Caffeine is a popular central nervous stimulant commonly found in coffee, tea, soda, as well as some food. Due to its large consumption around the world, caffeine has attracted considerable attention in the field of molecular physiology. Previous research has shown that caffeine consumption induces diuresis [excessive water loss] through the blockage of the adenosine A1 receptor. Surprisingly, the mechanisms by which caffeine acts on the kidneys to cause diuresis are still not fully understood, and more research needs to be done to uncover the clear physiological effects of caffeine.

In this study, we will [1] investigate the effect of acute and chronic caffeine consumption on the expression, localization and phosphorylation of NBCe1, and [2] determine whether metabolic acidosis due to disturbed NBCe1 protein follows long term caffeine consumption. We envision that this study will reveal the clear physiological role of NBCe1 in the regulation of caffeine-induced diuresis.

**ACKNOWLEDGEMENTS**
We would like to thank the UB center for Undergraduate Research and Creativity Activities (CURCA) and the UB Honors College for having sponsored this research initiative.

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**BACKGROUND**
Caffeine is used as a central nervous system stimulant to improve mental alertness. Its rapid action on the nervous system is such that it has become one of the world’s most widely consumed psychoactive drugs. Despite its tremendous contribution to our daily lives, caffeine comes with various side effects such as seizures, acid-base disorders, diuresis and natriuresis.

A common consequence of caffeine administration is the inhibition of fractional tubular reabsorption [resorption of the kidneys to reabsorb fluid from filtered blood plasma], which leads to diuresis.

A study conducted by Fenton et al. reported that blockade of the A1 receptors inhibits HCO3\(^-\) conductance and that pretreatment with DIDS\(^-\) [a NBCe1 antagonist] prevents caffeine-induced diuresis. Additionally, Trabulsi et al. presented a case where acidosis resulted from caffeine/lautine drinking.

NBCe1 (see figure 1) regulates the supply of HCO3\(^-\) to blood plasma, thereby maintaining plasma pH and increasing the osmotic driving force for water reabsorption. The two studies cited above have suggested the possible involvement of NBCe1 in the caffeine-induced diuresis.

![Figure 1: Acid-base-linked fluid reabsorption by the renal proximal tubule](image)

We believe that the SLC4A4 sodium-bicarbonate transporter is involved in the mechanism of caffeine-induced diuresis and natriuresis. However, it has yet to be determined whether the effect of caffeine on NBCe1 action is the result of [1] reduced activity of NBCe1, perhaps arising from phosphorylation change or [2] removal and degradation of NBCe1 from the plasma membrane.

We decided to center our study on detecting caffeine-induced changes in NBCe1 (and its apical counterpart NHE3) action/expression/modification in mice.

**GOAL OF THE STUDY**
The goal of this research is to reveal the clear effect of caffeine consumption on the sodium-bicarbonate co-transporter. This research will also aim to determine the effect of caffeine on renal acid-base balance.

The aim of the present project is to:
1. Study the effect of acute and chronic caffeine consumption on the expression, localization and phosphorylation of the sodium-bicarbonate cotransporter (NBCe1).
2. Explore the acid-base status of mice that are treated with a high dose of caffeine.
3. Investigate whether metabolic acidosis follows long-term caffeine consumption due to disturbed NBCe1 expression.

**EXPERIMENTAL METHODS**
The caffeine treatments were divided into three groups [1] acute [2] chronic (1 day) and [3] chronic (1 week).

1. Experimental animals were gavaged with 150 μL of caffeinated water (5 mg/mL) at final 45mg/kg body weight and sacrificed 15 min later. The control group of mice were gavaged with 150 μL of water (containing 5mg/mL mannitol as an osmotic balance) and sacrificed 15 min later.
2. Experimental animals were housed in metabolic cages (Figure 2) for 2 days to collect 24 hr urine output. On day 1 the mice had free access to non-caffeinated water. On day 2 the mice only had free access to caffeinated water (0.3g/L in tap water). Urine was collected at the end of both days. Blood was sampled from the mice by cardiac puncture at the end of the second day and the mice were sacrificed for kidney harvest. Control mice were treated the same but had free access to non-caffeinated water on both days.
3. Experimental animals were housed in metabolic cages for 5 days to collect 24 hr urine output. On all five days the mice had free access to caffeinated water (0.3g/L in tap water). Urine was collected at the end of all five days. Blood was sampled from the mice by cardiac puncture at the end of the fifth day and the mice were sacrificed for kidney harvest. Control mice were treated the same but had free access to non-caffeinated water on all days.

**RESULTS**
Table A represents the urine output and water intake of two mice (from group 3) treated with caffeine. The urine output and water intake were measured before and after they were given caffeine.

<table>
<thead>
<tr>
<th>Caffeine Treatment</th>
<th>Urine (g)</th>
<th>Water (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average pre-caffeine</td>
<td>0.51</td>
<td>4.10</td>
</tr>
<tr>
<td>Average post-caffeine</td>
<td>1.17</td>
<td>4.61</td>
</tr>
<tr>
<td>P-value</td>
<td>P=0.05</td>
<td>P=0.05</td>
</tr>
</tbody>
</table>

Table B represents the urine output and water intake of two control mice (from group 3) that received no caffeine.

<table>
<thead>
<tr>
<th>Without Caffeine</th>
<th>Urine (g)</th>
<th>Water (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average</td>
<td>0.74</td>
<td>3.97</td>
</tr>
<tr>
<td>Post-caffeine</td>
<td>0.74</td>
<td>6.87</td>
</tr>
<tr>
<td>P-value</td>
<td>P=0.05</td>
<td>P=0.05</td>
</tr>
</tbody>
</table>

**CONCLUSION AND FUTURE DIRECTIONS**
The protocol was sufficient to induce diuresis but no acidosis was observed. The C57BL/6J mice used in this study constitute suitable model. The next step will be to study the effect of caffeine on the phosphorylation and abundance of NBCe1.

**LITERATURE CITED**