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Interactions between adipose tissue and the immune system

BY CAROLINE M. POND

Department of Biology, The Open University, Milton Keynes MK7 6AA

Interactions entre le tissu adipeux et le système immunitaire

RÉSUMÉ

Un faisceau d'arguments indique l'implication du tissu adipeux dans la nutrition et la régulation du système immunitaire. L'anorexie accompagne presque invariablement les réponses immunitaires systémiques aiguës, qui, avec les modifications de tout le métabolisme des lipides du corps induits par l'infection, augmentent la lipolyse dans le tissu adipeux. Les acides gras ainsi relargués sont importants pour le tissu lymphoïde en tant que source d'énergie, composants de membranes et substrats pour la synthèse de cytokines complexes à base de lipides, telles que les leukotriènes, les prostacyclines, les thromboxanes et les prostaglandines. Des études *in vitro* ont démontré que les acides gras isolés peuvent agir aussi bien comme promoteurs que comme inhibiteurs de la prolifération lymphocytaire stimulées par un mitogène chez le rat et chez l'homme. Les acides gras non saturés sont sélectivement incorporés dans les lymphocytes en prolifération *in vitro*, et la composition en acide gras des lipides de l'alimentation affecte plusieurs aspects différents de la réponse immunitaire. Plusieurs travaux ont montré que les différentes cytokines sécrétées par les cellules lymphoïdes stimulent la lipolyse dans les adipocytes en culture. L'action du facteur de nécrose tumorale (TNF) semble dépendre de sa source dans le corps aussi bien que de sa concentration dans le sang veineux, impliquant fortement les interactions locales entre les tissus adjacents; cependant, il n'y a pas eu de tentatives systématiques pour identifier les différences spécifiques liées aux sites dans l'implication de différents dépôts adipeux dans les interactions avec le système immunitaire.

Les ganglions en tant que site important de prolifération et de dissémination lymphocytaire sont une caractéristique spéciale des mammifères adultes; on retrouve quelques structures similaires chez certains oiseaux mais les ganglions n'existent pas chez les vertébrés inférieurs. Entre l'anatomie du tissu adipeux des mammifères et celui des vertébrés inférieurs il existe un contraste très important dans sa fragmentation en nombreux dépôts, dont la plus grande partie contient des ganglions lymphatiques. Chez les mammifères, à peu près tous les gros ganglions lymphatiques, et un grand nombre de plus petits sont insérés dans du tissu adipeux. De petites quantités de tissu adipeux (quelques grammes ou moins) appraissent autour du ganglion poplité même chez des mammifères tels que les pinnipèdes, chez lesquels presque tout le tissu adipeux est spécialisé pour former de la graisse superficielle. Le tissu adipeux autour des ganglions est le dernier à disparaître chez les animaux sauvages très maigres. La littérature ancienne en anatomie mentionne fréquemment des associations étroites entre le tissu adipeux et les ganglions lymphatiques chez différents mammifères, y compris chez l'homme, dont beaucoup changent au cours d'états ontogéniques ou pathologiques. Une autre structure lymphoïde, le thymus, est aussi étroitement associée avec le tissu

adipeux chez les mammifères à la naissance, et est progressivement remplacé par lui, si bien que chez le cochon d'inde à l'âge d'un an, le site du thymus qui a disparu est constitué essentiellement de tissu adipeux. Bien connues des anatomistes, les propriétés biochimiques et physiologiques du tissu adipeux associé aux ganglions lymphatiques n'ont cependant jamais été étudiées.

Ce travail décrit les résultats *in vitro* des interactions locales entre les cellules lymphoïdes et le tissu adipeux de dix-huit différents sites définis par leurs relations *in situ* avec les ganglions lymphatiques (Pond & Mattacks, 1995). Il rapporte également les différences spécifiques selon les sites dans la composition des acides gras de triacylglycérol dans le tissu adipeux qui entoure les ganglions. Tous les explants de tissu adipeux inhibaient la prolifération lymphocytaire, qu'elle soit spontanée ou stimulée par le mitogène. Pour tous les dépôts contenant des ganglions, le tissu adipeux à côté d'un ganglion supprimait la prolifération des lymphocytes davantage que les échantillons des mêmes dépôts à des sites distants de 5–10 mm d'un ganglion. L'inhibition par le tissu adipeux provenant de tous les dépôts était presque totalement supprimée en présence de 500 μU d'insuline. Le tissu lymphoïde stimulait la lipolyse dans tous les tissus adipeux, excepté le tissu périrénal. Pour certains dépôts, les augmentations de la lipolyse, dans la petite fraction de la masse adipeuse qui entoure les ganglions, produites par la présence du tissu lymphoïde étaient bien plus importantes que celles dues aux agents lipogéniques systémiques tels que l'adrénaline. La capacité des différentes echantillons de tissu adipeux d'inhiber la prolifération lymphocytaire était proportionnelle à la lipolyse supplémentaire stimulée par la présence de cellules lymphoïdes. Pour tous les dépôts excepté ceux du mésentère, le relargage de glycérol stimulé par les cellules lymphoïdes était inversement proportionnel à la quantité mesurée dans des échantillons similaires de tissu adipeux incubés seuls dans les mêmes conditions.

