A STUDY OF NITROGEN BALANCE AND CREATINE AND CREATININE EXCRETION DURING RECUMBENCY AND AMBULATION OF FIVE YOUNG ADULT HUMAN MALES

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We hereby recommend that the thesis prepared under our supervision by Kathryn Baker Montgomery entitled A STUDY OF NITROGEN BALANCE AND CREATINE AND CREATININE EXCRETION DURING RECUMBENCY AND AMBULATION OF FIVE YOUNG ADULT HUMAN MALES

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The National Aeronautics and Space Administration has been involved for many years in a relentless search for answers to the many problems which are encountered as man projects himself into space.

The problem of immobility during space flights has long been under consideration, with bed rest studies as conducted in the Texas Woman's University serving as a useful means of obtaining information concerning the effects of immobilization upon the human body.

This particular study incorporates the use of Ca^{47} in conjunction with immobilization. The aspect of this study which will be covered in this report is the relationship of the levels of urinary creatine and creatinine excretions of the five experimental subjects to their body weight and total nitrogen output, both urinary and fecal.

The nutritional and biochemical aspects of past studies have served as guidelines for present and future studies. Past studies have shown increased urinary creatine and nitrogen excretion during periods of immobilization. Weight also is lost during immobility. Besides inactivity, stress has been indicated as a contributing factor to the metabolic changes occurring under a departure from the normal routine.

It is hoped that the information contained in this thesis may be of some value in future investigations.

LITERATURE

URINARY EXCRETION OF CREATINE AND CREATININE

CREATINE

Creatine is found in small amounts in the urine of normal adults, although it occurs in larger amounts in the urine of children and pregnant women. It formerly was believed that normal adult males excreted no creatine in their urine, but it has been shown by Wilder and Morgulis (50) and by Albanese and Wangerin (1) that a small creatine excretion is normal for healthy adult males.

Studies by Taylor and Cheu (42) demonstrate a small variable creatine excretion for young males 20 to 24 years of age.

Creatine is thought to constitute about six per cent of the total creatine-creatinine output, or about 60 - 150 mg. per day in males. Most women excrete about twice as much as males, and they excrete it more regularly. In about 20 per cent of females, the creatine excretion does not exceed that of the males. During pregnancy and for two or three weeks post partum, creatine is found in greater amounts than normal. Infants and children excrete creatine in large amounts in the urine.

Over a four-year period, Clark, Thompson, Beck, and Jacobson (11) observed 100 normal children between three and 18 years of age.

They excretion exceeded creatinine elimination at three years of age; but from that point it begins to decrease gradually, with a wide variance in the elimination by individual children. The obvious explanation for the larger amounts of creatine excreted by children and women is based on the fact that muscular efficiency is involved in the conversion of creatine to creatinine. The immature muscles of children and newly formed muscle tissue of pregnant women are not capable of optimum efficiency.

CREATININE

Creatinine, next to urea, is the most abundant nitrogen-containing compound in human urine. The excretion level is measured in terms of a 24-hour sample. "Creatinine coefficient" is a term applied to the daily excretion of creatinine expressed in mg. per kg. of body weight. Normal values for men are 20-26 mg. per kilogram of body weight per 24 hours; for women 14-22 mg. per kilogram of body weight is excreted per 24 hours. These values also may be expressed in relation to height according to Cantarow and Trumper (7). According to Arroyave (4) decreases in muscle mass are proportionately larger and their estimation more sensitive than decreases in body weight and body height. The expression "mg. of creatinine in 24 hr. per cm. of body ht." is to be preferred over that of "per kg. of body wt.", since variation in fat depots do not affect the .

common that urinary creatinine remained fairly constant and was a good indication of the accuracy of a 24-hour urine collection (19) (20). The current concept, however, is that constancy is dependent upon the individual. This idea was substantiated by a study of 18 men and women from 19 to 45 years of age which was reported by Vestergard and Leverett (45). Albanese and Wangerin (1) conducted an investigation on 30 normal males which showed an individual 24-hour total creatinine variation of 10 to 25 per cent. Chow et al (9) have supported the concept that creatinine excretion is as variable as that of other urinary components.

The amount of creatinine eliminated varies chiefly with the weight of the individual, unless he is obese. This is to say that it is related to the muscular mass. A round number for the daily excretion is approximately 1.5 gms.

EFFECT OF DIET

The prevalent feeling is that excessive dietary protein has little or no influence on the amount of excretion of creatine and creatinine (6) (17). In a study considering amino acid requirements of children (33), a change in urinary creatinine excretion was noticed when dietary adjustments were made.

Murlin, Hayes, and Johnson (32) conducted a study on the correlation etween the l value of protein and the amount of creatinine . a poor protein intake, the amount of nitrogen appearing in the urine is greater, and therefore, the creatinine protein of the total nitrogen constitutes a small percentage.

Hobson (24) found significant creatinuria in the case of adult male athletes in training on a high carbohydrate diet. Finkelstein (16) fed six young men liquid and solid diets of 1 gm. protein per kg. body weight. Urinary and fecal nitrogen and total creatinine were measured. There was no measurable effect of the consistency of the diet on the utilization of dietary nitrogen. These results support the use of liquid diets in nitrogen balance experiments in man.

Giving supplements of arginine and glycine to children with kwashiorkor caused an increase in the serum levels of creatine and creatine and creatinine, according to Srikantia et al (39). These changes, however, were not associated with parallel increases in urinary creatinine.

In a 15-month period, the addition of extra calcium to the diet of school children had no favorable effect on the growth rate or muscular development. The average amount of calcium per gram of creatinine corresponded with the amount of calcium in the diet (29). This observation thus supports the recommendation of this index as a measure for the calcium intake.

The tine body is derived, in part, from creatine in the diet. Dietary creatine does not normally appear either as extra creatinine or creatine in the urine. It apparently is incorporated into the body's supply of creatine.

Bleiler (6) found that the body synthesizes creatine in amounts necessary to meet metabolic needs when studying subjects on adequate protein but creatine-free diets. Walker (47) suggests that body synthesis of creatine is governed by the muscle content of phospho-creatine.

Harding and Gaebler (22) have found the creatine excretion to fluctuate with the dietary protein. They found that growing dogs on a high protein diet had an increase in creatine production. The maximum creatine production paralleled the maximum positive nitrogen balance. When the nitrogen intake was stabilized, an inverse relationship existed between creatine excretion and positive nitrogen balance.

Scrimshaw (36) states that there is a level of protein intake for each individual at which he not only is in nitrogen equilibrium, but his lean body mass is at a desirable maximum. If the diet contains too much protein, hypertrophy of the liver and kidney develops due to the necessity of excreting excesses of nitrogen material. Individual variation from set standards will occur due to differences in digestion and absorption. Requirements vary , pathological and psychological status as well as with the influence of the activity in which the individual is engaged. Nitrogen is lost in sweating and this must be considered.

The fact that ingestion of a gram or so of creatine does not normally lead to any change in urinary creatinine or creatine is used clinically in the so-called creatine tolerance test. In this test, a 24-hour sample, following the ingestion of a standard dose of creatine hydrate, is measured for urinary creatine excretion. Normally adult males excrete 20 per cent and females, 30 per cent of a test dose (7).

EFFECT OF STRESS

Scrimshaw (37) found the creatinine excretion of 26 college males increased during examination periods. This is interpreted as being related to the psychological stress of this period. Swartz and Shields (40) conducted a similar test with medical students. A rise in urinary creatinine again was noted.

Waldron (46) conducted a study on 11 females undergoing examinations. Creatinine excretion was significantly higher during this period than normally. Stress may affect muscle contraction thus causing increases in urinary creatinine.

EFFECT OF PATHOLOGICAL CONDITIONS

Creatinuria is the condition existing when the excretion of creatine is significant. The output of creatine is greater during starvation, carbohydrate deprivation, diabetes, muscular dystrophy, hyperthyroidism, fevers, and malnutrition. In all of these conditions there is an increased catabolism either of muscular tissue or of tissue proteins in general. Creatine elimination also is greatly increased in rheumatoid arthritis (28).

Very little that is important is known regarding the excretion of creatinine under pathological conditions. The creatinine content of the urine is said to be increased in conditions associated with increased tissue catabolism, such as fever. The output of creatinine is decreased in disorders associated with muscular atrophy and muscular weakness (34). Creatinine is an index of the gravity of chronic nephritis (28).

Dreyfus (15) observed an increase in serum enzyme, creatinine kinase, to be the major biochemical symptom in progressive muscular dystrophy. Creatinine kinase is present in high amounts only in skeletal muscle, heart, and brain.

Creatinuria is common in hyperthyroidism (48) (43). This has been demonstrated by the injection of thyroxine in rabbits (48). Low levels of creatine and phospho-creatine in the muscles were measured. It is the conclusion of Fitch (18) from studies using C^{14} labeled creatine that there is a block in the entry of creatine into skeletal muscles in subjects suffering from hyperthyroidism.

