Impact of Fasting and Consumption of Water, Coke and Gatorade on Urine Flow Rate and Urine Specific Gravity

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October 24, 2013
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Introduction

The body is constantly in flux trying to obtain a state of equilibrium known as homeostasis. The kidneys play one of the significant roles in the maintenance of homeostasis within the body. They regulate plasma volume, osmolarity, pH and ionic composition. In addition the kidneys remove waste products and other substances from the plasma as well as regulate blood pressure. The kidneys are able to do all of this through the functional unit of the renal system known as the nephron.

The nephron consists of two parts, namely the renal corpuscle and renal tubules. The renal corpuscle contains the glomerulus, which passively filters water and dissolved solutes from the plasma into the Bowman’s capsule space through a process known as glomerular filtration. Also located in the renal corpuscle is the Bowman’s capsule. Here, filtered water and solutes are transported from the glomerulus into the renal tubules. Renal tubules are a series of tubules that all have a separate and unique jobs. They consist of the proximal tubule, Loop of Henle, distal convoluted tubule and collecting duct. The nephron is able to carry out its function via filtration, reabsorption and secretion.

The nephron carries out its functions proficiently by three key renal exchange processes: glomerular filtration, tubular reabsorption and tubular secretion. First, glomerular filtration is the movement of protein free plasma from glomerulus to Bowman’s capsule. Here, urine formation begins. Tubular reabsorption is a selective uptake of water, ions and nutrients from tubules to peritubular capillaries. Lastly, tubular secretion is the selective discharge of water and ions from the peritubular capillaries into the renal tubules. Part of the secretion process is under hormone control which occurs in two areas within the nephron: the cortical collecting duct and distal convoluted tubules. Therefore a second factor enabling the renal system to carry out its functions are hormones.

There are three important hormones involved with the renal system, Anti-diuretic hormone (ADH), Aldosterone and Artial Natriuretic Peptide (ANP). ADH is secreted by the posterior pituitary gland and its function is to lower urine production and increase water absorption. It is triggered by the
decrease in plasma volume, blood pressure and an increase in plasma osmolarity. Aldosterone comes from the adrenal cortex and its function is to increase \( \text{Na}^+ \) reabsorption. It is triggered by the decrease of plasma osmolarity and plasma volume/pressure. Lastly, ANP comes from the cardia atrial muscle cells and its function will subdue the release of ADH and aldosterone by inhibiting \( \text{Na}^+ \) reabsorption and increasing \( \text{Na}^+ \) and water excretion. As a result there will be an increase in urine production and a decrease in urine specific gravity due to the decrease of blood volume, blood pressure and increase in plasma osmolarity. An experiment was conducted to test osmolarity regulation following ingestion of three different fluids.

The purpose of the experiment is to study the impact of fasting and consumption of water, Coke, Gatorade and not drinking fluids on urine flow rate and urine specific gravity through osmotic regulation of the renal system. In addition, the urine is tested for blood, ketone, glucose, protein and urine pH using Lapstix strips. There are three different fluids being ingested due to their different composition and the non-drinking group was used as a control. The hypothesis of this study is the Gatorade group will have the highest urine flow rate and the non-drinking group will have the highest corrected urine specific gravity.

**Methods**

Per the Biology 213 lab manual for the renal lab, prior to the experiment subjects are to refrain from exercise and consumption of large amounts of salt, sugar, caffeine and alcohol. Within five hours of the experiment subjects are to fast, with the exception of an 8oz glass of water during the first 2 hours of fasting to help prevent dehydration. One hour prior to the beginning of the experiment subjects are to completely empty their bladder and record exact time of void.

At the beginning of the experiment subjects are to be randomly divided into one of four groups: water, Coke, Gatorade and non-drinking group. The amount of fluid the subject has to ingest is based on their body weight and is calculated using the following formula:
mls of fluid intake = [body weight (lbs) x 7 mls/lbs] x 0.80 (Tidyman & Manuguid, Fall 2013)

At T = 0, all subjects are to use two urinary cups provided, go to the nearest restroom and void their bladders completely noting the exact time of void. After voiding, subjects are to bring back the sample and take temperature as soon as possible and record data in table of the lab manual. The drinking groups will then drink their calculated amount of test fluid within 15 minutes.

