

Cholesterol Has an Inflammatory Influence in the Effects of Fats on Metabolism in Endotoxemic Rats

Received: June 29, 2001

Abstract: We showed that butter suppresses metabolic responses to endotoxin in Wistar rats. Responses included increases in plasma caeruloplasmin and liver zinc and protein concentrations, and decreases in plasma zinc and albumin concentration. However, butter lost its suppressive effect at dietary concentrations above 90g/kg diet. The present study examined whether this phenomenon was due to the total dietary cholesterol intake rather than the intrinsic fatty acid composition of butter. Rats received diets, for four weeks, containing either 100g/kg corn oil, 90 g/kg butter, 190g/kg butter, or 90 g/kg butter supplemented with cholesterol to render the latter two diets identical in cholesterol content. All butter diets contained 10g/kg corn oil. Rats received an 800 µg/kg sub-cutaneous injection of *E. coli* endotoxin. Twenty-four hour post-injection measurement of plasma, cholesterol, albumin, and zinc, and liver cholesterol, glutathione, protein, and zinc was performed. The values were compared with those obtained from pair-fed controls. In rats fed butter (90g/kg), endotoxin had no effect except on liver glutathione, which increased by 85%. In rats

fed corn oil, butter (190g/kg) or butter (90g/kg) supplemented with cholesterol, the liver concentration of zinc increased by 20, 21 and 18% respectively, and that of protein increased by 30, 29 and 23% respectively. Cholesterol increased by 56, 35 and 14% respectively. Glutathione increased by 230, 38, and 65% respectively. In rats fed the diet containing butter (90g/kg), endotoxin had no effect on any of the parameters measured in plasma. However, in the three other dietary groups described above, the zinc concentration decreased by 38, 29 and 19% respectively, albumin decreased by 28, 20 and 15% respectively, caeruloplasmin increased by 142, 83, and 83%, and cholesterol increased by 91, 50 and 51% respectively. The results suggest that butter exerts two opposing influences of inflammation: a pro-inflammatory effect due to its cholesterol content and an anti-inflammatory influence which may be due to its fatty acid composition.

Key Words: Cholesterol, endotoxin, rats, zinc, protein, lipid, metabolism.

¹Department of Nutrition and Dietetics, School of Health Technology, Hacettepe University, 06100-Sihhiye, Ankara, Turkey

²Institute of Human Nutrition, School of Medicine, Biomedical Sciences Building, University of Southampton, Southampton SO16 7PX-United Kingdom

Introduction

Dietary fats exert modulatory influences on a wide range of activities by which the immune system responds to inflammatory stimuli. Following infection or injury, pro-inflammatory cytokines, such as interleukins-1 and -6 (IL-1, IL-6), and tumour necrosis factor-alpha (TNF) are released from cells of the immune system, endothelial cells and fibroblasts. These molecules exert widespread metabolic effects which help to destroy invading pathogens, initiate healing and provide substrate to support the activities of the acquired immune system (1). These effects include fever, anorexia, acute phase protein production, enhanced visceral protein synthesis, muscle proteolysis, enhanced gluconeogenesis, hypertriglyceridaemia and altered plasma cation concentration.

Aspects of both the innate and acquired response are influenced by the types of fats consumed (1-3). The production of IL-1 and IL-6 TNF, and target tissue sensitivity to these mediators, are influenced by the n-9 monounsaturated (n-9 MUFA), and n-3 and n-6 polyunsaturated fatty acid (n-3 and n-6 PUFA) composition of fat in the diet (1).

In a previous study (4), it was demonstrated that a number of metabolic changes associated with inflammation were altered in intensity by the intake of fats rich in n-6 PUFAs and n-9 MUFAs. The increase in the synthetic rate and concentration of protein in the liver, lung and kidney, which followed endotoxin injection in rats, was enhanced when corn oil was the major fat source in the diet and suppressed when olive oil or butter

was the major source of this macronutrient. The latter two diets contained 10g/kg of corn oil to prevent essential fatty acid deficiency. A similar pattern of modulation occurred with respect to the anorexia, the fall in plasma concentrations of albumin and zinc and the rise in plasma caeruloplasmin concentration and liver zinc content which followed exposure to endotoxin. When diets containing 50, 100 or 200 g/kg of fat were fed, prior to an endotoxin injection, the intensity of the responses increased in concert with dietary fat concentration when corn oil was the dietary fat source. Diets containing predominantly butter suppressed responses only at the two lower levels of intake of this fat source. However, when olive oil was the predominant fat source, responses were suppressed at all levels of intake. Oleic acid (OA) has the ability to suppress responses to TNF; thus the substantial concentration of this fatty acid in olive oil and butter (~690 and ~210g/kg respectively) may explain the anti-inflammatory influence of these fats (5). The high concentration of the n-6 PUFA, linoleic acid (LA) (~480g/kg), despite the presence of substantial amounts of OA (~290g/kg) in corn oil, may explain its pro-inflammatory influence. The appearance of responses to endotoxin of substantial magnitude in animals fed butter at the highest concentration was not explicable in terms of the LA (7g/kg) or OA (44g/kg) content of the diet. Another component of butter must therefore be exerting a pro-inflammatory influence. In addition to providing fatty acids, butter is unique among the fats used in the previous study in containing cholesterol. Fleet et al. (6) showed that the addition of cholesterol to the diet of rabbits enhanced the expression of mRNA for IL1 and TNF in the aorta wall, when the animals received an endotoxin injection. *In vitro* studies on monocytes (7) showed that the inclusion of cholesterol in the incubation medium increased the expression of human major histocompatibility complex D sub-region products. Cholesterol has also been shown to increase the proliferative response of human peripheral blood lymphocytes to phytohaemagglutinin (8). The cholesterol contained in butter may therefore exert a pro-inflammatory influence when the fat is present in the diet in substantial amounts, thereby overcoming the anti-inflammatory influence of the oleic acid contained therein. The present study examined this hypothesis by feeding rats, prior to an injection of endotoxin, diets containing butter at concentrations which would permit or suppress (190 g/kg or 90 g/kg, respectively) a metabolic response,

