Cantaurus, Vol. 4, 25-28, May 1996 © McPherson College Division of Science and Technology

Immunosuppression in Mice Consuming Elevated Levels of Dietary Saturated Fat

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ABSTRACT

It is known that lipids play a vital role in the human body. Different studies have shown that fats play an essential role in immune function. Alterations in the quality and quantity of dietary fat have been reported to influence the regulation of the immune system. The purpose of this study was to examine the possible immunosuppressive effects of mice ingesting diets high in saturated fat (coconut oil) for a period of 100 days. On day 93 all mice in both groups were injected with 1% chicken albumin in PBS (pH 7.2). Total white blood cell counts were done using a hemacytometer. Spleens were removed and weighed; lymphocytes were counted thereafter. It was found that there was a significant difference in white blood cell counts between the two groups. The control group, which consumed a 5% saturated fat diet had a mean white cell count of 2.708×10^6 cells/ml. The test group, which consumed a 30% saturated fat diet had a mean white cell count of 0.161×10^6 cells/ml. There was no significant difference between the mouse weight to spleen weight ratios of the two groups. Lymphocyte counts of the two groups were not found to be statistically significant. It was found that significant increases in dietary saturated fat does have an impact on white blood cell production in the laboratory mouse.

INTRODUCTION

Lipids play a vital role in the human body. Fatty acids constitute most of the lipids in food and in the body. The fatty acid consists of a long chain of carbons bonded to hydrogen atoms. If all of the bonds between the carbons are single bonds the fatty acid is referred to as being saturated (Wardlaw, 1993). It has been shown that the effect of fat on the immune system depends on the type of fat consumed, the duration of feeding, and the organ examined (Locniskar, 1983).

Different studies have shown that fats play an essential role in immune function. Alterations in the quality and quantity of dietary fat have been reported to influence the regulation of the immune system (Locniskar, 1983). The spleen (a major producer of lymphocytes) is an important site of antibody formation. It has been observed that substantial amounts of antibody are formed in areas where clusters of antibody forming cells come in contact with an antigen (Kabat, 1976). Increased fat intake has been shown to effect rat spleens by causing severe hyperplasia (Locniskar, 1983). Hyperplasia is an abnormal increase in the elements composing the cells of a tissue.

Essential fatty acids are those that cannot be made by the body and have to be obtained elsewhere (Wardlaw, 1993). Essential fatty acid deficiency can result in flaky, itchy skin, sores on the scalp, and retarded growth. Fatty acid deficiency reduces primary and secondary antibody responses. Reduction in primary antibody response would affect the initial encounter of an antigen-specific lymphocyte with the antigen. The overall effect would be a slower immune response due to the increased time span between the primary and secondary antibody responses (Kuby, 1994). The secondary response is the second contact with the antigen and causes an even greater immune response, but without the essential fatty acids this would be retarded as well.

It is unknown what impact saturated fat has on the immune system. We do know that it is a primary factor in those people suffering from heart disease (Wardlaw, 1993). We also know that major dietary changes can alter resistance to diseases in humans (Fernandes, 1994).

The purpose of this research is to understand more about the effects of saturated fat, specifically how elevated levels of saturated fat affect the immune system. We hypothesize that elevated levels of dietary saturated fat (high fat 30.26% saturated fat and normal fat 5.27%) introduced into the body over long periods of time will have a detrimental effect on the immune functioning of laboratory mice, and that high levels of saturated fat will cause a significant decrease in white blood cell production.

MATERIALS AND METHODS

In this experiment 24 female weanling CD1 mice (Charles River Laboratories Inc., Portage, MI) were randomly divided into two even groups. Each mouse was placed in its own cage. Group one was fed a control diet (TD 95146) consisting of 5.27% saturated fat (coconut oil). Group two was fed a diet (TD 95147) consisting of 30.26% saturated fat (coconut oil). Both diets were specially designed by and obtained from Harlan Teklad (Madison, WI). Both groups were fed their assigned diets for 100 days and tap water was provided ad libitum. Weights were taken on day one and every ten days thereafter.

On day 93 the mice in both groups were injected subcutaneously in the dorsal region with 1% chicken ovalbumin (Grade II) in PBS (pH 7.2). Injections were administered at 0.06ml/30g mouse weight. On day 101 all mice were injected with 1ml of 0.9% sodium heparin prior to sacrifice. Immediately after sacrifice blood samples were drawn and placed in vaccutainers.

Spleens were removed from all mice, weighed, and placed in individual vaccutainers containing Hank's solution. The following analyses were then performed.

