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The effects of feeding suet-enriched chow on site-specific differences in the composition of triacylglycerol fatty acids in adipose tissue and its interactions *in vitro* with lymphoid cells

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The effects of diet on the composition and properties of adipose tissue in relation to lymph nodes were studied in adult guinea-pigs. The proportions of monoenoic triacylglycerol fatty acids were constant in all sites in adipose tissue of similarly fed guinea-pigs, but were substantially greater in samples from guinea-pigs fed on suet-enriched chow. Triacylglycerols in adipose tissue from near nodes contained significantly fewer saturated fatty acids, and significantly more 18:2*n*-6 and 18:3*n*-3 than those in samples from sites remote from nodes within the same depot. Depots that interact most strongly with lymphoid cells *in vitro* had the largest and most consistent within-depot differences. The gradients of triacylglycerol fatty acid composition with distance from lymph nodes in two small intermuscular depots were similar in guinea-pigs fed on plain or suet-enriched chow. These findings are consistent with the hypothesis that adipose tissue around lymph nodes is specialized for local interactions with the lymphoid cells therein, and help to explain the variability of serial or duplicate measurements of adipose tissue composition. When cultured alone, lipopolysaccharide-stimulated lymph node lymphoid cells from suet-fed guinea-pigs incorporated as much labelled thymidine as the controls. Adipose tissue explants from suet-fed guinea-pigs inhibited lymphocyte proliferation much less than those of the controls, although the site-specific differences were similar. The pattern of site-specific differences in glycerol released from explants incubated alone was generally similar for both dietary groups, but except in the popliteal depot, the increases following co-culturing with lymphoid cells were smaller for samples from suet-fed guinea-pigs. These experiments show that minor changes in the fatty acid composition of the diet can substantially alter the interactions between adipose tissue and lymphoid cells.

Dietary fatty acids: Adipose tissue: Lymph nodes: Guinea-pig

By co-culturing adipose tissue explants with mixtures of lymphoid cells, Pond & Mattacks (1995) demonstrated within-depot and between-depots differences in the capacity of adipose tissue to interact with lymphoid cells. Samples of adipose tissue from near to lymph nodes were consistently more effective at curtailing spontaneous and mitogen-stimulated proliferation, with lipolytic products the most probable mediators of the effects. Similar experiments to study the action of single fatty acids (FA) showed that polyunsaturated fatty acids (PUFA) were most effective in inhibiting mitogen-stimulated proliferation of lymphocytes (Buttke, 1984; Calder *et al.* 1991), most readily incorporated into the membranes of these cells (Calder *et al.* 1994), may regulate gene action directly (Clarke & Jump, 1994; Hennig *et al.* 1996), and are essential precursors for the synthesis of eicosanoids. Such FA are always in the minority in adipose tissue triacylglycerols (TAG), particularly if they are also scarce in the diet.

If, as proposed by Pond & Mattacks (1995), the adipose tissue around lymph nodes is specialized to interact with adjacent lymphoid tissue, its efficiency in this role would be enhanced by an ability to release more PUFA, and to retain the capacity to do so even when

such nutrients are deficient in the diet. Local accumulation of PUFA, combined with the site-specific differences in lymphoid-cell-stimulated lipolysis demonstrated by Pond & Mattacks (1995) would together ensure that essential FA are available to the lymphoid system where and when required, and would protect these essential FA from being oxidized by tissues such as muscle that, especially during fasting, anorexia and/or fever, readily take up all species of FA from the circulation.

Although selectivity in lipolysis of TAG according to the chain length and degree of unsaturation of FA has now been demonstrated (Gavino & Gavino, 1992; Raclot & Groscolas, 1993), and site-specific differences in the response to changes in dietary lipids in growing animals have been reported (Colby & Pond, 1993), it is still believed that continuous lipolysis and re-esterification of adipose tissue TAG eventually homogenizes their composition throughout the adipose mass of adult mammals (Field & Clandinin, 1984; Malcom *et al.* 1989) except where deposition is biased by tissue temperature (Phinney *et al.* 1994). Exact congruence between the composition of TAG FA in adipose tissue and that of the diet is very difficult to demonstrate either in human subjects or in experimental animals. The data are always variable, even when a controlled diet is imposed strictly and for very long periods (Field & Clandinin, 1984; Valero-Garrido *et al.* 1990; Hunter *et al.* 1992; Lin *et al.* 1993; Tjønneland *et al.* 1993). It is not normally thought necessary, and may not be practical, to take serial biopsies and other kinds of samples of adipose tissue from sites that are exactly homologous for their positions in relation to lymph nodes (Hunter *et al.* 1992; Colby & Pond, 1993; Lin *et al.* 1993; Tjønneland *et al.* 1993).

As well as influencing the composition of storage TAG, dietary FA affect many aspects of immune function (Calder, 1995). For example, in young rats, diets rich in PUFA produce the most striking changes in the composition of membrane phospholipids in spontaneous and mitogen-stimulated proliferation of lymphocytes (Calder *et al.* 1994; Yaqoob *et al.* 1994), although such FA are always in the minority in adipose tissue TAG. Furthermore, the effects of manipulating the composition of dietary lipids cannot be satisfactorily predicted from studies of the action of single lipids on blood or blood fractions *in vitro* (Yaqoob *et al.* 1995). We have suggested an active role for adipose tissue in adjusting the temporal and spatial availability of FA to lymphoid cells (Pond & Mattacks, 1995; Pond, 1996*a*,*b*).

As part of an on-going investigation into the general principles that determine the gross distribution and internal organization of mammalian adipose tissue (Pond, 1996*a*,*b*), we describe the composition of TAG in adipose tissue from sites defined precisely by their anatomical relations to lymph nodes. We examine the effects of modification of the fat content of the diet on the tissue's composition and its interactions with lymphoid cells. We present data that suggest that the composition and the metabolism of adipose tissue are organized in relation to lymph nodes, and thus are able to offer some explanations for the apparent variability of the composition of adipose tissue TAG FA found in previous studies, and for the action of dietary lipids on immune response.

METHODS

Animals and tissue samples

Virgin female Bolivian guinea-pigs were born and raised at the Open University in standard cages (area: 0·45 m^2), at a room temperature maintained at 22–23°. Breeding-grade guinea-pig chow (supplied by Special Diet Services, Waltham, Essex), which contained 34 g crude lipid/kg, of which 20% was saturated FA (g/kg: 0·04, 12:0; 0·26,

14 : 0; 0·02, 15 : 0; 5·73, 16 : 0; 0·69, 18 : 0; 0·09, 20 : 0), 22·5% was monoenoics (g/kg: 0·56 16 : 1*n*-7; 6·66, 18 : 1*n*-9; 0·29, 20 : 1*n*-9; 0·14, 22 : 1*n*-9), 9·64 g/kg was 18 : 2*n*-6, 7·02 g/kg was 18 : 3*n*-3 and the remainder, longer-chain polyunsaturates. Water with added ascorbic acid (0·1 mg/ml) was available *ad libitum*. The guinea-pigs were given hay every day, and cabbage, carrot and apple on 5 d/week. They were transferred to permanent groups at weaning and used as mature adults aged 12–16 months, with body mass 900–1200 g. This age and size are the minima at which there is sufficient tissue in the minor depots for all the analyses. The body composition of guinea-pigs of the same strain raised under conditions similar to those used for these experiments is about 15–16% by weight dissectible adipose tissue (Pond *et al.* 1984*a*).

During the period December 1992 to December 1994, nineteen guinea-pigs were raised and maintained continuously on this diet, and used sequentially between February and December 1994. The manufacturers admit to minor differences in the ingredients of different batches of chow, so the exact composition of FA ingested over the guinea-pigs' life-span changed over this period of 2 years. Seven other guinea-pigs born early in 1994 were raised to adulthood (age 9–11 months) on the same diet as the controls, then fed *ad libitum* for 3–5 months (between February and August 1995) on similar chow mixed with beef suet (15 g Atora brand shredded suet, analysis given as 480 g saturated fat/kg (47 % 16 : 0; 45 % 18 : 0; 2 % 14 : 0), 321 g monoenoics/kg (89 % 18 : 1*n*-9; 6 % 16 : 1*n*-7) and 21 g 18 : 2*n*-6/kg), and 50 ml water to each 100 g powdered chow, plus the same rations of hay and vegetables as the controls. The recipe was designed to incorporate as much suet as possible, compatible with producing firm pellets that guinea-pigs would eat readily (Colby & Pond, 1993). The lipid content of the chow was thus increased from about 34 to 137 g/kg in the suet-enriched diet.

Guinea-pigs with visible local or systemic infections, imperfect coat or skin, or that showed signs of stress were rejected, as were those in which the lymph nodes were found to be enlarged at dissection. Adult female guinea-pigs live harmoniously in groups and very few were rejected on these grounds. Each animal was isolated with food and water available for 24 h before being killed, with minimal stress and excitement, by means of an intraperitoneal injection of pentabarbitone dissolved in sterile 25 mM phosphate buffered saline (PBS). The dissection was begun at once, and completed within a maximum of 1 h.