Dans presque tous les dépôts contenant des ganglions, les index de non-saturation des acides gras de triacylglycérol extraits du tissu adipeux à côté des ganglions étaient significativement plus élevés que ceux d'échantillons plus éloignés des ganglions dans le même dépôt, particulièrement chez les animaux chez lesquels les lipides de réserve étaient riches en acides gras saturés (Mattacks & Pond, 1996). L'augmentation de la proportion d'acides gras non saturés dans les triacylglycérols de réserve par une alimentation enrichie d'huile d'olive ou de tournesol pendant trois mois supprimait presque totalement les différences dans la composition des acides gras à l'intérieur des dépôts. Cette hétérogénéité de la composition des lipides du tissu adipeux à l'intérieur des dépôts peut aider à expliquer les contradictions qui apparaissent lorsqu'on mesure la composition en lipides des biopsies et d'autres petits échantillons de tissu adipeux.

Several different kinds of investigation suggest an important role for fatty acids (FA) in the nutrition and regulation of the immune system. They are a major fuel for lymphocytes and may be utilized in preference to glucose (Ardawi & Newsholme, 1985). Single free FA can act as both promoters and inhibitors of mitogen-stimulated proliferation of rodent and human lymphocytes *in vitro* (Buttke, 1984; Calder *et al.* 1991; Calder & Newsholme, 1992; Calder, 1993) and unsaturated FA are selectively incorporated into proliferating lymphocytes (Calder *et al.* 1994). At the 'whole-animal' level, altering the FA composition of dietary lipids over periods of weeks or months has been shown to affect various aspects of the response to infection and trauma (Erickson *et al.*

1992; Fernandes *et al.* 1992; Yaqoob *et al.* 1994). Much less is known about the source of such lipids *in vivo*, and about what is happening between the levels of cell biology and whole-body nutrition. Several lines of evidence point to a role for adipose tissue in the immune response.

During intensive immune activity, the contribution of ingested nutrients to the nutrition of the immune system is greatly reduced, sometimes eliminated entirely: anorexia almost invariably accompanies acute systemic immune responses (McCarthy *et al.* 1985) even though fever raises the metabolic rate, thus forcing the animal into dependence on its adipose stores. Chronic infections and immune disorders such as acquired immune deficiency syndrome (AIDS; Grünfeld & Feingold, 1991) stimulate major changes in whole-body lipid metabolism that also clearly implicate adipose tissue, but attempts to demonstrate its involvement directly have been contradictory or unconvincing. Some of the changes in blood chemistry can be explained by the direct and rapid effects of cytokines, including tumour necrosis factors (TNF), interleukin-1, and interferon-α released from activated immune cells, on lipogenesis and VLDL in the liver (Grünfeld & Feingold, 1992). 3T3 'adipocytes' respond to TNF in culture, albeit too slowly to account for the rapid responses observed *in vivo* (Patton *et al.* 1986; Hardardottir *et al.* 1992), but Grünfeld *et al.* (1989) were disappointed by their attempts to demonstrate a direct effect of TNF on the most conspicuous and commonly studied adipose depots of rats. With the data from intact perirenal and 'subcutaneous' (presumably inguinal) adipose tissue from young adult rats failing to support conclusions reached from experiments with 3T3 cell lines, the role of adipose tissue in the body's response to infection remains unclear.

The present review describes some long-established and newly investigated anatomical and physiological relationships between adipose tissue and the immune system that throw some light on the biological origins of the organization of adipose tissue in mammals. They also suggest some possible causes of the failure of *in vitro* studies on randomly chosen samples of adipose tissue to account fully for its observed involvement in immune function *in vivo*.

COMPARATIVE ANATOMY OF ADIPOSE TISSUE AND THE IMMUNE SYSTEM

In lower vertebrates, both the immune system (Kampmeier, 1969) and adipose tissue (Pond, 1978) are partially or completely localized into one or a few central organs. In all mammals, except certain marine species, adipose tissue occurs in many distinct depots, each ranging in size from up to 30% to less than 1% of the total adipose mass. Even in seals (Pinnipedia), in which almost all the adipose tissue is specialized to form superficial blubber, small quantities of adipose tissue (a few grams or less) are clearly visible around the popliteal lymph node.

Many features of the anatomical arrangement of mammalian adipose tissue and its selective depletion and expansion in fasting and obesity cannot readily be explained as adaptations to energy storage or to any of its proposed supplementary roles, such as thermal insulation or protection (Pond, 1992, 1994). The mammalian cellular immune system is also partitioned into numerous small entities, the gut-associated lymphoid tissue and lymph nodes, the latter being widely distributed throughout the body. Lymph nodes as major sites of proliferation and dissemination of lymphocytes are a special

feature of mammals: a few similar structures are found in certain birds but they are absent from lower vertebrates. Lymph nodes and adipose tissue often occur together, although most anatomical illustrations and models conceal rather than emphasize the fact. Most mammalian adipose depots contain at least one lymph node (Pond & Mattacks, 1995), and some of the largest and most consistently located nodes are found in minor adipose depots whose contribution to whole-body energy storage must be very small.

