Do Vivarium Conditions Influence Atherosclerotic Lesion Size?

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Atherosclerotic lesion measurement in mice has become a stalwart index as an experimental approach for mechanistic investigations. Currently, there are no generally accepted standards for measurement of lesions in this species. There are many other potential issues that contribute to the variability in lesion size and responses, including sex, genetic background, and diet composition. These factors may contribute to the highly variable and sometimes contradictory literature. An example of contradictory literature is the role of scavenger receptor class A, which has been shown to have positive, negative, and neutral effects on mouse atherosclerotic lesion size.

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Another potential variable in the development of mouse atherosclerosis is the infection burden of the colony. At one extreme, mice that are completely germ-free have reduced atherosclerosis. Conversely, increased atherosclerosis has been determined in mice with overtly induced infections, including Chlamydia pneumoniae, Porphyromonas gingivalis, and Helicobacter pylori. However, the majority of atherosclerosis studies are performed on mice that have an infection status between these 2 extremes. Vivaria that house these mice are characterized by a range of descriptors, including conventional, clean, specific pathogen-free, and barrier. However, there are no clear standards on the definition of these descriptors. This absence of uniformity of designation confounds the ability to assess vivarium housing conditions on atherosclerosis development.

Although it has not been extensively assessed, vivarium health status has been considered as a contributor to development of mouse atherosclerosis. In this issue of Arteriosclerosis, Thrombosis, and Vascular Biology, Srivastava et al provide extensive information on the development of atherosclerosis in 48 sex-matched inbred strains of mice that were housed in 2 rooms of differing health status levels. These analyses were facilitated by the extensive range of strains that can be monitored closely at the Jackson Laboratory. The strains were compared when housed in rooms defined as low or high health status. Although the difference between these rooms of differing status was not described, it was noted that a few opportunistic organisms were detected in the low health status room. There were a few mouse strains in which lesion formation was equivalent between rooms, with some strains exhibiting sex-specific effects. However, in most strains, lesions were only detected in the low health status room. This includes C57BL/6 mice, which are the most commonly used strain in atherosclerosis studies.

Overt pathological bacteria are monitored routinely in many vivaria through sentinel screening. Before the study of Srivastava et al, there were few direct comparisons of effects of vivarium housing conditions on atherosclerotic lesion size and mechanisms of formation. One example comes from the Mallat group, who demonstrated no significant difference in lesion size formation between C57BL/6 mice housed under 2 conditions that inferred a range of infective states. Conversely, deficiency of interleukin-10 had a much greater effect in accelerating atherosclerosis in mice housed under conditions more prone to bacterial infections. However, this study did not extend into factors causing the different atherosclerotic responses. Conversely, the study of Srivastava et al illustrates a systematic approach to determining the contribution of health status level to atherosclerosis.

Although the presence of a variable microbiological milieu may contribute to atherosclerosis development, there are many variables that exist among different vivarium housing conditions that may affect lesion formation. Caging systems may have several potential ramifications on environment and stress. Other potential parameters include sterilization status of food type, water source, and bedding composition.

Contemporary clean facilities commonly house mice in individually vented cages with high-efficiency particulate air-filtered air and direct cage exhaust. These conditions lead to reduced humidity and odors, including ammonia within cages. This dryness contributes to greater intervals between transferring mice to clean cages. Individually vented caging permits transferring mice to new cages only once every 2 weeks, whereas conventional caging is usually changed twice a week. The prolonged intervals between individual vented cage changes may lead to less disruption of social structure within the cage. Therefore, mice housed in cages of differing health status have many environmental and social changes that have undefined effects on lesion formation unrelated to infection burden.

Another common difference among facilities of various health status levels is the sterilization of diets. Mouse diets may be sterilized by autoclaving or irradiation to reduce bacteria content. Many of the saturated fat–enriched diets used in atherosclerosis studies cannot be autoclaved but may be irradiated. However, irradiation alters dietary constituents, in particular oxidation of fats present in atherogenic diets.
Water provided to mice may also differ greatly among vivaria. Use of local water provides constituents that vary by geographic region, whereas high health status facilities use sterilized drinking water. Therefore, the mineral content and pH of water may be different depending on source. Bedding is also typically sterilized for use in rooms of high health status.

Overall, it is clear that there are many variables that may impact the reported size of mouse atherosclerotic lesions. The comprehensive study of Srivastava et al\(^5\) demonstrates that differences in lesion size of the same mouse strain may vary as a function of health status housing. Although infection status of mice may influence the outcome of atherosclerosis studies, the housing conditions used in vivaria of differing health statuses could also directly influence lesion formation. In the broader context of atherosclerosis studies, this illustrates the benefits of authors providing complete information on housing conditions. For example, the Jackson Laboratory provides a description of operating procedures for their different vivarium conditions (http://jaxmice.jax.org/health/barrier.html). Given that most journals do not restrict the length of online supplements, authors should be encouraged to provide specific details of housing that includes caging and dietary parameters.

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**References**

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