or the latter diet to which cholesterol had been added to result in an intake of cholesterol similar to that of animals consuming the diet with the higher butter content. A small amount of corn oil (10g/kg) was added to each butter diet to prevent essential fatty acid deficiency. The study design also included animals which consumed a diet containing 100 g/kg fat in the form of corn oil, so that the influences of butter and corn oil at this level of fat intake could be compared.

Materials and Methods

Diets

The four pelleted diets (Table 1) contained respectively 100 g/kg corn oil (Corn oil diet), 90 g/kg butter (Low butter diet), 90 g/kg butter and 230 mg cholesterol (Low butter plus cholesterol diet), and 190 g/kg butter (High butter diet). The fat sources used were corn oil (CPC, Esher, Surrey, U.K.) and unsalted butter (Dairy Crest, Surbiton, Surrey, UK). All the butter diets contained corn oil (10 g/kg) to prevent essential fatty acid deficiency. The concentration of vitamin E in the synthetic diets was adjusted to 50 mg per kg of diet. The quantity of cholesterol added to one of the 90 g/kg fat butter diets was in an amount equal to the difference between the content of the sterol in the high and low butter diets. The low butter plus cholesterol and high butter diets contained 437 mg cholesterol/kg. In addition, all diets contained, per kg, 180 g casein, 3 g DL methionine, 100 g cellulose powder and 50 g of a vitamin and mineral mixture (Special Diet Services) to satisfy the animals' requirements (Special Diet Services, Witham, Essex, UK). The remainder of the diet was composed of an equal mix of sucrose and corn starch. Diets were stored at -20°C.

Animals and Experimental Protocol

All animal procedures were conducted in accordance with British Home Office regulations. Male weanling Wistar rats (weight 60 ± 3g) from the Southampton University Medical School colony were housed individually in stainless wire-mesh cages, in a humidity- (relative humidity 45 ± 5%) and temperature-controlled (23 ± 1°C) room. A diurnal light cycle of 12 hours light and darkness was maintained during the study. Initially, rats were fed standard laboratory chow (Special Diet Services, Cambridge, UK) for a three-day acclimatization period. After acclimatization, rats were randomly assigned, in groups of eight, to one of the four synthetic pelleted

Table 1. Dietary composition (g/kg)

Ingredients	Corn oil diet	Low butter diet	Low butter plus cholesterol diet	High butter diet
Casein	180	180	180	180
dl-methionine	3	3	3	3
Corn oil	200	10	10	10
Butter	0	90	90	190
Sucrose	233.5	283.5	283.5	233.5
Starch	233.5	283.5	283.5	233.5
Cellulose powder	100	100	100	100
Vitamin and mineral mix	50	50	50	50
Vitamin E	0.05	0.05	0.05	0.05
Cholesterol	0	0	0.23	0

diets. Rats were fed the diets *ad libitum*, with free access to tap water, for four weeks. Fresh diet was provided every other day and waste discarded. At the end of the feeding period, each dietary group was divided in two equal subgroups of four animals. One subgroup received a subcutaneous injection of 0.8 mg of *Escherichia coli* endotoxin/kg body weight (Difco, strain 055: B9). The other sub-group, after a delay of 24 h, with *ad libitum* access to food, received a subcutaneous injection of sterile non-pyrogenic saline (150 mmol/l) and was pair-fed the intakes of the corresponding endotoxin injected group, for a further 24-h period. Animals were killed by stunning and decapitation 24 h after injections. Blood was collected in heparinized tubes and placed on ice until centrifugation. Livers were rapidly removed, weighed and frozen in liquid nitrogen. Separated plasma and livers were stored at -70°C until analysis. Plasma was analysed for zinc, albumin, caeruloplasmin and cholesterol by atomic absorption spectroscopy, the bromocresol green method of McPherson and Everard (9), the oxidase activity method of Schosinsky *et al.* (10) and the method of Allain *et al.* (11) by using an analytical kit (Sigma Diagnostic Kit), respectively. Liver was assayed for zinc, protein and cholesterol content by atomic absorption spectroscopy (12), the bicinchoninic acid assay (13), and cholesterol methods, respectively. Liver was assayed for glutathione in fresh tissue immediately after dissection (14).

Statistical analysis

All results are expressed as the means and standard error of means (SEM) of each group. Data were analysed using three-way analysis of variance with treatment

(endotoxin) and the cholesterol content, and the type of fat as independent variables, followed by a Tukey test where interactions were considered significant. A probability of 5% or less was considered significant.

