

## The effects of dietary lipids on adrenergically-stimulated lipolysis in perinodal adipose tissue following prolonged activation of a single lymph node

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The effects of feeding beef suet (mostly saturated and monoenoic fatty acids), sunflower oil (rich in *n*-6 fatty acids) and fish oil (rich in *n*-3 fatty acids) on the response of mesenteric, omental, popliteal and perirenal adipocytes to experimentally-induced local inflammation were studied in adult guinea pigs. After 6 weeks on the experimental diets, the animals were fed standard chow, and lipopolysaccharide was injected unilaterally daily for 4 d to induce swelling of one popliteal lymph node. Basal lipolysis in the perinodal adipocytes of all depots studied was higher in the sunflower oil-fed animals than in the controls fed on standard chow, and lower in those fed on suet or fish oil. Dietary lipids altered rates of lipolysis during incubation with  $10^{-5}$  M noradrenaline in all samples studied from the locally-activated popliteal depot, but only in adipocytes within 5 mm of a large lymph node in the other depots. The fish-oil diet attenuated the spread of increased lipolysis within the locally-activated popliteal adipose tissue, and from this depot to other node-containing depots. These experiments show that *n*-6 polyunsaturated fatty acids promote and *n*-3 fatty acids suppress the spread of immune activation to adipocytes within and between depots, and alter the sensitivity of perinodal adipocytes to noradrenaline. Dietary effects are reduced or absent in adipocytes in sites remote from lymph nodes, and thus such samples do not adequately represent processes in perinodal adipose tissue. These results are consistent with the hypothesis that perinodal adipocytes interact with adjacent lymphoid cells during immune responses.

**Site-specific properties: Fish oil: *n*-6 Polyunsaturated fatty acids: Local interactions: Guinea pig**

Long-chain fatty acids are major metabolic fuels, as well as components of cell membranes and precursors of signal molecules secreted by immune cells. Fatty acids of particular chain length and unsaturation are much more efficient in the latter roles than others, and only minor intracellular modification is possible. Consequently, the lipid composition of the diet can alter many aspects of immune function (Calder, 2001). Most experimental investigations relate the current or very recent diet to immune processes, and assume that lymphoid cells acquire lipids directly from the blood or lymph, in competition with other tissues (Sanderson *et al.* 1995; Sadeghi *et al.* 1999; Wallace *et al.* 2001). Hypotheses concentrate on lipid metabolism in the immune cells themselves (Hwang, 2000). Anorexia is often associated with substantial or prolonged immune activation (Langhans, 2000), so stored nutrients may be as important as the current diet in determining the contribution

of nutrition to the course of inflammatory diseases (Grimble, 1998).

Adipocytes are now known to secrete and respond to many cytokines that were first described in the immune system (Coppack, 2001). Several cytokines stimulate the release of fatty acids and/or glutamine from adipocytes *in vitro*, but exactly where and how adipose tissue *in vivo* makes these essential nutrients available to lymphoid cells during periods of anorexia or malnutrition, or even normal metabolism, have been little explored. The site-specific properties of the adipocytes surrounding lymph nodes (perinodal adipose tissue) are consistent with the hypothesis that they are specialized to provision and regulate adjacent lymphoid cells (Pond, 1999, 2001). In certain depots, including the popliteal (POP), repeated immune stimuli induce properties characteristic of the perinodal adipocytes in adipocytes at least 10 mm distant from a

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**Abbreviations:** LPS, lipopolysaccharide; NOR, noradrenaline; POP, popliteal.

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major lymph node in guinea pigs (Pond & Mattacks, 2002). The triacylglycerols of perinodal adipocytes of all major node-containing depots contain proportionately more polyunsaturated fatty acids than those remote from lymph nodes and from nodeless depots (Mattacks & Pond, 1997). Adipose tissue explants from guinea pigs that were fed on suet-enriched chow for three months, interact much less strongly with lymphoid cells when co-incubated *in vitro* for 48 h than similar tissues from animals fed on standard chow. The manifestation of receptors to tumour necrosis factor  $\alpha$  on the surface of perinodal adipocytes in the hours after a simulated immune challenge is also reduced in rats that were fed suet-enriched chow (MacQueen *et al.* 2000). These experiments indicate that reception and/or secretion of cytokines that mediate local interactions between adipose and lymphoid cells are modulated by the fatty acid composition of the recent diet.

