

**Review Form 1.6**

Journal Name:	<a href="#">Asian Journal of Immunology</a>
Manuscript Number:	Ms_AJI_83640
Title of the Manuscript:	High fat diet triggers a prompt and transient increase in adipose tissue G-CSF and circulating myeloid cells in mice.
Type of the Article	Original Research Article

**General guideline for Peer Review process:**

This journal's peer review policy states that **NO** manuscript should be rejected only on the basis of '**lack of Novelty**', provided the manuscript is scientifically robust and technically sound. To know the complete guideline for Peer Review process, reviewers are requested to visit this link:

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PART 1: Review Comments

	Reviewer's comment	Author's comment (if agreed with reviewer, correct the manuscript and highlight that part in the manuscript. It is mandatory that authors should write his/her feedback here)
<b>Compulsory</b> REVISION comments	<p>Overall the study is addressing the short-term effect of high fat diet with focus on the effect on myelopoiesis, circulating neutrophils and the induction of G-CSF in an experimental obesity model. Therefore, I made considerations so that the authors can think about it and seek to improve the manuscript.</p> <p>1. "Microbiota has been suggested to play a role in HFD-induced inflammation via its production of endotoxin." The authors mention an important role of the microbiota in HFD induced inflammation. Why did the authors not assess the role of the microbiota in this model?</p> <p>2. Why only male mice were studied?</p> <p>3. The methodology is incomplete, important informations is missing, as the authors present graphs of body weight weekly and white tissue weight, however there is no information in the methodology that the weight was measured per week, which White adipose tissue compartments were weighed and used in the analyses. Please add this information.</p> <p>4. "At day 3, 8, 23, 30 and 37, eight mice fed HFD and four fed standard diet were weighed and anesthetized." Why did the authors use 8 animals for HFD and 4 animals for control? Some results show a high standard deviation in the control group, would the authors associate this with a reduced number of animals? Are four animals sufficient for the reliability of the results?</p>	<p>1. The role of the microbiota and LPS has been addressed by others, see e.g. Cani et al. 2007 and 2008. We rather build upon these data to investigate the cellular events and mechanisms.</p> <p>2. Female mice are known to vary according to their hormone cycle. Therefore we excluded female mice from the study.</p> <p>3. At each time point we weighted and took out visceral adipose tissue (VAT ) from 8 HFD and 4 control mice. We have tried to make this clearer in the manuscript (p. 5)</p> <p>4. We expected more variation in mice put on HFD, in particular in the beginning of the feeding regime. Therefore and because we wanted to address the events taking place when the mice were transferred to HFD, we chose more mice in this group. 12 animals were the highest number we could manage experimentally at a time. More animals would have been preferable but importantly ; we see interesting and consistent differences between the two groups with the used number of animals.</p>
<b>Minor</b> REVISION comments	<p>1. Please add male mice in the abstract.</p>	Is done
<b>Optional/General</b> comments		

PART 2:

	Reviewer's comment	Author's comment (if agreed with reviewer, correct the manuscript and highlight that part in the manuscript. It is mandatory that authors should write his/her feedback here)
<b>Are there ethical issues in this manuscript?</b>	<p>(If yes, Kindly please write down the ethical issues here in details)</p>	<p>We used mice for the study, but we did not do any intervention before the day they were put down. Here the mice were anestized before blood sampling and then euthanized. The mice were euthanized by cervical dislocation and tissue samples collected under sterile conditions. The experiment was carried out in accordance with the Council of Europe Convention European Treaty Series 123 on the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes, and the Danish Animal Experimentation Act (LBK 1306 from November 23, 2007) approved by the Animal Experimentation Inspectorate, Ministry of Justice, Denmark (Licence number: 2012-15-2934-00256 C1-6).</p>