SHORT COMMUNICATION

Effect of dietary linoleate content on the metabolic response of rats to *Escherichia coli* endotoxin

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Summary

1. Dietary fat influences many aspects of immune function. *Escherichia coli* endotoxin is a potent stimulator of interleukin 1 production from macrophages.

2. The present study examines the effect of feeding with fat diets rich (corn oil) and poor (coconut oil) in linoleate at high and low concentrations on responses to endotoxin.

3. Spleen phosphatidylcholine linoleate contents were higher in the corn oil than in the coconut oil group and arachidonate concentrations were highest in the group fed a high concentration of corn oil.

4. Coconut oil completely abolished the responses to endotoxin.

5. The inhibitory effects of coconut oil could largely be due to reduced prostaglandin and leukotriene synthesis.

Key words: dietary fats, eicosanoic acids, interleukin 1.

Abbreviation: IL1, interleukin 1.

Introduction

Far reaching metabolic effects occur as a consequence of exposure to bacterial endotoxins. Protein is lost from muscle, skin and bone, acute phase protein synthesis occurs in liver, serum zinc concentrations are depressed and glucocorticoid, insulin and glucagon concentrations are increased. Many of these actions have been ascribed to interleukin 1 (IL1), which is released from fixed and circulatory macrophages as a consequence of contact with endotoxins [1–3]. Dietary fat concentration and composition have been shown to modify immune function [4]. Dietary fat may influence immune function by producing alterations in the fatty acid components of membrane phospholipids, thereby altering the production of eicosanoids which are potent modulators of immune function [4]. As interleukin 1 is synthesized by cells of the immune system, its production and actions may thus be influenced by dietary fat concentration and composition. This study was undertaken to investigate this possibility.

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Methods

Animals and materials

Diets with high and low concentrations of polyunsaturated and saturated fatty acids were prepared in pelleted form. Four isoenergetic diets were prepared containing 20% of energy as protein in the form of casein supplemented with methionine. Vitamin and mineral mixtures [5] were added to the level of 2 and 4% respectively. The diets contained corn starch and sucrose in equal proportions and cellulose powder to a concentration of 10%. Two diets contained corn oil, at a concentration of 3 or 20%, and two diets, coconut oil, at concentrations of 2 and 19%. One per cent of corn oil was added to the latter two diets to prevent essential fatty acid deficiency.

Female Wistar rats from the Southampton Medical School animal colony were fed *ad libitum* for a 4 week period, before treatment with *Escherichia coli* endotoxin.

Animals were divided into control and test groups. Control groups received intraperitoneal

injections of sterile, non-pyrogenic saline (0.9% NaCl, 1 ml/kg body weight). Test groups received intraperitoneal injections of *E. coli* endotoxin (strain 055:B5, Difco). Two injections were given at 3 day intervals. The test group received doses of 400 μg/kg body weight initially and 200 μg subsequently. *E. coli* endotoxin injection produced reductions in food intake of approximately 50%, thus control groups were pair fed with test groups to eliminate the effect of reduced food intakes on the responses observed.

Rats were decapitated 24 h after *E. coli* endotoxin or saline treatment. Blood was collected, and the liver, pelt, a sample of thigh muscle and the tibia were rapidly removed and frozen in liquid nitrogen. Spleen was likewise collected from rats in the 20% corn oil and 2% and 19% coconut oil groups. Tissue samples and serum were stored at −20°C until analysed.

Analyses

Tissue samples were homogenized in 10% trichloroacetic acid. Precipitated protein was removed by centrifugation, washed with trichloroacetic acid (10 g/l) and dissolved in NaOH (0.3 mol/l) before measurement by the Folin Ciocalteau method [6]. Serum zinc concentrations and corticosterone were measured by atomic absorption spectroscopy and radioimmunoassay [7] respectively. Spleen phospholipids were extracted as described by Hyslop & York [8]. Phosphatidylcholine was separated by thin layer chromatography and after transmethylation its fatty acid composition was determined by gas–liquid chromatography.

Results

Significant differences occurred in spleen phosphatidylcholine fatty acid composition between the various dietary groups. The concentration of corn oil had effects in that the 20% group had significantly lower C16:0 (34.6 vs 47.7, $P<0.001$) and C16:1 (3.7 vs 6.7, $P<0.05$), and higher C18:0 (23.3 vs 12.6, $P<0.01$) and C20:4 (17.4 vs 10.2, $P<0.001$), than the 3% corn oil group. The 19% coconut oil fed group had significantly lower C16:1 (2.8, $P<0.01$), C18:0 (13.5, $P<0.01$), C18:2 (5.5, $P<0.001$) and C20:4 (9.3, $P<0.05$), and higher C16:0 (44.9, $P<0.001$) and C18:1 (23.3, $P<0.01$), contents than the 20% corn oil group. The 19% coconut oil group also showed significant differences from the 3% corn oil group, having a lower C16:1 (2.8, $P<0.05$), C18:2 (5.5, $P<0.001$) and higher C18:1 (23.4, $P<0.05$) concentration.