Total white blood cell counts were done using a hemacytometer and the results were recorded. Hemagglutination was used to study the serological aspects of the antigens. This assay is used to examine the serological aggregation of particulate antigens by a specific antibody. The aggregation of the particles is due to the formation of a lattice structure between the particles and antibody bridges. Each antibody molecule reacts with two antigenic sites, and, if these sites are located on different particles, sufficient reactions will cause a physical aggregation large enough to be visualized (Burrell, 1987). For each blood sample the following was performed: 0.08, 0.04, 0.02, 0.01, or 0.005ml of antiserum was placed in each assay well. To each well was added one drop (approximately 0.03ml) of antigen. The wells were observed for agglutination and the results were recorded.

Finally, spleens were processed to examine the lymphocytes. This was done by first extracting the spleen and placing it in 3ml of Hank's solution. The spleen was then ground into a single cell suspension by first grinding the spleen between two slides and placing it in Hank's solution once again. The suspension was then transferred to a clean test tube and allowed to settle. The supernatant was removed and the cells were counted using a hemacytometer. The results were observed and recorded.

Statistical testing was performed using the following tests. For the statistical significance of the white blood cell counts we used the Mann-Whitney Rank Sum Test ($\alpha = 0.05$). The splenic weights, the splenic white blood cell counts, and the spleen weight to body weight ratios were analyzed using a standard t-test. . The two weight gain curves were compared using the Kolmogorov-Smirnov test ($\alpha = 0.05$). The total white blood cells per milligram of spleen were analyzed for statistical significance using the Mann-Whitney Rank Sum Test ($\alpha = 0.05$).

RESULTS

White blood cell counts indicate that increased levels of dietary saturated fat have an immunosuppressive response on mice. The white blood cell counts were significantly different (Mann-Whitney Rank Sum Test, $P = <0.0001$). Mice consuming 5.27% saturated fat had a mean white blood cell count of 2.708×10^6 cells/ml. Whereas the mice consuming 30.26% saturated fat had a mean white blood cell count of 0.161×10^6 cells/ml (Figure 1). The splenic white blood cell counts were not statistically significant. The mean WBC count of the 5.27% group was 1.256×10^6 cells/ml and of the 30.26% group the mean WBC count was 0.902×10^6 cells/ml (Figure 1). Suppressed levels of white blood cells indicate that when an antigen was introduced, the mice consuming 30.26% dietary saturated fat were not able to fight off disease as well as the mice that were subjected to a normal dietary saturated fat level of 5.27%.

The splenic weights of the two groups were statistically significant (t-test, $P = 0.0192$). The mean weight of the 5.27% group was 0.104g, and the mean weight of the 30.26% group was 0.1325g (Figure 2). Analysis of the total white blood cells per milligram of spleen showed that there were more white blood cells produced per mg of spleen in the 5.27% diet than in the 30.26% diet (Figure 4). The mean white blood cell count per mg of spleen in the 5.27% group was 4.209×10^4. In the 30.26% diet the mean count per mg of

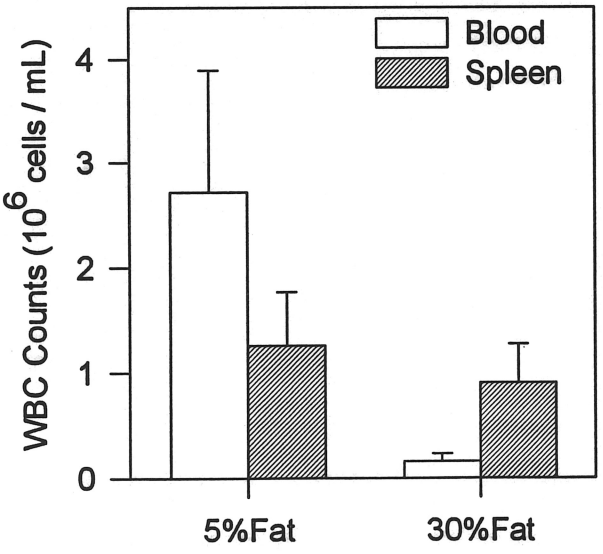

Figure 1. White Blood Cell counts (WBC) from blood and spleen samples of mice fed diets containing 5% or 30% fat for 100 days.

