Reviewer's report

Title: Suckling A Protein-Restricted Rat Dam Leads To Diminished Albuminuria In Her Male Offspring In Adult Life: A Longitudinal Study

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Reviewer: Mary Black

Reviewer's report:

General
This study looked at the effect of maternal protein restriction in utero or during lactation on albuminuria and creatinine levels later in life. Although there is mention that renal pathology was assessed, the details of how this was assessed and the reporting of the results were inadequate.

Major Compulsory Revisions (that the author must respond to before a decision on publication can be reached)

Major Compulsory Revisions:

Methods:
Many important details have been omitted from the Methods section. This section needs to be expanded.
Animals: The protocol used in the animal studies is not clear. For instance, why were there 24 control offspring, 16 recuperated offspring and 20 PLP offspring? If there were 5 dams fed the low protein diet during pregnancy and 4 males were used per litter doesn't this mean that there should have been 20 recuperated offspring? Also, were the control offspring cross-fostered? Indeed, it is important that the controls were treated in the same way as the other groups.
In my opinion, the decision to cease experiments at the time (10 months) when the first rat died was unusual and not well-justified. What did that rat die from? Ten months of age is far from the normal life span of a rat. I would expect that rats would normally live at least twice as long as this.
Laboratory Analyses: Creatinine was measured in the plasma and in the urine. What does the albumin/creatinine ratio represent? How was the creatinine clearance determined? These details should be included in the methods or references detailing these techniques should be referred to.
If there were 16 or more animals in all experimental groups, why weren't all the kidneys analysed at the termination of the experiment? In particular, why were the number of animals chosen to study kidney pathology different between the groups and why were only 5 kidneys from the recuperated animals investigated. For morphological/pathological analyses I would expect that you would need 8 to 10 kidneys per group. Was a power analysis performed to determine how many kidneys were to be assessed? Please elaborate on what parameters were investigated and the standard deviations associated with these techniques.
There is insufficient information to determine whether the pathological analyses of the kidneys were conducted correctly. When referring to pathological lesions in the kidneys what specifically was looked at and what was the criteria for classification of the lesions as minimal to mild, moderate or severe? Also, how were the kidneys sampled? How many sections and fields of view were assessed? It is essential that this information is provided.
It is deduced from the results section that many characteristics of renal pathology were investigated such as focal segmental sclerosis, tubular dilation, tubular atrophy, mononuclear cell inflammation, interstitial fibrosis, cell hyperplasia in the distal tubules and interstitial inflammation. In my opinion to adequately address many of these pathologies, specific histological stains or immunohistochemistry should have been utilised and simple analysis of haematoxylin and eosin stained sections would be inadequate.

Results:
For all data written into the text the means ± SEM should be included.
The way the results are written it is difficult to differentiate between which groups the significant differences lie. For instance, were the daily albumin excreations significantly different between the controls and the recuperated groups? Likewise were the daily albumin excreations different between the PLP and control groups? It would be better if the albumin data and the plasma creatinines, urine creatinines and creatinine clearance data was presented in table form with symbols showing between which groups the significant differences were detected.
I find it very difficult to understand why after 10 months of age the albumin excretion rates in the controls and recuperated animals was considerably reduced. Indeed, the results in the control animals do not fit in with previously published data from this group (with some of the same authors) published in the Am J
Physiol Renal Physiol 2006. In that published study in control male Wistar rats the urinary albumin excretion per 24 hr was about 20 mg at 7 months of age and this had increased markedly to 80 mg at 15 months of age. To the contrary in this study, the urine albumin excretion per 24 hrs was about 80mg at 7 months of age and had dropped to less than 20 mg at 10 months of age. These findings in relation to their previous findings should have been discussed in the discussion. Also, they should have been further discussed in relation to other published studies.

The renal histology section should be re-written. If all the parameters (focal segmental sclerosis, dilated tubules, tubular atrophy, interstitial fibrosis etc.) were all assessed in this study they should have been recorded and assessed separately. For instance, were the kidneys from the PLP group more susceptible to a particular renal pathology? This data could be reported in table form. Also, light micrographs of representative pathologies would be useful.

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Minor Essential Revisions (such as missing labels on figures, or the wrong use of a term, which the author can be trusted to correct)

Minor Essential Revisions:

Abstract:
It is incorrect to conclude that the slower rate of shortening in kidney telomere lengths (and increased lifespan) in rats exposed to maternal protein restriction during lactation was associated with diminished albumin excretion rats when you did not look at these associations in this study. You can only make conclusions on the parameters and data you investigated. You also cannot make the final statement in the conclusion. Firstly, you did not look at whether maternal protein restriction leads to nephron damage in utero. Indeed, I don't think that it is generally considered that maternal protein restriction in utero leads to nephron damage in the fetus. It generally leads to reduced nephron endowment in the offspring but that does not imply that the nephrons are damaged.

Background:
In the final paragraph of the Background, the aims were to investigate whether there were differences in renal function and pathology in male rats that were exposed to maternal protein restriction either pre-natally or whilst suckling. I do not think that renal function or pathology were adequately assessed. In rat studies it is possible to measure a number of parameters to assess renal function, such as glomerular filtration rate, renal blood flow, renal vascular resistance, filtration fraction and urine flow rate. Since these measures were not performed, it would be better to state the aims more specifically to indicate what parameters were investigated. Also, and more importantly, renal pathology did not appear to be adequately assessed. However, the description of how renal pathology was assessed in the methods and the outcomes were so poorly described that it is difficult to ascertain whether the analyses were performed correctly and what was measured.

Methods:
Laboratory Analyses: I think it is appropriate to reference the techniques for the immunosorbent assay for the measurement of albumin and to the Jaffe reaction for measuring creatinine.

Discussion:
First sentence: Second word â€œa€ should be deleted.
Most of the first paragraph describing results from a previous study should be either reduced or deleted.
Second paragraph: You cannot say that exposure to maternal protein restriction during the first few weeks of life led to significant reductions in urinary albumin excretion at 3 months of age. The changes in albumin excretion may be secondary to some other change in the kidney that has subsequently led to the changes in albumin excretion. You can state that it is linked but not that it â€œledâ€™ to the effects.
It is imperative that the authors discuss their albumin excretion results (especially in the control animals) in context with other published findings, including studies from their own laboratory. Are there other studies that have shown that in control animals or normal subjects that albumin excretion is reduced with age?
Conclusions: Much of what is stated in the conclusions is speculative and cannot be directly determined by the results presented in this study.

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Discretionary Revisions (which the author can choose to ignore)

What next?: Unable to decide on acceptance or rejection until the authors have responded to the major compulsory revisions

Level of interest: An article whose findings are important to those with closely related research interests
Quality of written English: Acceptable

Statistical review: Yes

Declaration of competing interests:
I declare that I have no competing interests.