

AD-A097 422

NORTH CAROLINA STATE UNIV RALEIGH

INFLUENCE OF GLYCERIDE STRUCTURE AND FATTY ACID COMPOSITION ON --ETC(U)

FEB 81 S B TOVE

DAAG29-78-6-0006

F/6 6/1

NL

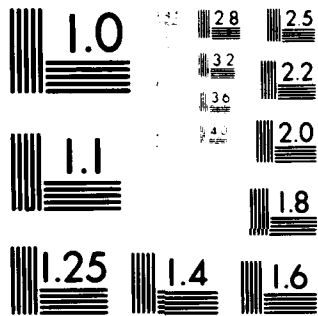
UNCLASSIFIED

ARO-14728.2-L

1 of 1
AD-A097 422



END
DATE
FILMED
5-81
DTIC



MICROCOPY RESOLUTION TEST CHART
NATIONAL BUREAU OF STANDARDS-1963-A

LEVEL

TITLE

✓ ARO 14728.2-L-

(18)

"Influence of Glyceride Structure and Fatty Acid Composition on Fat Nutrition"

TYPE OF REPORT (TECHNICAL, FINAL, ETC.)

Final Report

AUTHOR (S)

Samuel B. Tove

DATE

February 25, 1981

SECRET

U. S. ARMY RESEARCH OFFICE

CONTRACT/ GRANT NUMBER

DAAG29-78-G⁰006

INSTITUTION

North Carolina State University
Raleigh, NC 27650

APPROVED FOR PUBLIC RELEASE;
DISTRIBUTION UNLIMITED.

81 8 24 081

AD A 097422

DTIC FILE COPY

13V3

THE VIEW, OPINIONS, AND/OR FINDINGS CONTAINED IN THIS REPORT ARE THOSE OF THE AUTHOR(S) AND SHOULD NOT BE CONSTRUED AS AN OFFICIAL DEPARTMENT OF THE ARMY POSITION, POLICY, OR DECISION, UNLESS SO DESIGNATED BY OTHER DOCUMENTATION.

Unclassified

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

REPORT DOCUMENTATION PAGE		READ INSTRUCTIONS BEFORE COMPLETING FORM
1. REPORT NUMBER FINAL REPORT	2. GOVT ACCESSION NO. AD-A097 422	3. RECIPIENT'S CATALOG NUMBER 9
4. TITLE (and Subtitle) Influence of Glyceride Structure and Fatty Acid Composition on Fat Nutrition		5. TYPE OF REPORT & PERIOD COVERED Final Report. 5 Oct 77- 10/5/77 - 12/31/80 / 31 Dec 77
6. AUTHOR(s) Samuel B. Tove	7. AUTHORING OR PERFORMING ORGANIZATION NAME AND ADDRESS North Carolina State University Raleigh, NC 27650	8. CONTRACT OR GRANT NUMBER(s) DAAG29-78-G-008 04 20
9. PERFORMING ORGANIZATION NAME AND ADDRESS North Carolina State University Raleigh, NC 27650	10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS	11. CONTROLLING OFFICE NAME AND ADDRESS U. S. Army Research Office Post Office Box 12211 Research Triangle Park, NC 27709
12. REPORT DATE 2/24/81	13. NUMBER OF PAGES 7	14. MONITORING AGENCY NAME & ADDRESS (if different from Controlling Office)
15. SECURITY CLASS. (of this report) Unclassified		15a. DECLASSIFICATION/DOWNGRADING SCHEDULE
16. DISTRIBUTION STATEMENT (of this Report) Approved for public release; distribution unlimited		
17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report) NA		
18. SUPPLEMENTARY NOTES The view, opinions, and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy, or decision, unless so designated by other documentation.		
19. KEY WORDS (Continue on reverse side if necessary and identify by block number) Saturated Fat, Toxicity, Pneumonitis, Unsaturated Fat		
20. ABSTRACT (Continue on reverse side if necessary and identify by block number) We have shown that feeding palmitoyl glycerol to weanling mice results in death within a few days or poor growth in the survivors. Adding small amounts of unsaturated fats will prevent the toxicity. Free palmitic acid is not as toxic as palmitoyl glycerol, and the position of the palmitate on the glycerol does not affect its toxicity. Although linoleic and γ-linolenic afford the most protection, oleic acid is also protective. Changing the chain length, position of the double bond or its configuration greatly		

