Supplementation with Choline Chloride in a Liquid Rodent Diet: Recipe Development for Differing Caloric Intake in a Fetal Alcohol Model

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Background

- Fetal Alcohol Spectrum Disorders affect between 2-5% of children in the United States. [1]
- Choline supplementation of maternal diet during gestation and postnatal gavage of pups ameliorates fetal alcohol effects on offspring.[2]
- Alcohol and choline are generally supplemented via oral gavage, but this is not comparable to human consumption.
- Some studies have provided choline via saccharine-sweetened drinking water, but this does not allow ethanol administration.
- An issue we must overcome is that dams consume differing quantities depending on weight, stage of pregnancy, and diet content.

There is a need for a palatable method to deliver choline and ethanol to rat dams that more closely imitates maternal consumption in humans.

Objective

- Devise a means of delivering choline and ethanol to rat dams throughout gestation that is comparable to human consumption
- Develop a palatable choline-supplemented liquid ethanol diet
- Replicate previous choline supplementation doses of 250 mg choline per kg body weight per day (250 mg/kg/day)[3]

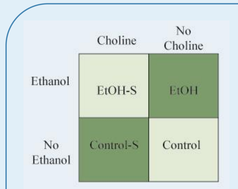
Objective Choline Dose:

250 mg choline
kg weight/day

Hypotheses

I. Choline supplementation will not affect consumption.
II. Pregnancy will have a significant effect on consumption.
III. Ethanol supplementation will have a significant effect on consumption.

Methods



Study Design
2x2 model illustrating four treatment groups:

1) *Ethanol with choline supplementation*
2) *Ethanol*
3) *Control with choline supplementation*
4) *Control*

Note: Each ethanol group also has a calorically matched pair-fed.

- Pilot studies in our lab found that sexually naïve female Wistar rats aged 100-120 days weigh an average 250 grams and eat approximately 65mL liquid diet per day.
- Throughout pregnancy, both weight and consumption change.
- Three choline recipes were designed for (1) pre-treatment, (2) pregnancy, and (3) ethanol supplementation during pregnancy.

	Recipe		
	(1) Pre-Treat	(2) Control-S	(3) Ethanol-S
Weight (kg)	0.250	0.288	0.3
Consumption (mL/day)	65	66	66
Consumption (mL/kg/day)	260[a]	229[b]	219[c]
Choline Supplementation (mg)	0.9615	1.0910	1.1402

Choline supplementation did not significantly affect consumption. However, pregnancy and ethanol supplementation during pregnancy did significantly decrease consumption relative to weight ($p<0.05$). The above weights and consumptions were used to devise recipes for choline supplementation.

- To insure biologically relevant ethanol consumption, blood ethanol concentration (k) was assessed from tail blood collected on GD15. Plasma was analyzed using an Analox GM7 analyzer.
- To assess choline uptake, liver samples were collected from dams on GD18 and processed for choline metabolites (e-j).

Results

		Control	Control-S	Ethanol	Ethanol-S	Control-S+
a	**Consumption, Pre-Treatment** (mL/kg)	341.2	268.0	268.3	280.4	291.4
b	**Consumption, Pregnancy** (mL/kg)	253.9	243.2	209.8	229.3	224.7
c	**Choline Supplementation, Pre-Treatment** (mg/kg/day)	--	257.7	--	269.6	280.2
d	**Choline Supplementation, Pregnancy** (mg/kg/day)	--	265.3	--	261.4	256.2
e	**Free Choline** (nmol/g)	30[a]	93[b]	--	92[b]	--
f	**Betaine** (nmol/g)	400[a]	794[b]	--	840[a]	--
g	**Phosphocholine** (nmol/g)	470[a]	1481[b]	--	1640[b]	--
h	**Glycerophosphocholine** (nmol/g)	135	102	--	98	--
i	**Phosphatidylcholine** (nmol/g)	16850	15271	--	16800	--
j	**Sphingomyelin** (nmol/g)	1515	1313	--	1260	--
k	**Blood Ethanol Concentration** (mg/dL)	3.2[a]	6.6[a]	66.2[b]	88.8[b]	7.2[a]

The table (above) shows average consumption and choline supplementation by group during (a,c) pre-treatment and (b,d) pregnancy. (e-j) show average choline metabolite concentrations by group from homogenized liver samples taken from pregnant dams at GD18. Free Choline and Phosphocholine were significantly increased in Control-S and Ethanol-S compared to Control. (k) shows average blood ethanol concentration by group from blood samples taken at GD15 via lateral tail vein nick two hours into the awake cycle.

Achieved Choline Dose:

258.38 mg choline
kg weight/day

Treatment	Choline Dose (mg/kg/day)
Control-S	269.85
Ethanol-S	251.71
Control-S+ (pair-fed to Ethanol-S)	237.35

The average choline dose across supplemented groups was 258.38 mg/kg/day. There were no significant differences between groups ($p>0.05$).

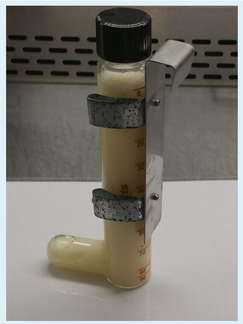


Image (left) shows a J-feeder used to administer diet to all animals through a week of pre-treatment and until GD20.

	Objective Dose:		***Average Weight:***		***Choline Needed:***		***Average Consumption:***	
Choline Recipe 1	$\frac{250mg\ choline}{kg\ weight/day}$	×	0.250 kg	=	$\frac{62.5mg\ choline}{day}$	÷	$\frac{65mL}{day}$	= $\frac{0.9615mg\ choline}{mL\ diet}$ $(Pre-treatment)$
Choline Recipe 2	$\frac{250mg\ choline}{kg\ weight/day}$	×	0.288 kg	=	$\frac{72.0mg\ choline}{day}$	÷	$\frac{66mL}{day}$	= $\frac{1.0910mg\ choline}{mL\ diet}$ $(Control-S)$
Choline Recipe 3	$\frac{250mg\ choline}{kg\ weight/day}$	×	0.301 kg	=	$\frac{75.3mg\ choline}{day}$	÷	$\frac{66mL}{day}$	= $\frac{1.1402mg\ choline}{mL\ diet}$ $(Ethanol-S)$

References

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