Hyperlipidemia in Rats Fed Retinoic Acid

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ABSTRACT

This report describes a series of experiments that attempt to characterize the lipidemia accompanying retinoic acid administration. After feeding young adult male Sprague-Dawley rats, 1.2 Retinol Equivalents (R.E.) retinyl acetate plus supplemental retinoic acid (100 μg/g dry diet) for three days and fasting for 6-8 hr, triglyceride, cholesterol, and phospholipid content of various serum lipoprotein fractions were determined. When compared to unsupplemented controls, both the serum very low density lipoprotein (VLDL) and the high density lipoprotein (HDL) fractions of the retinoic acid-fed rats were found to harbor an elevated triglyceride content. While VLDL cholesterol and phospholipid content were also elevated, total serum cholesterol and phospholipids were not statistically altered. The detergent Triton WR-1339 was used to depress serum triglyceride clearance in order to assess the effects of retinoic acid feeding on serum triglyceride levels. Triglyceride accumulation started earlier after Triton treatment and was greater when rats were fed 100 μg/g retinoic acid for three days prior to testing. Red and white gastrocnemius muscle, cardiac ventricular muscle, and perirenal adipose tissue were removed from rats following retinoic acid feeding. Analysis of these tissues for lipoprotein lipase (EC 3.1.1.3) activity showed a decrease in adipose tissue, a large depression in both areas of gastrocnemius muscle and no change in cardiac muscle as a result of retinoic acid feeding.

INTRODUCTION

Several reports have appeared documenting hypertriglyceridemia in rats fed or injected with supplemental vitamin A or retinoic acid (1-3). Previous work by Erdman et al. (4,5) has pointed to alterations in hepatic lipid synthesis in vitro when either retinyl acetate, retinol, or retinoic acid was added to a liver homogenate system, resulting in increased incorporation of lipid precursors into triglycerides. Setty and Misra (6) fed supplemental vitamin A for two days and observed a decreased uptake of intraportally injected palmitate-1-14C by heart, kidney, and adipose, implicating an impairment in the uptake of plasma lipids by extrahepatic tissues.

The experiments reported here were performed to study the mechanism whereby all-trans retinoic acid feeding results in hyperlipidemia. Young adult male rats were fed 100 μg retinoic acid/g dry diet for 3 days; a regimen which has been shown to consistently induce hypertriglyceridemia (7). A lipoprotein profile of the sera was done to determine the triglyceride, cholesterol and phospholipid content of lipoprotein fractions. Several experiments assessed the effect of retinoic acid on serum triglyceride accumulation when Triton WR 1339 was used to depress extrahepatic triglyceride breakdown. In another experiment, lipoprotein lipase (EC 3.1.1.3) activity was measured in several key tissue types, as an indicator of extrahepatic capacity for triglyceride uptake.

MATERIALS AND METHODS

Animals and Diets

Male Sprague-Dawley rats (Harlan Industries, Inc., Cumberland, IN) were placed into individual stainless steel cages in a controlled temperature environment, with a light-dark cycle of 0600 to 1800 hr light and 1800 to 0600 hr dark. Rats were fed daily 6% corn oil, 22% casein, 22% sucrose, 44% starch, 2% agar, 4% mineral mix, and 1% vitamin mix as gel diet ad libitum at the start of the dark cycle. All ingredients were supplied by Teklad of Madison, WI. Preparation of the diet as well as the composition of the vitamin and mineral mix has been described previously (3,8). All diets contained vitamin A as retinyl acetate (Sigma Chemical Company, St. Louis, MO) at a level suggested by the NRC, 1.2 Retinol Equivalents (R.E.)/g diet (9). (One R.E. is equal to the biological activity associated with 1 μg of retinol.) Crystalline all-trans retinoic acid, (gift of Hoffmann-LaRoche, Inc., Nutley, NJ) was dissolved in a small amount of 95% ethanol and added to the diets as needed to achieve a concentration of 100 μg/g dry weight of diet. An equivalent amount of the carrier ethanol was added to control diets.

Rats were acclimated to the control gel diet for several days prior to the experimental feeding period. During the 3-day experimental period, rats were fed 1.2 R.E./g dry diet with or without 100 μg/g dry diet all-trans retinoic acid. In all experiments, rats were fasted 4 to 8 hr prior to serum or tissue collection. Serum was obtained via cardiac puncture under light ether anesthesia. Tissue samples for enzyme analysis were removed postmortem, rinsed with
saline, blotted dry, and immediately frozen in either liquid nitrogen or an acetone/Dry Ice mixture, whereas serum and liver samples were stored at -20 C until analysis. Lipoprotein separation (experiment 1) was done on fresh serum. The average consumption of retinyl acetate and retinoic acid in all experiments can be found in Table 1.

Experimental Design

**Experiment 1.** Twenty-four male rats, ca. 300-500 g, were randomly assigned to one of two groups, and fed either control or retinoic acid-supplemented diets for 3 days. Following a 6-8 hr fast, serum was taken and lipoprotein analysis was done on 4 pooled serum samples per group. The sera from 3 rats were pooled to provide an amount sufficient for lipoprotein separation and analysis.

**Experiment 2.** Two experiments were conducted to assess the effect of retinoic acid on Triton WR 1339-induced hyperlipidemia. In the first experiment (experiment 2A), 30 male Sprague-Dawley rats, between 300-350 g, were fed either the basal or the retinoic-acid-supplemented diet for 3 days. Rats were injected ip with 800 mg/kg body weight of Triton WR 1339 (Sigma Chemical Co., St. Louis, MO) in 0.9% saline. Each rat was sampled for serum twice, such that the rats sampled at the time of Triton injection were sampled again 4 hr later, one group at 2 and 5 hr post-Triton, and another at 3 and 6 hr. A second experiment (experiment 2B) using sixty 250- to 300-g male Sprague-Dawley rats was conducted similarly, but modified so that the rats sampled at the time of Triton injection were sampled again 1.5 hr later. At this time, another group was sampled, and then monitored again in 1.5 hr. This pattern was repeated up to 7.5 hr post-Triton.

**Experiment 3.** Twenty 400-g male Sprague-Dawley rats were assigned to groups and fed one of the two previously described diets for 3 days. Serum and tissues (perirenal adipose, cardiac ventricular muscle, and red and white gastrocnemius muscle) were removed after the rats were fasted 4 to 6-1/2 hr.

**ANALYTICAL PROCEDURES**

Lipids were analyzed by methods previously cited (3), with the exception of lipoprotein cholesterol and triglyceride analysis, which made use of Agent Clinical Chemistry Reagent (Abbott Laboratories, Pasadena, CA) (10) and Beckman Enzymatic Triglyceride Reagent (Carlsbad, CA).