Numerous site-specific and species differences in the gross anatomy and histological structure of lymph nodes, and the abundance and arrangement of associated ducts have been described (Bélisle & Sainte-Marie, 1981), but they are no longer widely discussed in the immunological literature, which concentrates heavily on phenomena that can be monitored in blood samples, and on the properties of isolated molecules or cells *in vitro*. The structure and composition of the adipose tissue surrounding lymph nodes have not been investigated in detail since the work of Suzuki (1952): standard histological techniques revealed no site-specific differences other than adipocyte size, and by the time immunocytochemical methods became available, interest in the microscopic anatomy of adipose tissue had waned. Immunologists habitually begin all histological and physiological studies by isolating the node from its surrounding adipose tissue (Henry & Farrer-Brown, 1981; Kowala & Schoefl, 1986).

THE ROLE OF ADIPOSE TISSUE IN IMMUNE FUNCTION

By far the most thoroughly studied and widely discussed properties of adipose tissue are those that relate to its role as a general repository for triacylglycerols (TAG), and to its responses to insulin and other circulating hormones (Abate & Garg, 1995; Frayn *et al.* 1995). Within-depot (Mattacks *et al.* 1987; Pond & Mattacks, 1987*a*) and between-depot (Pond, 1992; Cousin *et al.* 1993) differences in adipocyte volume and in the activities of various enzymes and their genes have been reported but all are quantitatively quite small, at most a twofold difference between depots. They have been interpreted mainly as accounting for the selective expansion of the depots in obesity (Abate & Garg, 1995). Although *in vitro* studies have recently demonstrated that adipocytes secrete or respond to a variety of signal molecules (Ailhaud *et al.* 1992), including TNF (Hotamisligil *et al.* 1993), suggesting that adipose tissue may be involved in a wider range of metabolic functions, the emphasis is on the contribution of the adipose mass to whole-body metabolism and nutrient balance (Frayn *et al.* 1995), for which its partitioning into numerous scattered depots is an irrelevant curiosity. While no one doubts the importance of such properties, their prominence has detracted from investigations into possible local interactions, and of specialized, perhaps mutually exclusive, properties of certain depots or regions of depots.

The concepts of paracrine and autocrine interactions are now generally accepted for many mammalian tissues, but such ideas have hitherto been foreign to mainstream thinking about adipose tissue. Recent observations on pre-adipocytes in tissue culture indicate that such interactions could occur in developing cells (Ailhaud *et al.* 1992). The peculiar anatomy of mammalian adipose tissue suggests that autocrine relationships and local interactions with neighbouring structures may be important *modi operandi* for mature adipose tissue: partitioning of apparently similar tissues into depots that are distributed throughout the body is the ideal anatomical arrangement for autocrine

regulation and paracrine interactions, and most adipose depots have a diffuse blood supply shared with that of adjoining tissues. Local interactions are not easily detected *in vivo*: they are not readily monitored by analysis of blood samples taken from large superficial veins, and, since by definition they involve only a fraction of the adipose mass, they may have no manifestations in tissue taken from surgically convenient biopsy sites.

Research on the involvement of adipose tissue in immune function *in vivo* has focused on large responses such as septicaemia (Grünfeld & Feingold, 1992). But most immune responses are minor, transient and/or local. There are good reasons for keeping them so: the immune system uses large quantities of protein, rare fatty acids and other valuable resources (Woodward, 1992). Excess cytokines in the circulation, particularly TNFα, which is believed to be a major determinant of changes in lipid metabolism, can be highly toxic, leading to septic shock in the short-term and auto-immune disease in the long-term (Grimble, 1989; Grünfeld & Feingold, 1991). While it is well known that certain lymphoidal structures, such as the tonsils and the popliteal lymph node 'serve' a particular region of the body (Yoffey & Courtice, 1970), there has been little enthusiasm for the idea that adipose tissue functions in an analogous way, except possibly for the intermuscular and cardiac depots (Mattacks *et al.* 1987; Marchington & Pond, 1990; Pond *et al.* 1992).

LYMPHOID TISSUE REVEALS SITE-SPECIFIC DIFFERENCES IN ADIPOSE TISSUE

With these thoughts in mind, we designed some simple experiments that permitted the lymphoid tissue itself to point out the source of the adipose tissue with which it interacts most strongly (Pond & Mattacks, 1995). It was essential to use tissues from adult animals in which the lymph nodes would be fully functional. However, mature adipocytes are not easily cultured, so we devised a method of co-culturing, for up to 48 h, explants of adipose tissue, each containing about 1000 adipocytes, with 'lymphoid tissue', a mixture of T-lymphocytes, B-lymphocytes plus a few macrophages (Pond & Mattacks, 1995). Selective mitogens help to distinguish between the actions of different types of lymphoid cells; concanavalin A stimulates the proliferation of T-lymphocytes, and lipopolysaccharide activates macrophages and promotes proliferation of B-lymphocytes. Rats are not suitable for such investigations: their minor adipose depots, which contain most of the lymph nodes, are too small in unmanipulated adults to provide sufficient tissue for all the analyses planned. In guinea-pigs of body mass approximately 1 kg, the large cervical lymph nodes are big enough to yield over 100 identical portions of lymphoid cells, the smaller nodes in the omentum, mesentery and the superficial depots are readily located, and all the major depots are big enough for several well-separated, clearly defined samples of adipose tissue to be taken from two or more distinct sites defined by their anatomical relationship to nodes.