The adipose tissue samples were taken from sites homologous to those used for previous investigations into interactions with lymphoid cells in tissue culture (Pond & Mattacks, 1995). Samples of 20–30 mg were used for the analysis of TAG composition, and explants of about 1 mm^3 in volume for tissue culture. One set of samples from near to a conspicuous lymph node, and another from as far away as possible from any lymph nodes were examined from each of four superficial and two intra-abdominal depots that have previously been studied in guinea-pigs (Pond, 1992). The abundance and exact arrangement of most lymph nodes are variable in guinea-pigs, as they are in most other mammals, and published descriptions (Hadek, 1951; Maalouf *et al.* 1967) differ slightly in both terminology and the numbers and arrangements of lymph nodes reported. The dissection was always performed by the same experienced operator (CAM), who assessed each specimen carefully to ensure that the samples were taken from sites homologous for their anatomical relationships to lymph nodes.

The four superficial depots were: forearm, adipose tissue in front of the arm near the elbow, containing the cubital lymph node; behind arm, adipose tissue on and behind the upper segment of forelimb containing up to three axillary nodes grouped together; interscapular, with several small nodes of variable arrangement; inguinal containing two to five inguinal (subiliac) nodes in a tight group. Guinea-pigs of this strain and age are large

enough and fat enough for the 'far from node' samples to be taken from at least 10 mm away from the 'near node' ones. In such guinea-pigs, adipocytes are about 100–150 μm in diameter (Pond *et al.* 1984*a*,*b*), so this distance represents about 67–100 adipocyte-widths.

The two intra-abdominal depots were: mesenteric in the region of the small intestine, and omental from the greater omentum, each of which contain several nodes of various sizes, located near confluences of blood vessels. The 'far from node' samples were about 10 mm from the 'near node' ones. The third intra-abdominal depot, the perirenal, is the most massive in the guinea-pig body, accounting for about 26% of the total dissectible adipose tissue (Pond & Mattacks, 1991; Pond *et al.* 1992), but it does not encase any lymph nodes. There are a few small nodes associated with the aorta and posterior vena cava but they make contact only with the extreme edges of the depot (Hadek, 1951; Maalouf *et al.* 1967). Two sets of samples were taken from the perirenal depot at its thickest point around the kidneys, one from as far as possible from visible blood vessels and the other from near to a knot of blood vessels. The latter was chosen as the definition of a site comparable to that near a lymph node because most nodes occur at confluences of blood vessels.

Site-specific differences in the composition of adipose tissue TAG in relation to lymph nodes were studied in greater detail in two intermuscular depots: popliteal, containing the single large popliteal node, and cervical, the adipose depot medial to the anterior trapezius muscle of neck and on and around the serratus ventralis cervicis muscle, that contains one large and several smaller nodes, called by Cooper & Schiller (1975) the superficial dorsal cervical and deep cranial cervical node respectively. These depots change least in mass with changing fatness (Pond, 1994), and their lymph nodes are relatively large and are much more consistent in position than those in the other depots studied (Hadek, 1951; Maalouf *et al.* 1967). Such consistency makes it easier to define and identify exactly homologous sites within these adipose depots in different guinea-pigs. For seventeen of the guinea-pigs fed on unmodified chow and all those fed suet-enriched chow, six homologous samples of about 20 mg each were taken from different parts of the popliteal adipose depot defined by their anatomical relationships to the lymph node, and four from the cervical depot between the neck muscles.

When viewed from the side, the popliteal adipose depot is roughly triangular in shape, and thickest ventrally, near the gastrocnemius muscle (Pond *et al.* 1984*b*); the popliteal lymph node is located near its ventral posterior corner. The samples in which TAG FA were analysed were defined as follows: as near as possible to the node, from its distal and proximal sides; from the middle of the depot, between the node and the sciatic nerve about 4 mm anterior to the node, and from the more anterior end of the depot near where the sciatic nerve runs through it towards the gastrocnemius muscle, about 6 mm anterior to the node; as far as possible from the node going towards the anterior, behind the knee joint and as far as possible from the node (and from all visible nerves and blood vessels) going dorsally, where the adipose tissue tapers to a thin layer between the semitendinosus and biceps femoris muscles. The first of these 'furthest from the lymph node' was the greatest distance from the node (up to 10 mm away), but the other is furthest from visible blood vessels and nerves. One sample for tissue culture was from beside the node, and another from as far as possible from the node going towards the anterior. From the guinea-pigs fed on suet-enriched chow a third sample from the centre of the adipose depot, 4–6 mm from the node, was also studied.

The cervical adipose depot is roughly round in outline and thickest near its centre (Pond *et al.* 1984*b*). Two samples were respectively from near the large central node and the group of smaller nodes near the dorsal edge of the depot, and the two others were taken from opposite sides of the depot, as far away as possible (up to 5 mm) from the lymph

nodes. The average mass of each of the cervical and popliteal depots is about 1 g (Pond *et al.* 1984*b*), but the dimensions of the adipose tissue and the lymph nodes vary slightly with the body composition as well as with the overall size of the specimen, so the distances between samples and other structures are only approximate.

Tissue culture and analyses

Tissues from ten of the nineteen guinea-pigs fed continuously on plain chow, born in mid-1993, and from all seven of those fed on the suet-enriched diet were cultured together using the methods developed by Pond & Mattacks (1995). Lymphoid cells were isolated from the superficial ventral cervical, medial retropharyngeal and axillary lymph nodes (nomenclature after Cooper & Schiller, 1975) of the same guinea-pig from which the adipose tissue samples were derived. A mixed suspension of T-lymphocytes, B-lymphocytes plus much smaller quantities of macrophages and other leucocytes was extracted using standard procedures (Calder *et al.* 1991). The lymph nodes were dissected free of adipose tissue, washed in PBS and pushed through a sieve, thereby thoroughly mixing the cells from different nodes. The cells were spun at 400 ***g*** for 5 min, and washed twice in PBS before being resuspended in RPMI 1640 culture medium with 25 mM-HEPES, plus 100 ml fetal calf serum/l. The cells were layered onto 5 ml histopaque 1077 (Sigma, Poole, Dorset), and again spun at 400 ***g*** for 5 min. The lymphoid cells were collected as the middle layer and resuspended in fresh medium and serum.

After checking the cell count in 15 μl samples with a Coulter Counter, the suspension was diluted to produce approximately 10^6 cells/ml. Portions of lymphoid cells of volume 0·5 ml (i.e. approximately 5×10^5 cells) were cultured alone or with one explant of adipose tissue taken from the sites described above and 100 ml fetal calf serum/l, 2 mM-L-glutamine, antibiotics (penicillin: 200 units/ml; streptomycin: 100 units/ml) and 50 μg/ml of lipopolysaccharide (LPS), a mitogen that stimulates mainly B-lymphocytes and macrophages, although the latter do not divide. The total volume of the culture was approximately 1 ml. These concentrations of LPS and glutamine were determined from pilot experiments to produce maximal lymphocyte proliferation. The same medium was used for incubation of adipose tissue explants alone.

All incubations were carried out in quadruplicate in twenty-four-well tissue culture plates using four adipose tissue explants for homologous sites at 36·5–37·5° in CO_2–air (5 : 95, v/v) for 48 h. After this time, the adipose tissue explants were lifted out with a fine spatula. Two sets of wells were assayed for glycerol and for separation and identification of non-esterified FA. These samples of the incubated medium were stored in sealed tubes at −15°, for up to 3 months (in most cases, for less than 3 weeks). Portions of 3·7 KBq [^{3}H]thymidine (74 GBq/mmol; from Amersham International, Amersham, Bucks.) were added to the other pair of wells and the incubation was continued for a further 18 h. The cells from these wells were harvested onto filter paper using an automatic cell harvester and the incorporation of [^{3}H]thymidine into cells was measured (Calder *et al.* 1991).

Free glycerol was assayed in duplicate 10 μl samples using the glycerol kinase (*EC* 2.7.1.30) method developed by McGowan *et al.* (1983) for measuring glycerol at fairly low concentrations in small volumes of serum, as described in detail by Pond & Mattacks (1995).

Separation and quantification of fatty acids

Adipose tissue TAG FA were separated and identified by TLC and GC, using standard methods (Colby & Pond, 1993). The samples of fresh adipose tissue were stored at −15°

wrapped tightly in foil for up to 6 weeks (in most cases, for less than 3 weeks), and as fatty acid methyl esters (FAME) for a maximum of a further 6 weeks. All procedures were carried out in glass containers to avoid contamination with plastics. The twenty-four adipose tissue samples and the samples of culture media from each guinea-pig were analysed as a batch, running 'near node' and 'far from node' samples alternately but with pairs of samples in a random order.

TAG were extracted from samples of about 10 mg adipose tissue with a mixture of methanol–chloroform (containing 0·07 g butylated hydroxytoluene/l)–water (2 : 1 : 0·8, by vol.). The non-esterified FA from the incubation media were extracted using methanol and chloroform, with the medium serving as the aqueous phase. The TAG in the chloroform phase (without butylated hydroxytoluene) were isolated by TLC, using diethyl ether–hexane–acetic acid (14 : 10 : 0·8, by vol.) as the eluant on silica G60 plates. The FA were converted to methyl esters by heating each sample for 1 h at 70° in sealed ampoules containing BF_3 dissolved in methanol (140 g/l). The FAME were extracted into hexane using a mixture of hexane–water (2 : 1, v/v) and the hexane extract was dried under N_2 and tightly sealed. If necessary, the samples were stored as FAME for a maximum of 4 weeks (mostly about 2 weeks) at −15°. The standards were prepared in the same way and stored for the same lengths of time under the same conditions, and the whole set of samples from each guinea-pig was prepared, stored and analysed as a batch at the same time, and compared with standards prepared freshly each week.