In patients suffering from Graves' disease, it has been reported that there is creatinuria and diminished creatine tolerance (35) (38).

EFFECT OF IMMOBILIZATION

It has been known for many years that disuse of muscles leads to atrophy. As muscle contraction is directly responsible for the release of creatine and creatinine, the knowledge of weightlessness in space has led to many studies involving the inactivation of bed rest.

Although bed rest is necessary for convalescence in many diseases, the adverse effects must be recognized. Circulatory efficiency is impaired by weakened muscles thereby affecting the nutrition of the body.

In a study of four healthy young males immobilized for six to seven weeks by the use of bivalve casts extending from the toes to the umbilicus, Dietrick, Whedon, and Shorr (14) made several observations. There was a constancy in urinary creatinine excretion throughout the study and a day by day fluctuation in creatine excretion. There was a decrease in metabolic rate, nitrogen content of the body, and bone calcium. There was progressive reduction in creatine tolerance. The use of an oscillating bed in a later study conducted by the same research workers (49) showed that this limited stimulation greatly improved the metabolic findings.

Significantly higher amounts of urinary creatine during bed rest than before or after bed rest were the results noted in a study conducted by Heilskov and Schonheyder (23). Research conducted at Texas Woman's University is in agreement with these findings (8) (10) (25) (26) (44).

Cuthbertson (13) immobilized eight subjects in varying degrees, and found that the urinary creatinine excretion was fairly constant. There was an increase, however, in urinary nitrogen. Forty-eight young men were studied while working in flight simulators for 12 hours. This study did not indicate significant variations in creatinine excretion (21).

Metabolic studies were performed on 44 healthy men before and during bed rest at ground level or at simulated altitudes of 10,000 or 12,000 feet. The addition of 12,000 feet in simulated altitude significantly reduced the loss of total nitrogen (30).

The similarity between prolonged space flight and prolonged bed rest suggests that adverse physiological effects of bed rest also may develop during space flight. A decrease in exercise tolerance, orthostatic tolerance, muscle strength, and blood volume, coupled with an increase in urinary calcium excretion have been found to occur during prolonged bed rest (31).

INTERRELATIONSHIP WITH TOTAL NITROGEN

Much of the information on the metabolism of protein can be expressed in terms of nitrogen. The earliest method for studying these changes is based on the time-honored concept that nitrogen entering the body of mammals as food is ultimately stored in the form of body protein or eliminated, chiefly through urine and feces, as nitrogenous substances. Nitrogen loss also occurs through the skin (2).

Consolazio and associates (12) have demonstrated the finding that sweat losses can cause considerable variation in nitrogen excretion in studies involving men living and working in high environmental temperatures. It is observed that in studies of fairly inactive subjects living in air-conditioned wards that sweat losses probably are of little practical significance.

The creatinine excreted is about 3.6 per cent of the total nitrogen. Since most of the nitrogen of the diet represents protein, and most of the nitrogenous excretory products are derived from protein catabolism, it is apparent that there is a definite correlation to be found from the resulting excretion results.

PLAN OF PROCEDURE

PERIODS OF STUDY

Under the auspices of the National Aeronautics and Space Administration, the Texas Woman's University Research Institute has been conducting a series of bed rest studies. These studies are part of a vast research program being conducted in an effort to examine the response of subjects to conditions which simulate those which will be encountered in participation in the space flights.

This particular study lasted 97 days and included the following periods:

Equilibration Period, 29 days, June 19 - July 18, 1967.

Bed Rest Number One, 14 days, July 18 - August 1, 1967.

Interim Ambulatory Re-equilibration Period, 14 days, August 1 - August 15, 1967.

Bed Rest Number Two, 14 days, August 15 - August 29, 1967.

Post-Bed Rest Period, 26 days, August 29 - September 23, 1967.

SUBJECTS OF THE STUDY

Five adult male college students were chosen for this study. Before selection for participation in the study, these young men underwent extensive examinations, both physical and psychological, as have been described in previous reports. The following table shows their respective ages, heights and weights upon entering the study.

Subject	Age	Weight (lbs.)	Height (inches)
AA	24	155	70 1/4
BB	21	151	71 1/4
CC	21	138	66
DD	22	163	67
EE	21	182	73 1/2

REGIMEN AND DIET OF THE SUBJECTS

During the entire study, the subjects were housed and fed at the metabolic ward of the Nelda Childers Stark Laboratory for Human Nutrition Research at the Texas Woman's University Research Institute. Specially trained dietitians planned and supervised the preparation of the meals which were adequate in all nutrients, calcium being the nutrient which was variable. A careful record was kept of the food intake of the individual subjects throughout the study.

This study was conducted under close medical supervision. Periodic x-rays were made as well as various clinical laboratory tests. A record was made of weight changes throughout the study. Trained orderlies attended to the hygienic needs of the subjects when immobilized.

Equilibration Period

During this span of 29 days, the subjects led a normal life. They were engaged in various tasks in the laboratory eight hours daily. Their meals were planned to contain 800 mg. calcium daily during this period.

Bed Rest Period Number One

For a period of 14 days, the subjects were immobilized. They assumed a horizontal position on a single bed equipped with one pillow. They were encouraged not to lift their heads. Limited arm and leg movement was allowed. They were provided with hospital type television sets and given glasses with prismatic lens for reading. During this period the orderlies cared for the hygienic needs of the subjects, including bathing and tooth brushing. The men were spoon fed, and a careful record was kept of the individual intake of food. Ca^{47} was incorporated into their milk intake the first morning of this period to the extent of 50 microcuries. Diets were planned to contain 800 mg. calcium.

Interim Ambulatory Period

During this period, the men again were ambulatory. Four hours daily were spent in performing tasks assigned in the laboratory. Supervised physical activity was compulsory during the afternoons. Again, meals were served under the dietitian's supervision in the metabolic ward and were planned to contain 800 mg. calcium daily, with the quantity of other nutrients standardized as during the other periods of the study.

Bed Rest Period Number Two

The same conditions prevailed during this 14-day period as described under Bed Rest Period Number One, with the exception of the calcium content of the diet. In this phase of the study, the daily intake of calcium was lowered to 300 mg. As in the previous bed rest, the same level of Ca^{47} was incorporated into the milk during the first morning of recumbency.

Post-Bed Rest Period

Conditions during this period were similar to those which pertained during the interim ambulatory period with regard to work and physical activity. During this time, the calcium intake was varied as follows: August 29-September 13 (1500 mg.), September 13-16 (300 mg.), September 16-18 (1500 mg.), September 18-21 (300 mg.), and September 21-23 (1500 mg.).

PROCEDURE FOR DETERMINATION OF NITROGEN IN URINE AND FECES

Urinary and fecal types of nitrogen were determined by the Micro-Kjeldahl method as described by Archibald (3).

PROCEDURE FOR DETERMINATION OF CREATINE AND CREATININE IN URINE

The methods used for the collection, the storage, and the analytical procedure for creatine and creatinine were followed as have been used in previous studies conducted in the TWU laboratories (8) (10) (44).

Sample Collection and Storage

Urine specimens were collected on a 24-hour basis. The urine was measured and stored under refrigeration in clean polyethylene bottles. These bottles had been washed with a 10 per cent hydrochloric acid solution in an effort to prevent contamination of the urine by the enzyme creatinase which is most active at an alkaline pH (27).

Preparation of Reagents

1. Creatinine Standard Solution (0.5 mg./ml.) - 0.5 grams of creatinine anhydride were dissolved in distilled water and made up to volume of 1000 milliliters. This was stored under refrigeration.

2. Creatine Standard Solution (0.5797 mg./ml.) - 0.5797 grams of creatine anhydride were dissolved in distilled water and made up to a volume of 1000 milliliters. This was stored under refrigeration.

3. Picric Acid Solution (0.057 N) - 30 grams of crystalline picric acid were dissolved in 2000 milliliters of warm water. After cooling to

room temperature, this solution was refrigerated for 12 hours. As this is a supersaturated solution, crystals form which must be removed by filtration. In order to establish the normality, the filtrate was titrated with standardized NaOH using phenolphthaline as an indicator. The solution then was stored at room temperature.

4. 2.5 N NaOH Solution - 100 grams of NaOH crystals were dissolved in distilled water and made up to volume of 1000 milliliters. This was stored at room temperature.

Analytical Procedure

A color reaction for creatinine which first was described by Jaffe constituted the basis of a method developed by Folin in 1904 for the quantitative determination of creatinine in urine (41). Creatine was determined by converting this to creatinine since there is no test for creatine as such.

The method adopted for use in the TWU laboratories for the determinations of creatinine and creatine in the urine is the modified Folin procedure as developed by Biggs and Cooper (5).

DETERMINATION OF CREATININE

Into a clean 100 milliliter flask, pipette one milliliter of urine sample. To this add 10 milliliters 0.057 N picric acid and 1.5 milliliters of 2.5 N NaOH. Allow the mixture to stand 10 minutes for color development. Add distilled water to volume and shake the flask well. The absorbency of the solution is read on the Coleman Spectrophotometer at 540 millimicrons.