Results

Changes in body weight and food intake

Animals grew well on all diets and had similar body weight gains over the four weeks prior to injection. No significant differences in the food intake prior to injection were observed between the dietary groups. Mean daily food intakes (SEM) of animals receiving the high butter, or low butter plus cholesterol, or low butter, or the corn oil diets appeared to be 25.5 (± 1.0), 26.1 (± 0.7), 25.8 (± 0.7), and 25.9 (± 0.6) grams, respectively. At the end of the four-week period of feeding, no differences in body weights were observed between groups. Weight loss occurred during the 24 h after injection of endotoxin or pair feeding. The extent of weight loss which followed endotoxin injection was modulated by the type of fat contained in the diet (Table 2). A statistically significant weight loss of 6% occurred in animals fed the corn oil diet; however, in the groups fed butter diets, weight loss was not affected by the endotoxin injection. Injection of endotoxin resulted in a substantial decrease in voluntary food intake, the extent of which was modulated by the type and concentration of fat in the diet (Table 2). Loss of appetite was greater in animals fed the corn oil diet than in those receiving butter diets. Animals receiving the high butter, or low butter plus cholesterol diets exhibited a greater loss of appetite than those receiving the low

Table 2. Influence of Diets Containing Corn Oil, Butter and Butter Enriched with Corn Oil on Weight Loss and Food Intake Following Intake Following Injection of *E. coli* Endotoxin

	Injection	Corn oil diet 100g/kg	Low Butter Diet 90g/kg+	Low Butter Diet 90g/kg+plus Cholesterol#	High Butter Diet 190g/kg+
Pre-injection Weight (g)	Saline	297 ± 4 ^a	293 ± 4 ^a	287 ± 7 ^a	291 ± 6 ^a
	LPS	301 ± 2 ^a	301 ± 2 ^a	296 ± 5 ^a	302 ± 2 ^a
Post-injection Weight loss (g)	Saline	8 ± 0.5 ^a	5 ± 0.9 ^a	4 ± 1.2 ^a	4 ± 31.0 ^a
	LPS	17 ± 0.5 ^{**a}	8 ± 0.3 ^a	5 ± 0.9 ^a	10 ± 0.5 ^a
Food intake for 24 h post- injection(g)++	Both treatments	1.4 ± 0.2 ^a	3.2 ± 0.6 ^a	2.3 ± 0.1 ^a	2.2 ± 0.1 ^a

+ 10g corn oil added/kg diet to prevent essential fatty acid deficiency.

Additional cholesterol added so that diet contained identical amount to that in 190g/kg butter diet.

++ saline-injected animals in each dietary group were fed for final 24 h an intake identical to that consumed by LPS injected animal of the same dietary group.

Diets were fed for four weeks prior to subcutaneous injection of *E. coli* endotoxin (0.8 mg/kg). Values are means ± SEM (n= 4 per group).

*Significant difference between saline- and endotoxin-treated animals on the same diet (p< 0.05). Values on the same line not sharing the same superscript are significantly different (p< 0.05).

butter diet, although the values were not different statistically.

Plasma composition

The diets alone had no effect upon any of the parameters measured in plasma; however, the situation changed after endotoxin injection. Dietary fat type and cholesterol content altered the extent of the changes. In rats fed the corn oil diet, the low butter plus cholesterol diet and the high butter diet, values for plasma albumin and zinc were significantly decreased by endotoxin injection compared to saline-injected, control animals ($p<0.05$). The effect was greatest in the corn oil group, where a fall of 28 and 38% respectively in albumin and zinc occurred. Decreases of 15 and 19% respectively occurred in the animals fed the low butter plus cholesterol diet and of 20 and 29% respectively in the two parameters in rats fed the high butter diet (Table 3). Conversely, values for caeruloplasmin activity and cholesterol concentration were significantly greater than values in the control animals after endotoxin injection in rats from these three dietary groups ($p<0.05$). This effect was greatest in the corn oil group, where increases of 142 and 91% respectively occurred in the two

parameters. In the low butter plus cholesterol group, increases of 83 and 51% respectively occurred. Similarly, in the high butter group, increases of 83 and 50% occurred in the two parameters respectively (Table 3). All parameters were unchanged by endotoxin injection in animals receiving the low butter diet.

Liver weight, and protein, glutathione, zinc and cholesterol concentrations (Table 4)

Data for the saline-injected animals indicates that the various dietary treatments did not influence liver weight and changed composition only insofar as glutathione concentration. The animals receiving the low butter plus cholesterol diet and the high butter diet had a statistically significantly greater glutathione concentration than animals consuming the corn oil and low butter diets. Liver cholesterol concentration was influenced to a minor extent by the dietary intake of sterol. The mean values were highest in the low butter plus cholesterol and high butter groups but the values were not statistically greater than those in the low butter or corn oil groups. The period of reduced food intake experienced by these animals, however, may have modulated any influence of diet on liver composition.

