Dietary fat modifies some metabolic actions of human recombinant tumour necrosis factor α in rats

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To examine how fat might influence the metabolic effects of tumour necrosis factor α (TNFα), human recombinant TNFα was given intravenously to rats that had been fed for 12 weeks on diets containing (g/kg) 200 maize oil or 190 coconut oil + 10 maize oil. Rectal temperature and tissue composition measurements were made 8 and 24 h after injection. Ambient temperatures of 20° and 25° were employed to accentuate rectal temperature changes. Doses of 30 and 300 μg TNFα/kg body-weight were given, and brought about depression of serum zinc and albumin and elevation of copper. Muscle protein content was decreased and liver protein and Zn content enhanced by TNFα. Serum Zn and liver Zn content were negatively correlated 8 h after injections. Hypothermia developed within 1 h of injection. All responses except the rise in serum Cu and gain in liver Zn were more intense at the higher than at the lower dose of TNFα. Hypothermia was exacerbated by an environmental temperature of 20°. The coconut-oil diet blunted the hypothermia and likewise the changes in serum albumin and Cu content 8 h after injections and in muscle and liver protein after 24 h. Changes in eicosanoid metabolism may be involved in the modulatory effects of the coconut-oil-enriched diet.

Tumour necrosis factor α: Fat and tumour necrosis: Rat

Many of the responses to invasion by bacteria, viruses or parasites (Beisel, 1975) are initiated by the secretion of cytokines such as interleukin 1 (IL1) and tumour necrosis factor α (TNFα) from macrophages. The effects of IL1 and TNFα in vivo include fever, shock, protein loss from muscle and alterations of the pattern of protein synthesis in liver (Dinarello *et al.* 1986*a*; Dinarello, 1987). The production of acute-phase proteins is enhanced and that of serum albumin decreased (Perlmutter *et al.* 1986). Serum zinc concentration decreases due to uptake into liver and kidney (DiSilvestro & Cousins, 1984; Cousins, 1985). Serum copper rises due to an increase in the Cu-binding protein caeruloplasmin.

Prostaglandins (PG) and possibly leukotrienes (LT) may be involved in the mode of action of endotoxin, IL1 and TNFα on several tissues. PG are involved in fever (Bernheim *et al.* 1979; Sobrado *et al.* 1983; Dinarello *et al.* 1986*a*, *b*; Revhaug *et al.* 1988) and the profound fall in temperature caused by large doses of TNFα is prevented by cyclooxygenase and 5-lipoxygenase inhibitors (Kettlehut *et al.* 1987; Bibby & Grimble, 1989*a*). Muscle protein loss after endotoxin and IL1 injection is also blocked by cyclooxygenase inhibitors (Baracos *et al.* 1983; Wan & Grimble, 1986*a*) and increased PGE_2 production has been demonstrated in vitro, in skeletal muscle exhibiting increased catabolism in response to IL1 (Dinarello, 1987). The decline in serum Zn which followed endotoxin administration is inhibited by the 5-lipoxygenase inhibitor, AA861 (Wan & Grimble, 1986*b*).

Macrophage function is sensitive to nutritional factors. Kauffman *et al.* (1986) and Keenan *et al.* (1982) demonstrated a decreased ability of macrophages from malnourished patients to produce IL1. Dietary fat affects macrophage activities such as free radical

* For reprints.

Table 1. *Composition of diets (g/kg)*

Constituents	Maize oil	Coconut oil
Casein	224	224
DL-methionine	5	5
Maize oil	200	10
Coconut oil	—	190
Maize starch	206	206
Sucrose	205	205
Mineral mixture*	40	40
Vitamin mixture*	20	20
Cellulose powder†	100	100

* American Institute of Nutrition (1977).
† Solkafloc BW 40; Johnson, Jorgensen and Wettre Ltd, Wokingham, Berks.

production (Johnston & Marshall, 1984; Magrum & Johnston, 1985). The effects of fat on cytokine production have not been studied in detail, although monocytes from volunteers fed on fish-oil supplements show a reduced ability to produce IL1 and TNFα in response to endotoxin (Endres *et al.* 1989). In a previous study, we showed that giving rats diets low in linoleate blunted many responses to *Escherichia coli* endotoxin. Examination of phosphatidyl choline fatty acid compositions of spleen showed decreases in the concentration of arachidonic acid and its precursor linoleic acid (Wan & Grimble, 1987). As the former fatty acid acts as the parent compound for synthesis of the more potent forms of PG and LT (Moncada & Vane, 1983), it is possible that diets that were low in linoleate modified the stimulatory effect of endotoxin or endogenous cytokines (or both) on eicosanoid metabolism within target tissues. It was also possible that the diet may have modified cytokine release. Thus, either cytokine production, or actions, or both may have been modified. The second of the three possibilities is examined in the present study. Changes brought about by two doses of recombinant human TNFα over a 24 h time-course were studied in rats fed on diets rich and poor in linoleate content as in our earlier study (Wan & Grimble, 1987). Maize oil was the only fat source in the former diet. The fat source in the latter diet was predominantly coconut oil with a small addition of maize oil to prevent essential fatty acid deficiency. The variables studied were: liver and muscle total protein content; serum Zn, Cu and albumin concentrations; liver Zn content; and changes in body temperature.