While the drinking groups are drinking, the non-drinking group will proceed to measure and calculate the corrected urine specific gravity, urine flow rate and use the Labstix reagent to check for the presence of blood, ketone, glucose, protein and measure the pH in the urine sample.

To calculate the corrected urine specific gravity subjects must take temperature of urine with a thermometer and record reading in table. Next, transfer a portion of urine from the urinary cup into a urinometer cylinder and fill half full. Then carefully place a urinometer in the cylinder until it floats. Find the meniscus and obtain reading. This will be the specific gravity of the urine sample. Once temperature and specific gravity are collected then use the following formula to find the corrected specific gravity:

\[
\text{Measured urine temperature in } ^\circ\text{C} - 15^\circ\text{C} = Y^\circ\text{C} \\
Y^\circ\text{C} / 3^\circ\text{C} = Z \text{ (round to whole number)} \\
Z \times 0.001 = \text{factor to correct urine specific gravity} \\
\text{Measured urine specific gravity} + \text{factor to correct urine specific gravity} = \text{Corrected urine specific gravity} \text{ (Tidyman & Manuguid, Fall 2013)}
\]

To calculate urine flow rate take total volume measurement of the urine collected in the urinary cup in mL and divide the volume by the duration of time since last void in minutes.

\[
\text{Urine Flow Rate (mL/min)} = \frac{\text{volume voided (mL)}}{\text{duration of time since last void (min)}} \\ (\text{Tidyman & Manuguid, Fall 2013})
\]

To measure the presence of blood, ketone, glucose, protein and the pH of the urine sample use a Labstix strip and dip into urine and remove instantly to prevent any possible mixing of neighboring reagents. Then compare the strip against the color chart on the side of the Labstix bottle. Results from the test are to be recorded in a table.
Once subjects in the drinking groups have finished consuming their fluids they will proceed to measure and calculate the corrected specific gravity, urine flow rate and use the Labstix Strips as described above.

At T = 30, all subjects are to go to the nearest restroom, dispose of previous sample in the toilet, void and empty their bladders completely. Bring sample back, only measure and calculate the corrected urine specific gravity and urine flow rate of sample. Data is to be recorded in the table in the lab manual. Repeat these steps for T = 60, T = 90 and T = 120. If subject tests positive for any of the reagents in the Labstix test then they will be required to retest the Labstix test at T = 30. At the end of the experiment subjects are to dispose of urine in the toilet in the restroom. Labstix, urinometer, urinometer cylinder and thermometers are to be placed in proper receptacles.

Results

<table>
<thead>
<tr>
<th>Time Voided (Min)</th>
<th>Urine Flow Rate (mL/mins)</th>
<th>Non-Drinking</th>
<th>Water</th>
<th>Coke</th>
<th>Gatorade</th>
</tr>
</thead>
<tbody>
<tr>
<td>T=0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T=30</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T=60</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T=90</td>
<td></td>
<td>8.0</td>
<td>7.0</td>
<td>6.0</td>
<td>5.0</td>
</tr>
<tr>
<td>T=120</td>
<td></td>
<td>7.0</td>
<td>6.0</td>
<td>5.0</td>
<td>4.0</td>
</tr>
</tbody>
</table>

**Figure 1:** Class average comparison of urine flow rate (amount of mL of urine voided/ minutes since last void) over a 2 hour time span after drinking groups consumed predetermined fluid at T=15. All groups started at a slightly dehydrated state. Sample size for nondrinking group = 14-15, water =17-18, Coke =14, Gatorade=16.
In figure 1 urine flow rate (UFR) over time for all drinking groups increased and then decreased with a max UFR at T=90. The lowest UFR occurred at T=0 for all groups with a value of 1.00 ml/min or less. At T=30 there was a slight increase of UFR for all groups, the water group showed an increase from 0.36 mL/min to 1.04 mL/min. At T= 60, all drinking groups had substantial UFR increase, especially the Gatorade group with an increase from 0.93 mL/min to 4.56 mL/min. At T=90 the largest UFR occurred amongst all drinking groups with Coke having the highest UFR of 7.45 mL/min. At T=120 UFR dropped for all groups including the non-drinking group back to levels of T=60. Overall the group with the highest average UFR recorded was the Coke drinking group with the highest recorded value at T=90 of 7.45 mL/min. The group with the lowest average UFR recorded was the non-drinking group, with the lowest recorded value at T=120 of 0.59mL/min.

