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Accurate assessment of the quantitative significance of different sources of salt in the diet

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SUMMARY. A metabolic study was conducted to assess the validity of using lithium tagged salt as a technique for monitoring the sources of salt in the diet. Discretionary sources, table and cooking salt, were separately labelled and studied, the table salt being available *ad libitum* whereas cooking salt intakes were controlled. The study showed that lithium excretion in the urine did provide an accurate measure of the amount of the labelled salt ingested. Subsequent analysis suggest that Li is not excreted readily in sweat or faeces so it can be used on its own to ensure the completness of a series of 24h urines. Latin American studies on salt sources in the diet are needed as a base for programmes of primary prevention of hypertension.

RESUMEN. Valoración exacta del significado cuantitativo de las diferentes fuentes de sal en la dieta. Se llevó a cabo un estudio metabólico para evaluar si era válido utilizar sal marcada con litio como técnica para identificar las fuentes de sal en la dieta. La sal de mesa y la sal de cocina (fuentes discrecionales) fueron marcadas por separado. La sal de mesa fue utilizada *ad libitum* por los sujetos mientras que la cantidad de sal de cocina ingerida se controló cuidadosamente. El estudio mostró que la excreción de litio en orina proporciona una medida exacta de la cantidad de sal marcada que se ingiere. Análisis subsecuentes sugirieron que el litio no se excreta fácilmente en el sudor y las heces por lo que también puede ser utilizado como marcador para asegurar que una serie de colecciones de orina de 24h sean confiables. Se necesitan estudios latinoamericanos de las fuentes de sal en la dieta que orienten hacia las medidas pertinentes que deben adoptarse para la prevención primaria de la hipertensión arterial.

INTRODUCTION

The sodium ion has long been recognized as a possible nutritional risk factor in the development of hypertension. If advice to reduce sodium intake in the community is to be given, then it is important to know which sources of salt contribute most to the total intake. Unfortunately until recently there was no way of establishing how much of the 24h sodium intake was derived from different sources without undertaking exhaustive dietary studies to assess the origin of different dietary ingredients and then analysing the sodium content of the different ingredients. A new technique, involving the use of lithium as a marker of sodium, has however allowed a new approach. The concept, set out elsewhere(1) was based on the use of a small amount of lithium carbonate, fused with sodium chloride, as a tagged source of salt which, if handled by the body in the same way as sodium, would allow the quantitative tracking of salt used at home. By monitoring both urinary sodium and lithium we have a method for assesing both the absolute intake of sodium and that fraction derived from the lithium tagged source. This method has been successfully applied in previous studies in temperate climates(2-4). In regions where sweat losses may be considerable the method is not useful for tracking sodium losses in sweat since lithium is not excreted in the same proportion as sodium(5); nevertheless litium could be used as a marker for precise measurement of sodium intake from a particular salt source or food item and as a marker of completeness of urine collections. In this paper we explore the validity of this approach.

MATERIAL AND METHODS

The metabolic study was performed at the Dunn Clinical Nutrition Centre (DCNC) in Cambridge, England where it was approved by the Dunn Nutrition Unit's Ethical Committee. Five young male volunteers aged 19-30 years were recruited

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after a detailed explanation of the long experiment had been given and a medical examination had shown each volunteer to be physiologically acceptable. Highly co-operative, almost obssesive individuals had to be chosen to ensure the necessary degree of accuracy for the success of the experiment.

Experimental Design

The experimental design consisted of six periods with a total duration of 41 days. During this time the volunteers activities were restricted to the minimum compatible with carrying on their normal lives.

Period 1. Volunteers were given radio-opaque plastic pellets as faecal markers in capsule form three time a day for seven days while living in their own home and while taking their *ad libitum* diet. This allowed us to assess the time when, on entry to the Metabolic Suite, the faecal specimens no longer included components of their habitual home-based diets.