The ability of rats to develop hypothermia was tested by keeping rats at environmental temperatures of 20° or 25° after saline (9 g sodium chloride/l) or TNFα injections, since rats are less likely to develop fever in response to endotoxin at low environmental temperatures (Szekely & Szelenyi, 1979). The short-term effects of TNFα on tissue composition were examined in the groups maintained at a temperature of 20°. The long-term effects of TNF were examined in groups maintained at 25°.

MATERIALS AND METHODS

Animals

Male Wistar rats (3 weeks old) from the Southampton University Medical School colony were fed, *ad lib.*, on synthetic diets containing either 200 g maize oil/kg or 190 g coconut oil and 10 g maize oil/kg diet. Diet composition (Table 1) was the same as in the earlier study on the effects of endotoxin (Wan & Grimble, 1987) and the feeding period was 12 weeks.

Table 2. *Changes in food intakes and body-weights of rats raised on maize oil- or coconut oil-rich diets 24 h after intravenous injections of tumour necrosis factor α (TNFα) or sterile saline (9 g sodium chloride/l)*

(Values are means with their standard errors for six rats per group)

Diet... / Treatment	Period after injection (h)	Food intake 0–24 h after injection (g)				Δ Food intake 0–24 h after injection compared with pre-injection 24 h intake (g)				Body-wt at injection (g)				Δ Body-wt 24 h after injection or pair-feeding (g)			
		Maize oil		Coconut oil		Maize oil		Coconut oil		Maize oil		Coconut oil		Maize oil		Coconut oil	
		Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Saline control	8	—		—						441	12	422	12				
30 μg TNFα	8	—		—						444	20	432	19				
300 μg TNFα	8	—		—						462	10	429	12				
30 μg TNFα	24	9	2	11	1	−10	1	−8	1	439	9	455	10	−13	3	−5	1
300 μg TNFα	24	7	1	4	1	−7	1	−10	1	430	16	447	20	−14	1	−15	3
Pair-fed control (30 μg TNFα group)	—	9	2	11	1					434	9	454	9	−5	1	−3	1
Pair-fed control (300 μg TNFα group)	—	7	1	4	1					424	14	425	16	−8	1	−11	2

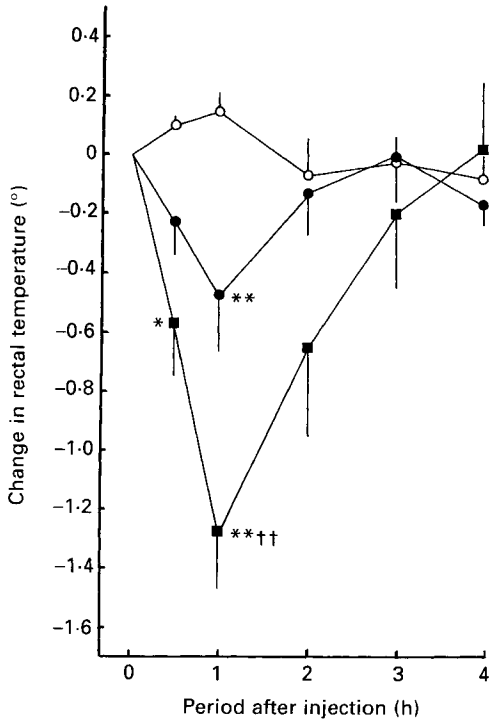

Fig. 1. Effect of intravenous injection of 30 (●) or 300 (■) μg recombinant human tumour necrosis factor α (TNFα)/kg body-weight, compared with sterile saline (9 g sodium chloride/l) (○), on rectal temperatures of rats fed from weaning for 12 weeks on a diet containing 200 g maize oil/kg. Temperatures measured at an ambient temperature of 25°. Values are means with their standard errors, represented by vertical bars, for groups of six rats. Values were significantly different from saline control (two-way analysis of variance): * $P < 0{\cdot}05$, ** $P < 0{\cdot}01$; and different from rats given 30 μg TNFα/kg: †† $P < 0{\cdot}01$.

