Monitoring of Sweat Calcium Using Skin Patches

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ABSTRACT

The purpose of this study was to determine whether a simple non-invasive sweat collection method using skin patches would be useful in monitoring sweat Ca and to determine changes in dermal Ca loss during a bed rest study testing a resistive exercise countermeasure. This study showed that the technique was highly reproducible: the mean intra-subject variation approached zero and the inter-individual variability (% CV) varied from 18-32% for the 3 anatomical regions (arm, chest, and back) tested. There was less than 10% difference in sweat Ca excretion from different skin regions within the same individual at a given time point. A calculated estimate of total body sweat excretion for 12 bed rest subjects was 35 ± 4 mg/day (mean ± SE), close to published whole body measurements. Bed rest testing showed no significant differences with or without exercise when conducted in a temperature controlled environment. We conclude that the skin patch technique is useful for monitoring changes in sweat Ca.

Key Words: Sweat patch, Calcium balance, Bed rest, Exercise, Dermal Ca loss

INTRODUCTION

Calcium (Ca) balance is defined as the difference between dietary intake and dermal, fecal and urinary loss (1). Ca balance, as part of research bed rest studies conducted by NASA, has been used for many years to detect early changes in whole body bone mass. Bed rest subjects routinely demonstrate a rapid increase in urinary and fecal Ca excretion that results in a negative Ca balance (2, 3). In most studies, the contribution from dermal loss is not measured and the contribution of sweat Ca to the overall Ca balance is assumed to be negligible. This assumption has rarely been tested, in part because of the difficulties with accurately measuring sweat calcium.

An important area of research is the effect of exercise as a countermeasure against disuse bone loss. Calcium loss in the sweat from an exercise program at normal room temperature can be significant. In one study, total body sweat Ca loss was measured during and after exercise in three male subjects (4). The mean value of sweat Ca loss was 47 mg/hour during one hour of running on a treadmill at room temperature, while the mean value for the entire day excluding running was 0.8 mg/hour. Others have found that sweat Ca loss was 25 mg/40 min during strenuous exercise compared to 16 mg /day for sedentary subjects (5). These data indicate that sweat loss of Ca could represent a significant fraction of the change in total body Ca output during exercise. In these studies the method for measuring sweat loss of Ca involved the cumbersome procedure of determining the Ca content of fluid used to wash sweat soaked clothing that was worn by the subjects.

One purpose of this study was to determine whether a simple non-invasive sweat collection method using skin patches would be useful in measuring sweat Ca. A second objective was to determine dermal Ca loss during a bed rest study testing a resistive exercise countermeasure during which the environmental temperature was maintained to minimize sweat loss.

EXPERIMENTAL SUBJECTS

The participants in an ongoing NASA-funded bed rest study, conducted in the Biomedical Research Bed Rest Facility at the Baylor College of Medicine, Houston, Texas, were recruited as subjects for the sweat Ca study. There were three different groups of subjects: group (1) performed a resistive exercise program with a workload that progressively increased throughout the bed rest period; group (2) did not exercise but took alendronate (Fosamax®), a pharmacological intervention, throughout the bed rest period; and group (3) neither exercised nor took alendronate and served as controls for comparison with the other two groups. To minimize sweating, the room in which the subjects exercised was maintained at a temperature of 18°C or less while the bed rest unit temperature was maintained between 23 and 26°C. There were 6 females (5 white and 1 black) and 10 males (9 white and1 black). They ranged in age from 22 to 48 years, in weight from 60 to 97 kg and in height from 157 to 185 cm. This study was approved by the Institutional Review Boards of Baylor College of Medicine and NASA Johnson Space Center. Informed consent was obtained from all subjects prior to participation in the study.

MATERIALS AND METHODS

Sweat calcium collection and measurement

Osteopatch™, a skin patch, made by Pacific Biometrics, Inc. was used to collect sweat from the participants. This patch has a fluid-absorbing pad, which is applied to the skin. The patch has an outer polyurethane adhesive layer and an inner cellulose collection pad with an absorbing area of 14 sq. cm. It is an FDA approved commercially available method for drug abuse testing. It absorbs the solid components of the sweat while fluid can evaporate out of the patch. Gloves were worn to apply and remove the patches from the skin. The skin area selected for application was cleaned with a 70% isopropyl alcohol swab and allowed to dry before applying the patch. After 24 hours, the patches were removed and the absorption pad portion of each patch was placed in a storage packet that was supplied by the manufacturer. The sealed packets were refrigerated until analytical analysis occurred.