The ultrastructure of the sympathetic innervation of adipocytes has been studied in detail (Cinti, 2001), but little is known about site-specific differences in neural control within or between depots. Although perinodal adipocytes apparently contribute little to the whole-body energy supply during fasting, they respond readily to catecholamines *in vitro*, reaching rates of lipolysis up to 20% higher than adipocytes from nodeless depots of the same animals (Mattacks & Pond, 1999). As well as regulating lipolysis and the secretion of several cytokines including tumour necrosis factor  $\alpha$  and interleukin 6 from adipocytes (Rayner, 2001), noradrenaline is also known to act on lymphoid cells, including spleen lymphocytes (Rogausch *et al.* 1999) and dendritic cells (Maestroni, 2000). Activation of immune cells is mediated primarily by cytokines, but there is also evidence for the involvement of the adrenergic system. Immune stimulants such as lipopolysaccharide (LPS) can alter the expression of  $\beta_3$ -adrenergic receptor mRNA in large murine adipose depots (Berkowitz *et al.* 1998), but no effects on sensitivity to catecholamines have been demonstrated. Metabolic regulators of adrenergic stimulation of lipolysis have been extensively studied and various agents have been identified (Björntorp, 1996; El Hadri *et al.* 1997; Orban *et al.* 1999), but dietary lipids were not thought to be among them: Mersmann *et al.* (1992, 1995) found only minimal effects of dietary fats on the abundance or functioning of adrenergic receptors

in pig adipocytes, although their experimental diets induced marked differences in the fatty acid compositions of the adipocyte membranes. However, these studies were limited to adipocytes from subcutaneous or large intra-abdominal depots that are not associated with lymphoid tissue.

In the present study, we explore the contribution of lipids previously sequestered from the diet in storage tissues to immune-stimulated and catecholamine-mediated interactions between lymphoid and adipose tissues following experimentally-induced local inflammation.

## Methods

### Animals

Bolivian guinea pigs were bred and raised at the Open University (Milton Keynes, Bucks., UK) in standard cages (area 0.45 m<sup>2</sup>), at a room temperature of 22–23°C, and were transferred to permanent groups at weaning. They were fed on breeding-grade guinea-pig chow (FD1; Special Diet Services, Witham, UK), which contained 34 g crude lipid/kg, available *ad libitum*, plus hay every day, and cabbage, carrot and apple on 5 d/week. Water with added vitamin C (0.1 mg/ml) was available *ad libitum*. Guinea pigs were rejected if they had visible local or systemic infections, imperfect coat or skin, or showed signs of severe stress.

At the age of 8–12 months (i.e. when fully mature), groups of nine or ten (six females + three or four males) were placed on lipid-enriched chow (the controls continued on the standard FD1 chow). The supplemented chows were made by crushing the FD1 chow pellets and reconstituting them with 100 g either shredded beef suet (Tesco's or *Atora* brand), sunflower oil (Tesco's or *Flora* brand) or fish oil (donated by Seven Seas Ltd, Hull, UK)/kg, which increased the lipid content of the chow to about 130 g/kg. The proportions of the major classes of fatty acids in the resulting mixtures are listed in Table 1. The lipid-enriched chows were freshly prepared twice per week, and after drying to a firm consistency for 2 d in air were eaten within 3 d. Any mixture that smelt strongly was discarded at once.

All the animals readily ate the modified chows, and there was no evidence that it was distasteful to them.

**Table 1.** Summary of the proportions (g/100 g total fatty acids extracted) of saturated monoenoic, *n*-6 polyunsaturated and *n*-3 polyunsaturated fatty acids in the triacylglycerols extracted from standard chow (34 g digestible fat/kg) and supplemented chows (134 g digestible fat/kg)\*

Diet	Saturated†	Monoenoic‡	<i>n</i> -6 PUFA§	<i>n</i> -3 PUFA	Unsaturation index¶
Standard chow	36.4	33.9	24.3	5.4	107
Chow+beef suet	47.4	38.8	12.7	1.1	71
Chow+sunflower oil	20.2	36.5	36.8	6.6	142
Chow+fish oil	23.6	34.1	30.8	11.6	152

PUFA, polyunsaturated fatty acids.