The presence of adipose tissue always curtails both spontanenous and mitogen-stimulated proliferation of lymphoctyes, but the extent of inhibition depends greatly on the source of the explants (Fig. 1). The least effective are those from the perirenal, which is the largest and one of the most intensively studied depots and does not enclose any lymph nodes. Lymph nodes often occur at confluences of blood vessels (this property helps us to locate them), so to demonstrate that site-specific differences were related to the nodes rather than to the blood vessels, we compared perirenal samples taken from

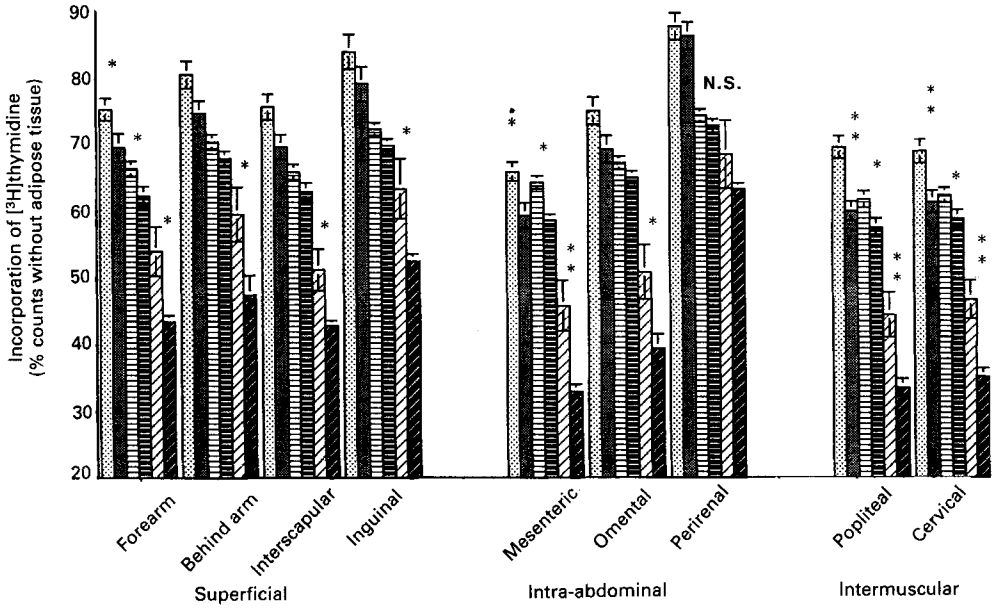

Fig. 1. Site-specific differences in the effects of adipose tissue explants on spontaneous and mitogen-stimulated proliferation of lymphoid cells in culture. Lymphocyte proliferation was measured as incorporation of [^{3}H]thymidine (% counts in portions of lymphoid cells incubated under identical conditions without any adipose tissue) into lymphoid cells incubated with an explant of adipose tissue from a site far from lymph node(s) or, in the case of the perirenal depot, away from knots of blood vessels (▨,▨,▨) and near to lymph node(s) or near to knots of blood vessels (■,■,■): (▨,▩) without mitogen; (▨, ■), with concanavalin A; (▨,■), with lipopolysaccharide. Pairs of samples were taken from four superficial, three intra-abdominal and two intermuscular adipose depots. Values are means with their standard errors represented by vertical bars. Mean values for samples taken from sites near to lymph node(s) or knots of blood vessels were significantly different from those of samples taken from sites far from lymph node(s) or knots of blood vessels for the same adipose depot under the same culture conditions: $*P<0{\cdot}05$, $**P<0{\cdot}01$. (From Pond & Mattacks, 1995.)

near and away from blood vessels; they were indistinguishable. All eight of the other depots studied contain one or more lymph nodes. In each case, the samples taken from near a lymph node were more inhibitory that those taken from elsewhere in the same depot. For the mesenteric, forearm, popliteal and cervical depots, such within-depot differences were significant in the presence of both mitogens, and with unstimulated lymphoid tissue (Pond & Mattacks, 1995; Fig. 1).

The capacity of explants of adipose tissue to inhibit lymphocyte proliferation is almost completely abolished by insulin, implicating lipolytic products as possible mediators (Pond & Mattacks, 1995). The presence of lymphoid cells stimulates lipolysis, measured as glycerol released, but, as in the case of the capacity to inhibit lymphocyte proliferation, the magnitude of the response differs greatly between samples of adipose tissue (Fig. 2). There are substantial site-specific differences in the quantity of glycerol released from adipose tissue explants incubated alone, but measurements from pairs of samples taken from different parts of the same depot are always similar. However, the presence of lymphoid tissue reveals within-depot as well as between-depot differences. The