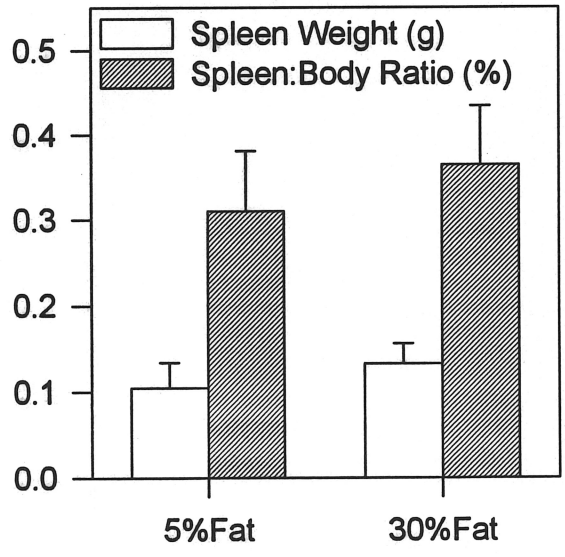

Figure 2. Spleen weights and spleen-to-body weight ratios for mice fed diets containing 5% or 30% fat for 100 days.

spleen was 2.007 X 10^4.

Consumption of a diet containing 30.26% saturated fat had no significant influence on body weight gain. The mean weight of the 5.27% diet was 33.63g and the mean weight of the 30.26% diet was 36.69g (Figure 3). Weight gains of the two groups over the 100 day period were not significantly different (Kolmogorov-Smirnov test, P = 0.076).

No correlation was found between the weights of the mice and their individual spleen weights (Figure 2). This leaves us to assume that increased saturated fat levels had no impact on splenic weights, however, it is possible because the difference in mean values of the two groups is not great enough to reject the possibility that the difference was due to random sampling.

Examination of the total number of white blood cells per mg of spleen in both groups showed that there was a significant difference (Figure 4). There was a suppressed level of white cells in the 30.26% fat group even though the spleens in the 30.26% group weighed more and produce on the whole, less white blood cells than the 5.27% group. When these counts were examined on the per mg of spleen basis it was clear that less cells per mg of spleen weight were produced in the 30.26% group which is what we had initially hoped to see.

The hemagglutination assay was inconclusive. Therewere no observable results; therefore there was no way to tell what kind of antibody response was present, if any. The protocol that we used in performing the hemagglutination did not specify a certain antiserum dilution, which is of great importance. If the proper dilution is not used then no precipitate will form, which is what we assume happened here. By the time this was discovered it was too late to perform another assay.

We calculated the required sample size for the significance of the t-Test given the observed means and variances, and $\alpha = 0.05$. From this we concluded that a sample size consisting of 55 mice would be needed in each group for the observed differences to be statistically significant.

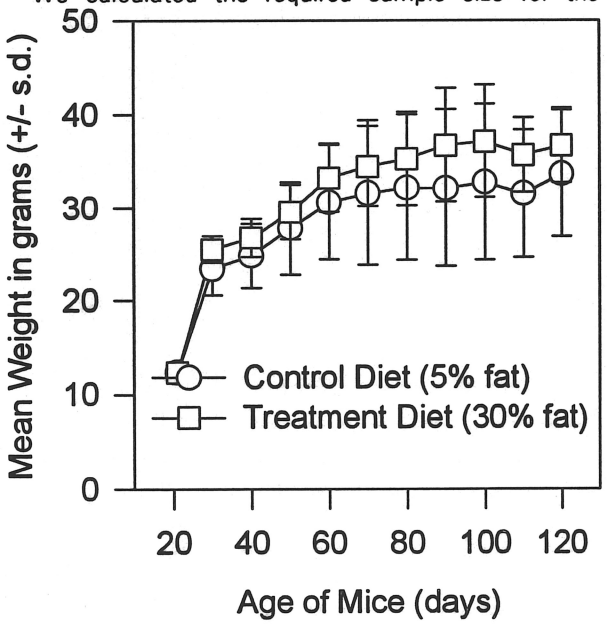

Figure 3. Mean weight gain of mice feds diets containing 5% or 30% fat for 100 days.

DISCUSSION

According to the Nutrition Committee of the American Heart Association, 30% of calories should come from fat and only 10% of daily calories should come from saturated fat. However, the typical U.S. diet contains 38% of calories from fat and 15.2% of the daily calories come from saturated fat (Wardlaw, 1993). The usage of saturated fat int he United States has been greatly reduced. The consumption of monosaturated and polyunsaturated fats, however, has either increased or people have substituted them in place of the saturated fats that they would normally consume.