Experimental protocol

Rats were housed in groups of three in wire cages and kept at ambient temperature of 25° on a 14 h light–10 h dark cycle. At the end of the feeding period rats were randomly assigned to subgroups of six rats, continuing on the same diet but being caged individually. After 3 d, the subgroups which were to have short-term effects of TNFα examined, and their controls, were placed in a room at 20°. Care was taken to minimize the stress of this manoeuvre by leaving the animals in their own cage and by gentle handling. Recombinant human TNFα (endotoxin < 137 pg/mg protein; BASF/Knoll AG, Ludwigshaven, West Germany), or sterile non-pyrogenic saline was given via the lateral tail vein to the subgroups from each dietary treatment at both environmental temperatures. Injection volumes of 1 ml/kg were used and doses of 30 or 300 μg TNFα/kg body-weight administered. Just before injections, food was removed from all cages and rectal temperatures measured using a plastic-coated probe inserted approximately 60 mm into the rectum. Temperatures were measured to the nearest 0·1° with an electronic thermometer (Light and Co. Ltd, Brighton). Injections commenced at 08·00 hours and were completed by 10·00 hours. Temperatures were monitored for 4 h. At 8 h after injection, half the TNFα- and saline-treated rats from each dietary group were stunned and decapitated. Blood was collected, and liver and tibialis muscle were rapidly removed, weighed, wrapped in aluminium foil and frozen in liquid nitrogen. Food was restored to all the remaining

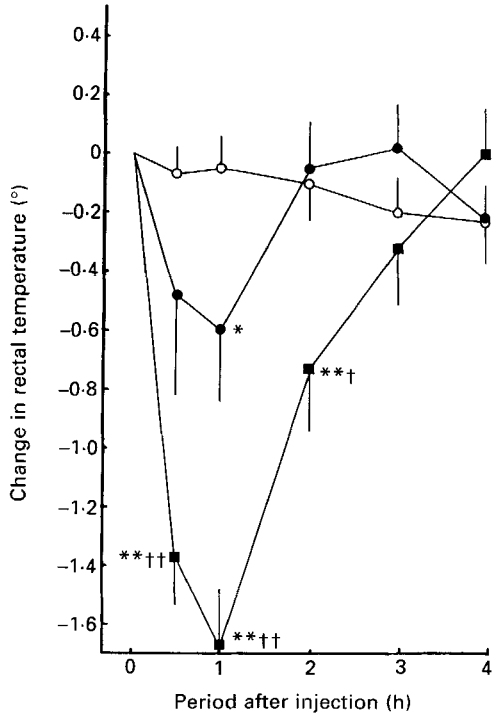

Fig. 2. Effect of intravenous injection of 30 (●) or 300 (■) μg recombinant human tumour necrosis factor α (TNFα)/kg body-weight, compared with sterile saline (9 g sodium chloride/l) (○), on rectal temperatures of rats fed from weaning for 12 weeks on a diet containing 200 g maize oil/kg. Temperatures measured at an ambient temperature of 20°. Values are means with their standard errors, represented by vertical bars, for groups of six rats. Values were significantly different from saline control (two-way analysis of variance): * $P < 0{\cdot}05$, ** $P < 0{\cdot}01$; and different from rats given 30 μg TNFα/kg: † $P < 0{\cdot}05$, †† $P < 0{\cdot}01$.

animals and the remaining TNFα-treated animals killed 24 h after injection and tissues collected as described earlier. As TNFα suppresses food intake, saline-injected pair-fed controls were prepared for each of the groups killed 24 h after TNFα injection by noting the weight of food consumed between 8 and 24 h after TNFα injection. A similar amount was given to pair-fed controls during the same period the following day. As this is normally the quiescent part of the rat's daily life-cycle they were not particularly voracious when food was re-introduced. The first 8 h of the day were spent without food. After the pair-feeding period rats were killed and processed exactly as the other rats in the study.

Tissues were stored at −20° until analysed. Tissue protein was measured by the Lowry technique (Lowry *et al.* 1951), after preparation as described by Garlick *et al.* (1980). Serum albumin was determined by the bromocresol green method (McPherson & Everard, 1972) and tissue and serum Zn and serum Cu by atomic absorption spectroscopy. Tissues were prepared for Zn analysis as described by Tocco-Bradley & Kluger (1984).

Statistical analysis

The values were examined by two-way analysis of variance for the effect of TNFα, of fat, of dose of TNFα, and TNFα × fat and dose × fat interactions. The relationship between serum and liver Zn was examined by linear correlation. Mean values with their standard errors are quoted throughout.

