



UC Davis MMPC-Live Protocol

CCK Inhibition of Diet Intake

Version: 1.0

Revision Date: 10/25/2023

Replaces version: None

Edited by: Michael Goodson - UC Davis Metabolism & Metabolic Health Core

[Summary](#)

[Reagents and Materials](#)

[Protocol](#)

[Reagent Preparation](#)

Summary:

The gut hormone cholecystokin (CCK) is secreted by I cells in the duodenum in response to the intake of fat and protein and binds to receptors on vagal afferent neurons as a satiety signal to trigger meal cessation. High fat diet feeding leads to CCK resistance which can be measured as reduced diet-intake in response to exogenous CCK hormone treatment.

Reagents and Materials:

<i>Reagent/Material</i>	<i>Vendor</i>	<i>Stock Number</i>
Cholecystokinin Octapeptide (sulfated)	Bachem	4033010
Sterile normal saline		

Protocol:

Conditioning

The ability of CCK to inhibit diet intake is particularly sensitive to stress. To get around this, mice are conditioned by injection with saline at least twice before the actual experiment.

1. Single house mice (they need to remain single housed for the duration of the study)
2. Within an hour of lights inject (intraperitoneal, IP) all mice with saline (10µl/g BW).
3. Two to three days later, repeat the IP injection with saline on all mice.

CCK Testing

CCK requires testing mice with both CCK and control (saline) injections. Before beginning the study, counterbalance mice in each group to receive either CCK or saline first. Also figure out the order of injections for each mice such that mice CCK and Saline injected mice from each group are grouped into sets (also counterbalancing) and the order of each group is varied between sets.

1. Fast mice into clean cages six hours before lights off. Weigh mice when fasting. Keep diet in weigh boats to be added once mice are injected with CCK.

2. Within one hour of lights out, dilute CCK to 0.3 ng/ μ l in saline. The dose of CCK is 3 μ g/kg BW.
3. Prepare insulin syringes for each mouse with 10 μ l/g BW of either diluted CCK or saline.
4. At lights out (under red light) begin the injections. Inject 1 mouse every two minutes.
5. After the mouse is injected, add the diet to the wire food hopper over a trashcan. Weight the diet and hopper together. Record this starting weight. Add the water to the cage.
6. Repeat for all mice.
7. Starting at 20 minutes. Weigh the hopper& diet. Repeat this for each mouse every two minutes. It may require more than one person to do injections and weigh diet if there are more than 10 mice.
8. Repeat the diet intake measurements at 40 and 60 minutes.
9. Wait 2-3 days and repeat this procedure (steps 1-8) reversing the CCK and saline injections.
10. For small effects on CCK sensitivity, it may be necessary to repeat both rounds a second time.

Data Analysis

CCK has a very short half-life, so normally the difference between groups is observed only in the 20 minute measurement.

1. To get the diet consumption at each time point, subtract the hopper/diet weight at each time point from the starting hopper diet weight.
2. To get the effect of CCK on diet intake at each time point, subtract the diet intake for the saline injection from the diet intake from the diet intake from the CCK injection for each mouse at each timepoint. The number should be negative if CCK is reducing diet intake.

High fat diet feeding can make mice more sensitive to stress, so overall (both CCK and valine injected) diet intake is lower for high fat vs low fat fed mice.

Reagent Preparation:

1 mg/ml CCK Stock: Dissolve CCK to a stock concentration of 1mg/ml in saline. Aliquot, snap freeze and store at -80°C. Dissolved CCK last approximately one year if stored at -80°C.