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Increased Dietary Fat Prevents Sleep Deprivation-induced Immune Suppression in Rats

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***Background and Purpose*: Fatty acid composition of rodent diets can affect baseline immune function as measured in vitro and in vivo. Stress, in a variety of forms, can also affect immune function. Possible interaction between diet and other stressors has not been fully explored. We examined the interaction between sleep deprivation stress and dietary fatty acid composition in altering lymphocyte responses to mitogen stimulation.**

***Methods*: Rats were fed diets containing various sources of fatty acids, then were subjected to sleep deprivation. Splenocytes were harvested and assayed for responsiveness to various mitogens, using a 72-h proliferation assay.**

***Results*: Rats subjected to sleep deprivation experienced significant suppression of in vitro proliferative response to various mitogens. This immune suppression was dependent on duration of sleep deprivation. Feeding sleep-deprived rats a diet enriched in fatty acids abrogated the effect of sleep deprivation.**

***Conclusions:* The fat content of rodent diets can have a marked effect on baseline and stress-modulated immune responses.**

Numerous reports have indicated that physical, chemical, or psychosocial stress can result in immune suppression and increased susceptibility to disease (1). That and other studies have led to the concept that interactions exist between the immune system and the central nervous system and that the immune system may be modified by stress to induce inappropriate changes in immunocompetence (2). Documentation of soluble mediators and receptors common to these two systems further supports this notion (3). Little information is available, however, regarding other interactions that may be involved in stress-induced immune modulation. Nutritional status of an individual can be an important component of the stress response, either being directly responsible for the stress (4, 5) or affecting the response to other stressors (6). While moderate dietary energy restriction prolongs life span and enhances immune responsiveness (7), protein energy malnutrition is associated with decreased lymphocyte proliferation-reduced cytokine release, lower antibody response to vaccines (8), and enhancement of tumor metastasis (9).

The amount and type of fatty acids in the diet can influence in vitro measures of immune function (10-12). In addition to being structurally important components of cell membranes and precursors of eicosanoids (prostaglandins, thromboxanes, leukotrienes [3, 14]), fatty acids also affect signal transduction and gene transcription (15). Eicosanoids can have varied effects on immune responses, and their synthesis is affect by fatty acid composition of the membrane. Eicosanoids formed from n-3 polyunsaturated fatty acids (PUFA) seem to be more beneficial to the immune system, compared with those derived from n-6 PUFA (16). Thus diets rich in n-3 PUFA are anti-inflammatory and immunosuppressive (17, 18). This has led to the suggestion that the amount and type of dietary fatty acids can influence immune function (19), and that dietary composition may need to be altered during stressful conditions (20). Thus, the objective of the study reported here was to investigate the interaction between dietary fatty acids and sleep deprivation-induced immune suppression.

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Materials and Methods

Animals. Male rats (Harlan Sprague-Dawley, Indianapolis, Ind.) weighing approximately 300 g were studied in all experiments. Rats were caged individually with ad libitum access to food and water in the AAALAC-approved vivarium at the Pennington Biomedical Research Center (Baton Rouge, La.). All procedures were reviewed and approved by the Pennington Biomedical Research Center's Institutional Animal Care and Use Committee and were in compliance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Euthanasia was achieved by use of CO_2 asphyxiation.

Sleep deprivation. Rats were sleep deprived by placing them into pedestal-style cages in which a small platform is surrounded by water (21). Rats were allowed ad libitum to food and water while on the platform but were not able to sleep. Rats were subjected to sleep deprivation for 24 to 72 h. Cage-control (CC) rats were kept in the same rooms, but housed in standard cages. A 12/12-h on/off light cycle was used throughout the experiment.

Diet. Rats were fed a low-fat diet (LF), a safflower oil diet (SO) high in n-6 PUFA, an olive oil diet (OO) high in monosaturated fatty acids, a coconut oil diet (CO) high in saturated fatty acids, and a menhaden oil (MO) diet high in n-3 PUFA (Research Diets Inc., New Brunswick, N.J.). All diets contain 4% corn oil in addition to the other oils that represented 30% of the calories in each of the diets. The LF diet had 4% corn oil as the only source of fat. All diets were isocaloric, with starch substituting for the fat in the LF diet. Rats were fed pelleted diets except while in the sleep deprivation tanks where treated and cage control rats were fed liquid diets. The liquid diets had the same nutrient composition as the pelleted diets. All rats were fed measured amounts of food each day, with residual body weight recorded before each day's feeding. Differences in feed consumption were not observed between any of the groups.