DETERMINATION OF CREATINE

Into a clean 100 milliliter flask, pipette one milliliter of urine sample. To this add 1 milliliter 0.057 N picric acid, 65 milliliters distilled water, and two glass beads. Boil the solution on an electric hot plate 40 minutes. Care should be taken that the volume in the flask does not fall below 20 milliliters. Additional distilled water is added as needed. After the solutions are cooled to room temperature, nine milliliters of picric acid and 1.5 milliliters of 2.5 N NaOH are added. Allow the solution to stand 10 minutes for color development and proceed as outlined in the determination of creatinine.

Calibration Curve for Urinary
Creatinine and Creatine

Since the colored compound formed does not follow Beer's Law, it is necessary to plot a graph and calculate results by reading from the graph. In order to construct a calibration curve, known amounts of the standard solutions are used to replace the urine samples and the determination procedures are applied to these standards.

The curves used were those made for concentrations of 0.5, 1.0, 2.0, and 3.0 milligrams of creatinine and creatine. This was done by pipetting 1.0, 2.0, 4.0 and 6.0 milliliters of the respective solutions into volumetric flasks and proceeding with the determinations.

Creatinine concentration may be read directly from the calibration curve. Creatine concentration is obtained by reading from the curve the value for the converted creatine plus creatinine. From this value is subtracted the creatinine concentration, and the difference is multiplied by 1.16, a correction factor.

$$\text{Creatine} = \left[(\text{Creatinine} + \text{Creatine}) - \text{Creatinine}\right] \times 1.16$$

AND DISCUSSION

EXCRETION OF NITROGEN

Excretion of urinary nitrogen for each subject is given on a daily basis in Table I (Appendix). Data on excretion of fecal nitrogen are outlined in Table II. Total nitrogen intake and outgo, together with the overall nitrogen balance are outlined in Table III.

Statistical comparisons of urinary nitrogen excretion between pairs of the different periods of the study are outlined in Table VI. Statistical analyses of fecal nitrogen for the different periods appear in Table VII. As in the case of Table I, these tables are given in the Appendix.

URINARY NITROGEN EXCRETION

The overall mean for the daily excretion of urinary nitrogen by Subject AA was 13.67 mg. per 24 hours during the first Bed Rest and 10.96 mg. during the Pre-Bed Rest Period, with a difference which was significantly higher ($P < 0.02$) during the Bed Rest Period.

Subject BB excreted an overall daily mean of 10.82 mg. during the first Bed Rest and 10.56 mg. during the Pre-Bed Rest. The difference was not statistically significant according to the "t" test. The mean was higher during the first Bed Rest than during the Pre-Bed Rest for

Subjects CC and DD, although again the differences were not statistically significant. In the case of Subject EE, 10.36 mg. of urinary nitrogen were eliminated during the Pre-Bed Rest and 12.45 during the first Bed Rest, with a highly significant difference ($P < 0.01$).

When the data on excretion of urinary nitrogen were pooled together for all five subjects, the amount excreted per 24 hours was significantly higher during the first Bed Rest than during the Pre-Bed Rest Periods ($P < 0.01$).

When the data for all five men were pooled, excretion of urinary nitrogen during Bed Rest 1 surpassed that during the Interim Ambulatory Period ($P < 0.001$).

For the data on all subjects pooled, Bed Rest 2 surpassed the Initial Ambulatory Period and the Interim Ambulatory Period, both by highly significant differences ($P < 0.001$), and the Final Ambulatory Period by a slightly significant difference ($P < 0.10$).

FECAL NITROGEN EXCRETION

During both Bed Rest Periods, an average of 96.6 per cent of the nitrogen was eliminated in the urine, and 3.4 per cent in the feces. Throughout the study, there were no statistically significant differences between fecal nitrogen excretion during any of the periods of the study.

NITROGEN BALANCE

All subjects remained in positive nitrogen balance throughout the study. This is understandable since the quantity of protein provided in the diet ranged from 90 to 110 grams daily. See Table III, which shows that the mean total nitrogen consumed ranged approximately from 14 to 18 grams/day per man.

EXCRETION OF CREATINE

Table IV gives the day-by-day quantities of creatine excreted in the urine. Table VIII outlines the statistical comparisons of urinary creatine excreted during the different periods of the study. With one exception (Subject BB) subjects excreted a higher amount of urinary creatine during Bed Rest 1 than during the Pre-Bed Rest Period. With the data of all subjects pooled, the quantity of creatine excreted in the urine during Bed Rest 1 surpassed that excreted during the Pre-Bed Rest Period by a statistically significant difference ($P < 0.05$).

Far less creatine, comparatively speaking, was excreted during Bed Rest 2 when 300 mg. of calcium were fed than during Bed Rest 1, when 800 mg. were provided. With the data for all subjects pooled, Bed Rest Period 1, with a mean creatine excretion of 110.3 mg./24 hours, surpassed that excreted during Bed Rest 2, with a mean of 38.3 mg./24 hours, by a difference was highly significant ($P < 0.001$).

Actually, the tine excreted in the urine was lower during Bed Rest 2 than during a period in the study.

EXCRETION OF CREATININE

The full daily data for creatinine excretion in the urine are given in Table V. Table IX shows the statistical comparisons between the amounts of urinary creatinine excreted between the various periods of the study.

Except for minor differences between the excretion levels during certain of the ambulatory periods, only small differences were found between the various periods of the study except in the case of Bed Rest 2. A slightly higher quantity of creatinine was excreted by all of the subjects during Bed Rest 2 than during the Interim Ambulatory Period.

COMPARISON OF HEIGHTS AND BODY WEIGHTS OF SUBJECTS

The heights and body weights of all subjects during the various periods of the study is shown in Table X.

SUBJECT AA

This subject weighed 150 pounds when the study began, and had a height of 5' 10 1/4". At the close of Bed Rest 1, he weighed 146.5 pounds, at the end of the Interim Ambulatory Period he weighed 148 pounds, after Bed Rest 2 142.75 pounds. At the close of the Post-Bed Rest Period he had gained slightly and weighed 146 pounds. Overall, therefore, he had lost four pounds, with no change in height.

SUBJECT BB

At the commencement of this study, Subject BB weighed 151 pounds, with a height of 5' 11.25". This subject lost four pounds during Bed Rest 1 but gained two pounds during Bed Rest 2. At the conclusion of the study he weighed 152 pounds, with no change in height.

SUBJECT CC

Subject CC who was 5' 6" tall, and weighed 138 pounds at the beginning of this study remained fairly constant in weight throughout the study fluctuating only one or two pounds. At the conclusion of the study this subject weighed 139 pounds, with no change in height.

SUBJECT DD

This subject weighed in at 163 pounds when the study began, and was 5' 7" in height. At the conclusion of Bed Rest 1 he had lost seven pounds. Two more pounds were lost during Bed Rest 2. Overall this subject lost 8 3/4 pounds during the study, with no change in height.

SUBJECT EE

At the onset of this study, this subject weighed 182 pounds and was measured at a height of 6' 1 1/2". At the conclusion of Bed Rest 1, this subject had lost 5.5 pounds. At the conclusion of the entire study, this subject exhibited an overall loss of four pounds.

SUMMARY

Five male university students participated in a study conducted at the Texas Woman's University Research Institute. The study lasted 97 days and included two 14-day Bed Rest Periods. A careful record was kept of the urinary nitrogen, fecal nitrogen, urinary creatine, and urinary creatinine excreted daily by each subject. The average excretion values for all subjects were within normal range.

Urinary nitrogen excretion for all subjects increased during Bed Rest 1 as compared to the Pre-Bed Rest Period, with a statistical significance of ($P < 0.01$) between the two means. The highest mean value among all subjects for urinary nitrogen excretion during the entire study was attained during Bed Rest 2, when the overall average excretion was 12.24 gms. per 24 hours. This mean value for all subjects for Bed Rest 2 was found to be highly significant ($P < 0.001$) when it was compared with the value for the Pre-Bed Rest Period.

Excretion values for fecal nitrogen varied only slightly between periods when statistical comparisons were made between the values for the various periods of the study. All subjects showed an increase in urinary nitrogen during the bed rest periods and a decrease in fecal nitrogen during both bed rest periods.

All subjects remained in positive nitrogen balance throughout the study. Of the total nitrogen excreted 96.6 per cent was found in the urine and 3.4 per cent in the feces.

All five subjects exhibited a generally minor weight decrease during Bed Rest 1. Two subjects lost weight during Bed Rest 2. Three subjects at the end of the study were lighter in weight than they had been upon entering this investigation.

During Bed Rest 1, the average excretion of urinary creatine for all subjects was 110.3 mg. daily as compared with 80.3 mg. daily during the Pre-Bed Rest Period. This had a statistical significance of ($P < 0.05$). During Bed Rest 2 the excretion of creatine fell to an average of 38.3 mg. per 24 hours. This drastic decrease in creatine excretion was found to be highly significant when compared to the excretion values for Bed Rest 1.