![Average Corrected Urine Specific Gravity Over Time](image)

**Figure 2:** Class average of corrected urine specific gravity in mL over a 2 hour time span after drinking groups consumed predetermined fluid at T=15. All groups started at a slightly dehydrated state. Sample size for nondrinking group= 8-14 , water= 11-18 , Coke= 9-14 , Gatorade=12-16.
In figure 2, the corrected urine specific gravity (CUSG) over time for all groups decreased and then increased. The drinking groups had the lowest values at T=90, while the non-drinking group had their lowest values at T=30 with a value of 1.023. T=0 was the highest CUSG for all groups; the water group recorded the highest average value of 1.028. At T=30, CUSG decreased slightly in all groups, with the largest decrease in the water drinking group. They went from a CUSG of 1.028 to 1.021. At T= 60, the drinking groups had a significant decrease in CUSG. The group with the largest decrease was the Coke drinking group; they went from 1.024 to 1.012. The non-drinking group had a relative constant value at 1.025. At T=90, the non-drinking group had a slight decrease in CSG from 1.025 to 1.024. The remaining groups continued to have a decrease in CUSG, with the lowest CUSG over time of about 1.009. At T=120, CUSG increased slightly for all groups. The group with the lowest average CUSG was water drinking group. They recorded a value of 1.009 at T=90. The group with the highest average CUSG was the non-drinking group. They had a recorded value of 1.0226 at T= 0.

Table 1: Labstix Test Results

<table>
<thead>
<tr>
<th>Test Reagent</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>a) Presence of Blood</td>
<td>Trace</td>
</tr>
<tr>
<td>b) Presence of Ketone</td>
<td>Negative</td>
</tr>
<tr>
<td>c) Presence of Glucose</td>
<td>Negative</td>
</tr>
<tr>
<td>d) Presence of Protein</td>
<td>Trace</td>
</tr>
<tr>
<td>e) Urine pH</td>
<td>6.0</td>
</tr>
</tbody>
</table>

Normal pH range is 4.5 - 8.0

Discussion

The hypothesis of this study is the Gatorade group will have the highest urine flow rate and the non-drinking drinking group will have the highest corrected urine specific gravity. Urine flow rate (UFR) is affected by the type of fluid ingested.
Within the experiment three fluids were ingested: water, Coke and Gatorade. First, water consists of just water with no added solutes, thus making it hypoosmotic relative to plasma. Coke consists of a high amount of glucose (sugar), water, a small amount of sodium and a small amount of caffeine making it hyperosmotic relative to plasma. Lastly, Gatorade consists of water, glucose and electrolytes such as sodium, chloride and potassium, which makes it isosmotic relative to plasma in an active state. The assumption is if in an active state the body would be losing water and solutes and thus Gatorade would replenish these items. Based on the fluid contents of the consumed substances, the functional mechanisms of the body and hormonal effects on average UFR and average USG are anticipated.

The expected results for the group with the highest UFR are the Gatorade group. At the beginning of the experiment all groups are in a slightly dehydrated state, but not too much so since subjects were instructed to ingest 8 oz of water within 2 hours of fasting. This slightly dehydrated state would result in the osmolarity increasing slightly by 2%. (S. Butt, Expected Experimental Results Handout 2013). Osmoreceptors located in the Supra-optic Nuclei (SON) and Para-ventricular Nuclei (PVN) monitor osmolarity. Thus when there is an increase in osmolarity the osomomreceptors will detect this increase and will cause the hypothalamus to secrete Anti Diuretic Hormone (ADH) from the posterior pituitary gland, (S. Butt, Lesson 3: Renal Physiology, slide#12-13, 2013) where it is stored, to begin to reabsorb water by inserting aquaporin channels into the collecting duct and distal convoluted tubule. The blood pressure and blood volume will be low and the barareceptors in the aortic arch and carotid sinus of the heart, which monitor blood pressure, will detect the low levels and help activate ADH. This will lead to the lowest UFR due to the body trying to reabsorb as much water as possible due to the slightly dehydrated state. ADH will continue to absorb liquid until about T=60 due to its ½ life and ANP will be more prevalent due to the consumption of Gatorade at T=15.
At T=15 a large amount of Gatorade containing glucose, water and electrolytes such as sodium, was consumed and it increased blood pressure and volume. Due to the subjects inactivity more solutes would be excreted since Gatorade function is to hydrate those in an active state when they are in loss of fluids and electrolytes. As the heart stretches due to the increase in blood volume the volume receptors in the atrial muscle will be stimulated to release Atrial Naturetic Peptide (ANP). ANP inhibits sodium reabsorption in the distal convoluted tubule and will increase excretion of sodium which is followed by water. Increasing the release of ANP will signal the increase in glomerulus filtration rate (GFR). Increasing GFR will filter sodium and water quicker resulting in a higher UFR. Consequently at T=90 the Gatorade group UFR would be increased as a result of three factors: glucose metabolism, which produces water as a byproduct, the natriuretic effects of ANP, and Gatorade itself contains water. Thus, the Gatorade drinking group is expected to have the highest UFR. However, the expected results did not match the experimental results.