The FAME were separated on a 30 m PEG-coated silica capillary column (ID 0·53 mm) in a Pye Unicam 4500 gas chromatograph (Pye Unicam, Cambridge, Cambs.) with a flame ionization detector, using H_2 as a carrier gas. Each sample was thawed and 10–100 μl dichloromethane was added, from which samples of 100–500 nl were injected manually into the GC column from a 1 μl microsyringe and run for 10 min. Peak integrations were performed with a JCL 6000 Jones chromatography data system (Jones Chromatography Ltd, Hengoed, Mid Glamorgan). Each peak was identified against commercially available standard FAME, PUFA mixtures 1 and 2 from Supelco (Sigma Aldrich Co. Ltd, Poole, Dorset) and from Sigma's FA standards that were prepared in the same way as the samples. The complete set of standards was run weekly, with those for 16 : 0 and 18 : 0 run each day to standardize the GC's operating conditions. Structural isomers were not identified separately.

The analytical methods used were intended to demonstrate major differences between and within adipose depots in the relative abundance of the eight commonest FA; we did not attempt to identify or quantify the minor components extracted by these methods. Four saturated (14 : 0 (myristic acid), 15 : 0 (pentadecanoic acid), 16 : 0 (palmitic acid), 18 : 0 (stearic acid), two monoenoic (16 : 1 (palmitoleic acid, 16 : 1*n*-7) and 18 : 1 (oleic acid, 18 : 1*n*-9)), one dienoic (18 : 2 (linoleic acid, 18 : 2*n*-6)) and one trienoic (18 : 3 (α-linolenic acid, 18 : 3*n*-3)) FA were present as 1 g or more/100 g of the total measured in the large majority of samples from all guinea-pigs examined. All the data refer only to the relative abundance of these fractions. Unsaturation index (UI) was calculated as: (% monoenoic + 2(% dienoic) + 3(% trienoic) ... etc).

Statistical analysis

The Tables show the means of data from homologous samples from all similarly treated specimens. In Tables 1–4 and 6, the means of the 'differences' between each pair of measurements from 'far from (lymph) node' and 'near (lymph) node' samples from the

same depot of the same specimen are also listed, and used as the basis for pairwise comparisons, using Student's two-sided t test, with $P < 0.05$ being taken as significant.

RESULTS

The guinea-pigs fed on the suet-enriched chow were not significantly heavier at the time of death than those fed on plain chow (body mass of controls: 1046 (SE 17) g; suet-fed: 1077 (SE 24) g; Student's t test: t 1·053, df 24, NS), so the adipose depots were similar in size and shape in the two groups, which facilitated locating exactly homologous sampling sites from each animal. The modified diet also did not affect the average incorporation of [^{3}H]thymidine into lymphoid cells stimulated with LPS and incubated without adipose tissue (incorporation into cells from guinea-pigs fed on unmodified chow: 17 541 (SE 817) disintegrations/min (dpm); those from animals fed on suet-enriched diets: 18 754 (SE 219) dpm; Student's t test: t 1·434, df 15, NS).

Site-specific differences in composition of triacylglycerol fatty acids

Measurable quantities of all eight major FA were found in over 95 % of the adipose tissue samples analysed. For some of the guinea-pigs fed on plain chow or suet-enriched chow that had a very low average proportion of PUFA in their adipose tissue TAG FA, 18 : 3n-3 was undetectable in both the perirenal samples and, less frequently, in the 'far from node' samples of the inguinal and 'behind arm' depots. Several repeated analyses using homologous samples of tissue confirmed the failure to demonstrate this FA. However, 18 : 3n-3 was invariably present in all the samples taken from near the lymph nodes of such guinea-pigs and throughout the mesenteric, omental, forearm and intermuscular depots.

Tables 1 and 2 show the relative abundance of saturated FA, monoenoic FA and two PUFA in TAG from adipose tissue in sites near to and far from a lymph node (or, in the case of perirenal, a knot of blood vessels) in four superficial and three intra-abdominal depots of the guinea-pigs, together with the means of the differences between each pair of samples from each sample site. TAG from all samples of those fed on the suet-enriched diet (Table 2) included a much greater proportion of monoenoic FA (overall mean: 41·3 (SD 4·5) g/100 g total fatty acids than those fed on plain chow (31·8 (SD 7·1) g/100 g total fatty acids; (Table 1), but within each set of data, there were no significant site-specific differences in the abundance of such FA.

The compositions of TAG FA in all the 'far from node' samples from similarly fed guinea-pigs were statistically indistinguishable. Within each depot, there were inverse relationships between the proportions of saturated FA, and dienoic and trienoic FA, with the former significantly more abundant in the 'far from node' samples, and the latter relatively more concentrated in the 'near node' samples. For the data from guinea-pigs fed on plain chow (Table 1), all three components of this pattern were significant for all node-containing depots and highly significant for the pairs of samples from the forearm, mesenteric and omental depots. The compositions of the pair of samples from near to and far from knots of blood vessels in the perirenal were indistinguishable from each other, and, in the samples from animals fed continuously on plain chow, from the 'far from node' samples of the other depots. For data from the suet-fed guinea-pigs (Table 2), these within-depot differences were highly significant for the mesenteric depot, and significant at $P < 0.05$ for the omental. The pairs of values of the two PUFA were significantly different in three superficial depots, but the compositions of the pairs of samples from the inguinal and perirenal depots were almost identical. The differences between the TAG FA

Table 1. *The proportions (g/100 g total fatty acids) of saturated fatty acids, monoenoic fatty acids, linoleic acid (18:2n-6) and α-linolenic acid (18:3n-3) extracted from the triacylglycerols in samples of adipose tissue from far from lymph node(s) or (in the case of perirenal) knots of blood vessels and near to lymph node(s) or knots of blood vessels from four superficial and three intra-abdominal depots of guinea-pigs fed on plain chow*†

(Mean values and standard deviations for nineteen guinea-pigs. The means and standard deviations of the differences between each pair of values are also shown)

		Saturated		Monoenoic		18:2*n*-6		18:3*n*-3	
Depot	Site	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Superficial									
Forearm	Far	51·0	15·1	31·8	8·6	14·5	7·2	2·7	1·6
	Near	40·1	12·7	31·9	5·5	23·6	8·2	4·4	2·3
	Difference	10·94***	6·96	−0·13	5·06	−9·07***	4·35	−1·73**	1·97
Behind arm	Far	53·4	17·1	31·7	10·3	12·7	10·0	2·3	2·1
	Near	45·7	14·0	34·3	7·7	16·8	8·0	3·2	2·3
	Difference	7·60**	7·82	−2·62	6·88	−4·13**	4·62	−0·93*	1·61
Interscapular	Far	47·9	14·1	32·5	6·4	16·1	8·2	3·5	2·3
	Near	41·7	14·0	31·2	8·3	22·0	7·9	5·2	3·3
	Difference	6·25**	8·06	1·31	7·50	−5·97***	3·96	−1·78**	2·35
Inguinal	Far	55·6	17·3	31·4	9·0	11·1	8·9	1·9	2·1
	Near	50·5	15·5	33·3	7·8	13·9	7·7	2·4	1·9
	Difference	5·08**	7·31	−1·86	4·80	−2·70**	4·07	−0·47	1·08
Intra-abdominal									
Mesenteric	Far	44·8	11·5	33·9	6·4	17·7	7·2	3·6	1·9
	Near	34·9	8·2	31·1	5·0	27·7	6·6	6·3	2·4
	Difference	9·94***	6·45	2·79*	4·35	−10·01***	6·51	−2·72***	2·54
Omental	Far	47·0	15·2	33·0	8·8	16·8	7·2	3·3	2·0
	Near	37·4	8·8	32·6	5·6	24·1	5·5	5·3	2·3
	Difference	9·62***	9·10	−0·30	6·67	−7·29***	4·90	−2·04***	1·81
Perirenal	Far	49·9	16·0	34·0	8·3	13·7	9·1	2·4	2·2
	Near	51·0	17·8	32·9	8·9	13·7	10·3	2·5	2·2
	Difference	−1·08	7·51	1·08	4·88	0·01	3·21	−0·03	0·84

Mean differences between the composition of the 'near node' and 'far from node' adipose tissue of the same depot were significant: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ (Student's two-tailed t test).

† A preliminary account of some of these data appears in Pond (1996*b*).

composition of the other 'far from node' samples from those fed on suet-enriched chow are discussed in connection with Table 5.

