High Fructose intake increases trafficking and phosphorylation of the Na/K/2Cl cotransporter (NKCC2) in rat thick ascending limbs

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Disclosure

Nothing to disclose
Background

Effect of self-reported added sugar consumption on cardiovascular disease risk

~25% of the adult population

Yang et al., JAMA Internal Medicine February, 2014
Background

- 50% of the calories from added sugars in the diet come from fructose.

- Up to 10 percent of the US population (30 million) consume 15-20% of their calories from fructose (NHANES).

- The effect of a fructose enriched-diet in blood pressure is controversial with some studies supporting a correlation whereas others showed no association. However, few studies in humans or animals have addressed the combined effect of a fructose-enriched diet and a high salt diet on blood pressure.

- In rats, fructose alone, at levels relevant to human consumption does not cause hypertension. However, it is not known whether it causes salt-sensitive hypertension.
Effect of 20\% fructose with or without high salt diet for 4 weeks on blood pressure

\[ \Delta \text{SBP at 4 weeks (mmHg)} \]

- Water
- Fructose 20\%
- High salt
- Fructose + High salt

* Ortiz, Garvin, Beierwaltes, unpublished results
Effect of 20% fructose with or without high salt diet for 1 weeks on blood pressure

Cabral PD et al., Hypertension. 2014 Mar;63(3):e68-73
Background

• The mechanism by which fructose rapidly induces salt-sensitivity is not clear.

• In animal models of salt-sensitive hypertension there is an increase in the activity of the Na/K/2Cl cotransporter NKCC2 in the thick ascending limb (TAL) that precedes the hypertension.

• NKCC2 activity is regulated by trafficking into and out of the apical surface and by phosphorylation at threonines 96,101 by SPAK and OSR1 kinases.

• It is not known whether a fructose-enriched diet (20% fructose) stimulates NKCC2 trafficking and phosphorylation in rat-TALs.
Hypothesis

A fructose enriched diet activates NKCC2 in rat TALs within a week by enhancing surface expression and phosphorylation.
Measurement of NKCC2 in the plasma membrane

**STEP 1**
- Rats
  - Control diet
  - 20% fructose drinking water
- TAL suspension
- 4 °C
- Wash & cell lysis
- NKCC2
- GAPDH

**STEP 2**
- Total pool of proteins
- Separation of biotinylated proteins with streptavidin beads
- Biotin
- Membrane proteins
- Intracellular proteins
- Western blot for NKCC2
Effect of 20% fructose for 1 week on Surface/total NKCC2 ratio

Surface/Intracellular NKCC2 (% of control)

- Control
- 20% Fructose

* Significance level
Representative molecular structure of NKCC2
Effect of 20% fructose for 1 week on NKCC2 phosphorylation at thr96/101

Control  20% Fructose

P-NKCC2/Total NKCC2 (% of control)

Thr96/Thr101  Total NKCC2

*
apical membrane

Recycling pool

Exocytosis

Endocytosis

NKCC2
Effect of 20% fructose for 1 week on phosphorylated NKCC2 at Thr96/101 in the surface

Control 20% Fructose

P-NKCC2 expressed at the Apical surface

![Diagram showing the effect of 20% fructose on phosphorylated NKCC2 at Thr96/101 in the surface. The control group shows a lower level of phosphorylated NKCC2 compared to the 20% fructose group, indicated by the higher bar in the graph.](image-url)
Effect of 20% fructose for 1 week on SPAK/OSR1 phosphorylation and total expression in TALs.
Summary

- 20% fructose in drinking water for a week:
  - Increases surface NKCC2 levels.
  - Increases NKCC2 phosphorylation at Thr96/101.

- 20% fructose in drinking water for a week:
  - Increases SPAK/OSR1 phosphorylation.
  - Total SPAK or its variants were not changed.
Conclusion

A fructose-enriched diet enhances NKCC2 trafficking and NKCC2 phosphorylation at Thr96/101 likely via activation of SPAK or OSR1 kinases.

Perspective

Fructose may stimulate NKCC2-dependent NaCl transport in TALs. Fructose-induced stimulation of NKCC2 may not be sufficient to cause hypertension but may increase blood pressure when combined with high salt intake.
Fructose (20%) but not glucose (20%) induces salt sensitivity indicating this effect is not due to an increase in caloric intake.
Effect of 20% fructose at 1 week on Surface/total NKCC2 ratio

- **Surface NKCC2**
  - Control
  - 20% Fructose

- **Total NKCC2**
  - Control
  - 20% Fructose

**Phospho Surface/Total NKCC2 (of control)**
- **Surface Phospho NKCC2**
  - Control
  - 20% Fructose

**Phospho Surface/Total NKCC2 (of control)**
- **Control**
- **20% Fructose**

* indicates statistical significance.
-A glass of fruit juice has about 25 gms of sugar which means it has about 12 gms of fructose. In comparison, a 12 ounce can of soda has about 40 gms of sugar and about 20 gms of fructose. However, what fruit has (rich sources of potassium, folate, fibre, antioxidants and bioactive phytochemicals) is not present in sodas.

-Moreover, Individuals who eat more fruit and vegetables (those who take care of their diet) are likely to have lower rates of smoking, a lower intake of salt and saturated fat, higher levels of physical exercise and are less likely to be overweight (a possible confounding).