Table 3. Influence of Diets Containing Corn Oil, Butter and Butter Enriched with Cholesterol on Plasma Composition of Rats Treated with *E. coli* Endotoxin

	Injection	Corn oil diet 100g/kg	Low Butter Diet 90g/kg+	Low Butter Diet 90g/kg+plus Cholesterol#	High Butter Diet 190g/kg+
Caeruloplasmin (Units/l)	Saline	38.7 ± 0.25 ^a	40.7 ± 0.52 ^a	43.1 ± 0.56 ^a	39.7 ± 0.35 ^a
	LPS	93.7 ± 0.44 ^{*a}	42.5 ± 0.36 ^d	78.7 ± 0.74 ^{*c}	86.3 ± 1.57 ^{*b}
Albumin (mg/ml)	Saline	39.1 ± 0.21 ^a	34.4 ± 0.24 ^a	35.2 ± 0.13 ^a	36.7 ± 0.15 ^a
	LPS	28.2 ± 0.45 ^{*c}	33.8 ± 0.12 ^a	30.6 ± 0.14 ^{*b}	29.4 ± 0.21 ^{*b}
Zinc (µg/ml)	Saline	2.60 ± 0.14 ^a	2.54 ± 0.16 ^a	2.45 ± 0.12 ^a	2.55 ± 0.10 ^a
	LPS	1.62 ± 0.13 ^{*c}	2.33 ± 0.12 ^a	1.98 ± 0.14 ^{*b}	1.82 ± 0.21 ^{*b}
Cholesterol (µmol/l)	Saline	2.85 ± 0.11 ^a	2.95 ± 0.10 ^a	3.05 ± 0.05 ^a	3.10 ± 0.10 ^a
	LPS	5.45 ± 0.15 ^{*a}	3.10 ± 0.10 ^c	4.60 ± 0.12 ^{*b}	4.65 ± 0.15 ^{*b}

+ 10 g corn oil added/kg diet to prevent essential fatty acid deficiency.

Additional cholesterol added so that diet contained identical amount to that in 190g/kg butter diet.

Diets were fed for four weeks prior to subcutaneous injection of *E. coli* endotoxin (0.8 mg/kg). Values are means ± SEM (n= 4 per group).

*Significant difference between saline- and endotoxin-treated animals on the same diet (p< 0.05). Values on the same line not sharing the same superscript are significantly different (p< 0.05).

Table 4. Influence of Diets Containing Corn Oil, Butter and Butter Enriched with Cholesterol on Liver Composition of Rats Treated with *E. coli* Endotoxin

	Injection	Corn oil diet 100g/kg	Low Butter Diet 90g/kg+	Low Butter Diet 90g/kg+plus Cholesterol#	High Butter Diet 190g/kg+
Liver weight (g)	Saline	9.71 ± 1.43 ^a	11.7 ± 1.07 ^a	10.8 ± 1.21 ^a	10.6 ± 1.03 ^a
	LPS	11.8 ± 1.31 ^{*a}	12.2 ± 1.28 ^a	11.3 ± 1.29 ^a	12.2 ± 1.43 ^a
Protein (mg/g tissue)	Saline	213 ± 5 ^a	207 ± 6 ^a	202 ± 5 ^a	208 ± 6 ^a
	LPS	277 ± 6 ^{*a}	218 ± 6 ^c	249 ± 5 ^{*b}	269 ± 6 ^{*a}
Glutathione (mg/g tissue)	Saline	2.36 ± 0.15 ^c	2.92 ± 0.22 ^b	3.53 ± 0.28 ^a	3.71 ± 0.32 ^a
	LPS	7.81 ± 0.24 ^{*a}	5.43 ± 0.16 ^{*c}	5.82 ± 0.14 ^{*b}	5.13 ± 0.17 ^{*d}
Zinc (µg/g tissue)	Saline	33.1 ± 0.94 ^a	33.4 ± 0.74 ^a	32.9 ± 1.12 ^a	34.1 ± 1.00 ^a
	LPS	7.81 ± 0.24 ^{*ab}	35.3 ± 1.02 ^c	38.7 ± 0.73 ^{*b}	41.2 ± 0.89 ^{*a}
Cholesterol (µmol/g tissue)	Saline	61 ± 3 ^a	64 ± 3 ^a	72 ± 4 ^a	74 ± 5 ^a
	LPS	95 ± 3 ^{*a}	75 ± 4 ^b	82 ± 2 ^{*b}	100 ± 3 ^{*a}

+ 10g corn oil added/kg diet to prevent essential fatty acid deficiency.

Additional cholesterol added so that diet contained identical amount to that in 190g/kg butter diet.

Diets were fed for four weeks prior to subcutaneous injection of *E. coli* endotoxin (0.8 mg/kg). Values are means ± SEM (n= 4 per group).

*Significant difference between saline- and endotoxin-treated animals on the same diet (p< 0.05). Values on the same line not sharing the same superscript are significantly different (p<0.05).

Endotoxin administration increased the mean liver wet weight in all groups but the difference was only statistically significant in the case of the animals fed the corn oil diet ($p < 0.05$). Liver protein and zinc concentration were higher after endotoxin injection in animals that had been fed the corn oil, low butter plus cholesterol or high butter diets, than in saline-injected animals ($p < 0.05$). Values were 30, 23, and 29% greater for protein concentration in these respective groups and 20, 18 and 21% greater for zinc concentration in the same groups respectively. In animals fed the low butter diet, endotoxin administration had no statistically significant effect upon protein and zinc concentrations. Reduced glutathione concentrations were significantly increased ($p < 0.05$) in the livers of rats that had received an endotoxin injection compared to values of saline-injected animals in all dietary groups. Values were highest in animals which had received the corn oil diet, where it was 230% greater than in the saline controls. Reduced glutathione concentration was 85, 65 and 38% greater in the low butter, low butter plus cholesterol and high butter groups respectively. Liver cholesterol concentration increased after endotoxin treatment in the corn oil, low butter plus cholesterol and high butter dietary groups. In endotoxin-treated animals, values were 56, 14 and 35% greater than in saline injected controls for these respective dietary groups. Although mean values were greater in animals receiving endotoxin than in saline-injected controls, in the low butter group the values were not statistically different.