When the mean creatinine excretions for all subjects during the different periods of the study were studied, it was noted that very little overall change took place.

The most significant finding in this study was the greater excretion of creatine during the First Bed Rest as compared to the Second Bed Rest when the calcium intake dropped from 800 mg. daily to 300 mg. per day. This was a finding for all subjects.

The results of this study suggest a possible connection between dietary calcium and creatine excretion. Since no studies were found in the literature to substantiate this finding, it is felt that further investigation as to the relationship between low dietary calcium intake and low creatine excretion is indicated.

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A P P E N D I X

NITROGEN

(gm.

PART A. SUBJECT AA

Equilibration Period	Bed Rest Number 1	Interim Ambulatory Period	Bed Rest Number 2	Post-Bed Rest
(1) 17.23	(1) 8.29	(1) 12.00	(1) 11.51	(1) 12.30
(2) 14.75	(2) 0.00	(2) 8.14	(2) 0.00	(2) 2.01
(3) 12.18	(3) 10.56	(3) 14.43	(3) 6.69	(3) 16.13
(4) 9.49	(4) 12.48	(4) 9.12	(4) 17.20	(4) 18.53
(5) 4.23	(5) 9.63	(5) 7.65	(5) 8.13	(5) 12.53
(6) 15.29	(6) 19.20	(6) 10.30	(6) 8.06	(6) 20.51
(7) 9.56	(7) 13.50	(7) 11.66	(7) 13.29	(7) 10.74
(8) 6.32	(8) 14.28	(8) 11.00	(8) 15.85	(8) 8.37
(9) 12.64	(9) 8.63	(9) 10.18	(9) 13.29	(9) 10.00
(10) 8.61	(10) 12.18	(10) 11.22	(10) 11.99	(10) 12.06
(11) 10.76	(11) 14.66	X	(11) 9.68	(11) 10.08
(12) 13.23	(12) 16.54	X	(12) 19.60	(12) 12.59
(13) 12.08	(13) 14.55	X	(13) 10.51	(13) 9.63
(14) 11.66	(14) 23.26	X	(14) 15.37	(14) 8.18
(15) 14.19	(15) 12.78	X	X	(15) 21.00
(16) 6.12	(16) 14.08	X	X	(16) 10.71
(17) 11.13	(17) 11.09	X	X	(17) 13.01
(18) 11.74	(18) 9.17	X	X	(18) 8.58
(19) 10.33	X	X	X	(19) 3.58
(20) 9.87	X	X	X	(20) 10.95
(21) 8.78	X	X	X	(21) 12.14
(22) 11.08	X	X	X	(22) 11.76
(23) 8.56	X	X	X	(23) 8.77
(24) 11.33	X	X	X	(24) 11.75
(25) 13.57	X	X	X	(25) 12.98
(26) 12.08	X	X	X	X
(27) 10.92	X	X	X	X
(28) 10.53	X	X	X	X
(29) 9.59	X	X	X	X

NITROGEN

PART B. SUBJECT BB

Equilibration Period	Bed Rest Number 1	Interim Ambulatory Period	Bed Rest Number 2	Post-Bed Rest
(1) 11.44	(1) 10.33	(1) 9.94	(1) 11.29	(1) 8.62
(2) 13.44	(2) 0.00	(2) 12.44	(2) 0.00	(2) 16.24
(3) 10.64	(3) 13.47	(3) 10.03	(3) 11.50	(3) 10.50
(4) 10.01	(4) 13.52	(4) 9.46	(4) 17.79	(4) 12.61
(5) 9.37	(5) 12.59	(5) 8.10	(5) 6.04	(5) 12.14
(6) 3.58	(6) 10.86	(6) 12.23	(6) 13.90	(6) 11.17
(7) 7.48	(7) 10.56	(7) 11.00	(7) 11.59	(7) 13.91
(8) 17.62	(8) 9.33	(8) 10.79	(8) 13.17	(8) 12.39
(9) 11.85	(9) 8.70	(9) 12.02	(9) 8.02	(9) 8.40
(10) 6.36	(10) 11.87	(10) 10.00	(10) 15.02	(10) 10.96
(11) 11.86	(11) 9.45	X	(11) 11.50	(11) 11.17
(12) 8.99	(12) 11.67	X	(12) 12.51	(12) 13.94
(13) 11.10	(13) 7.30	X	(13) 9.38	(13) 10.16
(14) 10.28	(14) 11.07	X	(14) 15.93	(14) 13.90
(15) 8.64	(15) 7.65	X	X	(15) 15.04
(16) 9.55	(16) 14.63	X	X	(16) 11.97
(17) 12.05	(17) 13.97	X	X	(17) 13.36
(18) 13.00	(18) 9.73	X	X	(18) 14.15
(19) 8.38	X	X	X	(19) 13.44
(20) 9.91	X	X	X	(20) 11.59
(21) 8.62	X	X	X	(21) 8.77
(22) 13.52	X	X	X	(22) 15.11
(23) 11.40	X	X	X	(23) 11.86
(24) 12.88	X	X	X	(24) 18.49
(25) 10.92	X	X	X	X
(26) 14.43	X	X	X	X
(27) 7.13	X	X	X	X
(28) 12.49	X	X	X	X
(29) 9.19	X	X	X	X

(gm. per 24 hours)

PART C. SUBJECT CC

Equilibration Period	Bed Rest Number 1	Interim Ambulatory Period	Bed Rest Number 2	Post-Bed Rest
(1) 13.89	(1) 9.18	(1) 13.04	(1) 7.80	(1) 11.07
(2) 12.55	(2) 0.00	(2) 7.38	(2) 0.00	(2) 14.32
(3) 15.79	(3) 11.06	(3) 5.10	(3) 11.11	(3) 18.24
(4) 10.71	(4) 11.99	(4) 6.43	(4) 12.48	(4) 7.65
(5) 10.28	(5) 16.43	(5) 6.00	(5) 8.46	(5) 7.36
(6) 16.85	(6) 12.06	(6) 10.77	(6) 13.17	(6) 5.73
(7) 14.46	(7) 10.42	(7) 5.82	(7) 9.22	(7) 9.06
(8) 10.27	(8) 8.94	(8) 2.79	(8) 14.84	(8) 10.43
(9) 9.38	(9) 8.39	(9) 4.96	(9) 10.23	(9) 10.94
(10) 10.67	(10) 9.34	(10) 10.60	(10) 11.92	(10) 8.21
(11) 11.28	(11) 10.56	X	(11) 10.17	(11) 6.94
(12) 9.88	(12) 12.18	X	(12) 13.66	(12) 7.70
(13) 13.90	(13) 10.04	X	(13) 11.22	(13) 11.44
(14) 10.95	(14) 13.66	X	(14) 13.75	(14) 9.03
(15) 9.83	(15) 9.78	X	X	(15) 11.87
(16) 10.74	(16) 8.16	X	X	(16) 11.98
(17) 9.90	(17) 11.25	X	X	(17) 9.23
(18) 9.81	(18) 4.41	X	X	(18) 10.87
(19) 8.06	X	X	X	(19) 6.70
(20) 8.89	X	X	X	(20) 9.07
(21) 7.57	X	X	X	(21) 9.27
(22) 10.46	X	X	X	(22) 11.12
(23) 8.82	X	X	X	(23) 15.17
(24) 11.13	X	X	X	(24) 8.55
(25) 11.26	X	X	X	(25) 8.74
(26) 10.01	X	X	X	X
(27) 9.07	X	X	X	X
(28) 10.98	X	X	X	X

(gm

D. SUBJECT DD

Equilibration Period	Bed Re Number	Interim	Bed Rest Number 2	Post-Bed Rest

(gm.

PART E. SUBJECT EE

Equilibration Period	Bed Rest Number 1	Ambula	Bed Rest Number 2	Post-Bed Rest

(mg. per 24 hours)

PART A. SUBJECT AA

Equilibration Period	Bed Rest Number 1	Interim Ambulatory Period	Bed Rest Number 2	Post-Bed Rest
(1) 000	(1) 000	(1) 982	(1) 439	(1) 000
(2) 630	(2) 000	(2) 000	(2) 000	(2) 570
(3) 183	(3) 1426	(3) 000	(3) 000	(3) 000
(4) 628	(4) 000	(4) 000	(4) 1541	(4) 000
(5) 667	(5) 648	(5) 000	(5) 000	(5) 1963
(6) 475	(6) 000	(6) 3514	(6) 000	(6) 000
(7) 419	(7) 000	(7) 000	(7) 1203	(7) 1047
(8) 000	(8) 1750	(8) 000	(8) 000	(8) 404
(9) 1185	(9) 000	(9) 2126	(9) 000	(9) 000
(10) 602	(10) 1177	(10) 000	(10) 000	(10) 767
(11) 000	(11) 1317	(11) 000	(11) 881	(11) 000
(12) 877	X	(12) 000	(12) 000	(12) 894
(13) 000	X	(13) 1398	(13) 857	(13) 000
(14) 000	X	(14) 000	X	(14) 326
(15) 000	X	(15) 000	X	(15) 000
(16) 4035	X	(16) 318	X	(16) 166
(17) 000	X	X	X	X
(18) 000	X	X	X	X
(19) 000	X	X	X	X
(20) 000	X	X	X	X
(21) 4047	X	X	X	X
(22) 000	X	X	X	X
(23) 703	X	X	X	X
(24) 000	X	X	X	X
(25) 000	X	X	X	X
(26) 000	X	X	X	X
(27) 2119	X	X	X	X
(28) 000	X	X	X	X
(29) 1645	X	X	X	X
(30) 000	X	X	X	X
(31) 550	X	X	X	X

Equilibration Period	Bed Rest Number 1	Interim Ambulatory	Bed Rest Number 2	Post-Bed Rest

(mg.