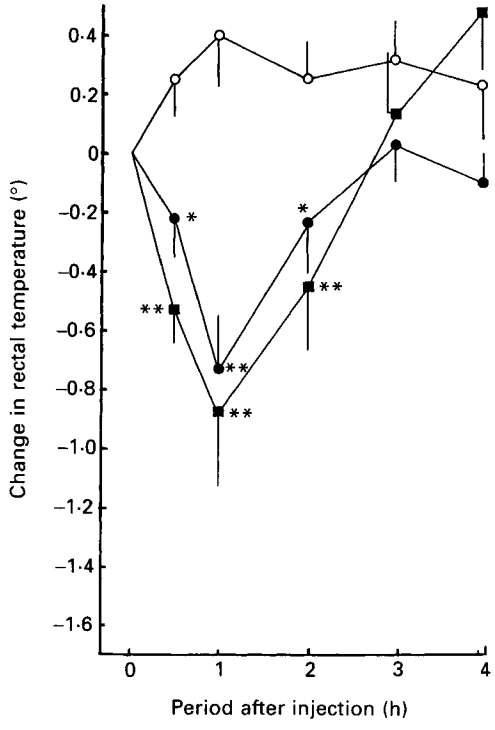

Fig. 3. Effect of intravenous injection of 30 (●) or 300 (■) μg recombinant human tumour necrosis factor α (TNFα)/kg body-weight, compared with sterile saline (9 g sodium chloride/l) (○), on rectal temperatures of rats fed from weaning for 12 weeks on a diet containing 190 g coconut oil and 10 g maize oil/kg. Temperatures measured at an ambient temperature of 25°. Values are means with their standard errors, represented by vertical bars, for groups of six rats. Values were significantly different from saline control (two-way analysis of variance): * $P < 0{\cdot}05$, ** $P < 0{\cdot}01$.

RESULTS

The changes in food intake and body-weight following TNFα administration are shown in Table 2. TNFα resulted in reductions in appetite and loss of body-weight at both doses employed. A rapid decline in rectal temperature occurred in all animals within 30 min of receiving TNFα. The decline continued until 1 h after injection, after which values returned towards those in the saline-treated animals as shown in Figs 1–4. The environmental temperature, type of diet and dose of TNFα influenced the size of the reduction in temperature below control values, to varying extents. At 25°, in rats fed on the maize oil-rich diet, falls of 1·3° and 0·5° were observed 1 h after injection of the high and low doses respectively. In rats fed on coconut oil the fall was similar in rats receiving either dose, and less severe at the higher dosage of TNFα, than in rats fed on maize oil. Significant falls of 0·9° and 0·7° were observed for the high and low doses of TNFα ($P < 0{\cdot}01$ and $P < 0{\cdot}01$ respectively).

An environmental temperature of 20° exacerbated the fall in temperature, particularly in rats fed on maize oil and receiving the highest dosage of TNFα. In these rats temperatures were 1·4°, 1·7° and 0·7° below starting values at 0·5, 1 and 2 h after injection respectively, whereas in rats at 25° falls of 0·6°, 1·3° and 0·6° were observed at these times. In animals given the lower dose of TNFα, a significant fall of 0·6° and 0·5° become apparent 1 h after injection at environmental temperatures of 20° and 25° respectively. Although the mean

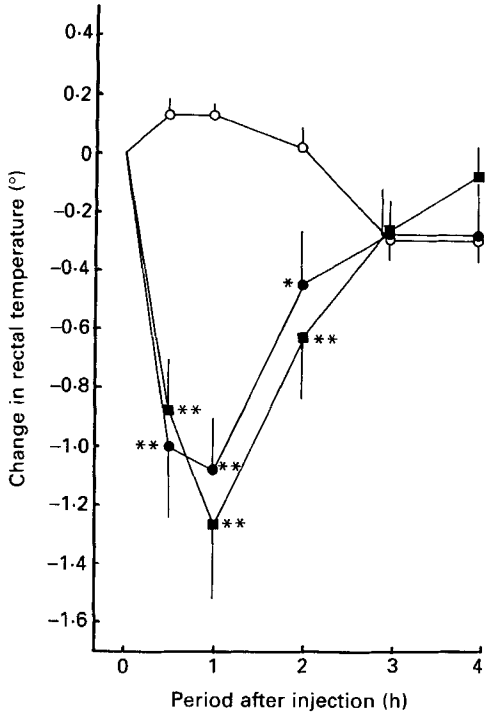

Fig. 4. Effect of intravenous injection of 30 (●) or 300 (■) μg recombinant human tumour necrosis factor α (TNFα)/kg body-weight, compared with sterile saline (9 g sodium chloride/l) (○), on rectal temperatures of rats fed from weaning for 12 weeks on a diet containing 190 g coconut oil and 10 g maize oil/kg. Temperatures measured at an ambient temperature of 20°. Values are means with their standard errors, represented by vertical bars, for groups of six rats. Values were significantly different from saline control (two-way analysis of variance): * $P < 0{\cdot}05$; ** $P < 0{\cdot}01$.

