

LYMPHATIC SYSTEM FUNCTION AND OBESITY — ARE THERE LINKS?

The work of Natasha Harvey et al in 2005 showed a strong link between lymphatic vascular defects and adult onset obesity (in mice), and Schneider et al (2005) commented that this, and other research, raised the spectre of significant involvement of the lymphatic system and its functioning (or more properly its inability to function well) and obesity.

When we follow the development of primary and secondary lymphoedemas of the limbs, we see a gradual progressive development of epifascial adipose tissue. It is not totally clear if they are new adipocytes or *in situ* ones becoming larger. Most lymphoedemas have slower than normal lymph flow, but the lymphatic system is usually structurally sound.

When we follow the development of lipoedemas, we find a similar epifascial fatty deposition, but it is limited to specific areas and there are underlying weaknesses (microaneurysms) of the lymphatic capillaries and the collectors leading to poor lymph flow.

When a patient with lymphoedema loses weight, the lymphoedema seems to resolve, but often when a patient with lipoedema presents, the lipoedema area generally does not resolve.

So, what is going on? What is the level of involvement of the lymphatic system, is there some relationship between a 'normally functioning' and structurally sound lymphatic system and control of adipose tissue, and a poorly functioning and structurally weak lymphatic system and excess adiposity? Does fast lymph flow make you skinny, and can slow or poor lymph flow make you fat? NP

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Wherever lymph flow is slow (and vessels weak) and or prolific (i.e. around most of the lymphatic collectors, especially the lymph nodes) there always seem to be fatty accumulations. So, what is adipogenic in lymph?

RD: The question is if lymph itself is adipogenic or is fat produced by the effect of inflammation due to stasis of macromolecules, accumulation of immune active substances and lymphocyte infiltration. In Chy mice (missing VEGFr3) there is proliferation of adipose tissue. Adipose tissue itself is a highly active organ with many functions, such as energy provision, thermoregulation, insulation, cytokine and growth factor generation and storage for fatty acids for cell membranes.

IFC: The formation of fatty (adipose) tissue is a complicated process. However, what we currently know is that the first stage in the process is the development of a mesenchymal stem cell into a pre-adipocyte and then the latter development into a mature fat cell (adipocyte). This developmental process is triggered and facilitated by many factors, including insulin-like growth factors, growth hormones and products from platelets. These factors, which like accumulated inflammatory mediators and proteins, can be expected to be in higher concentrations in lymphoedema when the lymph clearance is slow and/or if there are issues of lymphatic back-flow. In addition, as lymphoedema progresses we have increasing fibrosis within and around the fat cells, both individually and in groups, which can further exacerbate the accumulation of these factors in the tissues by slowing lymph flow.

There is also the possibility of microcirculatory defects in the affected tissues, whether this is the cause of the initial problem of more rapid maturation and proliferation of the stem cells or is a consequence of the cocktail of inflammatory and growth factors in the tissues is uncertain. Certainly, however, there appears to be some positive feedback cycle in the affected tissues.

NH: An intimate spatial association between lymph nodes, lymphatic collectors and adipose tissue has long been recognised (reviewed in Harvey, 2008). Work from Pond and Mattacks has demonstrated that

lymph-node associated adipose tissue is an important reservoir of energy deployed to power immune responses (Pond and Mattacks, 1998), illustrating that the spatial relationship between adipose tissue and lymph nodes is not merely coincidental. Intriguingly, in a mouse model devoid of lymph nodes, the lymph node-associated fat pads fail to develop (Eberl et al, 2004; Eberl, personal communication). This observation suggests that a lymph node/immune cell-derived stimulus is required to initiate the formation of lymph node-associated adipose tissue.