Period 2. Volunteers moved in to live in the Metabolic Unit. The experimental diet started and faecal and urinary collections were then begun. The faecal marker capsules were given orally three times daily from then on until the next dietary period when, once more, the marker was changed. The purpose of this period was for the volunteers to get used to the diet before starting the use of the lithium-labelled salt and to reach a baseline lithium equilibrium excretion.

Period 3. Lithium tagged salt was given to four subjetc for *ad libitum* use as table salt during 7 days. For each kg of common salt, 9.2 g of lithium carbonate (~250 µmol Li/g of salt) were used.

Period 4. A nine day «recovery» period was allowed so that the urinary lithium concentration could fall to background levels before testing with another labelled salt source.

Period 5. The lithium marker was again given but for six days; labelled cooking salt was used in this test under highly standardized cooking procedures..

Period 6. This period lasted six days at the end of which diet, urinary and sweat collections were discontinued. Faecal collections were extended a further three days to assure complete recovery of faecal markers.

Sweat losses and anthropometry

Sweat was collected for a total of ten days in each subjet, with a technique involving the use of a closely fitting two piece set of underwear, tennis shoes, cotton socks and towels which were submitted to a thorough cleaning with diluted nitric acid and deionised water. All the plastic material used in the cleaning was washed for a few minutes with concentrated nitric acid. Both the preparation of clothing and instructions to volunteers for the collection of sweat were based on the methods used by Dahl, Stall and Cotzias (6) for quantitating skin losses of electrolytes. Collections were made for the last two days each experimental period starting from period 2. Volunteers were requested to keep a daily record of their activities to verify compliance with a normal life-style. Incomplete collections of specimens, mistakes on diets or with the use of markers were also recorded. All subjects were measured daily for their body weight. Their height and skinfolds thickfolds thickness measurements were taken once using standard techniques.

The use of table salt

Table salt could be used *ad libitum* by the subjects throughout the study but labelled salt was substituted for one period. The table salt was provided in fridge-o-seal plastic salt cellars marked with the name of the volunteers to prevent confusion. Salt cellars were weighed at the end of each day on a set of Mettler P.L. 200 scales which were accurate to ± 2mg. In this way a preliminary estimate of salt consumption as indicated by cellar weight loss was recorded. Care was taken to reseal the salt containers after use, to prevent accidental loss and moisture uptake.

The use of cooking salt

Lithium tagged salt was used as cooking salt in two different ways depending on the food item: by adding a known amount of salt to individual items of cooked foods, e.g. jam tarts, omelette and gravy, and secondly in the cooking of vegetables where uptake by the food and losses in cooking water would resemble the normal routes for salt intake by the general population. Cooking salt was controlled throughout Periods 2 to 6. Tagged cooking salt was given in Period 5. Unlike the *ad libitum* use of table salt, cooking salt was used in a fixed daily amount throughout the study to test for possible variations between people in their handling of a constant load of lithium.

Cooking salt, labelled or unlabelled, was added directly to three items of the diet in the following total daily amounts: Gravy (8.6 mmol), jam tart (5.8 mmol) and Omellette (11.2 mmol). Each of these quantities was weighed with the utmost care in small plastic laboratory containers on a set of Mettler P L 200 scales accurate to ± 2mg. A total of 680 weighings were performed for the 5 volunteers. The weighed salt was then placed carefully into each individual portion of food. Some of the containers were randomly chosen and kept for later analyses of sodium and lithium residues.

The preparation of the vegetables proved a very laborious task. Each vegetable was cooked in known amounts of water, with the addition of either tagged -or untagged- salt which was weighed on a set of Mettler PL 200 scales. A detailed description of the methods used in the preparation of cooked foods is given else where (7).

Metabolic diet and supplementary food

A two-day rotating menu was chosen to simplify the diet but 22 items were used in each day's menu. The items for the two diets were similar, but a number of differences to improve acceptability had to be devised. The menus consisted of a basal diet which provided 11.3 MJ of energy and 144 mmol Na as estimated from food tables (7). Cooking and table sodium were not, of course, included in these estimates. Saltless supplements (bread and butter) of 1 MJ were designed to increase the energy intakes of individuals as required.