Discussion

The effects observed in the corn-oil-fed animals provide a 'benchmark' for the metabolic response of rats to an inflammatory stimulus. This diet provides a more than adequate quantity of the essential fatty acid, linoleic acid. Comparison of the data from the animals given a saline or endotoxin injection after being fed the corn oil diet shows many metabolic effects of the inflammatory response, which have been reported elsewhere by us and other groups. These effects include appetite and weight loss, an increase in liver weight and protein, zinc, glutathione and cholesterol concentration, an increase in plasma caeruloplasmin and cholesterol and a fall in plasma zinc and albumin (4,15-18). These results support the phenomenon seen in many other studies in which an inflammatory stimulus causes positive and negative

changes in the liver metabolism. The synthesis of liver structural and secretory proteins is enhanced, with the exception of that of serum albumin and other negative acute phase proteins, and liver and other visceral tissues increase the zinc concentration (often associated with enhanced metallothionein synthesis), while plasma concentrations decrease. These changes are the result of complex metabolic changes initiated by pro-inflammatory cytokine production, as outlined in the introduction.

Consistent with the studies by Di Silvestro *et al.* (18), Cousins *et al.* (19) and Mulrooney and Grimble (20), in the present study endotoxin injection resulted in increased liver zinc concentration and decreased plasma zinc concentration in rats on all of the dietary treatments with the exception of those on the low butter diet. Hyperlipidaemia, primarily due to the accumulation in plasma of very low density lipoproteins (VLDL), is generally associated with the acute-phase response. Beutler and Cerami (21) have proposed that TNF is responsible for the hyperlipidaemia that accompanies the inflammatory response. The administration of cytokines or endotoxin has been shown to decrease lipoprotein lipase activity, a key enzyme in the triglyceride catabolism that could impair the clearance of triglyceride-rich lipoproteins from the circulation and increase hepatic VLDL production, which could contribute to hyperlipidaemia (17,22). The increase in hepatic VLDL production, secondary to infection, may be due to an increase in fatty acid synthesis *de novo* in the liver (22). Associated with this effect, endotoxin administration has also been shown to produce a significant increase in serum cholesterol levels in rodents and rabbits (6,17).

It is tempting to speculate that the increase in hepatic cholesterol concentration induced by endotoxin treatment accounts for the increase in plasma cholesterol concentration. The present data is compatible with that from the study by Feingold *et al.* (17,22), in respect to tissue and circulating cholesterol levels. Several studies have pointed out that endotoxin can form complexes with lipoproteins or LPS-binding protein (17,23). Studies *in vitro* of mediator production by monocyte/macrophages show that binding of LPS (endotoxin) to HDL and other lipoproteins greatly reduces the production and release of TNF, IL-1 and IL-6 (23). Moreover, this binding can protect animals from the toxic effect of endotoxin. Hence, it may well be that there is a link between inflammation/infection and the lipid/lipoprotein

metabolism, as suggested by Feingold *et al.* (24). In the present study, the inflammatory process appears to be closely linked with the cholesterol metabolism in that highly significant correlations between plasma caeruloplasmin activity and cholesterol ($r = 0.96$, $p < 0.0001$), plasma caeruloplasmin activity and hepatic cholesterol concentration ($r = 0.77$, $p < 0.0001$), concentration of liver protein and plasma cholesterol ($r = 0.94$, $p < 0.001$), concentration of liver protein and liver cholesterol ($r = 0.92$, $p < 0.0001$) were noted. Significant negative relationships between the concentration of plasma albumin and plasma cholesterol ($r = -0.91$, $p < 0.001$) and concentration of plasma albumin and liver cholesterol ($r = -0.72$, $p < 0.018$) were also observed. Overall, these results would suggest that cholesterol mimics the group of proteins that are positively regulated, i.e., positive acute-phase proteins, by an inflammatory stimulus.

The liver is one of the most important organs involved in antioxidant defence via its major role in the glutathione metabolism. The organ is not only a major site of synthesis and utilization of glutathione, but also exports it into the blood for transport to other tissues such as the kidney and lung (25). Caeruloplasmin, also synthesized by the liver, contributes to anti-oxidant defence via its oxidase activity. In the present study, by comparing measurements from rats on the high butter diet with measurements from animals on the low butter diet in the presence and absence of added cholesterol, it is possible to differentiate between the modulatory effects of the fatty acids in butter and the effects of cholesterol on the inflammatory response to endotoxin.