The observation that lymphoedema is associated with adipose tissue deposition has also been well documented by clinicians. Our recent work in a mouse model provided the first evidence that lymphatic vascular rupture could result in adipose tissue accumulation, due to the promotion of adipogenesis by lymph-derived factors (Harvey et al, 2005). What is the identity of lymph-derived adipogenic stimuli? As yet, the factor or factors within lymph responsible for driving adipogenesis remain to be identified, but, at least *in vitro*, appear to be able to act synergistically with insulin to promote adipogenic differentiation (Harvey et al, 2005). The isolation and characterisation of lymph-derived adipogenic stimuli should prove valuable for the future development of molecular agents designed to minimise the symptoms of lymphoedema and obesity.

CP: Adipose tissue around lymph nodes has special properties that enable it to interact locally with lymphoid cells (Pond, 2005). Paracrine interactions between specialised perinodal adipocytes and lymph nodes, which may extend to lymph vessels as well as nodes, are comparable to those demonstrated between peri-vascular adipocytes and blood vessels (Yudkin et al, 2005). The presence of interchelated macrophages and other lymphoid cells in adipose tissue (Cousin et al, 1999) and the ability of adipocytes to secrete and respond to a variety of immune-specific cytokines (Mattacks and Pond, 1999) has persuaded some investigators to include adipose tissue as part of the immune system (Schaffler et al, 2007) and the lymphatic system (Rockson, 2004). Many, possibly all, of the fatty acids incorporated into membrane phospholipids of immune cells in lymph nodes (Pond and Mattacks, 2003) and dendritic cells permeating adipose tissue (Mattacks et al, 2004) are derived from contiguous adipocytes. The concept of paracrine interactions between lymphoid cells

RD: Very recent studies show no direct relationship between obesity and risk for breast cancer or recurrence.

IFC: If lymph flow is improved, the substances that are provoking fatty deposits could be cleared and their growth stopped.

NH: As yet, neither impaired lymphatic vascular function, nor mutations or polymorphisms in genes important for lymphangiogenesis, have been correlated with human obesity.

CP: So, lymph is not 'adipogenic', but the adipose tissue around its nodes (and probably also vessels) is integral to the nutrition of the immune system.

and contiguous adipocytes helps to explain some peculiarities of the arrangement of lymph vessels in adipose tissue (Heath and Spalding, 1987) and within the lymph nodes themselves (Gretz et al, 1997). So, lymph is not 'adipogenic', but the adipose tissue around its nodes (and probably also vessels) is integral to the nutrition of the immune system.

Investigations from Parbhoo et al (2002) showed that weight loss alone could result in a reduction of arm lymphoedema without other specific treatment. Our common knowledge from clinical trials show that when lymphoedema resolves, lymph flow improves and the fatty tissues recede. Does slow lymph flow make you fat, and can speeding up lymph flow help weight loss, or help reduce lymphoedema or gain control over obesity?

RD: Being obese or overweight is a risk factor for lymphoedema in breast cancer patients. Meeske et al (2008) showed that women who were obese (i.e. body mass index [BMI] >30) had a 2.5-fold greater risk of arm lymphoedema than lean women. Shaw et al (2007) showed that weight loss can lead to a reduction in the severity of breast cancer-related lymphoedema. In 1991, Werner et al identified being overweight as a risk factor in the development of lymphoedema in a prospective study of 282 patients with stage I or II breast cancer who received additional radiation therapy by measuring differences in treated and untreated arms.

IFC: There is a recent study from Shaw et al (2007) that shows that weight loss can significantly reduce lymphoedema volume in patients with breast cancer-related lymphoedema, and that a small reduction in weight has a good result in terms of volume reduction of the swollen arm.

Morbidly obese patients frequently have clinically severe lymphoedema with many dermal complications and fibrosis, although lymphoscintigraphy shows little lymphatic damage. Thus, it is a functional lymphoedema which improves when the weight is controlled. This is probably due to the fatty deposits around the vascular system which are increasing the resistance to the flow without any initial damage to it.

If lymph flow is improved, the substances that are provoking fatty deposits could be cleared and their growth stopped.