Lymphocyte proliferation. The spleen from each rat was obtained at necropsy following CO_2 asphyxiation. Single cell suspensions of each spleen were prepared by pressing the organ through a fine mesh cloth after having first nicked the capsule several times with a sharp blade. Erythrocytes were lysed by addition of an ammonium chloride solution (Sigma Chemical Co., St Louis, Mo.). Splenic lymphocytes (2×10^5) from individual rats were incubated for three days with mitogens (pokeweed [PWM], phytohemagglutinin [PHA], and concanavalin A [conA]; Sigma Chemical Co.), with or without recombinant human interleukin 2 (IL-2; Boehringer Mannheim, Indianapolis, Ind.) in 96-well U-bottomed plates (Costar, Corning Incorporated Life Sciences, Acton, Mass.) for three days at 37°C in a CO_2 incubator. Culture medium (RPMI-1640; Life Technologies, Rockville, Md.) was supplemented with 5% autologous rat sera obtained at the time of necropsy. Plates were pulsed for 4 h with 0.5 μCi of [^{3}H]thymidine (NEN Life Science Products, Inc., Boston, Mass.), and the DNA was harvested onto filter pads for liquid scintillation counting. Stimulation indices (SI) were calculated as counts per minute (CPM) of stimulated cultures divided by CPM of medium controls. All determinations were performed in triplicate.

Experimental design and analysis of data. Rats were fed the test diet two weeks prior to sleep deprivation and continued to consume the diet throughout the stress period. Control rats were housed in the same rooms, but were not sleep deprived. For time course experiments, control rats were sacrificed at the same time points as were sleep-deprived rats. Four rats were included in each group, and splenocyte cultures for each rat were set up in quadruplicate. Each experiment was performed thrice. Results represent the average CPM for the individual lymphocyte cultures from four rats. Results were analyzed, using one-way analysis of variance with post-hoc analysis and the Student Neuman Keuls multiple comparison test.

Results

Sleep deprivation resulted in a time-dependent decrease in the lymphoproliferative response to the test mitogens. Rats fed an LF diet were subjected to sleep deprivation of various durations. Statistically significant suppression of the response occurred following 48 h of sleep deprivation (Fig.1). Additional decrease was not evident after 72 h of sleep deprivation. Addition of recombinant IL-2 to the cultures failed to overcome the adverse effect of sleep deprivation on the proliferative response (Fig. 2).

Dietary fat affected the lymphoproliferative response to mitogens. As seen in Fig. 3, and reported by others (18), there was a significant ($P < 0.001$) difference in [^{3}H]thymidine incorporation by the PHA-stimulated splenocytes collected from rats fed the MO diet. Addition of IL-2 to the cultures enhanced the overall level of [^{3}H]thymidine incorporation among all groups, although that for the MO group remained lowest. Similar results were obtained with use of the other two mitogens (data not shown).

Dietary fat alters sleep deprivation inhibition of the lymphoproliferative response to mitogens. To determine the effect of diet on the SD-induced suppression of the lymphoproliferative response to PHA, rats were pre-fed the diets for two weeks, and were either sleep deprived for 48 h (SD) or were kept in their cages (CC). Again there was a significant ($P < 0.05$) dietary effect on the lymphoproliferative response of the CC rats fed the MO diet (Fig. 4). Sleep deprivation of the rats fed the LF diet resulted in characteristic suppression of the lymphoproliferative response to the mitogen ($P < 0.05$). By contrast, the mitogen responses of the rats fed the other high-fat diets were not affected by the sleep deprivation.