value for the temperature 0·5 h after injection was lower in rats at 20° than 25°, neither value was statistically different from those of the controls. In rats fed on coconut oil a difference of only 0·9°, 1·3° and 0·6° was noted following the higher dose of TNFα at an environmental temperature of 20°. As at 25°, raising the dose from 30 μg to 300 μg/kg did not exacerbate the hypothermia. The type of dietary fat, but not the environmental temperature, affected the speed with which rectal temperature returned towards values seen in the controls. While values had returned to normal 2 h after injection in rats receiving maize oil and 30 μg TNFα/kg, this did not occur until 3 h in those fed on coconut oil. After the 300 μg TNFα/g dose, values normalized 3 h after injections in both dietary groups at either environmental temperature.

TNFα brought about changes in tissue protein and trace element content in rats from both dietary regimens. While TNFα had no effect on the protein content of skeletal muscle 8 h after injection (values not shown), tibialis protein content declined and that of liver increased 24 h after injection (Table 3). The type of diet influenced the responses of liver and tibialis muscle in that, while the lowest dose of TNFα produced responses in rats receiving the diet rich in maize oil, it had no significant effect in animals receiving the diet containing coconut oil. High doses of TNFα, however, affected liver and muscle protein equally in both dietary groups. Tables 4 and 5 show serum Zn, Cu and albumin concentrations in rats killed 8 and 24 h after injection. TNFα brought about a fall in serum Zn and albumin and a rise in serum Cu. At 8 h after injection, serum Zn was significantly

Table 3. *Liver and tibialis muscle protein in rats raised on maize oil- or coconut oil-rich diets 24 h after intravenous injection with tumour necrosis factor α (TNFα) or sterile saline (9 g sodium chloride/l)*

(Values are means with their standard errors for six rats)

Diet	Injection	Mean	SEM	Injection	Mean	SEM	*F* values: TNFα dose	Fat	Fat × dose
Liver protein content (mg/liver)									
Maize oil	30 μg TNFα/kg	2450[b]	60	300 μg TNFα/kg	2720[c]	170	*	NS	NS
Coconut oil	30 μg TNFα/kg	2180[b]	100	300 μg TNFα/kg	2510[a]	120			
Maize oil	Saline	2290[b]	60	Saline	2410[b]	120			
Coconut oil	Saline	2340[b]	50	Saline	2110[b]	20			
F values									
TNFα		NS			*				
Fat		NS			NS				
Fat × TNFα		*			NS				
Liver protein concentration (mg/g wet wt)									
		Mean	SEM		Mean	SEM			
Maize oil	30 μg TNFα/kg	187[a]	4	300 μg TNFα/kg	187[a]	1	NS	*	NS
Coconut oil	30 μg TNFα/kg	173[b]	4	300 μg TNFα/kg	178[b]	5			
Maize oil	Saline	189[a]	5	Saline	189[a]	3			
Coconut oil	Saline	182[b]	5	Saline	181[b]	4			
F values									
TNFα		NS			NS				
Fat		*			*				
Fat × TNFα		NS			NS				
Tibialis muscle protein (mg/g)									
		Mean	SEM		Mean	SEM			
Maize oil	30 μg TNFα/kg	136[a]	3	300 μg TNFα/kg	136[a]	3	NS	*	NS
Coconut oil	30 μg TNFα/kg	158[b]	8	300 μg TNFα/kg	141[a]	3			
Maize oil	Saline	150[b]	3	Saline	149[b]	3			
Coconut oil	Saline	162[b]	4	Saline	154[b]	3			
F values									
TNFα		NS			**				
Fat		**			NS				
Fat × TNFα		NS			NS				

NS, not significant.

[a, b, c] Values with unlike superscript letters were statistically different: $P < 0{\cdot}05$.

Values examined for significant effects of TNFα, fat type and dose of TNFα by two-way ANOVA: * $P < 0{\cdot}05$, ** $P < 0{\cdot}01$.