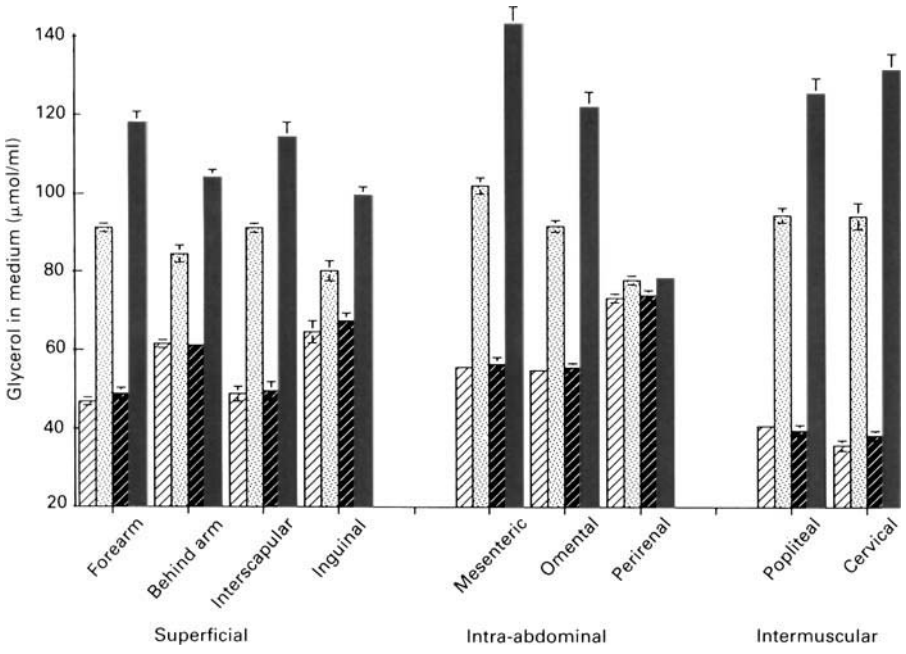

Fig. 2. Site-specific differences in spontaneous and lymphoid cell-stimulated glycerol release from adipose tissue explants. Glycerol concentrations in the medium after incubation with mitogen, lipopolysaccharide, for 48 h and an explant of adipose tissue from a site far from lymph node(s) or knots of blood vessels (▨,▩) and from near to lymph node(s) or knots of blood vessels (■,■) of four superficial, three intra-abdominal and two intermuscular adipose depots with (▩,■) or without (▨,■) lymphoid cells. All values are means with their standard errors represented by vertical bars for ten guinea-pigs. Pairs of samples from the perirenal were significantly different ($P<0{\cdot}05$). The differences between all other pairs of samples were highly significant ($P<0{\cdot}001$). (From Pond & Mattacks, 1995.)

highest rates of lipolysis in the presence of lymphoid tissue are found only in the samples of adipose tissue taken from sites immediately surrounding the lymph nodes, especially those of the small intermuscular popliteal and cervical depots. Lipolysis from such explants increases by up to threefold in the presence of mitogen-stimulated lymphoid cells, a greater increase than is observed when adipocytes isolated from similar sources are stimulated with supra-physiological concentrations of noradrenaline (Pond & Mattacks, 1989).

The interactions between lymph nodes and their surrounding adipose tissue are greatest and most consistent in mesenteric, omental, forearm, popliteal and cervical depots (Figs. 1 and 2). Site-specific differences in composition of TAG-FA are also greatest in these depots (C. A. Mattacks and C. M. Pond, unpublished results). The gross anatomy of these nodes and their surrounding adipose tissue suggests an explanation for the strong local interactions: the popliteal lymph node is the most distal of the lower limb nodes and drains the whole of the hindlimb below the knee (Yoffey & Courtice, 1970; Cooper & Schiller, 1975). The cubital lymph node (in the 'forearm' adipose depot) is also located at 'the end of the line' and performs similar functions for the distal part of the forelimb. The mesenteric nodes are the first to come in contact with newly absorbed materials from the gut, and the omentum is believed to remove debris

from the abdominal cavity (Kampmeier, 1969; Yoffey & Courtice, 1970). Hands and feet, paws and hooves, as well as guts, are continuously exposed to abrasion and assaults from parasites and pathogens, so the nodes that serve them are nearer 'the front line' in dealing with local, minor injuries, infections and inflammations than the more centrally located inguinal and axillary ('behind arm') nodes.

The adipose tissue surrounding lymph nodes that has these properties amounts to only a small percentage of the adipose mass, probably less than 5% of the total in a well-fed guinea-pig. Conversely, the whole of the massive perirenal depot, which accounts for about 26% of the total dissectible adipose mass, and large parts of the inguinal depot, have more conventional properties: they release more glycerol than the small intermuscular depots when incubated alone, suggesting that they make a large contribution to circulating lipolytic products, but in the presence of lymphoid tissue lipolysis increases by only about 25% in the inguinal and by less than 5% in the perirenal depot (Fig. 2; Pond & Mattacks, 1995).

Thus, incubation with lymphoid tissue reveals not only exceptionally large increases in lipolysis in certain depots, but also the greatest site-specific differences yet reported. The perirenal depot has been much studied: it has been found to respond substantially to nearly all the physiological conditions, circulating hormones and locally secreted agents known to affect any kind of white adipose tissue and is often regarded as representative of the adipose mass as a whole (Pond, 1992). Its minimal response to lymphoid cells makes it unlikely that these effects are mediated by any of the long-established lipolytic agents, such as noradrenaline. Lymphokines such as TNF and interleukins are obvious candidates, but, as the data in Figs. 1 and 2 clearly show, interactions with such agents are likely to be demonstrable only in tissue samples taken from beside lymph nodes.

Under these artificial culture conditions, the net effect of the presence of adipose tissue is inhibition of lymphocyte activity (Fig. 1). However, Calder *et al.* (1991) found that at low concentrations, certain FA stimulate lymphocyte proliferation to a small extent, although all those tested inhibited such activity at higher concentrations. We have no idea what the physiological concentration of FA released from adjacent adipose tissue might be at the sites of lymphocyte proliferation *in vivo*. But, because of the distance between the germinal centres in the node and the surrounding adipose tissue (Kowala & Schoefl, 1986), it is almost certainly lower than in this experimental situation. It is possible that under the artificial conditions of tissue culture the concentration of the products of lipolysis far exceeds the physiological concentrations, but that in the intact system stimulation of the adipose tissue might create an environment in which lymphoid cells are activated.