\* For details of diets, see p. 376.

† 14:0, 16:0 and 18:0.

‡ 14:1, 16:1 and 18:1.

§ 18:2, 18:3 and 20:3 and 20:4.

|| 18:3, 20:5 and 22:6.

¶ Unsaturation indices: monoenoic (g/100 g total fatty acids) + 2 × dienoic (g/100 g total fatty acids) + 3 × trienoic (g/100 g total fatty acids).

The animals remained on this diet for 6 weeks and were switched back to the unmodified, standard chow for the last 4 d before killing, during which time local inflammation was induced by daily injections of LPS. LPS dissolved in sterile 25 mM-PBS at pH 7.4 was injected subcutaneously (20 µg LPS/kg body mass) into the left lower leg about 8 mm cranial to the calcaneus, i.e. into the region of the skin drained by the left POP lymph node. Each animal was isolated, with food and water available, for 18 h before being killed at about 09.30 hours on the fifth day with minimal stress and excitement by means of an intraperitoneal injection of pentobarbitone.

### Tissue samples

The dissection was begun at once and completed within 30 min, following the protocol described previously (Mattacks & Pond, 1997; Pond & Mattacks, 2002) that distinguishes between perinodal adipose tissue from within 2 mm of a lymph node, 'remote' samples taken from about 10 mm away from any lymph nodes and 'middle' samples taken from about half-way between these sites about 5 mm from a lymph node. Such triplets of samples were collected from the stimulated (left) and unstimulated (right) POP depots separately. Perinodal and remote samples were also obtained from the mesentery and the equivalent material from the omentum: the slightly darker region in which assemblages of lymphoid cells form numerous 'milky spots' that are functionally similar to lymph nodes (Shimotsuma *et al.* 1993), and the paler region that has few milky spots. The sample sites were selected by an experienced operator (CAM) entirely on the basis of the adipose tissue's anatomical relations to lymph nodes. A sample was also taken from the perirenal depot, which in guinea pigs does not contain any lymph nodes. The single left and right POP lymph nodes, and four large conspicuous mesenteric nodes were also dissected out and weighed.

### Measurement of lipolysis

To isolate the adipocytes, each sample was cut into 1–2 mm fragments that were incubated at 37°C for up to 1 h in 10 ml Krebs buffer+bovine serum albumin (20 g/l) at

pH 7.4 and collagenase (1 mg/ml) in a gently shaking water-bath. Tissue debris was removed using a wide-mesh cell strainer. Adipocytes were collected on a 70 µm cell strainer and re-suspended and washed three times in 10 ml Hanks' balanced salt solution. The suspensions were allowed to stand for 10 min while adipocytes floated to the surface where they were removed with a pipette and re-suspended by gentle shaking in 1 ml Hanks' balanced salt solution. Three 10 µl samples of each suspension were removed for inspection and counting of adipocytes in a haemocytometer. The average of three estimations of cell number for each adipose tissue sample was used.

Basal and noradrenaline (NOR)-stimulated lipolysis was measured within 4 h of excision in duplicate 10 µl aliquots of each sample of adipocytes, diluted if necessary to produce approximately 2000 cells/20 µl well. The free glycerol released was assayed using the glycerol kinase method described in detail by Pond & Mattacks (1995, 1998). The numbers of adipocytes/ml to the nearest 100 cells was measured with a haemocytometer. These values were used to normalize the glycerol measurements, thus improving precision and reproducibility by correcting for minor differences in adipocyte concentration.

Data were analysed using the Statistical Package for Social Sciences (SPSS Inc., Chicago, IL, USA). *P* values refer to Fisher's *F* test or to Student's two-sided *t* test, with *P*<0.05 being taken as significant.