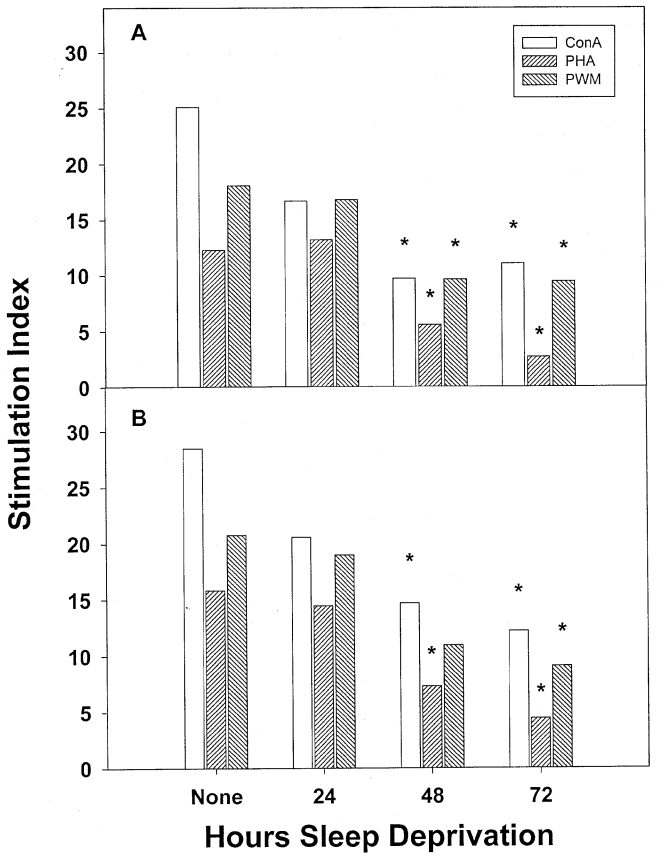

Figure 1. (A) Sleep deprivation results in an inhibition of the proliferative response to mitogens. Splenocytes were obtained from rats after 0 to 72 h of sleep deprivation. The cells were incubated in vitro with the various mitogens and pulsed for four hours with [^{3}H]thymidine. The results represent the average of individual cultures of four rats per treatment. Significance was determined at the $P < 0.05$ level by use of analysis of variance (ANOVA). (B) Supplementation of the cultures with interleukin 2 (IL-2) fails to overcome the effect of sleep deprivation. Rats were sleep deprived for 0 to 72 h and their splenocytes were incubated with mitogens, as before. Recombinant IL-2 (10 U/ml) was added to the cultures at the onset. The results represent the mean of individual cultures of four rats per treatment. Significance was determined at the $P < 0.05$ level by use of ANOVA.

Discussion

Overall, our results indicate the importance of dietary composition on immune responses and stress-induced immune modulation. Most rodent diets currently available through commercial sources contain low proportions of dietary fat (4 to 10% by weight). While this level will meet the daily energy requirements and the n-6 requirement of non-lactating rats, it does not result in maximal growth rate, as better results are obtained with diets containing 40% (22). However, multiple adverse health effects accompany the higher percentage fat diets and, thus, the lower fat concentrations are more widely used and recommended (22, 23). Diets rich in PUFA, especially n-3 PUFA, are anti-inflammatory and immunosuppressive (11, 18). As previously reported (11, 18), the splenocytes from the MO diet-fed control rats in this study also had decreased proliferative responses to the mi-

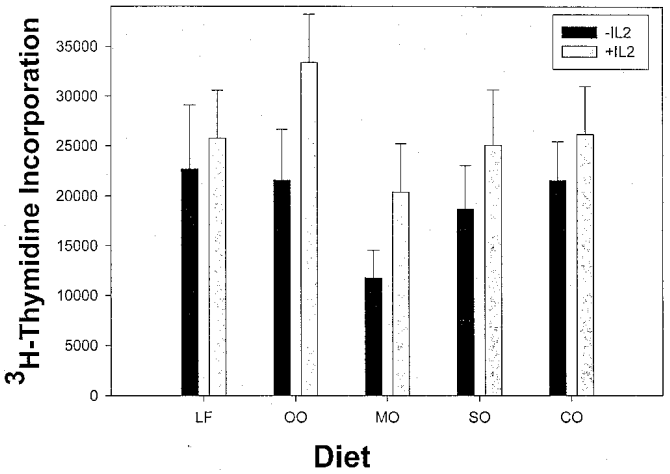

Figure 2. Dietary fat alters the proliferative response to phytohemagglutinin (PHA) and is unaffected by addition of exogenous IL-2. Rats were fed either a low-fat diet (LF), a safflower oil diet (SO) high in n-6 polyunsaturated fatty acids (PUFA), an olive oil diet (OO) high in monosaturated fatty acids, a coconut oil diet (CO) high in saturated fatty acids, or a menhaden oil (MO) diet high in n-3 PUFA. Following two weeks of consuming the diet, rats were sacrificed and their splenocytes were incubated with the PHA for 72 h in the presence or absence of IL-2 (10 U/ml). The results represent the mean (± SEM) of four rats per group.