NH: It would seem reasonable that improved lymphatic vascular function would result in the increased clearance of lymph-derived adipogenic stimuli and a concomitant decrease in adipogenesis. Speeding up lymph flow would, therefore, almost certainly help to reduce lymphoedema and could potentially contribute to weight loss. As slow lymph flow rates are associated with lymphoedema, it is plausible that aberrant lymphatic vascular function could be a predisposing, or contributing factor to obesity onset. Our work recently demonstrated that haploinsufficiency of the lymphatic master regulator gene, Prox1, was sufficient to mediate lymphatic vascular abnormalities that resulted in leakage of adipogenic lymph and subsequent obesity onset (Harvey et al, 2005). As yet, neither impaired lymphatic vascular function, nor mutations or polymorphisms in genes important for lymphangiogenesis, have been correlated with human obesity. No doubt future work will aim to investigate whether lymphatic vascular dysfunction is causally linked with human obesity.

CP: Experimental studies in rats show that chronic inflammation (lasting weeks or months) induces selective hypertrophy of this specialised perinodal adipose tissue (Sadler et al, 2005). Selective hypertrophy of certain adipose depots, often accompanied by regression of others, occurs in several disparate human disorders characterised by chronic low-grade inflammation (Pond 2001). Hypertrophied adipose depots also respond very weakly to weight loss. The 'fat wrapping' around inflamed parts of the bowel in Crohn's disease may be an example of the long-term outcome of such processes (Westcott et al, 2005). Recent research has demonstrated that chronic low-grade inflammation is a major factor in obesity (Yudkin, 2007). It may promote hypertrophy of perinodal adipocytes, which could expand and/or become numerous enough to restrict lymph flow. So, I do not think slow lymph flow *per se* induces local or whole body enlargement of adipose tissue, more the other way around. Weight loss could reverse these processes, especially if it is accompanied by more physical activity that improves lymphatic and venous drainage and removes lipolytic products.

Is there an inherited factor which makes some persons fat and keeps some skinny?

RD: No comment.

IFC: Yes, there are some inherited factors related to obesity. In addition to multifactorial obesity, there are single gene disorders with obesity as an isolated or predominant feature. Major obesity susceptibility loci appear to be located on chromosomes 4, 10, and 20 (Online Mendelian Inheritance in Man™, OMIM™). The obesity predisposing genotype is present in 10% of individuals.

NH: Mutations in several genes involved in the regulation of appetite, satiety and metabolism have been shown to be associated with human obesity (Flier, 2004). While in a minority of cases severe human obesity can be linked to a monogenic origin, in the great majority of cases, susceptibility to obesity onset is likely to be polygenic. As discussed above, whether human obesity may be caused, or contributed to, by lymphatic vascular dysfunction remains to be investigated.

CP: There are many such factors relating to metabolic rate, appetite control and exercise habits. Apart from that, I am not aware of any that specifically concern lymphatic vessels. But, few people have explored this topic.

Why doesn't lipoedema respond (or barely respond) to weight loss? Non-lipoedema areas reduce in size, while those with it remain much the same. Do variations in lymph flow and the lymphatic system functionality make the difference?

RD: First: adipose tissue in lipoedema has a different aetiology than 'normal excess of adipose tissue in obesity'. Therefore, dietary actions in reducing fat are often unsuccessful in the specific 'lipoedema regions', such as the hips, knees and malleolus. The second question is if there is a real lymphological problem in lipoedema? Some authors suggest that the lymphatics in lipoedema are initially not affected, as Földi showed with scintigraphy and which is also my opinion. In the latter stages, increased adipose tissue accumulation leads to dynamic lymph transport impairment and clinical signs of oedema.

RD: First: adipose tissue in lipoedema has a different aetiology than 'normal excess of adipose tissue in obesity'. The second question is if there is a real lymphological problem in lipoedema?

IFC: Many patients with lipoedema are misdiagnosed as being obese.

NH: The most effective current treatment for lipoedema appears to be liposuction...

CP: One of the special properties of the perinodal adipose tissue around lymph nodes is that it does not contribute much to whole body energy supplies.