## Results

### Structural results

Table 2 lists the morphological results obtained at dissection. The final body masses of the guinea pigs were not significantly altered by the experimental diets (ANOVA by diet: *F* 0.71, NS). The locally stimulated (left) lymph node and the conspicuous mesenteric lymph nodes were similar in mass under all the regimens (ANOVA by diet: left POP *F* 0.20, mesenteric *F* 0.512; NS). The masses of the right (non-stimulated) POP lymph node differed between experimental groups (*F* 10.83, *P*<0.001), being larger in guinea pigs fed on either the sunflower oil or the fish oil, though always much smaller than the homologous node that drained the skin that had been injected with LPS (Table 2). As we have found previously (Pond

**Table 2.** Effect of dietary lipids on body masses (g) and masses (mg) of the lipopolysaccharide-stimulated (left) and the unstimulated (right) popliteal lymph nodes, and the average of a sample of four mesenteric lymph nodes from each specimen of the four dietary groups†

(Mean values with their standard errors)

Diet	<i>n</i>	Popliteal lymph node (mg)										Stimulated popliteal nodes:mesenteric nodes			
		Body mass (g)		Stimulated				Unstimulated				Mesenteric lymph node (mg)		Mean	SE
		Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE				
Standard chow	9	886	75	14.1	1.37	1.9	0.13	7.81	0.90	4.29	0.85	3.33	0.09		
Chow+beef suet	10	1008	44	12.8	0.53	2.3	0.15	5.80	0.46	3.31	0.25	4.15	0.46		
Chow+sunflower oil	9	992	66	14.8	0.72	3.1*	0.26	5.10	0.56	3.69	0.27	4.26	0.46		
Chow+fish oil	10	964	66	15.5	0.79	3.1*	0.18	5.17	0.45	3.72	0.21	4.22	0.25		

\* Mean values are significantly different from the standard chow regimen *P*<0.05.

† For details of diets and procedures, see Table 1 and p. 376.

& Mattacks, 1998, 2002; Mattacks & Pond, 1999), the adipocyte isolation procedure yielded 1600–2100 intact cells/ml, with the perinodal samples, especially those from POP, consistently producing more adipocytes than others.

#### *Site-specific differences in rates of glycerol release*

Rates of glycerol release from the eleven samples of adipocytes from the guinea pigs that ate standard chow throughout are shown in left columns of Table 3. As we have reported previously (Mattacks & Pond, 1999), the 18 h fast produced similar rates of 'basal' lipolysis in the remote from node samples from the node-containing depots and the perirenal adipocytes. The application of NOR *in vitro* further raised the rate of glycerol release, and the maximum values in the presence of  $10^{-5}$  M-NOR are highest in the mesentery and omentum, and lowest in the perirenal. Site-specific differences in the rates of lipolysis in the node-containing depots after 4 d of stimulation with LPS were similar to those that develop within the first 24 h of an immune response (Pond & Mattacks, 2002). In the presence of NOR at the physiological range of concentrations ( $10^{-7}$  M), rates of lipolysis in all three samples of the stimulated POP were similar, but both basal and maximal lipolysis were higher in the perinodal than the remote samples ( $t$  2.789,  $P < 0.05$ ). Basal lipolysis from the perinodal samples was consistently higher than that from the remote samples of the same depot, with the middle sample similar to the perinodal in the stimulated POP depot but close to the values for the remote samples in the unstimulated leg. Rates of lipolysis in the mesenteric and omental perinodal samples were highly significantly higher ( $P < 0.001$ ) than in the corresponding remote samples under all conditions studied except omental in the presence of  $10^{-5}$  M-NOR.

#### *The effects of dietary lipids on site-specific differences in rates of glycerol release*

Table 3 also shows measurements from the homologous samples from guinea pigs that had eaten the lipid-enriched diets. Basal lipolysis was significantly higher in the perinodal sample of the LPS-stimulated POP from the sunflower oil-fed animals compared with the controls ( $t$  2.887,  $P < 0.02$ ), and lower in all samples from this depot from guinea pigs that ate fish oil ( $t$  3.491,  $P < 0.01$ ). Feeding suet or fish oil also significantly depressed lipolysis in the remote adipocytes compared with the homologous samples from the controls or sunflower oil-fed animals. Lipolysis in the middle samples was not significantly different from that in the perinodal adipocytes, but significantly different from the remote sample, in all samples from the stimulated POP except those from fish oil-fed guinea pigs. ANOVA for the effects of diet (Table 4) reveals a highly significant effect of diet on all POP samples from the LPS-stimulated leg under all conditions studied. Lipolysis in the perinodal samples under maximal stimulation with  $10^{-5}$  M-NOR was significantly higher for adipocytes from the guinea-pigs fed on standard chow or chow plus sunflower oil compared with adipocytes from elsewhere in the same depots and the homologous samples

from suet or fish oil-fed animals. Values from adipocytes incubated with  $10^{-7}$  M-NOR were intermediate, with those from the fish oil-fed guinea pigs significantly lower than those from the animals given sunflower oil or standard chow.