The experimental results, which deviated from the expected results, showed Coke as having the highest UFR amongst all groups. Coke had the highest UFR at T=30 with a value of 1.28mL/min and at T=90 with a value of 7.45mL/min. Coke contains more sugar and a little more caffeine than Gatorade. Thus, as in Shirley, Walter and Noormohamed research, caffeine had an effect on sodium excretion and associated water flow. This could be a cause of the variance in expected results. Gatorade was close to having the highest UFR on several occasions and succeeded once at T=60 with a value of 4.56mL/min. An instance where Gatorade was close to having the highest value was at T=90 when the UFR value of 7.41mL/min compared to the Coke drinking group value of 7.45mL/min. Since there were more instances of Coke having the highest UFR the first hypothesis is disproved; Gatorade drinking group does not have the highest UFR in this experiment. The second hypothesis was then examined.

The second hypothesis of this study is the non-drinking group will have the highest corrected urine specific gravity (CUSG). When in a dehydrated state osmolarity increases and blood pressure and
volume decrease causing a release in ADH to reabsorb water through the pathways discussed above. This process is an example of positive feedback. The ½ life of ADH would not decrease as we saw in UFR since the body would continue to detect a dehydrated state until liquid is consumed, which would change the blood volume. However, in the case of the non-drinking group, the blood volume would continue to be low during the experiment and ANP would be inhibited. The body will reabsorb water and will produce higher CUSG causing urine to be more concentrated and dense. There is an inverse relationship between UFR and CUSG. When UFR decreases, then CUSG will increase. If CUSG were to decrease, then the urine would be more dilute as it would be closer to the state of water density of 1.000. In the case of the non-drinking group we expect them to have the highest CUSG. The experimental results matched expected results.

The experimental results recorded the non-drinking group with the highest CUSG. The time at which their urine was the most concentrated was at T=0 with a value of 1.026. Over the duration of the experiment the values held relatively constant, varying the most by 0.003 at T= 30 with a value of 1.023. From the results shown, the hypothesis has been proved that the non-drinking group had the highest CUSG. In addition to the UFR and CUSG tests, a Labstix urinalysis test was done.

Labstix tests pH level and the presence of blood, ketone, glucose and protein within a urine sample. My results were negative for ketone and glucose, but had trace amounts for blood and protein. Normal range of pH level is 4.5-8.0 and my pH was 6.0, which is within normal range. Trace amounts of protein can be explained in that a small amount of albumin is expected to be present in urine (S. Butt, Notes on Labstix Analyses, Power Point slide# 3, 2013). With regards to trace amounts of blood this could be due to error. There are several potential sources for error for the Labstix and for this experiment.

Potential errors for this experiment could from different sources. First, for the Labstix test reagents could have mixed with nearby reagents causing false positives or in my case trace for blood. In
addition to Labstix there is also potential error in how the data was collected. Subjects may have not followed the protocol and consumed substances that were to be refrained from such as alcohol or caffeine. This could dehydrate the subjects more and cause subjects not to void. Also if subjects did not have an 8 oz glass of water within 2 hours of fasting the subject may be more dehydrated. Another source of error could have been with the collection of temperature data. Since the restroom was located about 5 minutes away from where the subjects read the temperature accurate reading may have not been obtained due to temperature drop. There are ways in which these potential errors could be prevented in the future and thus improving how the experiment is conducted.