IFC: Many patients with lipoedema are misdiagnosed as being obese. Weight loss can reduce the lymphatic vessels resistance to lymph flow (Van Geest et al, 2003) and improve the volume in the early stages of lipoedema, but, as a unique intervention, it does not significantly reduce the volume. In advanced stages, it is necessary to reduce weight, but this alone is not enough. In the early stages, the fatty deposits are not organised and fibrosed so they can be mobilised by physical therapies such as manual lymphatic drainage (MLD) and compression therapy (Földi and Földi, 2003; Schingale, 2003; Schmeller and Meier-Vollrath, 2005). With time, the transport of lymph decreases (Brauer and Brauer, 2005) and the lymphatics are damaged and the lipoedema progresses into advanced stages.

NH: Whether or not lipoedema is primarily caused by lymphatic vascular dysfunction can be debated. Work from a number of groups has shown normal, or only slightly reduced lymphatic function in lipoedema patients (Brautigam et al, 1998; Harwood et al, 1996), while studies from others have demonstrated slowing of lymphatic flow (Bilancini et al, 1995) and microlymphatic aneurysms (Amann-Vesti et al, 2001) in patients with lipoedema. It is entirely possible that the impairment of lymphatic flow and microlymphatic aneurysms seen in these patients could occur secondarily to adipose tissue deposition, as a result of the compression and obstruction of lymphatic vessels. The most effective current treatment for lipoedema appears to be liposuction, suggesting, together with the above data, that lipoedema may primarily be a lipodystrophy syndrome rather than a lymphatic vascular syndrome. The fact that abnormal adipose tissue deposition in lipoedema patients is generally restricted to the legs (but sparing the feet), points to the possibility of a locally acting adipogenic stimulus. This could potentially be one reason that weight loss does not resolve lipoedema.

CP: Selective hypertrophy of certain adipose depots, often accompanied by regression of others, occurs in several disparate human disorders characterised by chronic low-grade inflammation (Pond, 2001). Hypertrophied adipose depots also respond very weakly to weight loss. The causes of such 'redistribution' and the determinants of individual differences in distribution of adipose tissue generally remain obscure, in spite of years of intensive investigation.

One of the special properties of the perinodal adipose tissue around lymph nodes is that it does not contribute much to whole body energy supplies (Mattacks and Pond, 1999). Although *in vitro* perinodal adipocytes respond as well or better to noradrenaline than those from depots not associated with lymphatics, *in vivo* they are not activated as much during prolonged fasting. Lipolysis from perinodal adipose tissue seems to be controlled locally by paracrine signals from lymphoid cells (Mattacks et al, 2005) and its lipolytic products are taken up locally (Pond, 2003), with few entering the general blood circulation. Experimental inflammation of one or a few peripheral lymph nodes activates not only adjoining adipose tissue, but also perinodal adipose tissue around remote lymph nodes, especially those of the mesentery (Pond and Mattacks, 2002). Therefore, chronic inflammation contributing to this form of oedema may not be local; the adipose tissue may be responding to immune activation elsewhere in the body.

Is it possible that low-level anti-angiogenic treatment could inhibit the growth of new lymph vessels in developing lipoedema (where the vessels are inherently fragile and perpetuate the circle of problems), or is there some factor which encourages them to work better in lymphoedema patients which can make a difference to the development of these conditions?

RD: I know no figures in this perspective and would not wish to speculate. Animal experiments are performed but extrapolating it to human beings is not possible.

IFC: Advances in our understanding of the mechanisms underlying tumour progression suggest that angiogenesis plays a key role in malignancies. Vascular endothelial growth factor (VEGF) has emerged as an important therapeutic target, and a variety of strategies to inhibit VEGF are under investigation. Malignant tumours induce aberrant and dysfunctioning vessels that promote tumour growth. Some drugs with an anti-angiogenic action have shown a significant effect in normalising the microcirculation in tumours and inhibiting growth of metastases and are used with success in medical oncology (Yang et al, 2003; Whisenant and Bergsland,

2005; Katoh and Katoh, 2006). Probably some effect can be shown in lipoedema, but research is needed to prove that.