Corresponding measurements from adipocytes from the contralateral unstimulated POP depot present a qualitatively similar picture. Rates of lipolysis in the presence of  $10^{-5}$  M-NOR were almost identical in the two POP depots from the same guinea pigs, but with the lower dose of NOR, and in its absence, lipolysis in the perinodal adipocytes was lower in the unstimulated POP depot, and values from the middle samples were closer to those from the remote samples. Basal lipolysis in the unstimulated POP perinodal adipocytes of fish oil-fed guinea pigs was significantly lower than that from the suet-fed animals, although the values from the homologous samples of the LPS-stimulated POP were indistinguishable. The effects of diet on adrenergic-stimulated lipolysis were smaller in the unstimulated POP depot than in the corresponding samples from that containing the LPS-stimulated node, and none of the differences between values from the remote samples were significant.

All values for rates of lipolysis from mesenteric perinodal adipocytes were significantly higher than those for the corresponding remote samples. Dietary lipids had only a weak effect on basal and sub-maximal lipolysis (Tables 3 and 4) in remote mesenteric adipocytes, and none at all on maximal lipolysis. However, sunflower oil in the diet raised lipolysis in perinodal mesenteric adipocytes under all conditions studied, and fish oil curtailed maximal lipolysis in the presence of  $10^{-5}$  M-NOR. Values from the omentum present a similar picture, except that the suet-enriched diet appears to have a similar effect on maximal NOR-stimulated lipolysis in the 'around milky spot' samples as the fish-oil diet. The experimental diets induced minimal changes to basal and NOR-stimulated lipolysis in the omental adipocytes not associated with milky spots.

Administering LPS daily for 4 d induced a small increase in basal lipolysis and that with  $10^{-7}$  M-NOR in the perirenal depot of guinea pigs that had eaten sunflower oil over that of the animals on the other diets, but in contrast to the perinodal adipocytes, their rates of maximum lipolysis were not altered by any of these diet (Table 4).

## Discussion

### *Animals and diets*

Our lipid-supplemented diets induce substantial changes to the fatty acid composition of adipose tissue triacylglycerols in guinea pigs within 6 weeks (Colby & Pond, 1993), although they provide less lipid than those fed to mice by Wallace *et al.* (2000), or to rats by Sanderson *et al.* (1995) and Moussa *et al.* (2000), and more than those fed to weanling guinea pigs by Pöschl *et al.* (1999). In contrast to expectations from rat experiments (e.g. Harris *et al.* 1998), the final body masses of the guinea pigs fed on the enriched chows were not significantly different from those of the controls eating standard chow. In previous experiments (Colby & Pond, 1993; Mattacks &

**Table 3.** Basal and noradrenaline (NOR)-stimulated glycerol release ( $\mu\text{mol/h}$  per 100 adipocytes) from eleven samples of adipose tissue: perinodal, 2 mm ('middle') and 5 mm ('remote') from the single popliteal lymph node of the stimulated (left) leg and unstimulated (right) leg, perinodal and 5 mm ('remote') from conspicuous mesenteric lymph nodes, from the region of the omentum rich in milky spots, or with few milky spots ('remote') and from the nodeless perirenal depot†  
(Mean values with their standard errors)