The experiment could be improved by reducing possible error. One way to achieve this is by setting up a station to obtain temperature in the area in which urine void was collected. This would help ensure more accurate temperature readings, thus giving a more accurate CUSG. Another way in which the experiment could be improved is if the subjects were monitored more closely prior to the experiment to make sure the protocol was being followed. Lastly, if there was a larger sample of subjects this would allow more accurate data and help reduce possible error.
References

- S. Butt, Expected Experimental Results Handout 2013
- S. Butt, Lesson 3: Renal Physiology, PowerPoint slide# 12-13, 2013
- S. Butt, Notes on Labstix Analyses, PowerPoint slide# 3, 2013
Natriuretic effect of caffeine: assessment of segmental sodium reabsorption in humans

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ABSTRACT

In order to assess the intrarenal mechanisms responsible for the natriuretic action of caffeine, the renal clearances of $^{51}$Cr-EDTA (used as a measure of glomerular filtration rate (GFR)) and lithium (used as an index of end-proximal fluid delivery) were measured in eight healthy males before (control period) and immediately after (experimental period) a 400 mg oral dose of caffeine (given over 90 min) or placebo. In caffeine-treated subjects, the fractional excretion of sodium rose from $1.00 \pm 0.25\%$ in the control period to $1.47 \pm 0.18\%$ in the experimental period, while corresponding values on the placebo day were $1.04 \pm 0.16\%$ and $0.70 \pm 0.07\%$ respectively. GFR was unchanged following either caffeine or placebo. When compared with the placebo day, caffeine caused increases in lithium clearance (experimental period values: caffeine, $37 \pm 1$ ml/min; placebo, $28 \pm 2$ ml/min; $P < 0.001$), the fractional excretion of lithium (caffeine, $34 \pm 1\%$; placebo, $26 \pm 2\%$; $P < 0.001$) and the sodium/lithium clearance ratio (used as an index of the fraction of sodium delivered to the distal nephron that escapes reabsorption therein: caffeine, $4.4 \pm 0.3\%$; placebo, $2.8 \pm 0.2\%$; $P < 0.001$). These results suggest that reduced fractional sodium reabsorption in both the proximal tubule and the distal nephron contributes to the acute natriuretic effect of caffeine. The data also confirm the importance of controlling caffeine intake when investigating renal function using lithium clearance.

INTRODUCTION

It is now generally accepted that caffeine increases sodium excretion (e.g. [1,2]). However, despite its widespread use as a dietary constituent and drug adjuvant, the intrarenal mechanisms responsible for the natriuretic effect of caffeine remain to be determined, and conflicting views exist over the respective roles of altered renal haemodynamics and tubular reabsorption [1,3]. A suggestion that the proximal tubule might be involved came from an early study by Thomsen and Schou [4] in which it was reported that caffeine led to a rise in lithium clearance ($C_{Li}$), a variable now known to provide an index of end-proximal fluid delivery [5,6]. On the basis of that single report, caffeine-containing drinks have been prohibited in the vast majority of subsequent physiological and clinical studies involving measurement of $C_{Li}$. It is worth noting, however, that Thomsen and Schou [4] gave no details of the caffeine dosage, the magnitude of the change in $C_{Li}$ or the number of subjects studied. Moreover, a subsequent short report could find no effect of caffeine on $C_{Li}$ in healthy volunteers [7]. In view of these conflicting findings, and of the potential importance of the need to control caffeine intake in $C_{Li}$ studies, the present study was undertaken to determine the effect of a moderate dose of caffeine on the glomerular filtration rate (GFR) and tubular sodium reabsorption in healthy subjects. Sodium reabsorption in...
the proximal tubule and, by implication, in the distal nephron, were assessed through measurement of \( C_{Li} \).