COULD THE LIPOLYTIC PRODUCTS REACH THE LYMPHOID CELLS IN THE LYMPH NODES?

The exchange of materials between lymph nodes and adjacent tissues has never been studied experimentally. Histological examination (Suzuki, 1952; Henry & Farrer-Brown, 1981) of the outer capsule of lymph nodes reveals a fairly thin, loose layer of collagenous material. Blau (1977) described the arrangement of blood capillaries within guinea-pig lymph nodes as 'looped', with the highest density of capillaries in the primary follicles near the outer capsule of the node. Herman *et al.* (1972) studied the time-course of changes in the vascular architecture of the popliteal lymph node of young rabbits

following experimental infection with *Salmonella*. Even in the absence of immunological challenge, the arcade of capillaries just under the outer capsule is among the densest in the whole node and the earliest and most long-lasting changes in vasculature are observed following infection in this region of the node. Similar processes occur in the homologous node of the mouse, where they have been shown to depend on the presence of activated lymphocytes (Steeber *et al.* 1987). Thus, the periphery of the node, nearest to the adipose tissue that surrounds it, seems to be richly perfused. Heath & Brandon (1983) remarked on the large number of very fine afferent lymph vessels that branch from the main afferent vessel and enter the sheep popliteal node over almost its entire surface. Such tiny vessels are permeable to large molecules and even some kinds of small cells (Shields, 1992). Each must pass through the adipose tissue immediately surrounding the node, and may take up lipolytic products released by adjacent adipocytes into the extracellular space.

In handling these tissues it is clear that most nodes, including the popliteal and cervical, are completely enclosed by and firmly attached to the surrounding adipose tissue, but the moderate-sized nodes in the inguinal depots are less firmly embedded in the surrounding tissue. Even within the same animal, lymph nodes differ in their internal anatomy and fine structure (Bélisle & Sainte-Marie, 1981; Kowala & Schoefl, 1986), but nothing is known of how such differences correspond to their anatomical and physiological relationships to the surrounding adipose tissue.

LOCAL DIFFERENCES IN THE COMPOSITION OF FATTY ACIDS IN TRIACYLGLYCEROLS

Unsaturated FA are particularly important in the regulation of lymphocyte proliferation *in vitro*, and are readily incorporated into such cells *in vivo* (Calder & Newsholme, 1992; Calder *et al.* 1994). Local interactions with adipose tissue would be even more efficient if the adipocytes adjacent to the nodes could provide the lymphoid cells inside with a mixture of FA that was appropriate to their need for precursors for the synthesis of complex lipids as well as fuel.

It has long been assumed that continuous turnover of TAG-FA by hydrolysis and re-esterification would in time homogenize the composition of storage lipids throughout the adipose mass. This belief has endured in spite of many reports that the compositions of TAG-FA in serial or duplicate samples of adipose tissue is always somewhat variable (Valero-Garrido *et al.* 1990; Colby & Pond, 1993). In fact, the composition of adipose tissue around lymph nodes has not been studied: 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 sites far from such structures are usually selected for study (Lin *et al.* 1993). So we recently undertook a detailed study of the composition of the TAG-FA extracted from samples of adipose tissue defined by their anatomical relationship to lymph nodes (C. A. Mattacks and C. M. Pond, unpublished results).

As others have found, the composition of TAG-FA in adipose tissue samples taken from sites away from lymph nodes was a bit variable, but there were no consistent differences between depots. However, there were clear-cut within-depot differences: the unsaturation indices of the TAG-FA in adipose tissue near lymph nodes were consistently higher than those of samples obtained far from nodes in the same depot,

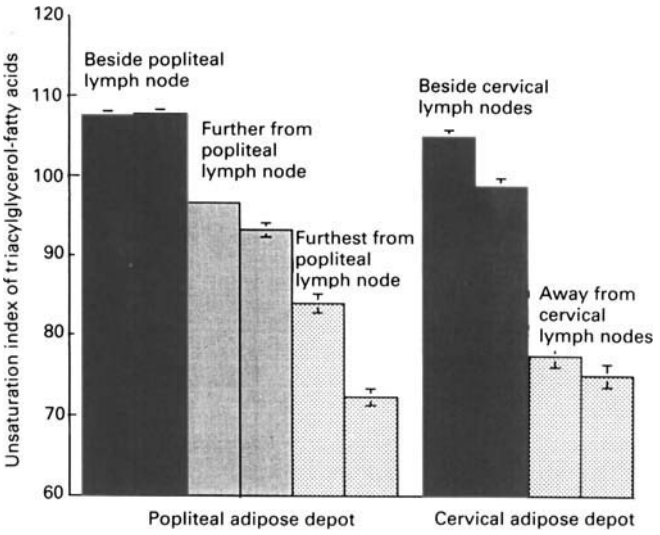

Fig. 3. Within-depot site-specific differences in the composition of triacylglycerol-fatty acids. Unsaturation indices (based on the abundance of eight fatty acids that comprised 1% or more of the total) of triacylglycerol-fatty acids extracted from samples of adipose tissue collected during the first hour *post mortem* from sites defined by their anatomical relationship to the lymph nodes enclosed within two small adipose depots. The six sample sites from the popliteal depot in the hind leg include two from opposite corners of the depot as far as possible (i.e. about 10 mm) from the popliteal lymph node, two from different sites adjacent to the node, and two intermediate sites. Those from the cervical depot were from opposite corners of the depot as far as possible (i.e. about 10 mm) from all lymph nodes and from near two different nodes. Values are means with their standard errors represented by vertical bars for eighteen guinea-pigs fed on an unmodified diet. (From C. A. Mattacks and C. M. Pond, unpublished results.)