The effects of feeding corn and coconut oil on the responses to endotoxin are shown in Table 1. The normal responses of a decrease in protein concentrations in muscle, skin and bone, a decrease in serum zinc and a rise in liver protein and corticosterone occurred in the group fed a diet of high corn oil content (20%). In the group receiving a low corn oil diet (3%) the expected changes in protein occurred in response to endotoxin, while changes in serum zinc and corticosterone did not. Responses to endotoxin were completely absent in the groups receiving coconut oil.

Discussion

Our study indicates that the ability of macrophages to produce IL1, the responsiveness of target tissues to IL1, or a combination of both, may be affected by dietary fat.

The fatty acid composition of dietary fat appears to be more significant in modulating effects of endotoxin attributed to IL1 than the total amount of fat fed. It should be noted that modulation of the effects of endotoxin could be related to the lineolate content of each diet. Both coconut oil diets contained 1% corn oil. Thus, for diets containing 1% corn oil, no effects were seen, for those containing 3%, effects involving protein metabolism were seen, and, for diets containing 20%, the full range of effects was observed.

Eicosanoids are intimately involved in the action of IL1 on target tissues [2]. It is possible that eicosanoid metabolism may have been affected by the diets. Rats fed diets where 40% of the energy was from coconut oil exhibited a lower urinary excretion of 6-ketoprostaglandin $F_{1\alpha}$ and production of thromboxane B_2 in blood than animals receiving safflower oil [9]. Substantial changes in plasma phospholipid arachidonate and its precursor, linoleate, occurred. In our study, significant changes in spleen phospholipid fatty acid composition were achieved by feeding coconut oil, most notably a reduction in linoleic acid. Increasing the concentration of corn oil in the diet from 3 to 20% resulted in a 73% increase in the arachidonate content of phosphatidylcholine fatty acids.

On the evidence of inhibitor studies it would appear that the majority of the effects of endotoxin examined in the present study are mediated by prostaglandins, with the exception of the depression of serum zinc and increase in liver protein content. Studies using indomethacin [10], ibuprofen [11] and sodium salicylate [12] have indicated that depressed serum zinc and enhanced liver protein metabolism are independent of prostaglandins. Serum zinc depression would appear to be dependent on leukotriene production [13] but the gain in liver protein would appear to be independent of leukotrienes. It could thus be postulated that all the effects of fat on endotoxin actions described in the

TABLE 1. *Effect of corn oil and coconut oil diets on metabolic responses to E. coli endotoxin*

Results are given as means ± SE ($n = 6$ per group). *, †, ‡ indicate a significant difference from saline values by Student's t-test of $P < 0.05$, $P < 0.01$ and $P < 0.001$ respectively. Diets were fed 4 weeks as shown before intraperitoneal injection of endotoxin. Parameters were measured 24 h after injection.

Diet...	Corn oil				Coconut oil			
	3%		20%		2%		19%	
Injection...	Saline	*E. coli*	Saline	*E. coli*	Saline	*E. coli*	Saline	*E. coli*
Pelt weight (g/kg body wt.)	180 ± 9	161 ± 7	168 ± 5	154 ± 2*	164 ± 5	153 ± 1	163 ± 4	156 ± 3
Liver total protein (g)	1.8 ± 0.07	2.0 ± 0.04†	1.8 ± 0.11	2.2 ± 1.10†	1.8 ± 0.09	1.9 ± 0.09	2.0 ± 0.04	2.0 ± 0.05
Protein concentration (g/kg)								
Muscle (thigh)	182 ± 6	148 ± 2‡	183 ± 5	142 ± 2‡	185 ± 6	182 ± 2	178 ± 5	173 ± 6
Skin (abdomen)	133 ± 2	127 ± 1*	141 ± 1	132 ± 2‡	126 ± 1	127 ± 6	129 ± 3	129 ± 3
Tibia	64 ± 1	57 ± 1†	58 ± 2	55 ± 1*	58 ± 2	58 ± 1	59 ± 1	60 ± 2
Serum zinc (μg/ml)	1.48 ± 0.06	1.36 ± 0.07	1.33 ± 0.01	0.79 ± 0.04‡	1.17 ± 0.07	1.25 ± 0.09	1.01 ± 0.04	0.99 ± 0.03
Corticosterone (ng/ml)	450 ± 45	520 ± 44	278 ± 19	858 ± 37‡	563 ± 71	365 ± 74	443 ± 66	393 ± 73
Body weight pre-injection (g)	218 ± 4		211 ± 3		198 ± 4		209 ± 3	
Change in weight after injection (g)	−6 ± 2	−12 ± 3	−9 ± 1	−11 ± 2	−7 ± 1	−7 ± 2	−10 ± 1	−7 ± 1

present study, with the exception of changes in liver protein, could be accounted for by effects on membrane phospholipid fatty acid composition and subsequent eicosanoid metabolism. It has been suggested [14] that enhanced glucocorticoid production plays a permissive role in the increase in liver protein content after endotoxin treatment. The inhibitory effect of coconut oil would not therefore be due directly to prevention of an increase in corticosterone, but rather to reduce amounts of some other stimulator of hepatic protein content.

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