored in amber bottles, adding 100 mg of Oxalic acid for every 25 ml of urine. Measured 2.0 ml of urine, 2.0 ml of working standard and 2.0 ml of water (as blank) into a glass-stoppered test tubes and to each added about 200 mg of the activated Decalso or permutit and mixed with 10 rapid shakes. The absorption was optimal at pH 3 to 6 which was assured by the use of oxalic acid. Now added about 8 ml acetic acid and mixed by inversion ten times. Allowed to stand for a short time and removed the supernatant fluid. Repeated this washing process, which was important since if ineffective, the final fluorescence has a silvery blue admixture to the true thiochrome mauve, thus giving unsatisfactory readings. Added 0.5 ml potassium chloride solution and shaken, gently taking care to avoid splashing the solid far up the side. Elution was completed within 30 seconds. Added 0.1 ml potassium ferricyanide and 0.25 ml sodium hydroxide shaking gently after each addition. Added 2 ml of isobutanol stoppered and shaken vigorously for about a minute. Allowed separation of the two phases and centrifuged. Transferred the supernatant fluid to a cuvette and read in the fluorimeter at 435 mm using excitation at 365 mm and zeroing with the blank extract.

ANNEXURE V

ESTIMATION OF N1-METHYL NICOTINAMIDE IN URINE (JOHNSON *et al.*, 1945) (VARLEY, 1980)

N1-Methyl nicotinamide was adsorbed at pH 4.5 on to a column of synthetic zeolite, eluted with potassium chloride, made alkaline and extracted with n-butanol. It is then compared fluorimetrically with standards.

Reagents:

These are same as Reagents 1 - 6 for Thiamine in Urine.

Technique:

Collected urine as for thiamine and proceeded as described for thiamine up to the point of washing the permutit or Decalso on to which the adsorption had been carried out, whereas thiamine was firmly adsorbed this was not so for N1-Methyl nicotinamide, which was washed off relatively easily. So washed only twice and adhered closely to a definite technique for test and standards. After elution with 0.5 ml potassium chloride, added 2 ml of iso-butanol into the tube, added 0.25 ml sodium hydroxide, stoppered at once and shaken up and down for about a minute.

Allowed to separate or centrifuged. Transferred 1 ml of the supernatant butanol layer to a cuvette and read in the fluorimeter at 460 nm using an excitation wavelength of 360 nm. Readings were taken not earlier than 5 min. shaking with the sodium hydroxide. For the working standard a concentration of 0.1 mg/litre was convenient. No blank correction was required so long as the urine concentration was below 15 mg/litre.