Diet ...	Standard chow (n 9)						Chow + beef suet (n 10)						Chow + sunflower oil (n 9)						Chow + fish oil (n 10)					
	Basal		$10^{-7}$ M-NOR		$10^{-5}$ M-NOR		Basal		$10^{-7}$ M-NOR		$10^{-5}$ M-NOR		Basal		$10^{-7}$ M-NOR		$10^{-5}$ M-NOR		Basal		$10^{-7}$ M-NOR		$10^{-5}$ M-NOR	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Stimulated popliteal depot																								
Perinodal	4.30	0.06	4.64	0.09	5.30	0.06	4.01	0.05	4.34	0.06	4.88	0.05	4.64†	0.03	4.83	0.03	5.28	0.03	3.91†	0.02	4.11†	0.03	4.83†	0.02
Middle	4.23	0.08	4.52	0.08	5.00	0.05	3.88	0.06	4.17	0.08	4.79	0.03	4.51	0.04	4.75	0.02	4.97	0.03	3.60*†	0.07	3.93†	0.06	4.73***	0.02
Remote	3.96	0.08	4.39	0.08	4.97	0.10	3.48†	0.09	4.03	0.06	4.75	0.03	4.12*	0.08	4.43***	0.03	4.84	0.02	3.31†	0.06	3.65†	0.06	4.62	0.03
Unstimulated popliteal depot																								
Perinodal	4.12	0.08	4.42	0.07	5.15	0.05	3.93	0.07	4.15	0.07	4.80	0.04	4.40	0.04	4.71	0.04	5.12	0.05	3.64†	0.07	4.00†	0.04	4.77†	0.04
Middle	3.44***	0.05	3.94*	0.06	4.87*	0.02	3.46***	0.06	3.98	0.07	4.69*	0.04	4.00	0.07	4.42	0.07	4.89	0.02	3.41	0.04	3.84	0.04	4.63	0.05
Remote	3.27	0.07	3.66	0.09	4.84	0.08	3.16	0.03	3.62	0.07	4.61	0.05	3.38	0.06	3.89	0.09	4.77	0.04	3.17	0.04	3.57	0.06	4.57	0.04
Mesenteric																								
Perinodal	4.10	0.07	4.66	0.09	6.24	0.05	4.40†	0.06	4.70†	0.03	6.12	0.08	4.82	0.07	5.10	0.08	6.59	0.10	4.28	0.07	4.59	0.08	5.82	0.07
Remote	3.51	0.04	3.92	0.08	5.55	0.07	3.64	0.03	4.20	0.04	5.50	0.03	3.67	0.04	4.33	0.09	5.52	0.05	3.54	0.03	4.04	0.06	5.41	0.06
Omental																								
Milky spots	4.36	0.05	4.83	0.14	5.56	0.10	4.33	0.03	4.58	0.04	5.33	0.05	4.80	0.09	5.15	0.08	5.68	0.05	4.40	0.07	4.76	0.08	5.34	0.05
Remote	3.60	0.05	4.04	0.10	5.32	0.07	3.62	0.02	4.24	0.04	5.26	0.04	3.67	0.08	4.25	0.06	5.39	0.05	3.54	0.03	4.09	0.05	5.10	0.05
Perirenal																								
Perirenal	3.84	0.04	4.04	0.07	4.35	0.06	3.76	0.02	4.06	0.05	4.26	0.03	4.04	0.05	4.26	0.03	4.41	0.03	3.79	0.04	4.04	0.04	4.28	0.04

Mean values were significantly different from the contiguous sample above it in the Table \* $P < 0.05$ , \*\*\* $P < 0.001$  (*t* test).

Mean values were significantly different from the value for the homologous sample from guinea pigs on the control diet † $P < 0.05$ .

‡ Guinea pigs were fed on standard or lipid-enriched chow for 6 weeks before the start of immune stimulation (for details of diets and procedures, see p. 376); all ate standard unmodified chow during the 4 d of immune stimulation with lipopolysaccharide and were fasted for 18 h before killing.

**Table 4.** Summary of effects of the experimental diets on lipolysis in the eleven samples of adipocytes stimulated *in vivo* by immune activation and by noradrenaline (NOR) *in vitro*\*†

	No NOR (i.e. basal lipolysis)		10 <sup>-7</sup> M-NOR		10 <sup>-5</sup> M-NOR		10 <sup>-7</sup> M-NOR (% basal)	
	F	P	F	P	F	P	F	P
Stimulated popliteal								
Perinodal	61.4	<0.001	32.3	<0.001	32.8	<0.001	18.4	<0.001
Middle	40.2	<0.001	31.7	<0.001	15.3	<0.001	27.1	<0.001
Remote	24.6	<0.001	36.4	<0.001	7.6	<0.001	16.5	<0.001
Unstimulated popliteal								
Perinodal	25.1	<0.001	24.4	<0.001	17.3	<0.001	9.1	<0.001
Middle	20.7	<0.001	17.3	<0.001	13.2	<0.001	11.5	<0.001
Remote	4.1	<0.05	3.5	<0.05	5.7	<0.01	2.4	NS
Mesenteric								
Perinodal	18.5	<0.001	9.5	<0.001	17.3	<0.001	10.9	<0.001
Remote	5.6	<0.01	6.5	<0.01	1.31	NS	2.8	NS
Omental								
Milky spots	7.1	<0.01	7.2	<0.01	7.1	<0.01	3.9	<0.05
Remote	1.5	NS	2.3	NS	5.9	<0.01	0.8	NS
Perirenal (no lymph nodes)	10.6	<0.001	5.00	<0.01	2.7	NS	2.4	NS

\* For details of diets and procedures, see Table 1 and p. 376.