PART C. SUBJECT CC

Equilibration Period	Bed Rest Number 1	Bed Rest Number 2	Post-Bed Rest

PART D.

Period	Bed Rest Number 1	Bed Rest Number 2	Post-Bed Rest

PART E. SUBJECT EE

Equilibration Period	Number	Bed Rest 2	Post-Bed Rest

TABLE III

NITROGEN BALANCE

PART A. SUBJECT AA

Period	Consumption					
		Ni	Nitrogen	Fecal		
		14.54			1	
t 1	91.3	14.61	13.67			
Interim Ambulatory	97.9	15.66	10.92	.596		
Bed Rest 2	103.8	16.61	12.40	.352	12.75	
Post-Bed Rest	112.8	18.05	12.31	.409	12.72	+5.33

TABLE III, CONTINUED

NITROGEN BALANCE

B. SUBJECT BB

	90.3	14.45	10.56			
Rest 1	0.2	14.43	10.82	.263		
	94.1	15.05	10.86	.387	11.25	
Bed Rest 2	102.5	16.40	12.13	.229	12.36	
Post-Bed Rest	111.5	17.84	12.08	.392	12.47	+5.37

TABLE III, CONTINUED

NITROGEN BALANCE

PART C. SUBJECT CC

	Consumption			Excretion		
		l	ry			
		.)	.)			
	90.7	.51	.94			
1	88.9	4.22	11.05	.369		
Interim Ambulatory	92.0	14.72	7.61	.	7.97	
Bed Rest 2	102.8	16.45	11.39	.357	11.75	+4.70
Post-Bed Rest	112.0	17.92	10.00	.336	10.34	+7.58

NITROGEN BALANCE

PART D. SUBJECT DD

Period	Consumption					
		Nitrogen	ry	Fecal Nitrogen		
Pre-Bed Rest	93.1	14.89				
Bed Rest 1	91.7	14.67	11.86	.376	12.	
Interim Ambulatory	98.8	15.81	10.24	.442	10.68	
Bed Rest 2	105.0	16.80	12.94	.270	13.21	+3.59
Post-Bed Rest	112.5	18.00	11.08	.458	11.54	+6.46

TABLE III, CONTINUED

NITROGEN BALANCE

PART E.

	umption					
	(gms.)		gen .)			
Pre-Bed Rest	90.6	14.4				
Rest 1	92.0	14.72	12.45	.691	13.	
Interim Ambulatory	98.7	15.79	10.91	.504	11.41	+4
Bed Rest 2	104.7	16.75	12.33	.548	12.88	
Post-Bed Rest	111.9	17.90	10.89	.394	11.28	+6.62

PART A. SUBJECT

	Bed	Rest Number 2	Post-Bed Rest
Period			

PART B.

libration Period	Bed Rest Number 2	Post-Bed Rest

PART C. SUBJECT CC

Equilibration Period	Bed Rest	Bed Rest Number 2	Post-Bed Rest

PART D.

libration Period	Bed	Bed Rest Number 2	Post-Bed Rest

PART E.

Equilibration Period	Bed Rest Number 2	Post-Bed Rest

)

PART A. SUBJECT AA

Equilibration Period	Bed	Bed Rest Number 2	Post-Bed Rest

PART B.

Equilibration Period	Bed	Interim	Bed Rest - Number 2	Post-Bed Rest

PART C.

Equilibration Period	Bed Rest	Bed Rest Number 2	Post-Bed Rest

PART D.

tion Period	Bed Rest	Bed Rest 2	Post-Bed Rest

PART E. SUBJECT

Period	Bed Rest Number 2	Post-Bed Rest

STATISTICAL

BETWEEN PERIODS

PART A. SUBJECT

Populations			"t"	Probability
Pre-Bed Rest Period Bed Rest No. 1		2.74 4.08	2.3980	$P < 0.02$
Pre-Bed Rest Period Interim Ambulatory		2.74 1.95	0.0523	N.S.
Pre-Bed Rest Period Bed Rest No. 2	10.96 12.40	2.74 3.71	1.3314	N.S.
Pre-Bed Rest Post-Bed Rest	10.9 12.31	2.74 4.86	1.1187	N.S.
Bed Rest No. 1 Interim Ambulatory	.67 .92	4.08 1.95	2.0940	$P < 0.05$
Bed Rest No. 1 Bed Rest No. 2	67 40	4.08 3.71	0.7698	N.S.
Bed Rest No. 1 Post-Bed Rest	3.67 .31	4.08 4.86	0.7396	N.S.
Interim Ambulatory Bed Rest No. 2	10.92 0	1.95 3.71	1.2142	N.S.
Interim Post-Bed	10.92 12.3	1.95 4.86	0.9325	N.S.
Bed Rest No. 2 Post-Bed Rest	0 .31	3.71 4.86	0.0489	N.S.

TABLE VI, CONTINUED

STATISTICAL COMPARISON OF URINARY NITROGEN

BETWEEN PAIRS OF THE DIFFERENT PERIODS

OF THE STUDY

PART B.

Populations C		Standard	"t"	Probability
Pre-Bed Rest Period Bed Rest No. 1	10.56 10.82	2.70 1.77	0.3134	N.S.
Pre-Bed Rest Period Interim Ambulatory	10.56 10.86	2.70 1.95	0.3552	N.S.
Pre-Bed Rest Period Bed Rest No. 2	10.56 12.13	2.70 3.07	1.5853	N.S.
Pre-Bed Rest Period Post-Bed Rest Ambulatory	10.56 12.08	2.70 2.18	1.8019	$P < 0.10$
Bed Rest No. 1 Interim Ambulatory	10.82 10.86	1.77 1.95	0.0411	N.S.
Bed Rest No. 1 Bed Rest No. 2	10.82 12.13	1.77 3.07	1.2228	N.S.
Bed Rest No. 1 Post-Bed Rest Ambulatory	10.82 12.08	1.77 2.18	1.5363	N.S.
Interim Ambulatory Bed Rest No. 2	10.86 12.13	1.95 3.07	1.1962	N.S.
Interim Ambulatory Post-Bed Rest Ambulatory	10.86 12.08	1.95 2.18	1.4772	N.S.
Bed Rest No. 2 Post-Bed Rest Ambulatory	12.13 .08	3.07 2.18	0.0461	N.S.

PART C. SUBJECT CC

Populations Compared		Standard	"t"	
Pre-Bed Rest Period Bed Rest No. 1	10.94 11.	2.08 2.07	0.1403	N.S.
Pre-Bed Rest Period Interim Ambulatory	10.94 7.61	2.08 2.94	4.0290	$P < 0.001$
Pre-Bed Rest Period Bed Rest No. 2		2.08 2.08	0.6046	N.S.
Pre-Bed Rest Period Post-Bed Rest Ambulatory	10.94 10.00	2.08 3.11	.1258	N.S.
Bed Rest No. 1 Interim Ambulatory	11.05 7.61	2.07 2.94	3.2374	$P < 0.01$
Bed Rest No. 1 Bed Rest No. 2	11.05 11.39	2.07 2.08	0.3874	N.S.
Bed Rest No. 1 Post-Bed Rest Ambulatory	11.05 10.00	2.07 3.11	0.9594	N.S.
Interim Ambulatory Bed Rest No. 2	7.61 11.39	2.94 2.08	3.5503	$P < 0.01$
Interim Post-Bed	7.61 10.00	2.94 3.	1.9817	$P < 0.10$
Bed Rest No. 2 Post-Bed Rest	11.39 10.00	2. 3.	1.2699	N.S.

PART D.