Tables 3 and 4 show data similar to those in Tables 1 and 2 for the detailed study of the two small intermuscular depots. The means and SD of the four groups of FA are shown, with the means and SD of the differences between each sample and that nearest to a large node (bottom row of each set of values). As for the superficial and intra-abdominal depots, all the samples of TAG FA from suet-fed specimens included a much greater proportion of monoenoics (mean 40·1 (SD 4·05) g/100 g total fatty acids) than those from plain-diet guinea-pigs (mean 30·8 (SD 5·18) g/100 g total fatty acids), but site-specific differences in the relative abundance of such FA were significant in only two of the sixteen pairs of samples. The proportions of all classes of FA in TAG from the pairs of samples taken from the two sides of the popliteal lymph nodes in guinea-pigs on both dietary regimens were similar, but the samples taken from beside the small dorsal cervical nodes contained fewer PUFA than those from beside the large central node. All the other pairs of samples in both sets of data revealed the same general pattern of site-specific differences in TAG FA

Table 2. *The proportions (g/100 g total fatty acids) of saturated fatty acids, monoenoic fatty acids, linoleic acid (18:2n-6) and α-linolenic acid (18:3n-3) extracted from the triacylglycerols in samples of adipose tissue from far from lymph node(s) or (in the case of perirenal) knots of blood vessels and near to lymph node(s) or knots of blood vessels from four superficial and three intra-abdominal depots of guinea-pigs fed on suet-enriched chow*

(Mean values and standard deviations for seven guinea-pigs. The means and standard deviations of the differences between each pair of values are also shown)

		Saturated		Monoenoic		18:2*n*-6		18:3*n*-3	
Depot	Site	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Superficial									
Forearm	Far	43·2	5·2	41·9	4·2	12·4	3·0	2·6	1·1
	Near	39·2	2·9	40·4	2·5	16·4	1·3	4·0	0·6
	Difference	4·00	6·02	1·43	3·24	−3·95*	3·67	−1·43*	1·21
Behind arm	Far	45·3	6·5	43·2	6·9	9·7	2·9	1·9	1·1
	Near	43·1	6·2	41·9	4·7	12·4	3·2	2·6	1·1
	Difference	2·16	6·48	1·26	4·89	−2·68*	2·62	−0·74*	0·77
Interscapular	Far	41·5	4·3	43·0	4·3	12·8	4·2	2·7	1·5
	Near	37·8	3·1	42·2	3·6	16·1	1·7	3·9	1·1
	Difference	3·76	4·31	0·80	2·58	−3·34*	4·01	−1·22*	1·20
Inguinal	Far	46·7	5·7	40·9	4·6	10·3	3·0	2·1	1·3
	Near	46·0	3·7	41·5	4·0	10·4	3·1	2·1	1·3
	Difference	0·74	6·30	−0·63	5·19	−0·14	1·43	0·08	0·28
Intra-abdominal									
Mesenteric	Far	39·9	2·2	41·8	3·3	14·8	2·3	3·6	1·2
	Near	32·6	3·1	41·5	1·9	20·9	2·0	5·1	0·9
	Difference	7·24***	1·98	0·31	2·60	−6·06***	1·77	−1·50**	1·05
Omental	Far	43·8	6·9	40·5	3·8	13·0	4·2	2·7	1·3
	Near	36·8	5·0	40·5	2·7	18·2	2·2	4·5	0·7
	Difference	7·07*	7·7	0·00	3·40	−5·28*	4·17	−1·78*	1·46
Perirenal	Far	50·3	3·7	40·4	4·7	7·8	3·8	1·4	1·6
	Near	47·6	3·8	42·7	6·1	8·3	3·6	1·4	1·5
	Difference	2·75	3·48	−2·30	2·62	−0·50	1·31	0·04	0·12

Mean differences between the composition of the 'near node' and 'far from node' adipose tissue of the same depot were significant: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ (Student's t test).

composition: saturated FA were more abundant, monoenoic FA constant (with the exceptions noted) and dienoic and trienoic FA less abundant in samples taken remote from lymph nodes than in those from beside a lymph node(s). In the case of the guinea-pigs fed on plain chow (Table 3), the statistical significance was consistently higher in samples taken from sites about 6 mm or more from nodes. In spite of the small size of these intermuscular depots, the differences in TAG FA composition between 'near node' and 'far from node' samples were as great as those observed between sample sites so defined in the much more extensive mesenteric and omental depots (Tables 1 and 2).

Interactions with lymphoid cells in vitro

Table 5 summarizes as unsaturation index (UI), a measure that takes account of changes in the proportions of all the FA, the compositions of TAG FA in the adipose depots studied in the guinea-pigs fed on plain chow that were used for these experiments, and in homologous samples from those fed for a few months on the suet-enriched diet. The UI were

Table 3. *The proportions (g/100 g total fatty acids) of saturated fatty acids, monoenoic fatty acids, linoleic acid (18:2n-6) and α-linolenic acid (18:3n-3) extracted from the triacylglycerols in samples of adipose tissue from sites defined by their relation to lymph node(s) in two small intermuscular depots of guinea-pigs fed on plain chow*†

(Mean values and standard deviations for seventeen guinea-pigs. Means and standard deviations of the differences between each pair of values are also shown)

	Saturated		Monoenoic		18:2*n*-6		18:3*n*-3	
Depot	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Popliteal								
Furthest from node, dorsally	46·5	13·3	30·4	6·0	19·4	7·3	3·7	2·1
Difference	−9·97***	8·26	−1·24	4·90	8·09***	4·97	3·18***	2·21
Furthest from node, anteriorly	52·6	15·5	31·3	6·6	13·6	8·4	2·5	2·0
Difference	−16·08***	8·01	−2·05	5·35	13·90**	4·92	4·32***	3·32
Centre, 6 mm anterior to node	41·2	11·0	31·8	4·7	22·7	6·6	4·3	1·9
Difference	−4·64**	5·00	−2·59**	2·78	4·86***	4·22	2·49***	2·22
Centre, 4 mm anterior to node	39·9	9·4	30·8	4·2	24·1	5·4	5·3	2·0
Difference	−3·36**	6·96	−1·59	3·61	3·41**	4·1	1·57**	1·89
Beside node, anterior	35·6	8·0	29·8	3·6	27·7	5·4	6·9	2·5
Difference	0·83	3·50	−0·60	1·58	−0·28	1·68	−0·11	0·78
Beside node, posterior	36·5	8·2	29·2	3·8	27·5	5·6	6·8	2·6
Cervical								
Furthest from nodes, ventrally	53·5	16·1	30·5	7·0	13·3	8·3	2·6	2·1
Difference	−16·2***	12·01	−1·00	6·53	13·15***	5·35	4·30***	2·90
Furthest from nodes, dorsally	50·4	15·3	31·4	6·1	15·5	6·1	2·8	1·9
Difference	−13·09**	11·46	−1·97	4·93	11·1***	6·63	3·98***	2·91
Beside small dorsal nodes	40·3	11·2	31·6	4·4	22·8	6·3	5·4	2·4
Difference	−2·99	6·06	−2·05**	2·70	3·79**	3·8	1·35*	2·60
Beside large central node	37·3	8·4	29·5	3·6	26·5	5·1	6·8	2·3

Mean differences between the composition of the 'beside node, posterior' samples of popliteal, or 'beside large central node' of cervical, and others from the same depot were significant: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ (Student's two-tailed *t* test).

† A preliminary account of some of these data appears in Pond (1996*b*).

significantly different between dietary groups for pairs of 'near node' samples from the perirenal and three superficial depots, with that for the inguinal depot being highly significant. The UI were highly significantly different for pairs of the 'far from node' samples taken from all the superficial depots and mesenteric, and significant for perirenal. The differences in UI of FA in TAG in homologous samples from the two groups of guinea-pigs were smaller in the 'near node' superficial samples (the clear trend for the 'near node' interscapular sample was not quite significant at $0{\cdot}1 > P > 0{\cdot}05$), and insignificant between both the intermuscular samples, and between those from near

Table 4. *The proportions (g/100 g total fatty acids) of saturated fatty acids, monoenoic fatty acids, linoleic acid (18:2n-6) and α-linolenic acid (18:3n-3) extracted from the triacylglycerols in samples of adipose tissue from sites defined by their relations to lymph node(s) in two small intermuscular depots of guinea-pigs fed on suet-enriched chow*

(Mean values and standard deviations for seven guinea-pigs. Means and standard deviations of the differences between each pair of values are also shown)

Depot	Saturated		Monoenoic		18:2*n*-6		18:3*n*-3	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Popliteal								
Furthest from node, dorsally	42·0	8·4	40·5	3·8	14·3	4·3	3·3	1·3
Difference	−8·88*	8·53	0·12	2·10	6·49*	4·90	2·27*	2·19
Furthest from node, anteriorly	47·0	9·6	39·9	5·4	11·0	4·6	2·1	1·4
Difference	−13·93*	10·67	0·80	4·34	9·72**	6·03	3·43*	2·48
Centre, 6 mm anterior to node	37·7	4·6	41·8	4·0	16·5	1·9	4·1	1·1
Difference	−4·58*	4·88	−1·12	3·29	4·25**	2·71	1·45*	1·31
Centre, 4 mm anterior to node	38·2	3·2	41·9	3·3	16·1	2·2	3·9	0·9
Difference	−5·10**	2·85	−1·23	2·57	4·64**	2·32	1·68*	1·24
Beside node, anterior	37·1	6·5	40·0	3·3	18·3	2·8	4·7	1·1
Difference	−4·15	5·05	0·62	2·11	2·49	2·71	0·89	1·54
Beside node, posterior	33·1	3·9	40·6	3·1	20·7	2·4	5·6	1·4
Cervical								
Furthest from nodes, ventrally	50·2	5·3	36·8	3·2	10·4	2·3	2·5	0·9
Difference	−15·11**	8·11	3·35	5·30	9·37**	4·43	2·39*	1·87
Furthest from nodes, dorsally	46·4	6·6	39·9	5·6	11·5	2·8	2·3	0·9
Difference	−11·23*	9·24	0·29	7·55	8·30**	3·95	2·66**	1·25
Beside small dorsal nodes	44·4	5·8	39·2	4·3	13·2	3·3	3·2	0·9
Difference	−9·32*	8·54	0·98	6·90	6·62**	4·77	1·72*	1·54
Beside large central node	35·1	4·8	40·2	4·0	19·8	2·7	4·9	1·2

Mean differences between the composition of the 'beside node, posterior' samples of popliteal, or 'beside large central node' of cervical, and others from the same depot, were significant: * $P < 0.05$, ** $P < 0.01$ (Student's two-tailed t test).

mesenteric and omental lymph nodes. Although there were consistent trends towards higher UI of TAG FA in the 'far from node' samples from the omental, popliteal and cervical depots, there was no hint of similar trends in the 'near node' samples of these depots or for the mesenteric depot.