Lymphocytes are the white blood cells responsible for the immune response (Wardlaw, 1993). There are many different kinds of white blood cells, and each one can tell more specifically the type of immune response that is taking place. The various types of lymphocytes include neutrophils, eosinophils, basophils, monocytes and lymphocytes. Granulocytes include neutrophils which make up 40-75% of the white blood cells in blood, eosinophils make up 1-6% and basophils <1%. The other class of white blood cells are agranulocytes these include lymphocytes which make up 20-45% of the white blood cells, and monocytes which constitute 2-10% of the white blood cells (Wheater, 1990). The amounts of these types of cells demonstrate at what level a specific immune response is occurring. Had a cell counter been available, the blood samples would have been counted differentially and a more detailed

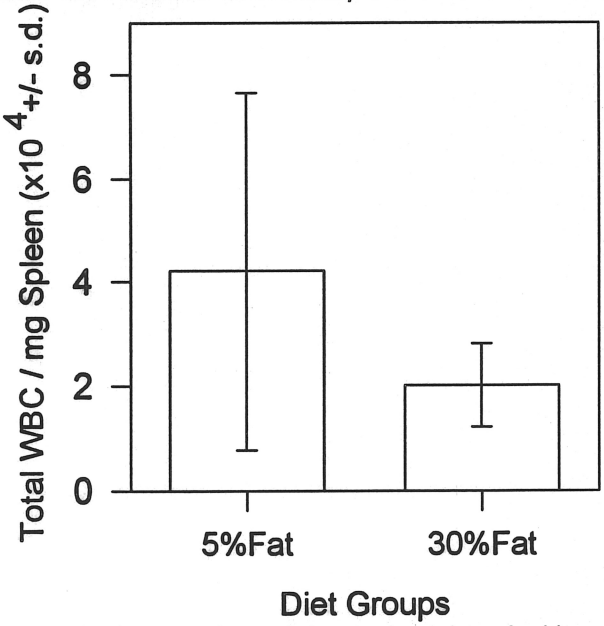

Figure 4. A comparison of the total number of white blood cells per mg of spleen for the 5% saturated fat and 30% saturated fat treatment groups.

assay on immune response would have been provided for study and analysis.

All literature studied was consistent with regard to weight gain. All studies supplied fats through diet. There were no statistical differences in animal growth rate in the dietary groups. The weights of the rodents at time of death were not significantly different as well.

Data obtained from these types of studies are often conflicting and difficult to interpret due to differences in the assay systems and the route of fat administration. Another conflicting factor shows that exogenous fatty acids inhibit or stimulate the response of lymphocytes to mitogens depending on the quality and quantity of fatty acid and the serum supplement used (Locniskar, 1983). Dietary lipids and antigens can modify the immunological function and can trigger or accelerate the disease processes (Fernandes, 1994).

This research differed considerably from that of other scientists who examined similar saturated fat questions. The major thing that this research focused on was that of suppressed white blood cell production and total white blood cell counts. Other research, such as that by G. Fernandes, looked at serum anti-dsDNA antibody levels, immune parameters and expression of oncogenes, TGFβ1 mRNA levels in the spleen, and antioxidant enzyme mRNA levels in the liver as well as a variety of other assays (Fernandes, 1994). Another study determined mitogen transformation response and cultured lymphocytes harvested from the spleen. They also harvested lymphocytes from he spleen and MLN to determine lymphocyte transformation response (Locniskar, 1983).

These different techniques produced much more detailed results. By looking at more specific details rather than just counting white blood cells the researchers were able to more accurately pinpoint the specific immune responses that were occurring, as a result of the treatment administered.

Manually counting white blood cells was not an entirely meaningful way to determine whether or not high levels of dietary saturated fat cause an immunosuppressive response in mice. However, it was a good initial representation of suppressed immune functioning.

Future research would put more focus on examining the more minute and significant aspects of immune function. The hemagglutination assay that was done in this research would definitely be repeated, and there would be four groups of mice instead of two (the other two not receiving injections of chicken ovalbumin). Another study that would be included in future research would be the examination of mitogen transformation response and histopathology of the spleens.

Alteration in the quality and quantity of dietary fat has selective and varying effects on the systemic and regional immune response (Locniskar, 1983). As was found with this research, the different assays which were performed show that the results vary as well as the methods and assays performed when compared to other studies. More detailed studies are necessary in order to determine if the results from this research were primarily immunosuppressive or not.

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ACKNOWLEDGEMENTS

The author would like to thank Dr. Jonathan Frye for all of his help and continuous enthusiasm throughout the past year. I would also like to thank Monica E. Embers for her assistance in the laboratory.