particularly in the small intermuscular depots that contain the large popliteal and cervical lymph nodes. These depots are convenient for detailed study because their lymph nodes are consistent in location, and the mass of the adipose tissue changes little with fatness (Mattacks *et al.* 1987; Pond, 1992, 1994), so it is possible to compare the composition of samples taken from exactly homologous sites (Fig. 3; C. A. Mattacks and C. M. Pond, unpublished results). These minor depots weigh approximately 1 g each and together account for only about 5% of the adipose mass, but there is nonetheless a large and consistent gradient of unsaturation indices of the adipose tissue TAG-FA: samples from near the nodes have higher unsaturation indices than those from only a few millimetres away. The proportion of monoenoic FA was almost constant throughout these depots (and in the other seven depots studied) but the proportion of polyunsaturated FA increased and that of saturated FA decreased with proximity to the lymph node (C. A. Mattacks and C. M. Pond, unpublished results). Feeding guinea-pigs for several months on diets containing FA of different composition showed that the FA composition of the TAG in the small areas of adipose tissue around the lymph nodes remained about constant but that of the rest of the adipose tissue followed the changes in the diet (C. M. Pond and C. A. Mattacks, unpublished results). This within-depot heterogeneity in the TAG-FA composition may help to explain inconsistencies in such measurements from biopsies and other small samples of adipose tissue taken from randomly chosen sites. The data on Fig. 3 show that differences in TAG-FA composition as large as many changes

induced by weeks on a controlled diet (Valero-Garrido *et al.* 1990; Colby & Pond, 1993; Lin *et al.* 1993) can be measured from samples collected simultaneously from within a single small depot.

These observations suggest that the adipose tissue around the nodes preferentially incorporates or selectively retains polyunsaturated FA more efficiently than the rest of the adipose mass does. Processes that could produce such heterogeneity in lipid composition of adipose tissue have recently been investigated: Raclot & Groscolas (1993) have demonstrated that isolated adipocytes stimulated with noradrenaline selectively retain longer-chain FA. 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). Unfortunately, these experiments involved only depots that have little or no specific interaction with lymphoid cells, so it is impossible to say whether selective uptake and mobilization have anything to do with making such FA available to the immune system. If the FA thus released near the nodes from such adipose tissue reflect the composition of those in its TAG, they would include a greater proportion of polyunsaturated FA than those released into the general circulation by the much larger quantities of adipose tissue that is not intimately associated with lymph nodes. Such FA could be essential for the synthesis of membrane lipids and lipid-based signal molecules such as leukotrienes, prostacyclins, thromboxanes and prostaglandins. 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 (Fernandes *et al.* 1992; Yaqoob *et al.* 1994) may be delayed or attenuated.

DISPENSABLE AND ESSENTIAL ADIPOSE TISSUE?

These highly site-specific properties have important implications for local interactions of adipose tissue with the immune system and for lipid homeostasis in the animal as a whole. The experiments suggest that certain adipose depots can interact in a paracrine manner with adjacent tissues, as well as, or possibly instead of, responding to the traditional endocrine agents. Adipose explants that respond most to lymphoid cells, such as those from near the nodes in the two intermuscular depots, have a lower rate of spontaneous lipolysis and, thus, would contribute less lipid to the general circulation. Nothing is known about how lipolysis in such adipose tissue is modulated by insulin or catecholamines: like the adipose tissue samples from which TAG-FA compositions are studied, responses to circulating agents have almost invariably been measured in samples taken from sites far from nodes. Competition between local interactions of adipose tissue with lymphoid cells and its contribution to whole-body energy supply might help to explain some of the effects of regular strenuous exercise on immune function (Keast *et al.* 1988).

Local interactions between lymphoid cells and the adipose tissue that surrounds them in the nodes would remove the need for abrupt rises in the concentration of cytokines in the circulation, with the risk of fever, septic shock and stimulating auto-immune disorders. The agent(s) involved in stimulating the very high increases in lipolysis around the mesenteric, cervical or popliteal lymph nodes (Fig. 2), even if they were to enter the circulation and reach other depots, would stimulate lipolysis in the large perirenal and

much of the inguinal depot to only a slight extent. The adipose tissue around the lymph nodes could respond rapidly and as extensively as required to nourish and/or regulate the lymphoid cells adjacent to them without involving the rest of the adipose mass, thus avoiding either flooding the circulation with FA, or, in periods of fasting or malnutrition, making its lipolytic products available to tissues such as muscle that would oxidize them in large quantities. A mechanism of local stimulation of lipolysis that meets local needs for lipolytic products could operate almost independently of the feedback between the brain and adipose tissue that regulates the adipose mass and body composition as a whole.