The capacity of explants of adipose tissue from different sources to inhibit LPS-stimulated lymphocyte proliferation is summarized in Table 6. All the homologous adipose tissue samples from guinea-pigs fed on suet-enriched chow were much less effective in inhibiting lymphocyte proliferation than the corresponding explants from those fed on plain chow. In tissues from the suet-fed guinea-pigs, the greatest inhibition observed (by the 'near node' samples from the mesenteric depot) was to 73 % of the control rate of

Table 5. *The composition of triacylglycerol fatty acids, summarized as unsaturation index, of adipose tissue taken from away from lymph node(s) or, in the case of the perirenal depot, far from knots of blood vessels and from near to lymph node(s) or near to knots of blood vessels from guinea-pigs fed on plain chow* (n *10) or suet-enriched chow* (n *7); homologous samples were taken from four superficial, three intra-abdominal and two intermuscular adipose depots*

(Values are means and standard deviations of one sample from each site of each guinea-pig, except those from popliteal and cervical depots which are the means of three and two samples respectively)

	Far from lymph node(s)				Near to lymph node(s)			
	Plain chow		Suet-enriched chow		Plain chow		Suet-enriched chow	
Depot	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Superficial								
Forearm	50·0	18·1	74·4**	8·9	74·4	14·2	85·1*	4·1
Behind arm	42·4	17·5	68·2***	8·5	59·4	14·8	74·5*	10·1
Interscapular	54·0	14·6	76·6**	10·2	72·8	22·6	86·2	5·3
Inguinal	36·7	16·8	67·8***	9·5	49·5	17·4	68·6**	8·2
Intra-abdominal								
Mesenteric	65·4	16·8	82·1**	5·4	97·3	11·3	98·4	6·5
Omental	60·3	22·9	76·7	13·4	89·1	12·3	90·4	8·2
Perirenal	46·7	19·3	60·3*	9·2	43·8	21·4	63·5*	7·3
Intermuscular								
Popliteal	57·1	21·7	68·3	15·9	97·2	11·8	98·8	7·2
Cervical	52·8	23·6	65·3	8·8	88·2	16·4	94·5	8·0

Mean values were significantly different from homologous samples for the plain chow group: * $P < 0·05$, ** $P < 0·01$, *** $P < 0·001$ (Student's two-tailed t test).

lymphocyte proliferation, compared with a maximum of 33–35 % by the corresponding depots for tissues from guinea-pigs fed on plain chow. As for the samples from guinea-pigs fed on plain chow, the samples from the perirenal and inguinal depots of the suet-fed guinea-pigs were the least inhibitory to lymphocyte proliferation; the mean values from incubations with the perirenal samples were barely significantly different (Student's t test: t 2·174, $P = 0·05$) from those of the control cultures incubated without adipose tissue.

The differences between the inhibitory capacity of the 'near node' and the 'far from node' samples were significant in all eight node-containing depots (i.e. except perirenal) of the guinea-pigs fed on plain chow (highly so for the intermuscular depots), but the values for the corresponding tissues from the suet-fed animals were lower, and were not significant for inguinal and 'behind arm' as well as for perirenal. In all cases, the 'near node' samples were more inhibitory than the 'far from node' samples. The capacity for greater inhibition of lymphocyte proliferation appears to be restricted to the adipose tissue located close to the lymph node: that produced by the middle samples from the popliteal depot was not significantly different from that of the 'far from node' samples, but was highly significantly less than that of the 'near node' samples (Student's t test: t 3·828, $P \ll 0·01$).

Table 7 shows the concentrations of free glycerol in the culture media after incubation for 48 h of adipose tissue explants from all the sites studied for Table 6 cultured alone, compared with those of adjacent explants after incubation with LPS-stimulated lymphoid cells. Large differences between, but not within, depots in the quantity of glycerol released from explants cultured with LPS but without lymphoid cells were found in the samples from guinea-pigs fed on the suet-enriched chow, as well as in those from specimens fed on plain chow. Samples from suet-fed guinea-pigs released significantly more glycerol after

Table 6. *Site-specific differences in the effects of adipose tissue explants from guinea-pigs fed on plain or suet-enriched chow on lipopolysaccharide-stimulated proliferation of their lymphoid cells in culture*†

(Mean values and standard deviations)

	Plain chow (n 10)						Suet-enriched chow (n 7)					
	Far from node		Near node		Differences		Far from node		Near node		Differences	
Depot	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Superficial												
Forearm	54·1	11·7	43·5	3·1	10·6*	12·3	83·7	3·4	80·8	4·8	2·88*	2·38
Behind arm	59·7	13·1	47·5	7·3	12·2*	15·0	89·4	3·4	87·8	4·6	1·69	2·22
Interscapular	51·4	9·8	42·9	2·5	8·4*	10·7	83·8	3·0	80·7	4·4	3·12*	2·45
Inguinal	63·6	14·2	52·7	3·2	10·9*	14·9	91·4	2·4	89·4	4·5	1·97	3·08
Intra-abdominal												
Mesenteric	45·9	12·0	33·1	3·4	12·9*	12·8	78·3	3·2	73·0	6·4	5·28*	4·07
Omental	51·0	12·9	39·5	6·7	11·5*	13·5	84·6	2·7	80·6	4·4	3·95*	3·02
Perirenal	68·7	16·1	63·5	3·1	5·2	15·2	93·4	2·2	94·2	5·0	−0·86	3·10
Intermuscular												
Popliteal	44·6	10·7	33·7	4·1	10·9**	9·5	87·7	6·2	75·0	3·9	12·7***	4·90
						Middle:	84·7	5·4				
Cervical	46·8	9·2	35·3	4·0	11·6**	10·4	78·7	3·1	74·9	3·1	3·83**	2·80

Mean differences between data from incubations with adipose explants from 'far from' and 'near to' lymph node(s) of the same depot of the same specimens were significant: * $P < 0{\cdot}05$, ** $P < 0{\cdot}01$, *** $P < 0{\cdot}001$ (Student's two-tailed t test).

† Cell proliferation was measured as incorporation of [^{3}H]thymidine into lymphoid cells incubated with one explant of adipose tissue taken from far from lymph node(s) or, in the case of the perirenal depot, away from knots of blood vessels and from near to lymph node(s) or near to knots of blood vessels. All values are percent of maximum incorporation into lymphoid cells incubated without adipose tissue, which for guinea-pigs fed on plain chow was 100 % = 17 541 (SD 470) dpm, and for those fed on suet-enriched chow 100 % = 18 754 (SD 219) dpm. Sets of samples were taken from four superficial, three intra-abdominal and two intermuscular adipose depots.

incubation alone than homologous samples from guinea-pigs fed on plain chow only in the cases of 'far from node' samples from the forearm, omental, popliteal and cervical depots; all nine pairs of values for homologous 'near node' samples were similar to each other.

Co-incubation with lymphoid cells was less effective in stimulating lipolysis in almost all explants from node-containing depots of the suet-fed guinea-pigs than in homologous samples from the controls (Table 7). The only 'near node' samples from both the plain-diet and suet-fed guinea-pigs to release similar quantities of glycerol were those from the popliteal depot: all other cultures with lymphoid cells from suet-fed guinea-pigs contained significantly less glycerol. All eight values from incubations with perirenal tissue were statistically indistinguishable, showing that glycerol release from perirenal adipose tissue was unchanged by either the modified diet or by co-incubation with lymphoid cells, although its composition and its capacity to inhibit lymphocyte proliferation were altered by enrichment of the diet with suet (Table 5). In having no detectable response to lymphoid cells, the perirenal stands out from all the other depots studied. Although often regarded as representative of other depots, the perirenal is clearly atypical in its interactions with lymphoid cells.