Although food tables (8) were used to calculate the nutrient content of the individual foods and estimate the likely intake, for balance measurements the sodium and lithium in the diets had to be analysed directly. Ten metabolic diets were homogenised for analysis with carefull weighing, homogenization in deionized water followed by sampling and freeze drying. Digestion in concentrated nitric acid was followed by dilution to volume and filtration before analysis of lithium and sodium in the spectrophotometer. Supplements were analyzed in a similar way. The basal diets were designed to have specified amounts of energy, protein, fat and carbohydrate (CHO) as well as of sodium, but the saltless supplements did not resemble the basal diet.

Most manufactured foods were purchased in bulk from the same supplier, whereas items such as milk, cream, fruit and vegetables were bought as necessary throughout the study. Meticulous steps were taken in cooking meat in batches and in preparing special gravy, tarts and omelette to allow the precise amount of salt to be tagged with lithium.

Fluids were given as deionised water or as milk. Deionised water was taken *ad libitum.* Twenty three weighings of food items/day/subject were performed making a total of 782 weighings in the whole study.

In order to minimise losses of sodium in plates («invisible return») the volunteers were asked to wipe their plates with the unsalted bread given as a supplement. Milk were also rinsed with deionised water and the washings drunk. Checks were also made on residual salt on plates.

Collections, sampling and analytical methods

Each subject was asked to collect «every single drop» of urine from the beginning of Period 2 until the end of the last Period. Complete 24 hour urine collections were required and were completed by collecting the first specimen on rising in the morning. The total 24 hours urine volumes were recorded and diluted to 2 litres with deionised water before four 20 ml aliquots were taken for storage at -20°C in plastic containers. Sodium and potassium were analysed in a flame photometer as presented in the Technicon Instrument Co. Ltd. Autoanalyser sheet Nº 11.07 (1971); creatinine by the method of Folin and Wu(9); chloride by the ferric ammonium sulphate/mercuric thiocyanate method devised by Davies and Taylor(10) and lithium was analysed in an SP9 Pye Unicam Flame Spectrophotometer. All the samples were analysed in duplicate to test the reproducibility of the methods as well as to test the sampling procedure.

Faecal collections

Volunteers took 10 radio-opaque pellets per capsule three times a day throughout the study to document not only stability of intestinal transit but also to allow a check on the complete collection of faecal specimens and if neccesary, allow the correction of mineral excretion in the different periods of the study (11). The shape of the markers given was changed at the beginning of each experimental period. Each stool was collected separately into a plastic bag which fitted into a specially designed collecting frame placed on the toilet seat. Stools were frozen at -20°C.

Method of analysis of faecal markers

Each stool was X-rayed using equipment from A.E. Dean and Company Ltd., Model D-44 and the number of markers and their type counted on the developed X-ray plates(12).

Faeces from each period identified by the specific faecal markers for the test period were pooled and homogenised with deionised water using a Silverson homogeniser for 10 minutes. Before being analysed for Li and Na aliquots were freeze dried and digested and processed in a manner analogous to that of the diets. Potassium was added to the aliquouts to overcome interfering ionisation effects.

RESULTS

Body weight remained stable during the study for most of the volunteers except for one subject who increased his weight by 3.8 kg (6%) and another who showed a small increase of 1kg.

Mineral content of metabolic diets

The analysis of sodium in ten metabolic diets, which included three supplements, and the basal diet gave a mean sodium content of 180.5 ± 7.2 mmol(SD). The contribution of cooking sodium to the prescribed total intake was 21.5% (39.8 mmol). The mean sodium content of the day 1 menu amounted to 184 ± 7.2 (SD) mmol whereas day 2 diets contained 177 ± 5.9 mmol Na(SD). The coefficient of variation of sodium content in the ten diets analysed amounted to 4%.

The two diets contained 499.5 and 466.3 µmoles lithium. Repeated analyses of lithium in 3 samples of homogenates from diets 1 and 2 in the appropriate lithium-labelled period of the metabolic study showed that the sampling plus analytical error for the lithium content was 2.5% and 1.4% for diets 1 and 2 respectively.