**METHODS**

**Subjects and protocol**

Experiments were performed on eight normotensive non-smoking subjects (males; age range 21–52 years) previously screened by medical examination and routine haematological and biochemical analyses. The study was approved by the local Ethical Committee (Riverside) and subjects gave informed, written consent. Each subject was studied on two occasions, separated by at least 1 week. For the 4 days leading up to each study day, subjects remained on their normal diet, but were asked to refrain from eating foods with particularly high or low sodium content (a list being given for guidance). To assess dietary sodium, urine was collected for the 24 h preceding each study day. During the 24-h urine collection, as well as on the study day itself, subjects abstained from alcohol and from all caffeine-containing foodstuffs (list provided). At 22.00 hours on the pre-study day, a single dose of 300 mg of lithium carbonate (Delandale Laboratories, Canterbury, Kent, U.K.) was taken. The next morning, after a light breakfast, subjects reported to the laboratory at 9.00 hours. An intravenous line was established in each forearm. Through one, a bolus dose of \( ^{51}\text{Cr-EDTA} \) (25 \( \mu \text{Ci} \); Amersham International, Aylesbury, Bucks., U.K.) was given, followed by a maintenance infusion of 0.2 \( \mu \text{Ci} / \text{min} \) in dextrose/saline at 2 ml/min. Immediately after the \( ^{51}\text{Cr-EDTA} \) bolus, each subject emptied his bladder, then drank a water load of 10 ml/kg body weight; thereafter, urine was collected every 30 min and, in order to maintain the water load, a volume of water equal to the urine volume was drunk. The water load was given to ensure a brisk urine flow rate and thereby eliminate bladder emptying errors. Subjects were supine except when micturating or drinking.

After 2 h, by which time the urine flow rate had stabilized, a 1 h (control) clearance period was initiated. At the end of the control period, a dose of 100 mg of caffeine (PP Products, Welwyn Garden City, Herts., U.K.) was taken, and this was repeated at 30-min intervals for 90 min (total caffeine intake of 400 mg). Clearance measurements were then repeated during the first 1 h after the discontinuation of caffeine (experimental period). Venous blood samples were taken at hourly intervals throughout.

Exactly the same procedures were performed on the second study day, except that placebo tablets were taken. The order in which the two study days were performed was varied from subject to subject.

**Analyses**

Sodium and potassium concentrations were measured by flame photometry (model 543; Instrumentation Laboratory, Warrington, U.K.); lithium concentrations by atomic absorption spectrophotometry (model 151; Instrumentation Laboratory); and \( ^{51}\text{Cr-EDTA} \) activities by \( \gamma \) spectroscopy (Autogamma 5550 series; Canberra Packard, Pangbourne, Berks., U.K.).

**Calculations and statistics**

Clearances (\( C_x \)) were calculated using the standard formula \( C_x = U_x \cdot V / P_x \), where \( U_x \) is the urine concentration of \( x \), \( V \) is the urine flow rate and \( P_x \) is the plasma concentration of \( x \). The renal clearance of \( ^{51}\text{Cr-EDTA} \) was taken to be a measure of GFR. Fractional excretions (\( \text{FE}_x \)) were calculated as \( C_x / \text{GFR} \).

Values are presented as means \( \pm \) S.E.M. Comparisons between the caffeine and placebo days, with respect to changes in a given variable with time (control period compared with experimental period), were made using two-way ANOVA with repeated measures. Post-hoc assessment was performed using the Student–Newman–Keuls test. Statistical significance was taken at the 5% level.

**RESULTS**

**24-h urine collections**

The sodium content of the 24-h urine sample collected immediately before the caffeine study day was 146 \( \pm \) 15 mmol, and that before the placebo day was 152 \( \pm \) 9 mmol. Respective 24-h creatinine excretions were 15.8 \( \pm \) 1.2 mmol and 14.8 \( \pm \) 1.0 mmol. On this basis, sodium intake was adjudged to be similar in the periods leading up to the two study days.

**Plasma electrolytes**

Table 1 shows the plasma concentrations of sodium, potassium and lithium, at the start of the control period and at the end of the experimental period, on the two study days.