The maximum stimulation of glycerol release (in the mesenteric 'near to node' explants) was reduced from 153 % in samples from the specimens fed on plain chow to 133 % for the suet-fed specimens. The inguinal and 'behind arm' samples from the suet-fed guinea-pigs resembled those of the perirenal in that the presence of lymphoid cells did not significantly increase glycerol release above the quantity produced by similar explants cultured alone. The mean quantity of glycerol released from the middle popliteal samples

Table 7. *Concentration of glycerol (μmol/ml) in the culture medium after incubation for 48 h, alone or with lipopolysaccharide-stimulated lymphoid cells, of explants of adipose tissue taken from away from lymph node(s) or knots of blood vessels or near to a lymph node or knot of blood vessels in four superficial, three intra-abdominal and two intermuscular depotsin of guinea-pigs fed on plain chow* (n *10) or suet-enriched chow* (n *7)*

(Mean values and standard deviations)

		Adipose tissue explants alone				Adipose tissue + lymphoid cells			
		Far from lymph node		Near to lymph node		Far from lymph node		Near to lymph node	
Depot		Plain	Suet	Plain	Suet	Plain	Suet	Plain	Suet
Superficial									
Forearm	Mean	46·9	55·4**	48·9	54·6	91·3	84·1**	118·2	106·2**
	SD	3·2	5·9	4·7	6·0	3·3	5·9	8·7	5·8
Behind arm	Mean	61·6	64·1	61·2	62·1	84·6	70·9**§	104·3	88·6**
	SD	3·2	5·4	3·2	7·1	7·0	8·8	6·1	6·0
Interscapular	Mean	48·8	54·1	49·6	55·9	91·3	73·1*	114·5	103·4*
	SD	6·0	7·4	7·4	6·4	3·7	11·8	11·9	7·6
Inguinal	Mean	64·5	67·1	67·5	66·1	80·3	70·1*§	99·6	81·7**
	SD	8·9	8·6	6·3	7·2	8·1	10·1	7·0	7·5
Intra-abdominal									
Mesenteric	Mean	55·7	54·5	56·5	55·9	102·2	92·1**	143·3	130·2**
	SD	3·2	4·8	5·4	2·5	6·6	3·2	14·2	4·6
Omental	Mean	54·9	58·9**	55·6	59·7	91·8	77·0**	122·3	106·6**
	SD	3·0	2·3	3·6	2·5	4·7	7·4	12·1	3·0
Perirenal	Mean	73·4	74·0	74·1	75·2	78·0§	75·4§	78·5§	79·0§
	SD	3·6	7·2	4·1	5·2	3·6	7·3	2·7	5·9
Intermuscular									
Popliteal	Mean	40·6	52·4**	39·6	42·7	94·6	85·9**	125·7	122·4
	SD	2·4	9·1	4·5	6·0	5·7	5·3	12·2	7·3
		Middle of depot	46·9 8·6			Middle of depot	97·6 4·9		
Cervical	Mean	35·7	41·2*	38·2	41·9	94·4	82·2**	131·6	115·5**
	SD	4·3	4·5	4·0	3·7	10·6	8·9	13·1	8·2

Mean values were significantly different from those for guinea-pigs fed on the plain diet, measured under similar incubation conditions: * $P < 0{\cdot}05$, ** $P < 0{\cdot}01$, *** $P < 0{\cdot}001$ (Student's t test).

§ Mean values were not significantly different from those for homologous samples incubated alone; all other values in this comparison were significantly different, $P < 0{\cdot}05$.

incubated alone was similar to that of the other popliteal samples, but in the presence of lymphoid cells, it was 12 % more than that produced by the 'far from node' sample (difference significant at $P \ll 0{\cdot}01$), and 20 % less ($P \ll 0{\cdot}001$) than the values from the 'near to node' samples.

Although substantial, these reductions in glycerol release following a suet-enriched diet were smaller than the reductions in incorporation of labelled thymidine (Table 6). Glycerol release was similar in all homologous 'near to node' samples from the two groups of guinea-pigs after incubation alone, but 'far from node' samples from the forearm, omental and intermuscular depots of suet-fed guinea-pigs produced significantly more glycerol (Table 7). The effects of adding lymphoid cells to the incubation medium on glycerol release were lower for tissues derived from suet-fed guinea-pigs for all samples except those from near to the popliteal lymph node (Table 7).

The effect of diet on the relationship between glycerol released and inhibition of lymphoid cells is summarized in Fig. 1. All values for the 'near node' samples formed

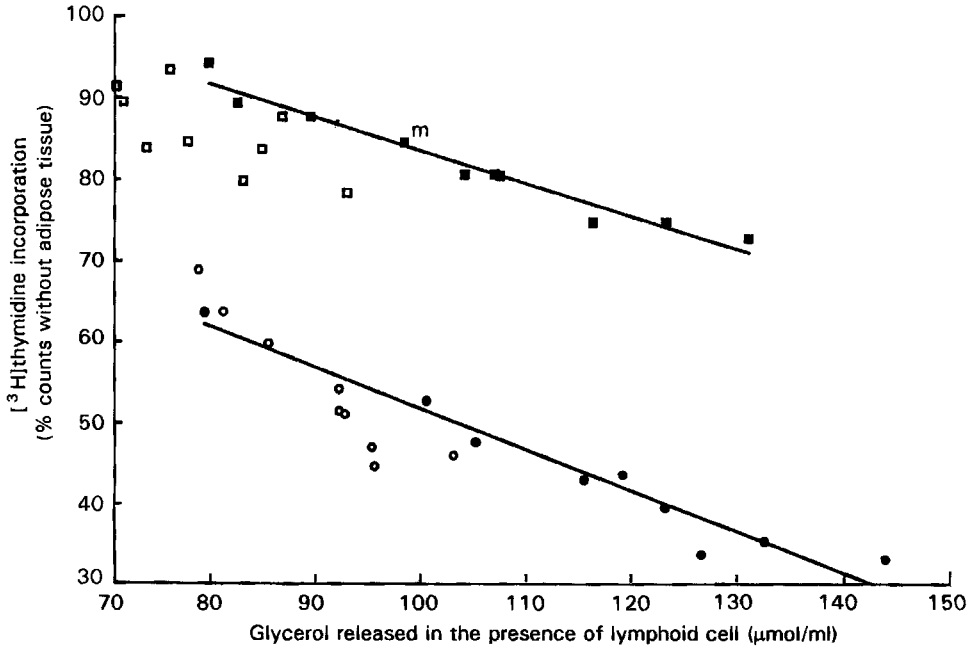

Fig. 1. The correlation between inhibition of lymphocyte proliferation (% maximum) and the total glycerol (μmol/ml) present in the culture media after incubation of adipose tissue explants from all depots studied with lipopolysaccharide-stimulated lymphoid cells (data from Tables 6 and 7). (●, ■), Adipose tissue from near to lymph nodes; (○, □), adipose tissue from far from lymph nodes. The point labelled 'm' is from the middle of the popliteal depot. (●, ○), Tissues from guinea-pigs fed on plain chow; 'near node' values fit the equation: $y = 101{\cdot}64 - 0{\cdot}50x$, r 0·972. (■, □), Tissues from guinea-pigs fed on suet-enriched chow for 3–5 months; 'near node' values fit the equation: $y = 123{\cdot}29 - 0{\cdot}40x$, r 0·978.

almost parallel straight lines while those for the 'far from node' samples were more variable. The 'far from node' adipose explants inhibited lymphoid cell proliferation slightly more than those from near nodes for similar quantities of glycerol release, especially for the samples from the suet-fed specimens. Extrapolation of the equations fitted to the data from the 'near node' samples shows that the glycerol concentration produced by tissues from the guinea-pigs fed on plain chow at which inhibition of lymphocyte proliferation would be completely abolished (i.e. [^{3}H]thymidine incorporation is 100%) is only 3 μmol/ml (95% confidence limits, 0–23 μmol/ml), essentially zero, but nearly twenty times higher, 58 μmol/ml (95% confidence limits, 44–71 μmol/ml), for tissues from suet-fed animals cultured under similar conditions.

The explants with the greatest capacity to inhibit mitogen-stimulated lymphocyte proliferation (Table 6) both released the largest quantity of glycerol when cultured (Table 7) and contained TAG with the largest proportions of PUFA (Tables 1–4). Site-specific differences in the composition of the free (i.e. non-esterified) fatty acids (FFA) in the tissue culture media corresponded closely to those of the TAG in the adipose tissue which had been incubated in it (Pond & Mattacks, 1995). So, to determine how FFA composition alone curtails lymphocyte proliferation, the ratio of percentage thymidine incorporation (Table 6) to glycerol concentration in the corresponding incubation medium (Table 7) had to be compared with the PUFA : saturated FA ratio in the incubation medium, as shown in Fig. 2. For tissue samples from both dietary groups, the slopes of the regression lines fitted to the values for 'near node' and 'far from node' samples were similar, although since the

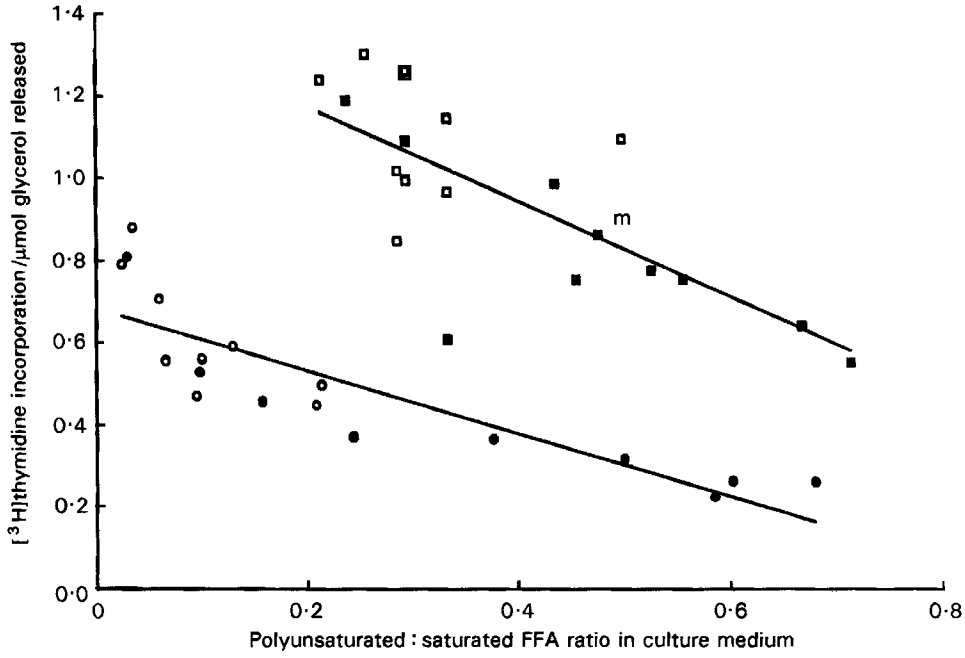

Fig. 2. The relationship between the polyunsaturated : saturated ratio of free fatty acid (FFA) released into the incubation medium from the adipose tissue explants, and the ratio of the incorporation of [^{3}H]thymidine into lymphoid cells to glycerol released (data recalculated from Tables 6 and 7). (●, ■), Adipose tissue from near to lymph nodes; (○, □), adipose tissue from far from lymph nodes. (●, ○), Tissues from guinea-pigs fed on plain chow; (■, □), tissues from guinea-pigs fed on suet-enriched chow. All eighteen values for the guinea–pigs fed on plain chow fit the equation: $y = 0{\cdot}684 - 0{\cdot}756x$, r 0·852; all nineteen values, including the point (labelled m) from the middle of the popliteal depot, from the guinea-pigs fed on suet-enriched chow fit the equation: $y = 1{\cdot}405 - 1{\cdot}14x$, r 0·735.