At the time of the elution procedure, forceps were used to transfer the pad from the storage packet to a tube with a small plastic cage at the top to hold the pad. The tube was dried for 30 minutes to remove all fluid from the patch and one ml of distilled water was added to the pad in the cage. After one hour, the tube was centrifuged at 3000 rpm for 10 minutes. This allowed the liquid to be collected at the bottom of the tube while keeping the solid pad in the cage at the top. The collected liquid was removed and the calcium concentration was determined using a Beckman-Coulter Synchron CX7. The elution efficiency of this technique was determined by adding known amounts of Ca to patches. The percent recovery of the added Ca was measured in duplicate with four different concentrations ranging from 0.1 to 0.25 mg/ml. The mean recovery was 87% with a standard error of 3%. There was no detectable association between the Ca

concentration added to the patch and the percent recovery.

Experimental protocol

Using the skin patch (OsteoPatch™) method, sweat Ca was collected from the study participants before and during bed rest with and without exercise. Six sweat patches were applied to the skin of the subjects in the following pattern: on the back, below the shoulder blade on each side of the midline, on the lower chest area between the nipple and the lowest rib on each side of the midline and on the upper part of each arm. After variability studies were completed, the number of patches was reduced from two to one per body region (arm, chest and back). Subjects wore the patches at 4 different time periods: once during the baseline ambulatory period and at 6, 12 and 17 weeks of bed rest. During each collection period patches were worn for 24-hours on two consecutive days. In the exercise group, during bed rest, the two consecutive days where exercise days. To obtain a non-exercise sweat collection during the bed rest period the exercise subjects wore patches for 24-hours on two successive Sundays. Exercise sessions were 6 days a week with none on Sunday.

Total body sweat Ca (mg/day) was calculated for the 12 subjects that wore 6 skin patches during the baseline collection period. An average patch Ca content was calculated for each site. The product of this average and the total body surface area in sq. cm. was divided by 14 (patch surface area in sq. cm). The total body surface area was estimated from the subjects' height and weight.

Statistical analysis

The effect of regional variation was determined from the differences between the three regions for the 12 subjects that wore six patches for sweat collection during the baseline collection period. Intra-individual variability within the same individual was determined by calculating the difference between 2 consecutive 24-hour measurements. Inter-individual variability (%CV) from the 3 different regions and consecutive measurements was calculated. A two-factor ANOVA for Repeated Measures (SPSS version 10) was used to determine mean differences between the 3 body regions (arm, chest and back) on two consecutive days. The variance accounted for by factors from the ANOVA was measured by eta-squared.

A single-factor between groups ANOVA was used to determine the effects of bed rest on sweat Ca loss in the non-exercise groups (control and alendronate, n=7). Changes between baseline and the different bed rest periods were tested separately for each of the three body regions (arm, chest and back).

Paired t-tests were used to examine the intra-subject difference for the exercise group (n=5) at the 12th week of bed rest. Differences between 2 consecutive days of exercise and between exercise and non-exercise days were tested. These intra-subject differences were tested separately for each of the three regions (arm, chest and back). Two-factor ANOVA (effect of days and sites) was not performed as the smaller sample size would elevate the risk of a type 2 error.

For the above statistical analysis a p value of ≤ 0.05 was used to determine significance. For data

presented in Tables 1, 2 and 3, the values for two consecutive days were averaged and the mean and standard error were calculated for each grouping.

RESULTS

Table 1 gives the baseline data for 12 subjects (9 non-exercise and 3 exercise). Data was collected for 6 regions (duplicates for three body sites) and 2 consecutive days during the baseline period. There were no differences between the duplicate measurements from a given region (arm, chest, and back) and the average for the duplicates are shown. There was a small but statistically significant effect for body regions (p=0.013). Statistically significant effects were detected for back vs arm and back vs chest but not chest vs arms by ANOVA post hoc testing. No statistically significant effect was found for consecutive days. There was no detectable interaction or change in the relationships between the 3 body regions over the 2 days. Eta squared determined that 58% of variability was attributed to site, 0.3 % to day and 10% to site by day interaction. The range of %CV representing inter-individual variability during the baseline time period varied from 18 to 32% for the 3 regions. The % CV for arms, chest and back (combined over days) were 20, 18 and 24% respectively and the % CV for days (combined over sites) were 20 and 29%.

Seven non-exercise subjects (control and alendronate) had data collection for the four time periods. Shown in Table 2 is the data for these subjects. No statistically significant difference was found between baseline and the other time periods (6, 12, and 17 weeks of bed rest).

Five exercise subjects had measurements made on exercise and non-exercise days during the bed rest period. Shown in Table 3 is the data for these subjects. No statistically significant differences were detected between exercise and non-exercise days and between 2 consecutive exercise days for the same individual.