Table 4. *Serum copper and albumin concentrations in rats raised on maize oil- or coconut oil-rich diets 8 and 24 h after intravenous injection with tumour necrosis factor α (TNFα) or sterile saline (9 g sodium chloride/l)*

(Values are means with their standard errors for six rats)

		Serum copper (μg/l)					*F* values		
		Cu (8 h)			Cu (8 h)				
Diet	Injection	Mean	SEM	Injection	Mean	SEM	TNFα dose	Fat	Fat × dose
Maize oil	30 μg TNFα/kg	2080[a]	60	300 μg TNFα/kg	1950[b]	140	NS	*	NS
Coconut oil	30 μg TNFα/kg	1717[b]	80	300 μg TNFα/kg	1850[b]	100			
Maize oil	Saline	1512[b]	103	Saline	1650[b]	50			
Coconut oil	Saline	1570[b]	50	Saline	1570[b]	90			
F values									
TNFα		**			NS				
Fat		NS			NS				
Fat × TNFα		*			NS				
		Cu (24 h)			Cu (24 h)				
		Mean	SEM		Mean	SEM			
Maize oil	30 μg TNFα/kg	3050[a]	240	300 μg TNFα/kg	3120[a]	100	NS	NS	NS
Coconut oil	30 μg TNFα/kg	2860[a]	220	300 μg TNFα/kg	2710[a]	220			
Maize oil	Saline	1870[b]	110	Saline	1930[b]	110			
Coconut oil	Saline	1860[b]	190	Saline	1960[b]	120			
F values									
TNFα		**			**				
Fat		NS			NS				
Fat × TNFα		NS			NS				

cont. on p. 662.

Table 4. (*cont.*)

		Serum albumin (g/l)					*F* values		
		Albumin (8 h)			Albumin (8 h)				
Diet	Injection	Mean	SEM	Injection	Mean	SEM	TNFα dose	Fat	Fat × dose
Maize oil	30 μg TNFα/kg	45·3[a]	0·4	300 μg TNFα/kg	42·1[b]	0·6	**	NS	**
Coconut oil	30 μg TNFα/kg	43·5[a]	0·7	300 μg TNFα/kg	43·6[a]	0·2			
Maize oil	Saline	44·6[a]	0·6	Saline	43·9[a]	0·2			
Coconut oil	Saline	43·0[a]	0·3	Saline	45·0[a]	0·3			
F values									
TNFα		NS			*				
Fat		**			**				
Fat × TNFα		NS			NS				
		Albumin (24 h)			Albumin (24 h)				
		Mean	SEM		Mean	SEM			
Maize oil	30 μg TNFα/kg	44·4[a]	0·5	300 μg TNFα/kg	41·4[b]	0·9	*	NS	NS
Coconut oil	30 μg TNFα/kg	43·8[a]	0·5	300 μg TNFα/kg	40·5[a]	1·4			
Maize oil	Saline	45·4[a]	0·9	Saline	45·9[a]	0·6			
Coconut oil	Saline	44·9[a]	0·7	Saline	43·8[a]	0·8			
F values									
TNFα		NS			**				
Fat		NS			NS				
Fat × TNFα		NS			NS				

NS, not significant.
[a, b] Values with unlike superscript letters were statistically different: $P < 0{\cdot}05$.
Values examined for significant effects of TNFα, fat type and dose of TNFα by two-way ANOVA: * $P < 0{\cdot}05$, ** $P < 0{\cdot}01$.

Table 5. *Serum zinc concentration in rats raised on maize oil- or coconut oil-rich diets 8 and 24 h after intravenous injections of tumour necrosis factor α (TNFα) or sterile saline (9 g sodium chloride/l)*

(Values are means with their standard errors for six rats per group)

		Serum Zn (μg/l)					*F* values		
		Zn (8 h)			Zn (8 h)				
Diet	Injection	Mean	SEM	Injection	Mean	SEM	TNFα dose	Fat	Fat × dose
Maize oil	30 μg TNFα/kg	870[a]	20	300 μg TNFα/kg	720[c]	20	**	NS	NS
Coconut oil	30 μg TNFα/kg	870[a]	40	300 μg TNFα/kg	630[c]	20			
Maize oil	Saline	1620[b]	40	Saline	1660[b]	40			
Coconut oil	Saline	1380[b]	40	Saline	1740[b]	100			
F values									
TNFα		**			**				
Fat		NS			NS				
Fat × TNFα		NS			NS				
		Zn (24 h)			Zn (24 h)				
		Mean	SEM		Mean	SEM			
Maize oil	30 μg TNFα/kg	1530[a]	40	300 μg TNFα/kg	1400[a]	80	NS	NS	NS
Coconut oil	30 μg TNFα/kg	1450[a]	30	300 μg TNFα/kg	1310[a]	120			
Maize oil	Saline	1430[a]	50	Saline	1530[a]	50			
Coconut oil	Saline	1290[b]	40	Saline	1470[a]	50			
F values									
TNFα		**			NS				
Fat		**			NS				
Fat × TNFα		NS			NS				

NS, not significant.