† Values for Fisher's *F* test were computed by ANOVA by dietary group.

Pond, 1997), we also found that guinea pigs do not fatten when fed additional lipid; the contrast may be a species difference, perhaps arising from the fact that sedentary adult guinea pigs on standard chow are about 150 g dissectible adipose tissue/kg (Pond *et al.* 1984), fatter than normal laboratory rats. The constancy of final body mass in all experimental groups (Table 2) eliminates obesity or changes in total body composition as the cause of the effects described in Tables 3 and 4.

The differences between dietary groups in the swelling of the lymph nodes (Table 2) must arise from persistent metabolic or structural changes induced by the diets, possibly alterations to the fatty acid composition of membrane phospholipids, since at the time of immune stimulation all the animals were eating the same standard chow. The larger size of the right (non-stimulated) POP lymph node in animals that had eaten either of the diets rich in polyunsaturated fatty acids was not expected: feeding fish oil to mice for 5 weeks diminishes, while sunflower oil enhances the inflammatory response to LPS (Sadeghi *et al.* 1999). Swelling of the rat POP lymph node in response to grafting of foreign tissue was reduced by feeding fish oil compared with other diets supplemented with *n*-6 polyunsaturates or monounsaturated fatty acids (Sanderson *et al.* 1995), apparently because it curtails lymphocyte activation and proliferation.

#### *The response of adipose tissue to prolonged immune stimulation*

Maximum rates of lipolysis in the perirenal depot and in the 'remote' samples from the other depots studied are similar for all dietary groups to those reported by Pond & Mattacks (1991). The site-specific rates of lipolysis in adipocytes from the node-containing depots after administration of LPS for 4 d (Table 3) were very similar to those found after only 24 h of LPS-stimulation of guinea pigs fed on standard chow (Pond & Mattacks, 2002). The activation

of perinodal adipocytes is thus concentrated into the early stages of the immune response, which is consistent with the hypothesis (Pond, 1999, 2001) that the role of these specialized adipocytes is to provision the proliferation and maturation of lymphoid cells. The higher rates of basal lipolysis in the perinodal samples than in the remote from node and the perirenal represent the sum of that induced by the endocrine conditions of fasting and by stimulation of the immune system (Pond & Mattacks, 2002). During immunological quiescence, perinodal adipocytes release less glycerol than those not associated with lymph nodes (Mattacks & Pond, 1999).

As previously reported (Pond & Mattacks, 2002), the time courses of activation of lipolysis in mesenteric and POP adipocytes and their maximum responses are different. Even prolonged immune stimulation over 4 d failed to induce high rates of lipolysis in the 'remote' samples from the mesentery or omentum. Under normal conditions, these adipocytes may not be involved in local inflammation, though they may become so in certain pathological states (Pond, 2001).

#### *The effects of diet*

The pro-inflammatory diet of sunflower oil raised maximum lipolysis in the perinodal adipocytes, and promoted the spread of immune-stimulated lipolysis within and between node-containing depots (Table 3). The fish oil and suet diets did the opposite, although the swelling of the lymph nodes in response to the immune stimuli was similar following all the diets studied (Table 2). These effects on adipocytes may be secondary to the well established action of dietary lipids on the response of the lymph node lymphoid cells to the inflammatory stimuli (Yaqoob *et al.* 1994; Sanderson *et al.* 1995, 1997). The responses to the suet-enriched chow are consistent with previous reports of this diet reducing the interactions between lymphoid cells and explants of perinodal adipose tissue

*in vitro* (Mattacks & Pond, 1997), and the expression of receptors to tumour necrosis factor  $\alpha$  on the perinodal adipocytes following LPS-stimulated immune responses. The manifestation of tumour necrosis factor  $\alpha$  receptor type 1 on POP adipocytes is delayed and attenuated in rats fed on suet-enriched chow compared with controls fed on standard chow, but no change in receptor expression following a similar period on a diet enriched with sunflower oil could be detected (MacQueen *et al.* 2000).