When the predominant fat in the diet is butter at a concentration of 90 g/kg (low butter diet), the majority of these changes (with the exception of loss of appetite and increase in liver glutathione), are absent. However, the metabolic effects of endotoxin were similar qualitatively but not quantitatively in animals fed the low butter plus cholesterol diets and in those fed the corn oil diet. Clearly these two diets were neither similar in fatty acid composition, the former being poor in linoleic and the latter rich in this fatty acid, nor in cholesterol content, the latter diet being devoid of this sterol. When the high butter diet was fed, in which the content of this fat in the diet is more than double that of the low butter diet, all of the responses also become apparent, to a level much closer to that seen in the animals fed the corn oil diet. The concentrations of liver glutathione is the exception to this

general change in that it was elevated in response to endotoxin in all dietary groups. The magnitude of liver cholesterol concentrations of animals, in response to an endotoxin injection, was modulated by the amount of cholesterol in the diet, but not the concentration of butter fat (comparing data from the low butter group with that from the low butter plus cholesterol and high butter groups). It is unclear whether this phenomenon is due to a direct dietary effect of cholesterol or an indirect effect since the response is greater in rats consuming the corn oil, which is devoid of cholesterol, than in the high butter group. As indicated above, the increase in liver cholesterol may simply be a further index of the inflammatory response of the animals, in a similar manner to changes in liver zinc, and protein, or plasma caeruloplasmin or albumin.

Dietary cholesterol may be acting in this secondary role by exerting an oxidative stress. Certainly the enhancement of liver glutathione and the raised (although not significantly) plasma caeruloplasmin activity concentration, caused by inclusion of cholesterol in the low butter diet in the saline controls, suggest that this dietary addition may act in this way.

The high concentration of LA (48 g/kg of diet) in the corn oil diet may explain the pro-inflammatory influence of the diet despite the presence of substantial amounts of OA (29 g/kg of diet). The responses to endotoxin in rats fed the highest concentration of butter could not be simply explained by the OA (44 g/kg) and LA (7 g/kg) content of the diet. It is well known that dietary fatty acid composition can exert an anti-inflammatory effect. Diets enriched with n-3 polyunsaturated fatty acids have exhibited anti-inflammatory properties and survival advantages in experimental models of inflammation and shock (26). Furthermore, essential fatty acid deficiency (EFAD), i.e., an insufficient intake of linoleic acid, in which the accumulation of the 20-carbon tri-unsaturated acid (Mead acid, 20:3 n-9) (which only occurs in trace amounts in healthy tissue) has been reported, has been shown to prevent and ameliorate inflammation and consequent tissue damage (27). In addition to the changes in fatty acid intake, changes in cholesterol intake have also been reported to influence immune response and the inflammatory process (3). In a study on rabbits, hypercholesterolaemia induced by feeding 500mg cholesterol/kg diet for eight weeks elevated TNF-alpha production and mortality rates after endotoxin injection (28).

Although the mechanisms underlying the anti-inflammatory effect of dietary fatty acid manipulation have not been wholly elucidated, the possible primary mechanism proposed for the observed anti-inflammatory effects may be changes in plasma membrane lipid composition resulting in generation of an altered eicosanoid profile (29-31), changes in other cellular signalling mechanisms such as protein kinase A and C and G protein activation (29,32) and alterations in membrane fluidity and function. Fatty acids (particularly palmitic, stearic, oleic, linoleic, and arachidonic acid) and cholesterol are major lipid constituents of cell membranes. Generally the unsaturation index and the cholesterol to phospholipid ratio of the membrane correlate well with many membrane-related activities and membrane fluidity (33). Dietary fatty acids are readily incorporated into the phospholipid (PL) fraction of cell membranes. It has been shown that increasing or decreasing either the degree of unsaturation in membrane PL, or the ratio of cholesterol to phospholipid has an effect on cell surface-related events, including membrane fluidity, cell signal transduction via receptor mediated responses to extracellular stimuli or in providing local signalling molecules, e.g., cAMP, Ca²⁺, phospholipase C or protein kinase C (PKC) and phospholipase A₂ (34-38). These signalling molecules have been demonstrated to participate in the inflammatory response. Activation of PKC has been shown to be involved in several macrophage functions including eicosanoid, oxygen radical, and cytokine (particularly TNF- α production) (39,40). In previous studies, we showed that feeding corn oil resulted in lower lateral fluidity in the cell membranes of macrophages and hepatocytes than when butter was fed (32,41). The diets were identical to the corn oil and low butter diets in the present study. While no data was available for G protein activity, cAMP production or TNF receptor binding affinity in hepatocytes from these studies, in peritoneal macrophages all of these parameters were higher in animals fed corn oil than in cells from those fed butter. Thus the lower rigidity of cell membranes, TNF receptor affinity and activity of intracellular mediators observed in butter-fed rats could contribute to the lower responsiveness to endotoxin in the present study. Addition of cholesterol to membranes is known to decrease membrane fluidity. Incubation *in vitro* microsomal preparations with cholesterol were shown to