<table>
<thead>
<tr>
<th>Electrolyte</th>
<th>Placebo Control period</th>
<th>Placebo Experimental period</th>
<th>Caffeine Control period</th>
<th>Caffeine Experimental period</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na(^{+}) (mmol/l)</td>
<td>137 ( \pm ) 1</td>
<td>135 ( \pm ) 1</td>
<td>136 ( \pm ) 1</td>
<td>135 ( \pm ) 1</td>
</tr>
<tr>
<td>K(^{+}) (mmol/l)</td>
<td>4.4 ( \pm ) 0.3</td>
<td>3.9 ( \pm ) 0.2</td>
<td>4.2 ( \pm ) 0.1</td>
<td>3.7 ( \pm ) 0.1</td>
</tr>
<tr>
<td>Li(^{+}) (mmol/l)</td>
<td>1.17 ( \pm ) 0.11</td>
<td>0.96 ( \pm ) 0.7</td>
<td>1.12 ( \pm ) 0.7</td>
<td>0.85 ( \pm ) 0.3</td>
</tr>
</tbody>
</table>

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study days. The fall in plasma lithium concentration with time was significantly greater ($P < 0.05$) on the day on which caffeine was ingested.

### Clearance data

Sodium excretion rates are shown in Figure 1. Baseline values were similar on the two days, but during the period 30–60 min after the start of caffeine ingestion sodium excretion was already significantly elevated; it remained elevated throughout the study period. Subsequent data are presented only for the control and experimental periods.

During the experimental period, $\text{FE}_{\text{Na}}$ was significantly higher on the caffeine day than on the placebo day, and there was a significant interaction between treatment and time (Figure 2), indicating that the natriuresis resulted, at least partly, from a reduction in fractional tubular reabsorption. Fractional water excretion also increased after caffeine administration. However, fractional potassium excretion was not significantly altered.

GFR, calculated from the renal clearance of $^{51}$Cr-EDTA, was stable on both days and was unaffected by caffeine (Figure 3). During the experimental period, $C_{\text{Li}}$ (an index of end-proximal fluid delivery) was significantly higher on the caffeine day than on the placebo day, and there was a significant interaction between treatment and time. These comments also applied to $\text{FE}_{\text{Li}}$. Finally, $C_{\text{Na}}/C_{\text{Li}}$, used as an index of the fraction of sodium delivered to the distal nephron that escapes reabsorption therein, was also significantly elevated after caffeine, and again there was a significant interaction between treatment and time.

### DISCUSSION

The present study confirmed that a moderately high dose of caffeine, ingested over a 90-min period, caused a substantial acute increase in sodium excretion and an accompanying diuresis. Caffeine is a major dietary constituent, and its natriuretic and diuretic effects have been recognized for some time, yet only fragmentary evidence is available concerning its renal site(s) of action. The present study has attempted to clarify this issue by assessing the effect of caffeine on the renal clearance of $^{51}$Cr-EDTA, a more accurate measure of GFR than that usually employed (creatinine clearance), and by using the $C_{\text{Li}}$ technique to assess segmental sodium reabsorption. The use of $C_{\text{Li}}$ as a measure of the volume of glomerular
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Figure 3  Effects of caffeine on (a) $^{51}$Cr-EDTA clearance (GFR), (b) $C_{Li}$, (c) $FE_{Li}$ and (d) the $C_{Na}/C_{Li}$ ratio
Measurements were made during the 1 h control period (C; open circles) and the 1 h experimental period (E) following oral caffeine (closed circles) or placebo (shaded circles) administration. Values are means ± S.E.M. ($n = 8$); *$P < 0.001$ for caffeine compared with placebo. NS, not significant.

filtrate reaching the end of the proximal tubule is based on the premise that lithium is reabsorbed in the proximal tubule in the same proportion as water, but that none is reabsorbed in the loop of Henle or beyond [8]. Although micropuncture studies have shown that lithium reabsorption in the proximal tubule actually lags slightly behind that of water, and that a small proportion of filtered lithium is reabsorbed in the loop of Henle [9–11], these small errors largely cancel out and the consensus is that, under normal conditions, $C_{Li}$ does fulfil its purpose as a reasonable estimate of end-proximal fluid delivery [5,6]. An exception to this is under conditions of sodium restriction, when lithium can be reabsorbed additionally in the collecting duct, at least in rats [12]; however, none of the subjects in the present study was on a low sodium intake, as indicated by the 24-h excretion rates.

Our finding of a substantial increase in sodium excretion without any measurable change in GFR indicates that the caffeine-induced natriuresis resulted largely from inhibition of fractional tubular reabsorption, as confirmed by the marked increase in $FE_{Na}$. This finding, together with the previous demonstration that caffeine is without effect on renal plasma flow in humans [1], challenges the widely held view that renal haemodynamic effects are involved in the natriuresis [3,13] (although we cannot discount the possibility that higher doses of caffeine might increase GFR).