Populations			"t"	Probability
Pre-Bed Rest Period Bed Rest No. 1		1.66 2.62	1.1728	N.S.
Pre-Bed Rest Period Interim Ambulatory	 4	1.66 3.17	1.0272	N.S.
Pre-Bed Rest Period Bed Rest No. 2	11.04 12.94	1.66 2.63	2.6932	$P < 0.01$
Pre-Bed Rest Period Post-Bed Rest Ambulatory	11.04	1.66 2.73	0.0623	N.S.
Bed Rest No. 1 Interim Ambulatory	11.86 1 .24	2.62 3.17	1.3424	N.S.
Bed Rest No. 1 Bed Rest No. 2	11.86 12.94	2.62 2.63	0.9675	N.S.
Bed Rest No. 1 Post-Bed Rest	6 11.08	2.62 2.73	0.7196	N.S.
Interim Ambulatory Bed Rest No. 2	.24 .94	3.17 2.63	2.2285	$P < 0.05$
Interim Post-Bed Rest	10.24	3.17 2.73	0.7161	N.S.
Bed Rest No. 2 Post-Bed Rest	12.94 11	2.63 73	1.7046	N.S.

PART E.

Populations			"t"	Probability
Pre-Bed Rest Period Bed Rest No. 1	1	1.99 1.88	3.0536	$P < 0.01$
Pre-Bed Rest Period Interim Ambulatory	10.36 10.9	1.99 1.79	0.8386	N.S.
Pre-Bed Rest Period Bed Rest No. 2	10.36 12.33	1.99 1.93	2.8595	$P < 0.01$
Pre-Bed Rest Period Post-Bed Rest Ambula	10.36 10.89	1.99 2.06	0.7970	N.S.
Bed Rest No. 1 Interim Ambulatory	12.45 10.91	1.88 1.79	2.0189	$P < 0.10$
Bed Rest No. 1 Bed Rest No. 2	12.45 12.33	1.88 1.93	0.1447	N.S.
Bed Rest No. 1 Post-Bed Rest	12.45 10 89	1.88 2.06	1.9304	$P < 0.10$
Interim Ambulatory Bed Rest No. 2	10.91 12.33	1.79 1.93	1.8400	$P < 0.10$
Interim Post-Bed	.91 .89	1.79 2.06	0.0213	N.S.
Bed Rest No. 2 Post-Bed	12. 89	1.93 2.	1.4385	$P < 0.10$

PART F. ALL SUBJECTS

Populations Compa	Means	ndard	"t"	Probability
Pre-Bed Rest Period Bed Rest No. 1	10.77 11.97	2.29 .82	3.2278	P< 0.01
Pre-Bed Rest Period Interim Ambulatory	10.77 .10	2.29 2.74	1.8462	P< 0.10
Pre-Bed Rest Period Bed Rest No. 2	10.77 12.24	2.29 2.81	3.9489	P< 0.001
Pre-Bed Rest Period Post-Bed Rest	1 11	2.29 3.26	1.3114	N.S.
Bed Rest No. 1 Interim Ambulatory	11.97 10.10	2.82 2.74	3.8403	P< 0.001
Bed Rest No. 1 Bed Rest No. 2	11.97 12.2	2.82 2.81	0.5300	N.S.
Bed Rest No. 1 Post-Bed Rest Ambula	11.97 27	2.82 3.26	1.3278	N.S.
Interim Ambulatory Bed Rest No. 2	10 12	2.74 2.81	4.3970	P< 0.001
Interim Post-Bed Rest	10.10 11.27	2.74 3.26	2.2909	P< 0.05
Bed Rest No. 2 Post-Bed Rest	12.24 .27	2.81 3.	1.8353	P< 0.10

NITROGEN EXCRETION

PERIODS

PART A.

Populations Compared		Standard	"t"	Probability
Pre-Bed Rest Period Bed Rest No. 1	647 451	1062 642	0.6094	N.S.
Pre-Bed Rest Period Interim Ambulatory	647 596	1062 1032	0.1436	N.S.
Pre-Bed Rest Period Bed Rest No. 2	647 352	1062 521	0.9437	N.S.
Pre-Bed Rest Period Post-Bed Rest Ambulatory	647 409	1062 544	0.7806	N.S.
Bed Rest No. 1 Interim Ambulatory	451 596	642 1032	0.4133	N.S.
Bed Rest No. 1 Bed Rest No. 2	451 352	642 521	0.4178	N.S.
Bed Rest No. 1 Post-Bed Rest Ambu	451 409	642 544	0.1772	N.S.
Interim Ambulatory Bed Rest No. 2	596	1032 521	0.7334	N.S.
Interim Ambulatory Post-Bed Re	596 409	1032 544	0.5719	N.S.
Bed Rest No. 2 Post-Bed Res tory	352 409	521 544	0.2701	N.S.

EXCRETION

PART B.

Populations		Standard	"t"	Probability
Pre-Bed Rest Bed Rest No. 1		721 658	0.4658	N.S.
Pre-Bed Rest Period Interim Ambulatory	6	74 982	0.2090	N.S.
Pre-Bed Rest Period Bed Rest No. 2	446 229	721 451	0.9874	N.S.
Pre-Bed Rest Period Post-Bed Rest Ambula	446 392	721 614	0.2347	N.S.
Bed Rest No. 1 Interim Ambulatory	334 387	658 982	0.1560	N.S.
Bed Rest No. 1 Bed Rest No. 2	334 229	658 451	0.4593	N.S.
Bed Rest No. 1 Post-Bed Rest	334 392	658 614	0.2276	N.S.
Interim Ambulatory Bed Rest No. 2	387 229	982	0.5097	N.S.
Interim Post-Bed Rest	387 392	982 614	0.0145	N.S.
Bed Rest No. 2 Post-Bed	229	451 614	0.7552	N.S.

PART C.

Populations			"t"	Probability
Pre-Bed Rest Period Bed Rest No. 1		581 387	0.8803	N.S.
Pre-Bed Rest Period Interim Ambulatory		581 589	0.8369	N.S.
Pre-Bed Rest Period Bed Rest No. 2		581 387	1.1149	N.S.
Pre-Bed Rest Period Post-Bed Rest Ambulatory		581 330	0.9596	N.S.
Bed Rest No. 1 Interim Ambulatory	 3	387 589	0.0451	N.S.
Bed Rest No. 1 Bed Rest No. 2	369 32	387 387	0.2663	N.S.
Bed Rest No. 1 Post-Bed Rest	369 	387 330	0.0338	N.S.
Interim Ambulatory Bed Rest No. 2		589 387	0.1620	N.S.
Interim Ambulatory Post-Bed Rest Ambula	359 364	589 330	0.0228	N.S.
Bed Rest No. 2 Post-Bed Rest	3 3	387 330	0.2592	N.S.

BETWEEN PAIRS OF THE DIFFERENT PERIODS OF THE STUDY

PART D. SUBJECT

Populations		Standard	"t"	Probability
Pre-Bed Rest Period Bed Rest No. 1		654 658	0.7497	N.S.
Pre-Bed Rest Period Interim Ambulatory	544 442	654 729	0.4000	N.S.
Pre-Bed Rest Period Bed Rest No. 2	 270	654 561	1.2848	N.S.
Pre-Bed Rest Period Post-Bed Rest Ambulatory	544 488	654 517	0.2724	N.S.
Bed Rest No. 1 Interim Ambulatory	376 442	658 729	0.2321	N.S.
Bed Rest No. 1 Bed Rest No. 2	376	658 561	0.4264	N.S.
Bed Rest No. 1 Post-Bed Rest	376 488	658	0.4772	N.S.
Interim Ambulatory Bed Rest No. 2	442 27	729 561	0.6486	N.S.
Interim Ambula Post-Bed	442 488		0.1863	N.S.
Bed Rest No. 2 Post-Bed Rest	270 488	561 517	.0154	N.S.

EXCRETION

PART E. SUBJECT

Populations			"t"	Probability
Pre-Bed Rest Period Bed Rest No. 1	1	898 998	0.1897	N.S.
Pre-Bed Rest Period Interim	504	898 694	0.4436	N.S.
Pre-Bed Rest Period Bed Rest No. 2	630 548	898 966	0.2631	N.S.
Pre-Bed Rest Period Post-Bed Rest	630 394	898 395	0.9310	N.S.
Bed Rest No. 1 Interim Ambulatory	691 504	998 4	0.5342	N.S.
Bed Rest No. 1 Bed Rest No. 2	691 548	998 966	0.3581	N.S.
Bed Rest No. 1 Post-Bed Rest	691 394	998 395	0.9906	N.S.
Interim Ambulatory Bed Rest No. 2	504	694 966	0.1279	N.S.
Interim Ambulatory Post-Bed	504 394	694 395	0.4909	N.S.
Bed Rest No. 2 Post-Bed Rest Ambula	548 394	966 395	0.5274	N.S.

