# Review of: "L-citrulline ameliorates pathophysiology in a rat model of superimposed preeclampsia"

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Potential competing interests: The author(s) declared that no potential competing interests exist.

The aim of the study by Man et al. was to determine the effects of maternal L-citrulline supplementation in pregnant Dahl salt-sensitive rat DSSR), a model of preeclampsia. L-citrulline was administered in drinking water (2.5g/L) from the time of conception throughout gestation, and pregnant dams were sacrificed either at day 12 or 21 of gestation. The authors found that citrulline supplementation significantly reduced gestational hypertension, proteinuria, improved vascular endothelial function and placental insufficiency through enhanced angiogenesis and decreased placental fibrosis, resulting in improved fetal growth. The authors conclude that in the DSSR model, citrulline supplementation is able to mitigate many of the alterations observed in preeclampsia, and therefore is a promising candidate for treating preeclampsia in human pregnancies.

Main comments:

1. Preeclampsia is one of the most common complications of pregnancy worldwide, is associated with significant morbidity and mortality in mothers, and results in preterm delivery or intrauterine growth restriction (IUGR), which entails increased cardiometabolic risk in the future adult. Moreover, there is no effective treatment except for planned preterm delivery. Preeclampsia therefore is a major public health concern. The search for innovative treatments therefore clearly is warranted. Trying citrulline as a putative therapeutic agent also makes sense, as citrulline has been shown to be bioavailable in rats and humans, and to positively impact placental function and fetal growth in animal models of IUGR. Yet to our knowledge there is no animal study investigating the effect of citrulline supplementation in preeclampsia. The rationale of the study is sound, and the authors should be commended for a timely study. Their investigation of placental function is thorough. Yet the results would be more convincing if the design was described in more detail.
2. First, limitations of the Dahl salt-sensitive rat (DSSR) model should be addressed. For instance, hypertension is present before gestation in DSSR model, whereas it may only appear in the $2^{nd}$ or $3^{rd}$ trimester of pregnancy in human preeclampsia. This timing is very relevant since the human fetal weight triples in the $3^{rd}$ trimester of pregnancy (just like fetal weight increases nearly 10-fold in the $3^{rd}$ week of gestation in rodents).
3. In addition, an ideal study design would have included a true control group with no hypertension, i.e., a group of Dahl salt-resistant pregnant rats.

4. As citrulline was provided in drinking water, monitoring of water intake clearly was needed to calculate the actual dose of citrulline ingested. Assuming an average maternal body weight of 300g (0.3 kg), and an average fluid intake of 60 mL/kg/d (0.06L/kg/d), the average daily dose of citrulline would an average rat would ingest 0.06 Lx0.3kgx2.5g/L=0.045g/kg/d, *i.e.*, 40 times lower than the ≈2g/kg/d dose used in other studies in IUGR or undernourished rats, Did the authors observe a dose-response curve? This discrepancy in dosage should be addressed.
5. Were plasma citrulline concentrations measured in maternal and fetal plasma?
6. What was the rational for using the in the umbilical vein (Fig 2H) rather than another vessel to assess acetylcholine-induced contraction?

Minor comments

1. On page 11, line 10: replace ‘assocaited’ with ‘associated’
2. On page 11, line 13: replace ‘health pregnancy’’ with ‘healthy pregnancy’