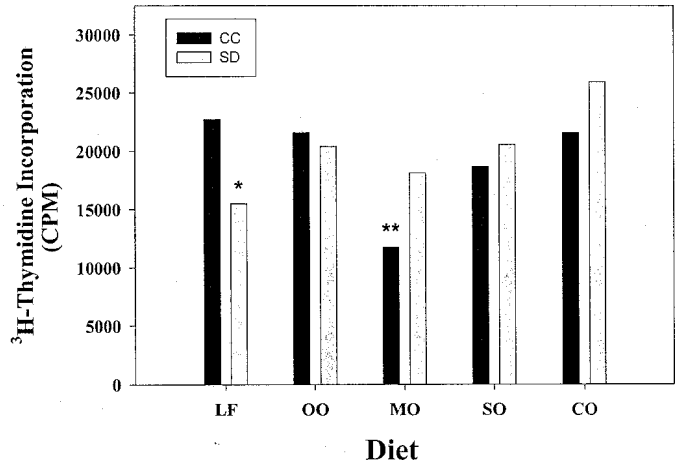

Figure 3. Dietary fat alters the effect of sleep deprivation on the proliferative response to mitogens. Rats were fed their respective diet two weeks prior to 48 h of sleep deprivation and continued to consume the same diet while being sleep deprived. Cage controls (CC) were fed the same diets, but were not sleep deprived. Splenocytes were incubated with PHA and pulsed with [^{3}H]thymidine, as previously described. *Significantly ($P < 0.05$) different from values for CC; **significantly ($P < 0.05$) different from values for LF group.

togens. This effect was most apparent when the splenocytes were cultured in media containing autologous sera, though similar results were obtained for the cells cultured in medium containing fetal bovine serum (FBS [data not shown]). Results of a previous study had indicated an even greater effect of FBS on these responses (24).

Though the MO diet decreased the lymphoproliferative response of the cage-control rats, it also prevented suppression of the proliferative response by sleep deprivation. The effect of sleep deprivation on immune responses is itself controversial (25, 26). Modest disturbances of sleep induce a reduction of natural killer (NK) cell activity and lymphocyte proliferative responses in humans (27, 28). Although there is a report of sleep-deprivation exacerbating viral infections (29), others failed to find a similar association (30, 31). We report that rats fed LF diet for two weeks then subjected to sleep deprivation developed signs of SD-induced immunomodulation, as evidenced by decreased in vitro lymphoproliferative responses to the mitogens tested. Rats fed the high-fat diets did not have suppressed lymphoproliferative response following sleep deprivation.

These results are similar to reports of dietary supplementation with n-3 PUFA preventing immunosuppression after surgical (20, 32) and burn trauma (33). Given the immunosuppressive nature of these diets, such protective effect seems paradoxical. Nevertheless a similar effect of dietary fat on exercise-induced immune modulation also has been reported (34).

The mechanism of dietary fat effects on immune function remains uncertain. While eicosanoid pathways can be altered by increasing n-3 PUFA, it is also clear that these fatty acids can elicit their effects by eicosanoid-independent mechanisms (10). Alterations in CD^4-to-CD^8 ratios have been associated with feeding diets high in n-3 PUFA (16). Although we did not examine lymphocyte subset distributions in the current study, future studies will address this question. Likewise possible effects of the diet on macrophage function were not assessed, though others have reported significant alterations (35). Analysis of IL-2 receptor expression by use of flow cytometry indicated no differences in receptor expression following mitogen stimulation between any of the sleep-deprived groups or the controls (data not shown). Since IL-2 receptor expression is a sign of cellular activation and thus consistent with the successful engagement of the T cell receptor-signaling apparatus, it is likely that the suppression of the proliferative response we observed in this study involves a later step in this response.

The mechanism of the immunoprotective effect of n-3 PUFA also remains controversial (36, 37). Alteration in eicosanoid pathways caused by substituting n-3 for n-6 PUFA could account for some of the effects observed here and elsewhere (38). Since n-6 PUFAs play a significant role as precursors of those eicosanoids that induce immunosuppression, platelet aggregation, and excess or chronic inflammation in acute stress responses, increasing n-3 PUFA content could give rise to those eicosanoids that are less inflammatory and may, thus, limit severity of the stress response (38). Since we observed an immunoprotective effect with use of all of the high-fat diets, including the SO diet (high in n-6 PUFA content), it seems unlikely that alterations in eicosanoid pathways accounted for the effects we observed. The precise mechanism remains unknown and is the subject of current investigation.

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