'far from node' samples from the suet-fed animals inhibited lymphocyte proliferation so weakly (Table 6), the data from these incubations were more variable. Fig. 2 shows that FFA in the tissue culture medium with a greater proportion of polyunsaturates were more effective per unit of lipolysis for inhibiting lymphocyte proliferation, whether they came from adipose tissue that was located near to or far from nodes. The minor changes in FFA produced by the modified diet greatly altered the capacity of lymphoid cells to respond to the presence of adipose tissue.

DISCUSSION

The effects of the suet-enriched diet on body mass were minimal, and those on the TAG FA composition were small, because the guinea-pigs were fully mature before the modified diets were applied, and turnover of storage TAG was not hastened by prolonged fasting or forced exercise, as was imposed on rabbits by Lin *et al.* (1993). As well as being raised on a normal diet, the suet-fed guinea-pigs also received all the nutrients in the normal chow, albeit smaller quantities because, since they did not become heavier, they probably ate less of the enriched chow and maintained constant body composition. Nonetheless, the high proportion of saturated and monoenoic FA, especially oleic acid (18 : 1*n*-9), and the lack of PUFA, especially 18 : 3*n*-3, in beef suet (Paul *et al.* 1980) are reflected in the composition of all samples from the guinea-pigs fed on suet-enriched chow. The exact cause of the greater variation between specimens fed on plain chow in the average composition of TAG

FA in all adipose tissue samples (Tables 1–4) is not known, but it was probably due mainly to changes during the guinea-pigs' lifetime in the lipid composition of the chow (the manufacturers admit to minor differences in the ingredients of different batches).

Composition of adipose tissue triacylglycerol fatty acids

The absence of significant differences in the composition of the TAG FA between samples taken from 'far from' the lymph nodes of different adipose depots (Tables 1–4) are consistent with many previous studies in both human subjects and animals (Field & Clandinin, 1984; Malcom *et al.* 1989; Calder *et al.* 1992; Colby & Pond, 1993). The trend towards more saturated FA in the inguinal depot has been found in previous studies of guinea-pigs (Colby & Pond, 1993), and there are hints of a similar situation in the homologous depot of women (Phinney *et al.* 1994). To minimize injury, biopsy sites are normally chosen to avoid blood or lymph vessels and lymph nodes (Hunter *et al.* 1992; Tjønneland *et al.* 1993), and in *post mortem* investigations, 'pure' adipose tissue samples, taken from as far as possible from such structures, are usually selected for study (Malcom *et al.* 1989; Valero-Garrido *et al.* 1990; Frank, 1991; Calder *et al.* 1992; Colby & Pond, 1993; Lin *et al.* 1993). So the composition of the TAG FA extracted from adipose tissue near lymph nodes has never been studied systematically in any species.

The close similarity in the TGA FA composition of the two samples from the perirenal depot indicates that the key feature associated with the site-specific properties is a lymph node, not a confluence of blood vessels. The 'near node' samples from the mesenteric, omental, forearm, popliteal and cervical depots, that contain large or numerous nodes, consistently contained significantly more PUFA and fewer saturated FA than the 'far from node' samples. The position of the lymph nodes in the intermuscular adipose depots was consistent enough to demonstrate gradients of TAG FA composition with distance from nodes. In spite of the small size of the popliteal and cervical depots (Pond *et al.* 1984*b*; Pond & Mattacks, 1995), the ranges of values for the proportions of PUFA and saturated FA within them (Tables 3 and 4) were as great as any of the differences measured among all the other samples studied.

The patterns of site-specific differences were similar for homologous samples from the two dietary groups, indicating that local determinants of TAG FA composition can transcend substantial changes in the average composition of TAG FA. Site-specific differences in adipocyte volume (Pond *et al.* 1984*b*) and glycolytic enzyme activity (Mattacks *et al.* 1987) have been demonstrated in the guinea-pig popliteal adipose depot. The sites of those samples correspond approximately to those studied here (Tables 3 and 4), but nothing is known of how such structural features and metabolic properties relate to TAG FA composition.

The steep and consistent gradation in TAG FA composition in the intermuscular depots (Tables 3 and 4) emphasizes that only a small proportion of any adipose depot has the properties associated with lymph nodes, even in moderately large depots that contain several nodes, such as the omental and mesenteric. We estimate that, in guinea-pigs, only a small fraction of the total adipose tissue, probably less than 5%, interacts strongly with lymphoid tissue (Pond & Mattacks, 1995; Pond, 1996*a*) and contains PUFA-enriched TAG FA. The presence of TAG containing FA with a higher proportion of PUFA in the small fraction of adipose tissue associated with lymph nodes would not make much difference to the overall abundance of such FA in the body as a whole: but if the adipose tissue around the lymph nodes makes them preferentially available to lymphoid tissue, supplies of rare or essential FA could be maintained for the immune system, even when such lipids are scarce

or absent in the diet. FA have a wide range of subtle effects on lymphocyte function (Calder, 1995), so a 'nurse' tissue around lymph nodes that actively sequesters and controls the release of such rare but essential metabolites may make an indispensable contribution to immune responses.

The site-specific differences in responses of overall TAG FA composition to dietary change (Table 5) correspond exactly to those found by Colby & Pond (1993) in much younger guinea-pigs: in the brief period for which the suet-enriched diet was imposed, the FA compositions of adipose tissue TAG changed significantly in the perirenal and inguinal depots, but those of the omental and intermuscular depots were not detectably altered. The data presented here also demonstrate within-depot differences in the effects of diet on the composition of adipose tissue TAG FA: that of 'far from node' samples changed more during the period for which the diet was imposed than that of the 'near node' samples, with UI of samples from near lymph nodes in the omentum, mesentery and intermuscular depots not significantly different from that of the corresponding samples of the controls. Colby & Pond's (1993) data, and the fact that the 'near node' samples from three superficial depots were significantly different in the two dietary groups, suggest that if continued for long enough, the suet-enriched diet would alter the FA composition of all adipose tissue TAG.

There are several reports of the site-specific differences in composition of adipose tissue TAG FA. In Svalbard reindeer (*Rangifer tarandus platyrhynchus*), which remain active throughout the year in a cold climate, TAG in the adipose tissue near the skin contain a greater proportion of unsaturated fatty acids than those in the deeper adipose tissue in the same depot (Pond *et al.* 1993). Such phenomena and the site-specific differences in TAG FA composition in human adipose tissue (Phinney *et al.* 1994; Seidelin, 1995) have been explained as adaptations to tissue temperature, probably associated with the need to retain fluidity at lower temperatures.

Such an explanation clearly cannot account for the within-depot differences (Tables 1–4), which were found to be greatest in internal depots such as the mesentery, omentum and intermuscular depots that must be at almost uniform temperature. Our finding that the relative abundance of saturated FA and PUFA reciprocate, while that of monoenoics is constant for all depots of the same animal (Tables 1–4), contrasts with previous investigations into site-specific differences in TAG FA composition. Comparison between composition of deep, superficial and distal human tissues led Seidelin (1995) to conclude that there are small but significant differences in the saturated : monoenoic FA ratio (mostly 18 : 0/16 : 1) in relation to tissue temperature, but that linoleic acid is uniformly deposited in TAG over all body sites.

We propose that the site-specific differences in TAG FA composition reported here are adaptations to local interactions with lymphoid cells in lymph nodes. Explants of adipose tissue from depots in which the 'near node' samples consistently contain significantly more PUFA and fewer saturated FA than the 'far from node' samples (Tables 1–4) interact most strongly with lymphoid cells *in vitro* (Pond & Mattacks, 1995; Tables 6 and 7), increasing the rate of lipolysis by up to 245 %. PUFA are more strongly implicated in the direct action of FA on the genome than saturates or monoenoic FA (Clarke & Jump, 1994) and the PUFA, linoleic acid, has been identified as a specific activator of nuclear transcription factors (Hennig *et al.* 1996). Furthermore, this FA has been shown to amplify the action of the lymphokine tumour necrosis factor-α (TNF-α) at least in cultured cells (Toborek *et al.* 1996). If the FA thus released near the nodes from such adipose tissue reflect the composition of those in the TAG, they would include a greater proportion of PUFA than those released into the general circulation by the much larger quantities of adipose tissue that are not intimately associated with lymph nodes. The limited information about blood

flow between lymph nodes and adjacent tissues, and other mechanisms that could contribute to local interactions, is reviewed elsewhere (Pond, 1996*a*,*b*).