FECAL NITROGEN EXCRETION

OF THE STUDY

PART F. ALL SUBJECTS

Populations Compared	Means	Standard Deviation	"t" Value	Probability
Pre-Bed Rest Period Bed Rest No. 1	559 463	806 767	0.7565	N.S.
Pre-Bed Rest Period Interim Ambulatory	559 443	806 773	1.0553	N.S.
Pre-Bed Rest Period Bed Rest No. 2	559 344	806 622	1.9382	N.S.
Pre-Bed Rest Period Post-Bed Rest Ambulatory	559 409	806 493	1.4547	N.S.
Bed Rest No. 1 Interim Ambulatory	463 443	767 773	0.1505	N.S.
Bed Rest No. 1 Bed Rest No. 2	463 344	767 622	0.9397	N.S.
Bed Rest No. 1 Post-Bed Rest Ambulatory	463 409	767 493	0.4778	N.S.
Interim Ambulatory Bed Rest No. 2	443 344	773 622	0.8429	N.S.
Interim Ambulatory Post-Bed Rest Ambulatory	443 409	773 493	0.3155	N.S.
Bed Rest No. 2 Post-Bed Rest Ambulatory	344 409	622 493	0.6854	N.S.

OF CREATINE EXCRETION

BETWEEN PERIODS

OF THE STUDY

PART A. SUBJECT AA

Populations Compared		Standard		Proba
Pre-Bed Rest Bed Rest No. 1	62.3 112.1	79.5 53.3	1.7772	$P < 0.10$
Pre-Bed Rest Period Interim Ambulatory	62.3 62.2	79.5 104.8	0.0042	N.S.
Pre-Bed Rest Period Bed Rest No. 2	62.3 62.1	79.5 63.7	0.0078	N.S.
Pre-Bed Rest Period Post-Bed Rest Ambulatory	62.3 94.7	79.5 112.3	1.0536	N.S.
Bed Rest No. 1 Interim Ambulatory	112.1 62.2	53.3 104.8	1.2738	N.S.
Bed Rest No. 1 Bed Rest No. 2	112.1 62.1	53.3 63.7	1.6112	N.S.
Bed Rest No. 1 Post-Bed Rest	112.1 94.7	53.3 112.3	0.4327	N.S.
Interim Ambulatory Bed Rest No. 2	62.2 62.1	104.8 .7	0.0020	N.S.
Interim Ambulatory Post-Bed Rest Ambula	62.2 94.7	104.8 112.3	0.8090	N.S.
Bed Rest No. 2 Post-Bed Res	62.1 94.7	63.7 112.3	0.8873	N.S.

EXCRETION

BETWEEN PAIRS OF THE DIFFERENT PERIODS

OF THE STUDY

PART B. SUBJECT BB

Populations		Standard	"t"	Probability
Pre-Bed Rest Period Bed Rest No. 1	54.3 45.0	56.7 53.3	0.4924	N.S.
Pre-Bed Rest Period Interim Ambulatory	54.3 48.9	56.7 70.9	0.2594	N.S.
Pre-Bed Rest Period Bed Rest No. 2	54.3 13.2	56.7 33.8	2.3993	$P < 0.02$
Pre-Bed Rest Period Post-Bed Rest	54.3 21.5	56.7 34.5	-1.9677	$P < 0.05$
Bed Rest No. 1 Interim Ambulatory	45.0 48.9	53.3 70.9	0.1518	N.S.
Bed Rest No. 1 Bed Rest No. 2	45.0 13.2	53.3 33.8	1.7520	$P < 0.10$
Bed Rest No. 1 Post-Bed	45.0 5	53.3 34.5	1.3214	N.S.
Interim Ambulatory Bed Rest No. 2	48.9 1 .2	70.9 .8	1.5792	N.S.
Interim Ambulatory Post-Bed	48.9 5	70.9 34.5	1.2436	N.S.
Bed Rest No. 2 Post-Bed Rest	13.2 21.5	33.8 34.5	0.6075	N.S.

OF CREATINE EXCRETION

BETWEEN PAIRS OF THE DIFFERENT PERIODS

OF THE STUDY

PART C. SUBJECT CC

Populations Compared		Standard	"t"	Probability
Pre-Bed Rest Period Bed Rest No. 1	89.4 123.6	90.2 122.8	0.9783	N.S.
Pre-Bed Rest Period Interim Ambulatory	89.4 6	90.2 75.3	0.4368	N.S.
Pre-Bed Rest Period Bed Rest No. 2	89.4 24.5	90.2 42.3	2.4500	P $<$ 0.02
Pre-Bed Rest Period Post-Bed Rest Ambula	89.4 49.9	90.2 112.4	1.2017	N.S.
Bed Rest No. 1 Interim Ambulatory	123.6 76.6	122.8 75.3	1.1317	N.S.
Bed Rest No. 1 Bed Rest No. 2	123.6 24.5	122.8 42.3	2.6503	P $<$ 0.02
Bed Rest No. Post-Bed Rest	123.6 .9	122.8 112.4	1.5691	N.S.
Interim Ambulatory Bed Rest No. 2	76.6 24.5	75.3 42.3	2.0971	P $<$ 0.05
Interim Post-Bed Rest	76.6 49.9	75.3 112.4	0.6951	N.S.
Bed Rest No. 2 Post-Bed Rest	24.5 49.9	42.3 112.4	0.7412	N.S.

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CREATINE EXCRETION

BETWEEN PAIRS OF THE DIFFERENT PERIODS

PART D.

	Means	rd	"t"	Probability
Pre-Bed Rest Period Bed Rest No. 1	104.5 130.2	98.7 98.3	0.7629	N.S.
Pre-Bed Rest Period Interim Ambulatory	104.5 103.5	98.7 89.6	0.0303	N.S.
Pre-Bed Rest Period Bed Rest No. 2	104.5 43.3	98.7 64.4	2.0213	P < 0.05
Pre-Bed Rest Period Post-Bed Rest Ambulatory	104.5 50.1	98.7 53.2	1.9057	P < 0.10
Bed Rest No. 1 Interim Ambulatory	130.2 103.5	98.3 .6	0.6963	N.S.
Bed Rest No. 1 Bed Rest No. 2	130.2 43.3	98.3 64.4	2.5682	P < 0.02
Bed Rest No. Post-Bed	2 5 .1	98.3 53.2	2.5595	P < 0.02
Interim Ambulatory Bed Rest No. 2	103.5 43.3	89.6 64.4	1.8960	P < 0.10
Interim Post-Bed	103.5 50.1	89.6 53.2	1.8291	P < 0.10
Bed Rest No. 2 Post-Bed	43.3 50.1	64.4 53.2	0.2914	N.S.

EXCRETION

BETWEEN PAIRS OF THE DIFFERENT PERIODS

OF THE STUDY

PART E. SUBJECT EE

Populations Compared			"t"	Probability
Pre-Bed Rest Period Bed Rest No. 1	90.9 139.8	97.7 122.4	1.3424	N.S.
Pre-Bed Rest Period Interim Ambulatory	90.9 8	97.7 78.0	0.0925	N.S.
Pre-Bed Rest Period Bed Rest No. 2	90.9 48.2	97.7 71.6	1.3914	N.S.
Pre-Bed Rest Period Post-Bed Rest Ambulatory	90.9 42.9	97.7 60.6	1.6639	N.S.
Bed Rest No. 1 Interim Ambulatory	139.8 88.0	122.4 78.0	1.2387	N.S.
Bed Rest No. 1 Bed Rest No. 2	139.8 48.2	122.4 71.	2.2421	P $<$ 0.05
Bed Rest No. Post-Bed	139.8 42.9	122.4 60.6	0.9036	N.S.
Interim Ambulatory Bed Rest No. 2	88.0 48.2	78.0 7 .6	1.3053	N.S.
Interim Ambulatory Post-Bed Re	88.0 42.9	78.0 60.6	1.6252	N.S.
Bed Rest No. 2 Post-Bed Rest	48.2 42.9	71.6 60.6	0.2024	N.S.

BETWEEN PAIRS OF THE DIFFERENT PERIODS

OF THE STUDY

PART F. ALL SUBJECTS

Populations Compa	ns	Standard	"	Probability
Pre-Bed Rest Period Bed Rest No. 1	3 110.3	88.0 106.1	2.1645	P < 0.05
Pre-Bed Rest Period Interim Ambulatory	80.3 .8	88.0 86.7	0.3456	N.S.
Pre-Bed Rest Period Bed Rest No. 2	80.3 38.3	88.0 59.6	3.5821	P < 0.001
Pre-Bed Rest Post-Bed Rest	80 3 51	88.0 84.6	2.2827	P < 0.05
Bed Rest No. 1 Interim Ambulatory	110.3 75.8	106.1 86.7	2.0730	P < 0.05
Bed Rest No. 1 Bed Rest No. 2	110.3 38.3	106.1 59.6	4.8811	P < 0.001
Bed Rest No. 1 Post-Bed Rest Ambulatory	110.3 8	106.1 84.6	3.6295	P < 0.001
Interim Ambulatory Bed Rest No. 2	75.8 38.3	86.7 59.6	2.9444	P < 0.01
Interim Post-Bed Re	75.8 51.8	86.7 6	1.6642	P < 0.10
Bed Rest No. 2 Post-Bed Res	38.3 51.8	59.6 84.	1.0932	N.S.