A mean difference of 1.5% was shown between the sodium content of two samples of homogenates taken from diet 1. The difference was 1.8% for diet 2. Analytical variation in three subsamples of the sample homogenates varied from 0 to 5% for diet 1 and from 0 to 3.5% for diet 2.

The CV between four different Day 1 diets was 3.5% and for Day 2 diets was 2%. This means that the greatest error in the estimate of nutrients arose from true variations in the composition of foods. Reducing the error from variation in the composition of diet would require more samples for analysis as suggested by Isaksson et al (13).

The amount of sodium in supplements was minute. A total of ten polled supplements contained 3.6 mmol Na. Therefore each supplement increased the sodium content of basal diets by 0.2% of total Na in the diet.

The washings of five lots of dishes used throughout a 24 hour period by one subject gave a sodium content of 0.13 ± 0.03 mmol (SD). The sodium left in the dishes by another subject was checked throughout the study. Thirty-four 24 hour washings gave a mean total sodium loss («invisible return») of 0.29 ± 0.22 mmol (SD)/day. These results confirm the extreme care of the subjects in wiping out their plates.

Mineral excretion

The group's mean 24 hour urinary volume was 1.45 ± 0.40L with a sodium output ranging from o182.7 ± 32.9 to 282 ± 51.7 mmol (Mean ± SD) (Table 1). Urinary volume was found to relate directly to the amount of ingested Na when individual mean data are compared: each litre of urine contained on average 125 mmol Na. Chloride excretion was concordant with but 3% greater than that of sodium. Potassium output was about half that of sodium and had a group mean of 75.3 ± 4.4 mmol (SD). The potassium excretion during period 1 ranged from 4.1 ± 0.4 to 5.3 ± 0.8 µmol Li (Mean ± SD) with a coefficient of variation between individual of 9.2%. In the recovery periods there was a progressive decline in lithium urinary output with an exponential pattern. During the 6-day period with labelled cooking salt urinary lithium excretion was remarkably similar from subject to subject which reflected the constant amounts of cooking salt used throughout the study.

TABLE 1
THE URINARY VOLUME, SODIUM INTAKE, EXCRETION AND RECOVERIES IN THE FIVE SUBJECTS

Subject	Daily Urinary Volume (l) Mean± SD	Total Intake (mmol) Mean± SD	Total Excretion (mmol) Mean± SD	Urinary Excretion (mmol) Mean± SD	Sodium Retention (mmol) Mean± SD	Recovery (%)
N.G.	1.22 ± 0.20	197.8 ± 5.1	190.7 ± 27.3	186.1 ± 27.5	7.15 ± 27.4	96.4
R.T.	2.09 ± 0.45	292.7 ± 39.1	287.7 ± 51.6	282.2 ± 51.7	4.98 ± 47.3	98.4
J.P.	1.16 ± 0.15	197.5 ± 5.3	194.8 ± 21.8	187.6 ± 21.8	2.75 ± 21.2	98.6
J.W.	1.60 ± 0.35	218.4 ± 10.8	210.7 ± 31.1	203.2 ± 31.2	7.68 ± 31.8	96.5
R.G.	1.21 ± 0.30	208.6 ± 12.2	194.1 ± 32.7	182.7 ± 32.9	14.50 ± 33.9	93.0

Urinary creatinine

At the time of the study biological markers to assess the completness of urine collections were not available so urinary creatinine was used as an index of completness of collections. Urinary creatinine excretion was remarkably stable during the 34 day experiment in 4 of the 5 subjects. One subject RG immediately revealed his unreliability because 23.5% of his urine collections had to be excluded from the calculations because of intermitent very low creatinine output values.

Sweat measurements

Sodium excretion in fifty 24 hour sweat collections showed a short range: from 3.1 to 7.4 mmol Na (mean of 4.4 ± 0.8 mmol Na (SD). The daily coefficient of variation in sweat sodium in different subjects ranged from 24 to 37% (Table 2). Baseline sweat lithium output was also stable at 1.7 ± 1.0 µmol Li (SD) (range 0.54 - 3.2). During the first lithium feeding period, lithium sweat losses clearly increased above baseline levels in 4 of the subjects, but no increase was detected in subject JP.