If the adipose tissue around lymph nodes is adapted to interact with the lymphoid cells rather than serve as a general lipid store, its contents might be selectively conserved during fasting. In naturally-obese wild mammals, depots such as the mesenteric, omental and popliteal that contain many and/or large lymph nodes undergo less extensive changes in mass than the larger depots that contain few or no lymph nodes (e.g. perirenal): node-containing depots are relatively large in lean individuals, but in very obese specimens they never become proportionately as massive as depots without nodes (Pond, 1994; Pereira & Pond, 1995). In very lean wild mammals, the lipid in the adipose tissue immediately surrounding the nodes appears to be the last to be reclaimed.

These properties make sense if a major, possibly the principal, function of the small depots that contain large nodes is selective storage and release of lipids which are utilized by the immune system, and if the interaction could be impaired by excessive expansion. Studies on the homologous depots of *ad lib*.-fed laboratory rodents show that, far from being metabolically inert, these depots contain a higher proportion of protein (Mattacks *et al.* 1987) and are more sensitive to noradrenaline and insulin (Pond & Mattacks, 1991). This principle is particularly clear in primates where the perirenal and superficial abdominal paunch that lack lymph nodes may expand enormously in well-fed captive lemurs (Lemuridae; Pereira & Pond, 1995) and monkeys (*Macaca* spp.; Pond & Mattacks, 1987*b*) and in well-nourished middle-aged humans (Björntorp, 1987), while the smaller depots around lymph nodes remain about the same size as in leaner conspecifics.

THE EVOLUTION OF LOCAL INTERACTIONS BETWEEN ADIPOSE TISSUE AND LYMPH NODES

What circumstances might have promoted the evolution of this curious arrangement? An explanation might lie in the uniquely mammalian ontogenetic changes in diet. Mammals have a high-fat diet during suckling, when the thymus is active and the lymph nodes are immature. Both adipose tissue and the immune system grow and mature rapidly during this period (Morris, 1986). At least in humans reproduction imposes heavy demands on the mother's reserves of lipids, especially essential FA, which are depleted to support the growth of the offspring (Al *et al.* 1995). With a few exceptions, the diets of most adult mammals are low in lipids, especially polyunsaturated lipids, and rich in carbohydrates. Weaning marks an abrupt transition from a high-fat diet to a high-carbohydrate diet, and triggers major changes in adipose tissue metabolism (Girard *et al.* 1992). After weaning, exposure to irritants and infections that stimulate immune responses is as bad as, or worse than, during suckling. Just at the time when the lipid content of the diet falls abruptly, the need for strenuous exercise increases and, in many cases, total nutritional

intake is so compromised that the weanling becomes lean, often thin. A few rare FA tucked away in the adipose tissue just where they are most needed by the immune system might come in very handy during this period of nutritional insecurity that is often combined with immunological challenge. Local sequestration of FA required by the immune system and local interactions between adipose tissue and lymph nodes would also be useful to adult mammals during periods of nutritional stress, so there would be no reason to abandon such adaptations in adulthood.

One of the chief advantages of the mammalian reproductive system over those in which the parents do not feed their offspring or, as in most birds, where the parents bring locally collected food to the nestlings is that nutrients required by the young can be stored in the mother's body (Pond, 1977). Thus, the family is partially emancipated from reliance on all the appropriate nutrients being available at the time and in the locality where breeding takes place. The capacity to manage indefinitely on monotonous and/or low-nutrient content diets is a much underestimated but important adaptive advantage that mammals have over birds and lower vertebrates (Pond, 1983) which has enabled them to maintain permanent breeding populations in low-diversity and disturbed ecosystems, to which many birds are only seasonal visitors.

As Frayn *et al.* (1995) have emphasized, mammalian adipose tissue (i.e. the large depots) regulates the whole-body lipid supplies very efficiently. The specialized site-specific properties of certain minor depots may further improve lipid economy by promoting the efficient use of rare but essential nutrients: indispensable nutrients are retained where they are needed for synthesis, while commoner and/or less-important molecular species are released for oxidation. Indeed, adaptive local interactions with peripheral lymph nodes might be a major reason for the evolution of the partitioning of mammalian adipose tissue into so many small depots (Pond, 1978). Small adipose depots may not be just inferior large depots: they may have different but complementary roles in the metabolic efficiency of mammals.

CONCLUSIONS

Adipose tissue is heterogeneous, in metabolism as well as in composition. Sites may look alike in microscopic structure (or differ in apparently irrelevant properties such as adipocyte volume), but detailed studies on living adipose tissue reveal major site-specific differences in its capacity to interact with the immune system. Because so much research on adipose tissue is directed towards controlling obesity, there is a strong temptation to believe that bigger depots are somehow 'more important'. The opposite applies to interactions of adipose tissue with the immune system: immediate, controllable access to lipolytic products of appropriate composition is probably much more important for local interactions with lymphoid cells in nodes than access to large quantities of lipid.

We find that interactions with lymphoid cells are most prominent in the minor depots, and may be almost absent in the largest, most accessible and thus most frequently studied depots. Large mammals including humans have many such minor depots widely distributed around the body. They may contribute less (perhaps nothing at all) to the general lipid supply for tissues such as muscle, but could be specialized to nourishing or regulating cells of the immune system. We urge that, in selecting samples for the study of the involvement of adipose tissue in sepsis, cachexia of cancer and auto-immune diseases, more attention is paid to their anatomical relations to lymph nodes: the

perirenal or epididymal fat pads of young rats are the tissues least likely to provide data pertinent to these important topics.

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