It has long been assumed that continuous turnover of TAG FA by hydrolysis and re-esterification of FA would in time homogenize the composition of all storage lipids. However, processes that could produce the heterogeneity in lipid composition of adipose tissue summarized in Tables 1–4 have recently been demonstrated (Lin *et al.* 1993; Raclot & Groscolas, 1993, 1994). Isolated rat epididymal adipose tissue selectively releases 20 : 4*n*-6 (arachidonic acid) and 18 : 3*n*-3, while retaining other unsaturated FA (Gavino & Gavino, 1992). Adipocytes isolated from the perirenal depot of rats and stimulated with noradrenaline selectively release more unsaturated and shorter chain FA, thereby selectively retaining longer chain FA (Raclot & Groscolas, 1993). Such selective uptake and/or selective release of FA could maintain the site-specific differences in TAG FA composition described here. Unfortunately, these observations were made only on the perirenal, inguinal or epididymal adipose depots, which we find to have little or no specific interaction with lymphoid cells (Pond & Mattacks, 1995), so it is impossible to say whether selective mobilization has anything to do with making such FA available to the immune system.

If the contribution of the adipose tissue around the nodes to the nutrition and regulation of the lymph nodes is large compared with that of the rest of the adipose mass and nutrients in the general circulation, attempts to modulate immune function by dietary manipulation (Yaqoob *et al.* 1994) may be delayed or attenuated. Several diverse kinds of proteins contribute to the uptake of lipids into and around cells (Fielding, 1993), and the metabolic fate of FA in cells can depend on their mode of delivery and uptake (Teruya *et al.* 1995). The small mass of the adipose tissue associated with lymph nodes and the possibility of local transfer mean that FA released from such sources would be unlikely to produce detectable effects on the composition or abundance of non-esterified FA in the general blood circulation. So the composition of FA measured in blood samples (Erickson *et al.* 1983; Haugen *et al.* 1994) may not accurately reflect that of the FA actually available to the lymphoid cells in the nodes.

The site-specific differences in composition of TAG FA within and between adipose depots demonstrated by these data (Tables 1–4) may help to explain the variable and inconsistent results that frequently emerge for studies in which insufficient attention is given to the exact anatomical sources of adipose tissue samples from different specimens, or from the same specimen taken sequentially (Valero-Garrido *et al.* 1990; Hunter *et al.* 1992; Lin *et al.* 1993; Tjønneland *et al.* 1993). If the samples compared were from parts of adipose depots that differed in their anatomical relations to major lymph nodes, the FA composition of the TAG in them would be different, whatever the effects of diet. Similar explanations may account for the poorly defined site-specific differences in TAG FA composition that have been reported (Malcom *et al.* 1989; Calder *et al.* 1992; Phinney *et al.* 1994). Such measurements might be more reliable and produce more clear-cut conclusions if adipose tissue samples from exactly homologous sites were compared.

Interactions between adipose tissue and lymphoid cells in vitro

The suet-enriched diet fed to these adult guinea-pigs produced few detectable changes in the metabolism of either lymphoid cells or adipose tissue when each was cultured alone. LPS-stimulated lymphocytes from both dietary groups proliferated to a similar extent when incubated without adipose tissue, although in young rats, dietary lipids alter the

composition and properties of lymphoid cells (Anel *et al.* 1990*a*; Calder *et al.* 1994; Yaqoob *et al.* 1994).

The large differences between depots in glycerol released from adipose tissue explants incubated with LPS but without lymphoid cells described previously for guinea-pigs fed on normal chow (Pond & Mattacks, 1995) were also found in homologous samples from those fed on the suet-enriched diet (Table 7). Spontaneous lipolysis produced the same quantity of glycerol from all homologous 'near node' samples from normal and suet-fed guinea-pigs, but there were some differences in the quantity of glycerol released from homologous 'far from node' samples from the two groups of guinea-pigs that could have been due to differences in handling the animals as they were killed. Noradrenaline, which is released within seconds of the animal experiencing fear or excitement and can remain active for hours, is a major determinant of lipolysis in such adipose tissue (Pond & Mattacks, 1991). Consistent with this interpretation is the fact that differences between tissues from the two dietary groups were significant for samples from forearm, popliteal and cervical, the depots in which the adipocytes were found to be most sensitive to noradrenaline (omental was not studied) (Pond & Mattacks, 1991). The effects of agonists such as insulin and noradrenaline on the adipose tissue that surrounds lymph nodes have never been studied. But the fact that the quantity of glycerol released after the incubation without lymphoid cells from all eight such explants is almost identical for homologous samples from both dietary groups (Table 7) is consistent with the suggestion that this adipose tissue, which is specialized to produce glycerol at a very high rate in the presence of lymphoid cells (Pond & Mattacks, 1995), is less responsive to such circulating agonists than that remote from lymph nodes.

Although both adipose tissue explants and lymphoid cells from animals fed on the different diets behaved similarly when each was studied alone, co-incubation revealed that the suet-enriched diet greatly modified the interactions between them (Tables 6 and 7). There are clear discrepancies between the effects of diet on the FA composition of adipose tissue TAG (Table 5), glycerol release (Table 7) and capacity to inhibit lymphocyte proliferation (Table 6). The compositions of TAG FA, and hence that of the FA released with the glycerol, were not significantly altered by the suet-enriched diet in the omental and intermuscular depots, but their capacity to inhibit lymphocyte proliferation was nonetheless substantially altered by the experimental diet, so FFA in the media cannot be the only cause of retention of the within-depot differences. The middle popliteal sample was not significantly more effective in inhibiting lymphocyte proliferation than the 'far from node' sample, although its glycerol production and its TAG FA composition (Tables 3 and 4) were intermediate between those of the samples from nearest to and furthest from the node.

As summarized in Fig. 1, a significant relationship between glycerol release and inhibition of lymphocyte proliferation remained in guinea-pigs fed on the suet-enriched chow, at least for adipose tissue from near to lymph nodes, although the inhibitory capacity of the adipose tissue explants was greatly reduced. These effects are unlikely to be due simply to the fact that the experimental diet contained a greater proportion of lipid, because feeding chow enriched with olive oil and sunflowerseed oil for a similar period does not significantly alter the interactions between adipose tissue and lymphoid cells *in vitro* (Pond, 1996*b*). The action of the suet-enriched diet on the functional relationship between adipose tissue and lymphoid cells suggests that either the diet has altered the capacity of the lymphocyte membrane to take up FA, or that some factor other than lipolytic products mediates adipose tissue's inhibition of lymphocyte proliferation and was affected by the diet. The case for the former explanation is strengthened by Fig. 2, which shows that the PUFA : saturated FA ratio in the FFA available to the lymphocytes in the culture medium

correlated with inhibition per unit of glycerol released to a similar extent in the tissues from suet-fed guinea-pigs and in those from the controls, and applied to both 'near node' and 'far from node' explants. By the same reasoning, it is unlikely that all the effects of the suet-enriched diet were due to the larger proportion of monoenoic FA: all adipose tissue samples from the suet-fed guinea-pigs contained more monoenoic FA (Tables 1–4), but inhibition of lymphocyte proliferation per unit of glycerol released was just as sensitive to the PUFA : saturated FFA ratio (Fig. 2).

Lymphocyte phospholipids exchange FA with those in their surroundings within 48 h (Yaqoob & Calder, 1993; Yaqoob *et al.* 1994), much more quickly than FA corresponding to the diet appear in detectable quantities in adipose tissue TAG. Although activation of desaturase enzymes is an integral part of the response of lymphocytes to mitogens, their transformations only 'fine tune' the proportions of FA in the membranes. Their composition in activated lymphocytes is due mainly to uptake of FFA from the surroundings (Anel *et al.* 1990*b*). A wide variety of lipids affect the activity of protein kinase C, including FA as FFA or esterified as diacylglycerols (Merrill & Schroeder, 1993), and can thereby affect lymphocyte metabolism (May *et al.* 1993). The lipid composition of the diet also affects the capacity of macrophages (Yaqoob & Calder, 1995*a*) and T-lymphocytes (Yaqoob & Calder, 1995*b*) to produce certain cytokines, although no changes that could explain the action of the particular diet used for these experiments were reported.

We wish to emphasize that whatever the cause of the changes in properties, they could be demonstrated only by examining the interaction between the two tissues: no effects of diet were detected when adipose tissue or lymphoid cells were studied in culture alone. Minor changes in the FA composition of dietary lipids alter the overall proportions of the major FA in the adipose tissue TAG, but the pattern of within-depot site-specific differences remains. Diets high in saturated and monoenoic FA impair the action of adipose tissue on lymphoid cells, and to a lesser extent the inverse action. Interactions with lymphoid cells were most efficiently retained in sites that interact most strongly *in vitro* with lymphoid cells from guinea-pigs fed on plain chow. If the interactions between adipose tissue around lymph nodes and lymphoid cells studied here are physiologically important *in vivo*, e.g. during undernutrition, fever or anorexia, their alteration by dietary lipids may impair the capacity of adipose tissue to contribute to the regulation of the immune response.

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