[a, b] Values with unlike superscript letters were statistically different: $P < 0.05$.

Values examined for significant effects of TNFα, fat type and dose of TNFα by two-way ANOVA: ** $P < 0.01$.

Table 6. *Liver zinc in rats raised on maize oil- or coconut oil-rich diets 8 and 24 h after intravenous injections of tumour necrosis factor α (TNFα) or sterile saline (9 g sodium chloride/l)*

(Values are means with their standard errors for six rats per group)

		Zn concentration (μg/g wet wt)					*F* values		
		Zn (8 h)			Zn (8 h)				
Diet	Injection	Mean	SEM	Injection	Mean	SEM	TNFα dose	Fat	Fat × dose
Maize oil	30 μg TNFα/kg	37[a]	1	300 μg TNFα/kg	36[a]	1	NS	NS	NS
Coconut oil	30 μg TNFα/kg	37[a]	1	300 μg TNFα/kg	37[a]	1			
Maize oil	Saline	30[b]	2	Saline	30[b]	1			
Coconut oil	Saline	33[b]	1	Saline	31[b]	1			
F values									
TNFα		**			**				
Fat		NS			NS				
Fat × TNFα		NS			NS				
		Zn (24 h)			Zn (24 h)				
		Mean	SEM		Mean	SEM			
Maize oil	30 μg TNFα/kg	38[a]	4	300 μg TNFα/kg	41[c]	3	**	*	NS
Coconut oil	30 μg TNFα/kg	35[a]	3	300 μg TNFα/kg	40[c]	2			
Maize oil	Saline	30[b]	4	Saline	33[b]	1			
Coconut oil	Saline	31[b]	2	Saline	33[b]	2			
F values									
TNFα		**			**				
Fat		NS			NS				
Fat × TNFα		NS			NS				

Table 6. (*cont.*)

		Zn concentration (μg/g wet wt)					*F* values		
		Zn (8 h)			Zn (8 h)				
Diet	Injection	Mean	SEM	Injection	Mean	SEM	TNFα dose	Fat	Fat × dose
Maize oil	30 μg TNFα/kg	544[a]	28	300 μg TNFα/kg	535[ac]	31	NS	*	NS
Coconut oil	30 μg TNFα/kg	477[a]	33	300 μg TNFα/kg	477[a]	14			
Maize oil	Saline	418[b]	18	Saline	439[b]	20			
Coconut oil	Saline	413[b]	28	Saline	393[b]	21			
F values									
TNFα		**			**				
Fat		NS			*				
Fat × TNFα		NS			NS				
		Zn (24 h)			Zn (24 h)				
		Mean	SEM		Mean	SEM			
Maize oil	30 μg TNFα/kg	574[a]	26	300 μg TNFα/kg	600[a]	37	NS	NS	NS
Coconut oil	30 μg TNFα/kg	552[a]	16	300 μg TNFα/kg	563[a]	23			
Maize oil	Saline	419[b]	22	Saline	418[b]	23			
Coconut oil	Saline	460[b]	19	Saline	396[b]	21			
F values									
TNFα		**			**				
Fat		NS			NS				
Fat × TNFα		NS			NS				

NS, not significant.
[a, b, c] Values with unlike superscript letters were statistically different; $P < 0{\cdot}05$.
Values examined for significant effects of TNFα, fat type and dose of TNFα by two-way ANOVA: * $P < 0{\cdot}05$, ** $P < 0{\cdot}01$.

lower in rats treated with TNFα than in those given saline. The higher dose of TNFα brought about the largest fall. At 24 h after injection, serum Zn values were mostly similar to those of the pair-fed saline-injected controls. Dietary fat had no influence on the fall in serum Zn concentration.

Serum Cu was significantly elevated 24 h after injection of TNFα, both doses being equally effective. The type of diet had no influence on the value achieved; however, 8 h after injection of the lower dose of TNFα an elevation occurred only in the animals fed on maize oil.

Serum albumin was reduced 8 and 24 h after the highest dose of TNFα only in rats fed on the diet rich in maize oil. In rats raised on the diet rich in coconut oil, however, the highest dose only produced a reduction in concentration at the latter time.

The concentrations and total contents of Zn in livers 8 and 24 h after injections is shown in Table 6. Values were raised at both times, in all rats injected with TNFα. Both doses of TNFα were equally effective, and the type of diet did not influence events.

