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Examining the effect of a nutrition intervention on immune function in healthy humans: what do we mean by immune function and who is really healthy anyway?[1,2]

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Designing studies to examine the role of nutrition in disease prevention can provide interesting challenges. For example, assessing the effect of nutritional interventions on immune function is often a daunting prospect for those of us in nutrition. The field of immunology is moving so rapidly that it is difficult for immunologists to keep up, let alone the rest of us. What aspect of immune function should we examine? Which assay should we use? A second daunting prospect in such studies is that we may want to work in a population at high risk of disease. This raises the question of how to design and evaluate our intervention to account for this potentially confounding variable. (Occurrence of disease is often an outcome variable, of course, but may also be a confounding variable.) In this issue of the Journal, Walrand et al (1) faced both of these issues—Which immune function test do I use? and How will underlying disease affect the interpretation of my data?—when they chose to examine the effect of a nutritional stress (a 36-h fast) on immune function in young and elderly persons. The choices Walrand et al made in designing their study and interpreting their results can be instructive for those of us facing similar study design issues.

But first, why examine a nutritional stress? These authors postulate that healthy elderly subjects may not handle such a common stress (eg, decreased food intake during a brief illness) as well as their younger counterparts would. This is reasonable, but does raise the question of which factor—nutrition or stress—is the real independent variable of interest.

To deal with the issue of underlying disease, Walrand et al made the excellent choice of following the SENIEUR protocol (2) to select elderly subjects. This protocol establishes admission criteria for immunologic studies in healthy elderly persons that are based on clinical information and laboratory data. Use of prescription drugs is also considered. This protocol is not the only way to define *healthy*, but it is well established and clear in its criteria so that readers can understand what *healthy* means in studies following this protocol. Often, the issue of underlying disease is dealt with in a perfunctory manner that leaves more questions than answers about the health status of the subjects in published studies.

Walrand et al then moved on to the next issue: How to assess immune function? Protection against infectious diseases is the principal job of the immune system and is a common goal of nutritional interventions in elderly populations. Which assays of immune function tell us something about such protection? Assays that look at effector mechanisms of the immune system tell us about protection against disease. Which effector mechanisms are important? For the adaptive immune system, one example is neutralizing serum antibody. Such antibodies inactivate infectious agents. Thus, the antibody response to a viral vaccine would be a good functional endpoint and has often been used in comparing immune response between elderly and young subjects (3). For the innate immune system, functional studies of phagocytic cells (including neutrophils and monocytes) collected from the peripheral blood can reveal useful information about the ability of these cells to kill ingested organisms.

Walrand et al chose to look at measures of both the innate and adaptive immune system, because both are crucial to protection against infection (4). Neutrophils act in the blood, spleen, and tissue to ingest and kill microorganisms. Thus, their numbers are important and are regulated by production in the bone marrow. The nutritional stress of a 36-h fast increased the number of neutrophils in the peripheral blood in both the elderly and young adult subjects in Walrand et al's study. Is that good? Why would fasting increase blood neutrophils? The increase probably is good, in the sense that more neutrophils means greater antimicrobial capacity, and the bloodstream is one site of action for neutrophils. In answer to the second question, fasting is a plausible stimulus for increasing the blood neutrophil count when we realize that a short-term fast can induce a stress response that may activate the innate immune system. Fasting is certainly a physiologic stress in that it can increase cortisol concentrations in humans (5), just as a traditional experimental stress (restraint stress) can increase cortisol concentrations in experimental animals (6). Restraint stress also increases blood neutrophil numbers (by mobilizing reserves) and cortisol appears to be the mediator of this increase (7). In addition, the neutrophils from the fasted subjects in Walrand et al's study had greater unstimulated superoxide anion production than was found before or after the fast. This may indicate that the oxidative metabolism of the neutrophils was increased to be ready to kill ingested pathogens. Thus, fasting, a nutritional insult, appears to have the paradoxical result of activating the innate immune system, probably by

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activating a cortisol-mediated physiologic stress response. The same results may not be seen during a longer fasting period when nutritional status begins to suffer.

What about the adaptive immune system? Is it activated by fasting or stress? The answer appears to be yes. Although Walrand et al did not look at functional indicators of adaptive immunity (as their options were limited by a short intervention period), they did find that peripheral blood lymphocyte numbers (eg, numbers of $CD4^+$ lymphocytes) decreased significantly during fasting. Although short-term changes in peripheral blood lymphocyte counts are not useful in inferring changes in the pool size of lymphocytes in the body, these numbers may be telling us something useful about redistribution of lymphocytes in response to stress. For example, restraint stress causes the same phenomenon (lymphopenia) within minutes and is rapidly reversible with alleviation of the stress (6). Increased plasma cortisol triggered by the restraint stress causes lymphocytes to be drawn into regional lymph nodes (presumably by regulation of adhesion molecule expression), apparently to be ready for an immunologic challenge (8). Thus, the adaptive immune system is on alert, as is the innate immune system, during the stress of short-term fasting.

In conclusion, when we plan nutritional interventions that involve measuring immune function in populations at risk of disease, we need to think clearly about the underlying health of our subjects and about exactly what we want to measure with regard to immune response. Functional measures of the immune system are best, although limitations inherent in human studies require us to look creatively at some more readily available measures, such as peripheral blood lymphocyte counts. Such measures can provide useful information, as the study by Walrand et al has shown.

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