Both $C_{Li}$ and $FE_{Li}$ increased substantially after caffeine. This indicates that at least part of the overall reduction in sodium reabsorption occurred in the proximal tubules, leading to an increase in end-proximal fluid delivery. One factor underlying the reduction in proximal tubular reabsorption might be the well documented pressor effect of acute caffeine administration [1,3]. Increases in renal perfusion pressure, by increasing renal interstitial hydrostatic pressure, can inhibit proximal tubular reabsorption, at least in deep nephrons [14]. However, the dose of caffeine used in the present study results in only a small change in mean arterial pressure [1], and this mechanism is unlikely to be the sole cause of the increase in $FE_{Li}$ [15]. Caffeine is known to be a non-specific antagonist of adenosine receptors [3], and it is reasonable to propose that $A_2$ receptor antagonism contributes to its proximal effect: in vivo clearance and micropuncture studies in rats have indicated that specific $A_2$-adenosine receptor antagonists can inhibit proximal
tubular reabsorption [16–18], through mechanisms as yet unknown. A further effect of $A_2$-adenosine receptor antagonism is inhibition of the tubulo-glomerular feedback mechanism [18–20]. This action could explain how an increase in the volume of fluid arriving at the distal tubule was sustained in the present study without eliciting a tubulo-glomerular feedback-mediated reduction in GFR.

As well as reduced proximal tubular reabsorption, our results also point to an effect of caffeine on the distal nephron. Although absolute sodium reabsorption in the distal nephron increased substantially (otherwise the raised distal delivery would have resulted in a vastly greater increase in sodium excretion than that observed), the marked increase in the $C_{Na}/C_{Li}$ ratio (an index of the fraction of sodium delivered to the distal nephron that was excreted in the urine) indicates that the fractional distal reabsorption of sodium fell following caffeine administration. The increase in absolute distal sodium reabsorption would be anticipated, partly as an inherent response of the distal nephron to the increased load and partly as a consequence of $A_2$-adenosine receptor antagonism: *in vitro* evidence suggests that $A_2$ receptor stimulation by adenosine can inhibit reabsorption in the thick ascending limb of the loop of Henle [21] and in the cortical and medullary collecting ducts [22,23]. Indeed, long-term treatment with specific $A_2$-adenosine receptor antagonists is associated with increased fractional sodium reabsorption in the distal nephron to the extent that, despite sustained inhibition of proximal tubular reabsorption, overall sodium excretion returns to normal (see [24]). The present finding that, in the acute situation, fractional distal sodium reabsorption was reduced suggests that, in the case of caffeine, additional factors (possibly related to its $A_2$-adenosine receptor antagonism) must have been operating to offset the above effects. Caffeine has no inhibitory effect on either plasma renin activity [1,2,25] or aldosterone secretion [2,26], effects. Caffeine has no inhibitory effect on either plasma renin activity [1,2,25] or aldosterone secretion [2,26], an increase in the volume of fluid arriving at the distal tubule would be anticipated, partly as an inherent response of the distal nephron to the increased load and partly as a consequence of a tubulo-glomerular feedback-mediated reduction in GFR.

Finally, the practical implications of the present findings for the use of the $C_{Li}$ technique in physiological and clinical investigations should be considered. In 1984, Thomsen [27] recommended that coffee, tea, cola, cocoa and other caffeine-containing beverages be avoided in all studies of $C_{Li}$, on the basis of anecdotal evidence that caffeine increases $C_{Li}$. Since then, in hundreds of $C_{Li}$ studies in patients and healthy subjects, this stricture has been followed. Our findings indicate that the recommendation was sound: despite the inconvenience caused, avoidance of caffeine (or, alternatively, standardization of caffeine intake) is necessary if this factor is not to bring unwanted variability to $C_{Li}$ values. Our findings also provide a basis for earlier claims [28,29] that variations in caffeine intake can influence the plasma lithium concentration in patients receiving prophylactic lithium treatment and thereby either reduce the effectiveness of treatment or precipitate a lithium overdose.

**REFERENCES**

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Received 25 February 2002; accepted 24 July 2002.