significantly increase the cholesterol to phospholipid ratio, which resulted in a decreased membrane fluidity. Similarly, cholesterol feeding increased the accumulation of cholesterol, raised the cholesterol to phospholipid ratio, and, subsequently, reduced the fluidity of microsomal membranes (33). Membrane-bound enzyme activity (42) is also altered when the concentration of cholesterol and membrane fluidity changes. Although we did not measure membrane cholesterol content in the present study, this effect could arguably contribute to the restoration of the inflammatory response in animals fed the low butter diet to which cholesterol had been added and in rats fed the high butter diet. Eicosanoids exert an important influence on the inflammatory response leading to changes in cytokine biology. It has also been proposed that this is one of the main mechanisms by which fatty acids can alter inflammation in the host (2). Generally, exogenous pathogenic agents such as LPS frequently result in weight loss, anorexia and finally cachexia. Cachexia denotes the development of a chronic catabolic state, a characteristic of certain infections; it results in loss of appetite and weight. Cytokines such as TNF and IL-1 produced by endotoxin-stimulated macrophages have been implicated in these catabolic processes (16,43). In the present study, animals fed the corn oil diet had the greatest decline in food intake and body weight when compared with those fed the diets containing butter fat. This is not surprising since the appetite response has been shown to be mediated by members of the prostaglandin family whose production is determined by the availability of precursor arachidonic acid, and ultimately, linoleic acid intake. It has also been demonstrated that stimulation of cytokines induces increased phospholipase A₂ activity, which enhanced eicosanoid production (37). Inhibition of eicosanoid production by cyclooxygenase and lipoxygenase inhibitors eliminates hypothermia, loss of appetite and weight loss associated with endotoxin and cytokine administration (16,38,43). These observations, together with the observation of suppression of the responses in the low butter diet and their re-emergence when the high butter diet was fed, suggest two counterposing effects of butter: the first being a predominant anti-inflammatory effect due to its fatty acid composition and the second a pro-inflammatory effect due to its cholesterol content. The latter influence only becomes apparent at high levels of intake.

Abbreviations

TNF, tumour necrosis factor- α ; IL1, interleukin 1; IL6, interleukin 6; n-9 MUFA, n-9 monounsaturated fatty acids; n-3 PUFA, n-3 polyunsaturated fatty acid; n-6 PUFA, n-6 polyunsaturated fatty acid; OA, oleic acid; LA, linoleic acid; VLDL, very low density lipoprotein; HDL, high density lipoprotein; LPS, endotoxin; PKC, protein kinase C; PL, phospholipid.

Correspondence author:

Halit Tanju BESLER

Hacettepe University, School of Health Technology,

Department of Nutrition and Dietetics

Sihhiye, Ankara - TURKEY

References

- Grimble RF. Dietary lipids and the inflammatory response. *Proc Nutr Soc.* 57: 1-8, 1998.
- Calder PC. Dietary fatty acids and the immune system. *Nutr Revs.* 56: S70-S82, 1998.
- Kelley DS, Daudu PA. Fat intake and immune response. *Prog Food Nutr. Sci.* 17: 41-63, 1993.
- Besler HT, Grimble RF. Comparison of the modulatory influence of maize and olive oils and butter on metabolic responses to endotoxin in rats. *Clin Sci.* 88: 59-66, 1995.
- Mulrooney HM, Grimble RF. Oleic acid as a determinant of the difference between the suppressive effects of coconut oil and butter on responses to tumour necrosis factor- α in rats. *Proc Nutr Soc.* 51: 89A, 1992.
- Fleet JC, Clinton SK, Sakloman RN. Atherogenic diets enhance endotoxin-stimulated interleukin-1 and tumor necrosis factor gene expression in rabbit aortae. *J Nutr.* 12: 294-298, 1992.
- Hughes DA, Townsend PJ, Haslam PL. Enhancement of antigen presenting functions of monocytes by cholesterol: possible relevance to inflammatory mechanisms in extrinsic allergic alveolitis and atherosclerosis. *Clin Exp Immunol.* 87: 279-286, 1992.
- Wilsher ML, Hughes DA, Haslam PL. Immunoregulatory properties of pulmonary surfactant: influence of variation in phospholipid profile. *Clin Exp Immunol.* 73: 117-122, 1988.
- McPherson IG, Everard BW. Serum albumin estimation: Modification of the bromocresol green method. *Clin Chim Acta.* 37: 117-119, 1972.
- Schosinsky KH, Lehmann HP, Beller ME. Measurement of caeruloplasmin from its oxidative activity in serum by use of O-dianisidine dihydrochloride. *Clin Chem.* 29: 1556-1563, 1976.
- Allain CA, Poon LS, Chan CSG, Richmond W, Fu PC. Enzymatic determination of total serum cholesterol. *Clin Chem.* 20: 470-475, 1974.
- Tocco-Bradley R, Kluger MJ. Zinc concentration and survival of rats infected with salmonella typhimurium. *Infect Immun.* 45: 332-338, 1984.
- Smith TJ, Krohn RI, Hermanson GT. Measurement of protein using bicinchoninic acid. *Anal Biochem.* 950: 76-85, 1985.
- Beutler E, Duron O, Kelly BM. Improved method for the determination of blood glutathione. *J Lab Clin Med.* 61: 882-888, 1963.
- Bibby DC, Grimble RF. Temperature and metabolic changes in rats after various doses of tumour necrosis factor. *J Physiol.* 490: 367-380, 1989.
- McCarthy DO, Kluger MJ, Vander AJ. The role of fever in appetite suppression after endotoxin administration. *Am J Clin Nutr.* 40: 310-316, 1984.
- Feingold KR, Hardardottir I, Memon R, Krul EJT, Moser AH, Taylor JM, Grunfeld C. Effect of endotoxin on cholesterol biosynthesis and distribution in serum lipoproteins in Syrian hamsters. *J Lipid Res.* 34: 2147-2158, 1993.
- Di Silvestro RA, Cousins RJ. Mediation of endotoxin-induced changes in zinc metabolism in rats. *Am J Physiol.* 247: E436-E441, 1984.
- Cousins RJ, Leinart AS. Tissue-specific regulation of zinc metabolism and metallothionein genes by interleukin 1. *FASEB J.* 2: 2884-2890, 1988.
- Mulrooney H, Grimble RF. The influence of butter and corn, coconut and fish oils on the effects of recombinant human tumour necrosis factor alpha in rats. *Clin Sci.* 84: 105-112, 1993.
- Beutler B, Cerami A. Cachectin and tumor necrosis factor as two sides of the same biological coin. *Nature* 320: 584-588, 1986.
- Feingold KR, Staprans I, Memon RA, Moser R, Shigenaga JK, Doerrfer W, Dinarello CA, Grunfeld C. Endotoxin rapidly induces changes in lipid metabolism that produces hypertriglyceridemia, low doses stimulate hepatic triglyceride production while high doses inhibit clearance. *J Lipid Res.* 33: 1765-1776, 1992.
- Levine DM, Parker DS, Donnelly TM, Walsh A, Rubin AL. In vivo protection against endotoxin by plasma high density lipoprotein. *Proc Natl Acad Sci (USA).* 90: 12040-12044, 1993.
- Feingold KR, Hardardottir I, Grunfeld C. Beneficial effects of cytokine-induced hyperlipidemia. *Z Ernahrungswiss.* 37: 66-74, 1993.
- Beutler E. Nutritional and metabolic aspects of glutathione. *Ann Rev Nutr.* 9: 287-302, 1989.
- Grimble RF. The modulation of immune function by dietary fat. *Br J Intensive Care.* 4: 159-167, 1994.
- Lefkowitz JB, Morrison A, Lee V, Rogers M. Manipulation of the inflammatory response by dietary polyunsaturated fatty acid modulation. *J Immunol.* 945: 1523-1529, 1990.