BETWEEN PAIRS OF THE DIFFERENT PERIODS

OF THE STUDY

PART A. SUBJECT AA

Populations Compa	Means	Standard	"t"	Probability
Pre-Bed Rest Period Bed Rest No. 1	2205 1982	529 600	1.1757	N.S.
Pre-Bed Rest Period Interim Ambulatory	 1895	529 378	1.8734	P< 0.10
Pre-Bed Rest Period Bed Rest No. 2	2205 2152	529 650	0.2675	N.S.
Pre-Bed Rest Period Post-Bed Rest Ambulatory	2205 1999	529 257	1.3604	N.S.
Bed Rest No. 1 Interim Ambulatory	1982 1895	600 378	0.4270	N.S.
Bed Rest No. 1 Bed Rest No. 2	1982 2152	600 650	0.6679	N.S.
Bed Rest No. 1 Post-Bed Rest Ambu	1982 1999	600 257	0.0924	N.S.
Interim Ambulatory Bed Rest No. 2	1895 2152	378 650	1.1888	N.S.
Interim Ambulatory Post-Bed	1895 1999	378 257	0.8090	N.S.
Bed Rest No. 2 Post-Bed	2152 199	650 257	0.7860	N.S.

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STATISTICAL COMPARISON OF CREATININE EXCRETION BETWEEN PAIRS OF THE DIFFERENT PERIODS OF THE STUDY

PART B. SUBJECT BB

Populations Compared	Means	Standard	"t"	Probability
Pre-Bed Rest Period Bed Rest No. 1	1833 1732	259 349	1.0165	N.S.
Pre-Bed Rest Period Interim Ambulatory	1833 1738	259 313	0.9945	N.S.
Pre-Bed Rest Period Bed Rest No. 2	1833 1912	259 541	0.6132	N.S.
Pre-Bed Rest Period Post-Bed Rest	1833 1861	259 298	-0.3093	N.S.
Bed Rest No. 1 Interim Ambulatory	1732 1738	349 313	0.0508	N.S.
Bed Rest No. 1 Bed Rest No. 2	1732 1912	349 541	0.9735	N.S.
Bed Rest No. 1 Post-Bed Rest Ambulatory	1732 1861	349 298	1.0026	N.S.
Interim Ambulatory Bed Rest No. 2	1738 19	313 541	0.9641	N.S.
Interim Post-Bed Rest	1738 1861	313 298	1.0055	N.S.
Bed Rest No. 2 Post-Bed Rest	1912	541 298	0.6486	N.S.

OF CREATININE EXCRETION

BETWEEN PAIRS OF THE DIFFERENT PERIODS

OF THE STUDY

PART C. SUBJECT CC

Populations	Means	Standard	"t"	Probability
Pre-Bed Rest Period Bed Rest No. 1	1795 1708	155 308	1.1609	N.S.
Pre-Bed Rest Period Interim Ambulatory	1795 1481	155 565	2.6203	$P < 0.01$
Pre-Bed Rest Period Bed Rest No. 2	1795 1896	155 253	1.5360	N.S.
Pre-Bed Rest Period Post-Bed Rest Ambulatory	1795 1644	155 524	1.3517	N.S.
Bed Rest No. 1 Interim Ambulatory	1708 1481	308 565	1.2236	N.S.
Bed Rest No. 1 Bed Rest No. 2	1708 1896	308 253	1.6356	N.S.
Bed Rest No. 1 Post-Bed Rest Ambulatory	1708 1644	308 524	0.3683	N.S.
Interim Ambulatory Bed Rest No. 2	1481 1896	565 253	2.3257	$P < 0.05$
Interim Post-Bed Rest	1481 1644	565 524	0.7507	N.S.
Bed Rest No. 2 Post-Bed Rest	18	253 524	1.5166	N.S.

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STATISTICAL COMPARISON OF CREATININE EXCRETION

BETWEEN PAIRS OF THE DIFFERENT PERIODS

OF THE STUDY

PART D. SUBJECT DD

Populations Compared	Means	Standard	"t"	Probability
Pre-Bed Rest Period Bed Rest No. 1	2330 2056	404 648	1.6097	N.S.
Pre-Bed Rest Period Interim Ambulatory	2330 2117	404 405	1.5464	N.S.
Pre-Bed Rest Period Bed Rest No. 2	2330 2314	404 307	0.1280	N.S.
Pre-Bed Rest Period Post-Bed Rest Ambulatory	2330 2057	404 527	1.8181	$P < 0.10$
Bed Rest No. 1 Interim Ambulatory	2056 2117	648 405	0.2769	N.S.
Bed Rest No. 1 Bed Rest No. 2	2056 2314	648 307	1.2491	N.S.
Bed Rest No. 1 Post-Bed Rest Ambula	2056 2057	648 527	0.0054	N.S.
Interim Ambulatory Bed Rest No. 2	2117 2314	405 307	1.3476	N.S.
Interim Post-Bed Rest	2117 2057	405 527	0.3168	N.S.
Bed Rest No. 2 Post-Bed Rest Ambulatory	2314 2057	307 527	1.4796	N.S.

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STATISTICAL COMPARISON OF CREATININE EXCRETION

BETWEEN PAIRS OF THE DIFFERENT PERIODS

OF THE STUDY

PART E. SUBJECT EE

Populations Compared	Means	Standard Deviation	"t" Value	Probability
Pre-Bed Rest Period Bed Rest No. 1	1931 2188	281 533	1.9633	$P < 0.05$
Pre-Bed Rest Period Interim Ambulatory	1931 2187	281 628	1.7476	$P < 0.10$
Pre-Bed Rest Period Bed Rest No. 2	1931 2240	281 274	3.2526	$P < 0.01$
Pre-Bed Rest Period Post-Bed Rest Ambulatory	1931 2039	281 255	1.1940	N.S.
Bed Rest No. 1 Interim Ambulatory	2188 2187	533 628	0.0060	N.S.
Bed Rest No. 1 Bed Rest No. 2	2188 2240	533 274	0.3012	N.S.
Bed Rest No. 1 Post-Bed Rest Ambulatory	2188 2039	533 255	0.9036	N.S.
Interim Ambulatory Bed Rest No. 2	2187 2240	628 274	0.2710	N.S.
Interim Ambulatory Post-Bed Rest Ambulatory	2187 2039	628 255	0.7820	N.S.
Bed Rest No. 2 Post-Bed Rest Ambulatory	2240 2039	274 255	1.9062	$P < 0.10$

CONTINUED

STATISTICAL COMPARISON OF CREATININE EXCRETION BETWEEN PAIRS OF THE DIFFERENT PERIODS OF THE STUDY

PART F. ALL SUBJECTS

Populations Compared	Means	ndard		Probability
Pre-Bed Rest Period Bed Rest No. 1	2019 1933	409 540	1.2749	N.S.
Pre-Bed Rest Period Interim Ambulatory	019 1884	409 538	2.0161	P< 0.05
Pre-Bed Rest Period Bed Rest No. 2	2019 2067	409 509	0.7464	N.S.
Pre-Bed Rest Period Post-Bed Rest Ambulatory	2019 1920	409 422	1.6582	P< 0.10
Bed Rest No. 1 Interim Ambulatory	1933 1884	540 538	0.5355	N.S.
Bed Rest No. 1 Bed Rest No. 2	1933 2067	540 509	1.4912	N.S.
Bed Rest No. Post-Bed Rest	1933 1920	540 422	0.1606	N.S.
Interim Ambulatory Bed Rest No. 2	1884 2 7	538 509	2.0444	P< 0.05
Interim Ambulatory Post-Bed Rest Ambulatory	1884 1920	538 422	0.4488	N.S.
Bed Rest No. 2 Post-Bed Rest Ambulatory	2067 20	509 422	1.8739	P< 0.10

TABLE X

HEIGHT AND BODY WEIGHT OF SUBJECTS

Period	Subject	Weight	Height
Pre-Bed Rest	AA	150	5' 10 1/4"
	BB	154 1/2	5' 11 1/4"
	CC	138	5' 6"
	DD	157 1/2	5' 7"
	EE	179	6' 1 1/2"
Bed Rest 1	AA	146 1/2	5' 10 3/4"
	BB	150 1/2	5' 11 1/4"
	CC	136 1/2	5' 6 1/4"
	DD	156	5' 8"
	EE	176 1/2	6' 2"
Interim Ambulatory	AA	148	5' 10 1/2"
	BB	150 1/2	5' 11 1/4"
	CC	136 1/2	5' 6"
	DD	154 1/2	5' 7"
	EE	178	6' 1 1/2"

TABLE X, CONTINUED

HEIGHT AND BODY WEIGHT OF SUBJECTS

Period	Subject	Weight	Height
Bed Rest 2	AA	142 3/4	5' 10 1/2"
	BB	152 1/4	5' 11 1/2"
	CC	137	5' 6"
	DD	154	5' 7 1/2"
	EE	176 1/2	6' 2 1/4"
Post-Bed Rest	AA	146	5' 10 1/2"
	BB	152	5' 11 1/4"
	CC	139	5' 6"
	DD	154 1/2	5' 7"
	EE	178	6' 1 1/2"