The effects of the experimental diets on NOR-stimulated lipolysis in adipocytes from within 5 mm of lymph nodes (i.e. perinodal and middle samples) were not anticipated from previous studies (Mersmann *et al.* 1992, 1995) on the action of dietary lipids on the adrenergic receptors of adipocytes themselves. Recent discoveries about the relationship between membrane structure and its capacity to support receptors and secretion vesicles (Simons & Ikonen, 1997) suggest that substitution of fatty acids in membrane phospholipids may underlie the effects reported here. Another possibility is a direct action of fatty acids on the production and manifestation of receptors (Grimaldi, 1999). However, such mechanisms should affect all adrenergically-controlled adipocytes about equally, but we found major effects of dietary lipids on NOR-stimulated lipolysis *per se* only in adipocytes that are known to be sensitive to local lymphoid secretions (Table 4).

These site-specific differences suggest a more complicated mechanism, involving diet-induced changes in the adjacent lymphoid cells rather than just on the adipocytes themselves. Calder (2001) and Wallace *et al.* (2000) have described various effects of dietary lipids on the secretions of lymphoid cells in response to immune stimulation. The lymph vessels divide into many fine branches within a few millimetres of major lymph nodes (Heath & Brandon, 1983), an arrangement that would bring mobilized lymphoid cells into close proximity with the perinodal adipocytes, and enable them to take up fatty acids and signal molecules (Pond, 1996). The intracellular pathways of adrenergically-stimulated lipolysis are distinct from those of cytokine-mediated lipolysis (Londos *et al.* 1999), so may be independently modulated by the diet. The similarity in the effects of the suet and fish oil diets in all samples studied (Table 3), in spite of large differences in their fatty acid composition (Table 1), is also consistent with the interpretation that the primary effects are on the interaction between lymphoid cells and adjacent adipocytes.

Certain fatty acids are substrates for the prostanoid pathway in lymphoid cells, and act directly on transcription factors and genes (Grimble, 1998; Jump & Clarke, 1999). The high flux of fatty acids into and out of adipocytes is probably why most of their genes are refractory to long-term regulatory control by fatty acids (Bernlohr *et al.* 1997). This role may be among the reasons for the higher proportion of polyunsaturated fatty acids and fewer saturated fatty acids in the triacylglycerols of perinodal adipocytes (Mattacks & Pond, 1997). This gradient in the composition of storage lipid persists in guinea pigs fed for several weeks on a suet-enriched diet, but the absolute amounts of polyunsaturated fatty acids in the perinodal adipocytes fall, and interactions *in vitro* with lymphoid cells are impaired (Mattacks & Pond, 1997).

According to the hypothesis that the adipose tissue around lymph nodes interacts *in vivo* with adjacent lymphoid tissue (Pond & Mattacks, 1995, 1998, 2002; Mattacks & Pond, 1999), the effects described here would contribute to the known actions of dietary lipids on immune function summarised by Calder (2001). The contrasting action of sunflower and fish oils parallels Guthrie & Carroll's (1999) conclusion that *n*-6 fatty acids promote the development of mammary, colonic and pancreatic cancer, while large quantities of *n*-3 fatty acids suppress it. The capacity of sunflower oil to amplify the response of adipose tissue to immune stimuli may be among the ways in which diets rich in *n*-6 fatty acids promote atopic disease in children (Calder & Miles, 2000) and hyperinsulinaemia, obesity, atherosclerosis and some forms of cancer in adults (Yam *et al.* 1996).

We conclude that the fatty acid composition of the diet before an immune response is elicited determines the extent of involvement of adipose tissue in node-containing depots. Fish oil attenuates, and sunflower oil enhances, the spread of increased lipolysis within the locally-activated POP adipose tissue, and from this depot to other lymphoid tissue-containing depots. Both paracrine interactions with the lymphoid cells (mediated by cytokines and/or eicosanoids) and the adrenergic pathway are modulated by dietary lipids. These site-specific differences in the control of short-term activation of adipose tissue may contribute to the long-term changes in its distribution that are associated with chronic immune disturbance (Pond, 2001).

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