28. Brito BE, Romano EL, Grunwald C. Increased lipopolysaccharide-induced tumour necrosis factor levels and death in hypercholesterolemic rabbits. *Clin Exp Immunol.* 101: 357-361, 1995.
29. Tappia PS, Grimble RF. The relationship between altered membrane composition, eicosanoids and TNF-induced IL1 and IL6 production in macrophages of rats fed fats of different unsaturated fatty acid composition. *Mol Cell Biochem.* 165: 135-143, 1996.
30. Broughton KS, Whelan J, Hardardottir I, Kinsella JE. Effect of increasing the dietary (n-3) to (n-6) polyunsaturated fatty acid ratio on murine liver and peritoneal cell fatty acids on eicosanoid formation. *J Nutr.* 121: 155-164, 1991.
31. Wahle, KW. Fatty acid modification and membrane lipids. *Proc Nutr Soc.* 42: 273-287, 1983.
32. Tappia PS, Ladha S, Clark DC, Grimble RF. The influence of membrane fluidity, TNF receptor binding, cAMP production and GTPase activity on macrophage cytokine production in rats fed a variety of fat diets. *Mol Cell Biochem.* 166: 135-143, 1997.
33. Huang YS, Koba K, Horribin DF, Sugano M. Interrelationships between dietary proteins, cholesterol and n-6 polyunsaturated fatty acid metabolism. *Prog Lipid Res.* 32: 123-137, 1993.
34. Awad AB, Ntanios FY, Fink CS, Horvath PJ. Effect of membrane lipid alteration on the growth, phospholipase C activity and G protein of HT-29 tumor cells. *Prost Leuk Essent Fatty Acids.* 55: 293-302, 1996.
35. Rando RR. Regulation of protein kinase c activity by lipids. *FASEB J.* 2: 2348-2355, 1988.
36. May CL, Southworth AJ, Calder PC. Inhibition of lymphocyte protein kinase C activity by unsaturated fatty acids. *Biochem Biophys Res Commun.* 995: 822-828, 1993.
37. Kinsella JE. Lipids, membrane receptors, and enzymes: effects of dietary fatty acids. *JPEN.* 14: 2005-2175, 1990.
38. Bibby DC, Grimble R. Eicosanoids and the hypothermic effects of tumour necrosis factor alpha. *J Biomed Sci.* 9: 30-37, 1990.
39. Nishizuka Y. Intracellular signalling by hydrolysis of phospholipids and activation of protein kinase C. *Science.* 258: 607-614, 1992.
40. Sweet MJ, Hume DA. Endotoxin signal transduction in macrophages. *J Leuk Biol.* 60: 8-26, 1996.
41. Clamp AG, Ladha S, Clark DC, Grimble RF, Lund EK. The influence of dietary lipids on the composition and membrane fluidity of rat hepatocyte plasma membrane. *Lipids.* 32: 179-184, 1997.
42. McMurchie EJ. Dietary lipids and the regulation of membrane fluidity and function. *Advances in membrane fluidity*, in *Physiological regulation of membrane fluidity* (Aloia RC, Curtain GC, Gordon LM, eds.), pp189-237, Alan R. Liss, New York.
43. McCarthy DO, Kluger MJ, Vander AJ. Suppression of food intake during infection: is interleukin-1 involved? *Am J Clin Nutr.* 42: 1179-1182, 1985.