Serum Zn concentration and liver Zn content were found to be negatively correlated, in all rats, from each dietary group, 8 h after injection, with r values of $-0{\cdot}656$ and $-0{\cdot}623$ ($P < 0{\cdot}01$, n 18) for rats receiving diets containing maize oil and coconut oil respectively. No relationship existed between serum Zn concentration and liver Zn content 24 h after injections had taken place.

DISCUSSION

The present study confirms some of the effects of TNFα in vivo previously reported.

Kettlehut *et al.* (1987) observed hypothermia in rats 1 h after lethal doses of TNFα. We have observed a similar but transient hypothermia following large but sub-lethal doses of recombinant human TNFα (Bibby & Grimble, 1989*b*). The dose used in the present study (300 μg/kg) represents 43% of the LD_{50} for human recombinant TNFα (Tracey *et al.* 1986).

Our results confirm that TNFα has complex actions on liver protein metabolism. Serum albumin concentration was depressed, serum Cu elevated and serum Zn depressed in conjunction with an increase in hepatic Zn content. Since serum Cu is indicative of the Cu-binding acute-phase protein caeruloplasmin, the first two changes suggest that the switch in type of hepatic protein synthesis, reported by Perlmutter *et al.* (1986) in vitro, also occurs in vivo. DiSilvestro & Cousins (1985) showed that metallothionein synthesis was stimulated by IL1 and endotoxin, leading to an increase in hepatic Zn content and a reciprocal fall in serum Zn concentration. We have demonstrated that TNFα produces a similar reciprocal relationship between metallothionein and serum Zn content, (Grimble & Bremner, 1989) and between hepatic Zn content and serum Zn concentration (Bibby & Grimble, 1989*b*).

The results of the present study and those reported by us and other workers elsewhere suggest that TNFα, like endotoxin, can induce muscle protein loss in vivo and that a decrease in protein synthetic rate may be responsible (Jepson *et al.* 1986; Charters & Grimble, 1989).

All responses to TNFα, in the present study, showed a degree of dose dependency, with the exception of the rise in serum Cu and gain in liver Zn. Coconut oil reduced the sensitivity to TNFα insofar as only the high dose produced a reduction in protein in muscle and a gain in the liver. Furthermore, the high dose produced a fall in serum albumin only in rats fed on maize oil. Similarly, serum Cu was elevated 8 h after TNFα injection in rats fed on maize oil but not in those given coconut oil. In rats given maize oil either increasing the dose of TNFα or reducing ambient temperature intensified the hypothermia. However, in rats receiving coconut oil, only the latter produced this phenomenon. In addition, at both environmental temperatures, the extent of the fall brought about by the highest dose of

TNFα 1 h after injections was less in the rats receiving coconut oil ($P < 0.05$ at both temperatures).

What mechanisms could underly the blunting actions of coconut oil? Alterations in eicosanoid production within target tissues may be partly responsible since eicosanoids are implicated in many of the actions of endotoxin or cytokines such as fever, hypothermia and muscle protein loss. However, a direct role for PGs in the stimulation of liver protein metabolism by endotoxin or pure cytokines is much less likely since the increase in liver protein content (Wan & Grimble, 1986*a*) and non-secretory protein synthetic rate after endotoxin or IL1 treatment is not blocked by cyclooxygenase inhibition (Sobrado *et al.* 1983). Likewise cyclooxygenase inhibitors are ineffective at blocking the increase in plasma C-reactive protein (Revhaug *et al.* 1988) or serum amyloid P protein (Poole *et al.* 1984).

There is a substantial body of research which suggests that fats with low linoleate contents may alter membrane phospholipid fatty acid composition and subsequent eicosanoid production in target tissues (Croft *et al.* 1984).

We found that diets of the same composition as those in the present study produced substantial reductions in the arachidonic acid concentration of spleen membrane phosphatidyl choline (Wan & Grimble, 1987). Diets containing 40% of energy in the form of coconut oil produced substantial reductions in plasma phospholipid arachidonate and linoleate. Reduced urinary excretion of 6-keto PGF_1 and production of thromboxane B_2 in blood occurred. Johnston & Marshall (1984) showed that $PGF_{2\alpha}$ and PGE_2 production were significantly reduced in liver, thymus and spleen of rats that had been fed on diets containing differing abundances of linoleate for 2 months. Membrane compositions in brain and muscle are also sensitive to change by dietary fat in the short term (Bourne *et al.* 1988; Jackson *et al.* 1988).

The observations of similarities in the blunting effects of coconut oil feeding on the actions of TNFα and of endotoxin (a stimulator of endogenous cytokine production) also suggest that the oil affects target-tissue sensitivity to cytokines.

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