The total body sweat Ca was estimated using the formula described in the methods section. The total body sweat Ca for 12 subjects during baseline data collection was 35 ± 4 mg/day (mean ± SE). The average of the three sites was used for this calculation. The mean values for the arm, chest and back were 33, 35 and 37 mg/day respectively.

DISCUSSION

The small variations of repeated measurements by day and site indicate the repeatability of the patch collection method to measure sweat calcium loss. There was a small but significant difference when the back was compared to the arm or chest regions. This agrees with a published study, which found a higher evaporation rate on the back than on the chest or arms (6). Since dermal Ca loss was not determined during profuse sweating, it is unknown what the variability of the skin patch method would be under that circumstance.

A method for estimation of dermal Ca loss as part of a combined Ca balance and radioactive Ca kinetic study has been reported (1). When 17 normal subjects were studied their dermal Ca loss ranged from 36 to 96 mg/day with a mean value of 64 mg/day. Our baseline mean, determined for 12 subjects, was 35 mg/day and the values ranged from 22 to 73 mg/day. A review of published literature reports a wide variation in the estimated values for daily dermal Ca loss (5). When conditions were similar to the baseline period of our study, that is sedentary subjects at an environmental temperature of less than 28° C, the reported values ranged from less than 10 to 148 mg/day.

With a temperature controlled environment there was no significant difference between baseline and bed rest values of sweat calcium loss or between exercise and non-exercise days for subjects that performed resistive exercise during bed rest. The largest mean difference between exercise and non-exercise days was 0.005 mg for patches worn on the chest. The statistical power to detect this mean difference with a SE of .007 and a sample size of 5 would be very small, only 0.088. Sweat Ca increases significantly with aerobic exercise at room temperature (4, 5). The

lack of an effect on sweat Ca in this study is attributable to the cool environment in which the exercise was performed, 18°C, but also may result from decreased sweating associated with resistive versus aerobic exercise.

The small but significant difference found for the back as compared to the arms would increase the total body Ca loss by 4 mg/day. This is a small amount when compared to the negative Ca balance that has been reported for two of the bed rest groups studied here, -213 and –62 mg/day, control and alendronate respectively (7). For bed rest studies, net Ca balance is reported, that is the difference between a baseline control period and the bed rest period. Since there was no change in sweat excretion of Ca with bed rest, no error would be introduced in the Ca balance study by not measuring sweat Ca. This may not be true for clinical balance measurements, where the purpose is to measure absolute balance and not a change in balance.

Other investigators have designed a gauze sponge patch to determine sweat Ca and while their technique yielded realistic values their sample collection times were limited to 20 minutes due to saturation of the gauze with sweat (8). Although the skin patch method reported here has been used for certain medical purposes, it has never been used for collecting sweat Ca. The study results show that, under our study conditions, the skin patch can be used as a non-invasive and convenient method to determine the amount of calcium in human sweat and that the collection period can be up to 24-hours. It also indicates that the changes in sweat calcium from bed rest alone, with or without a brief period (1hr/day) of resistive exercise performed in a cool (18° C) environment, would not significantly affect total body Ca balance.

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Table 1. Sweat Ca loss during pre bed rest baseline data collection

	Mg Ca per patch (24 hour collection)	
Patch Site	Day 1	Day 2
Arm	0.023 ± 0.002	0.023 ± 0.001
Chest	0.024 ± 0.002	0.023 ± 0.001
Back	0.027 ± 0.003*	0.026 ± 0.003*

n = 12 (subjects from all three groups)

* The back was different from the other two sites at the $p \leq 0.05$ level.

Table 2. Sweat Ca loss for non-exercise subjects expressed as mg Ca per patch (24 hour collection)

		Weeks of Bed Rest		
Patch Site	Baseline	6	12	17
Arm	0.024 ± 0.001	0.023 ± 0.001	0.022 ± 0.001	0.025 ± 0.002
Chest	0.025 ± 0.001	0.025 ± 0.001	0.023 ± 0.002	0.024 ± 0.002
Back	0.027 ± 0.001	0.026 ± 0.001	0.025 ± 0.002	0.027 ± 0.002
n	7	7	7	7

Table 3. Sweat Ca loss in the exercise group during bed rest

	Mg Ca per patch (24 hour collection)	
Patch Site	Non-Exercise Day	Exercise Day
Arm	0.023 ± 0.001	0.023 ± 0.002
Chest	0.025 ± 0.002	0.030 ± 0.004
Back	0.026 ± 0.002	0.027 ± 0.002

n = 5