TABLE 2
SODIUM LOSSES IN SWEAT A 24 HOUR PERIOD

Subject	Total sweat sodium (mmol) Mean ± SD (CV%)	Range of sodium output (mmol)
N.G.	3.9 ± 1.2 (31)	3.1 - 6.0
R.T.	3.5 ± 1.1 (31)	2.4 - 5.0
J.P.	4.9 ± 1.8 (37)	2.8 - 6.9
J.W.	5.5 ± 1.5 (27)	3.9 - 7.4
R.G.	4.2 ± 1.0 (24)	3.4 - 4.9

Faecal specimens

A total of 152 faecal specimens were obtained from the 5 volunteers during 34 days. Total marker recovery was 99% in those 4 subjects who continued collecting faeces for 4 days at the end of the study. The mean daily excretion of sodium in the pooled collections for each period ranged from 0.7 to 2.5 mmol Na in 4 subjects but the fifth excreted 7.3 ± 1.0 (Mean ± SD). Daily lithium faecal excretion was 2.0 ± 0.3 μmol Li. Faecal lithium was very unresponsive to the intake of Li tagged salt.

Sources of total sodium intake during the metabolic study

Table salt intakes assessed by the weighing of salt loss from salt cellars in the five subjects varied from one individual (JP) who used 0.99 ± 0.31 g(mean ± SD) to another (RT) who used 6.51 ± 2.27 g(mean ± SD) NaCl and showed a much greater daily variation in use. The average contribution of the different sources of dietary Na to the total intake is shown for each subject in Table 3. The proportions were, of course, to a large extent determined by the amount of table salt used, the rest of the sodium intake being standardized. Nevertheless this variability had the effect of changing the contribution of sodium intake being standardized. Nevertheless this variability had the effect of changing the contribution of sodium during food processing from 73.5% in one case to 49.6% in another individual.

TABLE 3
PERCENTAGE CONTRIBUTION OF SOURCES OF DIETARY SODIUM TO TOTAL INTAKE DURING A METABOLIC STUDY

Subject	Na in natural and processed food	Na added during cooking	Na added at the table
NG	73.4	17.8	8.8
RT	49.6	12.1	38.3
JP	73.5	17.9	8.6
JW	66.5	16.2	17.3
RG	69.6	16.9	13.5
Mean± SD	66.5 ± 9.9	16.2 ± 2.4	17.3± 12.3
CV%	14.9	14.7	71

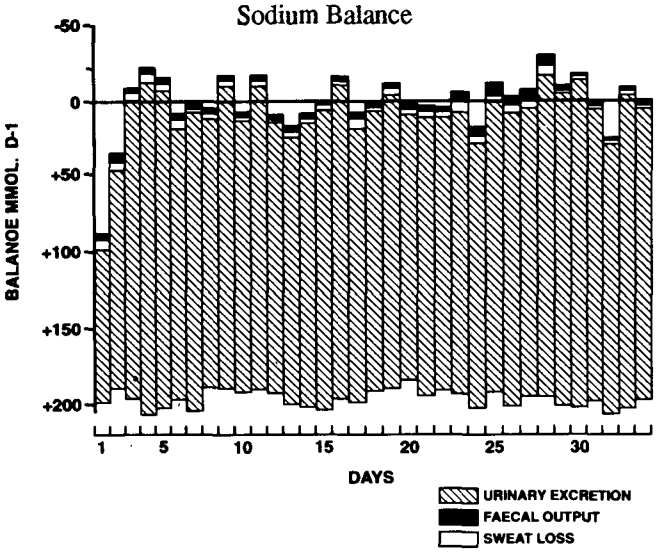
Total sodium and lithium recoveries

The sodium was excreted by three routes but the urinary excretion of sodium dominated. Total recovery of excreted sodium in the five subjects was 96.6 ± 2.2% with subject RG again showing the lowest recovery (Table 1). Total recoveries of lithium used as cooking salt were within 2.5% of that expected except for one subject where only 91.7% of the added lithium was recovered. Of the total Li output, 93.2 ± 1.4% of table salt and 96.2 ± 4.2% of cooking salt was excreted through the kidneys.