DD FORM 1473 EDITION OF 1 NOV 65 IS OBSOLETE

Unclassified

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

259300

4pg

Unclassified

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

decreases or eliminates the protective effects of unsaturated fat. Animals that die show a severe inflammatory reaction in their lungs that we have termed interstitial pneumonitis. The interstitium of the lungs is so filled with macrophages and leukocytes that severe alveolar stasis and collapse occurs, resulting in death, probably from congestive heart failure. In collaboration with Dr. Ralph Snyderman of Duke University, we have shown that extracts of the affected lung contain a compound(s) that is chemotactic toward murine macrophages. Although the precise mechanism behind the toxicity is unknown, present evidence points to a defect in the metabolism of arachidonic acid.

Accession For	
DTIS GRA&I	☒
DTIC TAB	☐
Unannounced	☐
Justification	
By _____	
Distribution/	
Availability Codes	
Dist	Avail and/or Special
A	

Unclassified

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

ABSTRACT

The triacylglycerols of milk fat of most animals have the majority of the palmitate at the 2-position, whereas the triacylglycerols of the other tissues have the majority of the palmitic acid at the outer positions. For example, in mouse milk more than half of the palmitic acid is found in the 2-position of the triacylglycerols, whereas the triacylglycerols of adipose tissue have only about 20% of the total palmitate esterified at the 2-position. This difference in distribution coupled with the age effect prompted an investigation to determine if palmitate at the 2-position would also be toxic. In order to block the rapid isomerization of a 2-monoacylglycerol to a 1(3)-monoacylglycerol, we acetylated the two open hydroxyl groups of sn-2-palmitoyl glycerol. As a control, we prepared diacetyl rac-1(3)-palmitoyl glycerol but found that this compound was much less toxic than rac-1(3)-palmitoyl glycerol. In contrast, diacetyl-sn-2-palmitoyl glycerol was as toxic as rac-1(3)-palmitoyl glycerol. Thus the position of the palmitate does not affect the toxicity of palmitoyl glycerol. In vivo absorption studies and in vitro experiments with pancreatic lipase indicated that the major protective effects of acetylating rac-1(3)-palmitoyl glycerol resulted from lipolysis of the acetylated palmitoyl glycerol to the less toxic free palmitic acid.

During all of these studies we had not identified the target tissue or immediate cause of death. Extensive histopathology by Dr. Robert Kanich, Director, Department of Pathology, Rex Hospital, Raleigh, NC, showed that the gastrointestinal tract, liver, heart, spleen, kidneys, adrenals, and brain were free of any pathological lesions. However, the lungs of those animals that died showed varying degrees of vascular congestion, edema, and interstitial infiltration with lymphocytes and histiocytes, and we have termed this constellation of changes interstitial pneumonitis. The spectrum of changes varied in extent and severity. A minimal extent would involve only focal areas of pulmonary tissue, while most extensive changes seen only in animals dying during the course of the experiment would involve entire lung lobes. Grading of the lesion was based upon the severity of the inflammatory changes, not upon the amount of pulmonary tissue involved, but generally the two correlated highly. The lowest grades of pathological changes would involve edema, slight capillary dilation, and an increased number of interstitial lymphocytes. The most severe changes were characterized with massive vascular dilatation and dense infiltration of the interstitium with histiocytes and lymphocytes. The alveolar spaces usually were obscured by the severe interstitial changes. In about 75% of the animals that die, the interstitium is so filled with macrophages and leukocytes that severe vascular stasis and alveolar collapse occurs, resulting in death, possibly from congestive heart failure. The onset of this syndrome is exceedingly rapid. Most animals succumb within 2-4 days after receiving the toxic diet and the histopathology has been observed nine hours after the mice were fed the diet. In rats, although death did not occur, a growth depression was encountered, and a focal interstitial pneumonitis was observed.

The adverse effects of the toxic diet do not appear to be associated with an infectious agent. We have not isolated or observed a bacterium or virus upon culture of the lung tissue and a wide spectrum of antibiotics (penicillin, tetracycline, streptomycin, sulfamethazine and sulfaguanidine) given parenterally or orally were without effect. Obviously these findings do not prove the absence of an infectious organism, but when coupled with the rapid onset of the toxic syndrome and its prevention by unsaturated fat, we are led to seek a more direct relationship between the toxic syndrome and palmitoyl glycerol.

Because palmitic acid is the major fatty acid in pulmonary surfactant, we considered the possibility of a connection between the toxicity and the pulmonary surfactant. However, this does not appear to be the case. Even just prior to death there are no overt signs of respiratory distress in that the mice do not exhibit the tachypnea which is characteristic of this process. Moreover, we have not observed the hyaline membrane expected for respiratory distress syndrome in the lungs of animals that die of the saturated fat toxicity. To further ascertain the absence of surfactant involvement, we examined the phospholipids of the lungs from mice 4.5 hours after gavage with rac-1(3)-[1-¹⁴C]-palmitoyl glycerol. The percentage of ¹⁴C of the lung phospholipids in phosphatidyl choline was $48 \pm 8\%$ (mean $\pm$ S.D.) for mice fed 30 mmoles of palmitoyl glycerol/100 g diet without safflower oil and $57 \pm 13\%$ when the diet was supplemented with 4% safflower oil. Moreover, there was no difference in the proportion of palmitate at the 2-position of the lung phosphatidyl choline from mice fed the two diets ($30 \pm 12\%$ vs. $29 \pm 12\%$).

The intense inflammatory infiltrate in the interstitium of the lung raised the question of whether the toxicity of palmitoyl glycerol might be due, at least in part, to the accumulation of some phlogistic product(s) in the lung which initiates the pneumonitis. This possibility led to collaboration with Dr. Ralph Snyderman of Duke University. We sought to determine if, after consuming the toxic diet, lungs from mice contained an extractable substance(s) capable of initiating the accumulation of inflammatory cells in vivo or initiating the chemotaxis of leucocytes in vitro. Homogenates of lung from mice fed 45 mmoles of rac-1(3)-palmitoyl glycerol/100 g diet for 6 hours did contain chemotactic activity for murine macrophages. In contrast, neither the palmitoyl glycerol itself nor lungs from animals fed stock diet before the toxic diet were chemotactic. Although some chemotactic activity was present in other tissues (liver, kidney) from mice fed palmitoyl glycerol, lung tissue had the greatest activity. Animals which received 4% safflower oil had no chemotactic activity in the lung but a marginal level was seen in the kidney and gut.

Preliminary results from a study on the properties of fatty acid unsaturation have provided some interesting leads on the mechanism behind the protective effects of adding certain unsaturated fatty acids to the diet. In these experiments a high level of palmitoyl glycerol was fed (60

mmoles/100 g of diet) in order to shorten the feeding period to one week. Since the unsaturated fatty acids were fed at the marginal protective level of 2%, some mortality occurred on all diets. The results showed that protection was afforded only when the double bond(s) was of the cis configuration. Of the monoene fatty acids, increasing the chain length (11-eicosaenoic acid), decreasing the chain length (9-hexadecenoic acid), and changing the position of the double bond (6-octadecenoic acid) reduced the protection as compared to oleic acid. With the polyene fatty acids, all were effective with 6,9,12-octadecaticenoic acid and 9,12,15-trienoic acid equaling the protective effects of linoleic acid, whereas 11,14-eicosadienoic acid was less protective. Taken as a whole, these results point to the possibility that a prostaglandin or fatty acid hydroperoxide is involved in the protective mechanism.

PUBLICATIONS:

1. Schnitzer-Polokoff, R., and Tove, S. B. (1979) "Toxicity of *rac*-1(3)-palmitoyl glycerol in weanling mice." J. NUTR. 109:1358-1367.
2. Schnitzer-Polokoff, R., Kanich, R. E., and Tove, S. B. (1980) "Interstitial pneumonitis induced by ingestion of palmitoyl glycerol." J. NUTR. 110: 2396-2408.

PARTICIPATING SCIENTIFIC PERSONNEL:

Robin Schnitzer-Polokoff	Ph.D.	1979
Rebecca Gooding	M. S.	(In Training)
Samuel B. Tove		

EN
DAT