Sodium balance

The cumulative sodium metabolic balance of one of the subjects over the 34 days is shown in Figure 1. The mean retention for the group (Table 1) was 7.4 ± 4.4 mmol Na or 3.4 ± 2.2% of the total intakes. One explanation of the higher apparent retention in subject RG could be excessive losses through skin on some of the days when sweat was not collected. The subject used to cycle for miles (22 miles during the study + 19 miles walking), whereas the rest of the group had a more sedentary life.

FIGURE 1
Sodium Balance



The figure shows the sodium balance of subject JP. Intakes are denoted by the bottom line each day with the urinary excretion as indicted in graph plotted up from this line. The thin horizontal line shows the zero balance point so that loss shown above the line means negative balance; that below the line signifies sodium accumulation within the body.

Discussion on the application of the lithium marker technique

This study suggested that lithium could prove of value as a simple marker of discretionary salt use in an epidmiological context and this proved feasable. We used a 12 day protocol to assess salt use over a full week having had two preliminary 24h urine samples to estimate the background excretion of Li (3). A further 3 day period was allowed to collect the Li excreted during the wash-out phase and to ensure that we had complete Li recovery. This approach has been simplified by Ferro-Luzzi and her colleagues (14) who used only one preliminary 24h collection and 3 full 24h urinary collections timed to coincide with the plateau excretion 4 days after Li tagged salt had replaced that normally used in cooking and at the table. They also simplified the process of fusing the lithium carbonate with the sodium chloride, and by diluting the fused salt they improved the pouring qualities of the tagged product.

By choosing only the three days at the time of a plateau in Li excretion they reduced the need for compliance, but added the newly available PABA test to ensure that urinary collections were coplete (15).

Regarding sodium and Li excretion in sweat, Verboven et al (15) studied twelve male volunteers who were divided into two groups one of which ran more than 8 kms per day. There was a marked difference between the sweat sodium output of the physically active and the sedentary groups which could be estimated at mmol/day but there was no difference in sweat lithium excretion so that in both the active and sedentary groups 95% of the lithium was recovered in the urine. This show that Li and sodium are not handled equivalently by the sweat gland so Li is probably a very reliable food marker of tagged salt or food intake whatever the climate or activity of the subjects. Thus, in metabolic or epidemiological studies where the intake of a particular food or salt source is under scrutiny then by labelling the item with lithium the total intake of the source can be assessed indirectly but accurately from the urinary lithium output.

Since lithium does not track salt losses in sweat, the ratio of Li/Na in urine reflect both the diluting effects of additional Na ingested from non-tagged sources and the effects of the selective loss of sodium from the skin during sweating. Careful balance studies by Ashworth and Harrower(16) showed that unacclimatized subjects sweating with heavy physical activity in tropical environment can lose on average nearly 60 mmol Na per day but, within a week of acclimatization, this loss will fall. To cope with assessing the impact of sweat losses other tests by direct measurement would require the possible use of the heavy isotope of chloride i.e ^{35}Cl. The fraction of a standard dose of ^{35}Cl given by mouth which was recovered in the urine could be used to assess the impact of sweat losses on Na output and allow appropriate correlations to be developed in studies on hypertension.

The data on lithium demonstrates that because of the astonishingly high recovery rates in urine whatever the rate of sweating, Li can also be used as an alternative to PABA as a marker of completeness of urine collections. Since PABA is excreted rapidly it has to be taken in 3 doses per day to ensure that the whole 24h excretion is covered; with Li the issue is quite different because Li is only slowly washed out of the body water pool and therefore Li would be a particularly good marker when a series of complete sequential urines have to be collected.

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