NH: Work recently published by Tammela et al (2007) certainly lends promise to the development of therapies to treat secondary lymphoedema by stimulating lymphangiogenesis. Tammela et al showed that the treatment of mice that had undergone lymph node excision with adenovirally delivered VEGF-C or VEGF-D, stimulated the growth of new lymphatic vessels and improved the success of lymph node transplantation. These studies lend weight to the possibility that lymph node transplantation and lymphangiogenic therapy could be useful in the prevention of secondary lymphoedema following cancer surgery. Whether lymphangiogenic therapy could be used in primary lymphoedema patients to regenerate or repair dysfunctional lymphatic vessels remains to be fully explored, although work in mouse models suggests that this approach may be feasible (Karkkainen et al, 2001; Saaristo et al, 2002). Whether anti-angiogenic/lymphangiogenic treatment might be effective in preventing the onset of lipoedema requires more investigation into the cause of this disease, together with the development of an appropriate mouse model in which to investigate lipoedema and potential therapeutic regimens.

CP: In Crohn's disease, perinodal adipose tissue around mesenteric lymph nodes excised from near the chronically inflamed bowel lacks the usual composition of triacylglycerols fatty acids (Westcott et al, 2005). Probably as a consequence, fatty acid precursors of eicosanoid and docosanoid signal molecules, especially arachidonic acid, are abnormally low in the lymphoid cells within the contiguous lymph node. Persistent 'distress' signals from the lymph nodes starved of appropriate, necessary fatty acids may induce the 'fat wrapping' that is so characteristic of Crohn's disease. Incompetent perinodal adipose tissue may be the primary defect of Crohn's disease. Although its intestinal symptoms are the most conspicuous and troublesome, abnormal immune processes can occur in other organs and tissues (Hyams, 1994), suggesting general impairment of lymphatic-adipose interactions. Selective hypertrophy of adipose tissue could happen in a more diffuse and generalised way in some forms of obesity involving chronic inflammation and sub-optimal interactions between lymph vessels and their associated

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IFC: Compression seems to be the most effective intervention to improve lymph flow...

NH: It remains to be established whether a proportion of obesity in the human population can be attributed to lymphatic vascular defects.

CP: ... it would be unrealistic to suggest that such measures could substitute for the traditional prevention and cure for obesity, i.e. improved diet and more exercise.

adipocytes. In other words, I am suggesting that many of the symptoms of Crohn's disease arise from severe impairment of the paracrine relationships between lymphatic and adipose tissues, which in a milder and probably commoner form produce lymphoedema-associated obesity. If there is any truth in this hypothesis, then dietary changes, especially increasing the availability of arachidonic acid (Treble et al 2004), may be of benefit.

Is the same possible to help reduce obesity? What can we do to help the lymph flow better in obese patients and is it likely that this will help manage their obesity (and any lymphoedema that they might have)?

RD: Very little literature is published in the field of lipoedema. I think in the question there is too little discrimination between various patho-physiological backgroups of lymphatic impairment. In my opinion, the oedema seen in obesity or lipoedema is more from a dynamic failure (high output failure), whereas in 'true lymphoedema' there is a static impairment (low output failure). So, in true lymphoedema more adipogenic features are available leading to adipose hypertrophy/plasia. The oedema component in obesity is based more on increased lymph production due to increased filtration and overburdening of the lymphatic system, which does not need to be defective. In this respect, compression therapy is very effective in reducing oedema.

IFC: Compression seems to be the most effective intervention to improve lymph flow, as the evidence base for other physical therapies needs more well-designed studies to show their effectiveness (Badger et al, 2004). Obese patients would benefit from compression stockings to control oedema in their lower limbs and to prevent the appearance of lymphoedema.

NH: The Prox1 heterozygous mouse model described above, in which mice become obese due to lymphatic vascular defects (Harvey et al, 2005), provides a model that could be used to test whether lymphangiogenic therapy or therapy targeted at lymph-derived adipogenic factors is able to combat obesity. As discussed, it remains to be established whether a proportion of obesity in the human population can be attributed to lymphatic

vascular defects. If this turns out to be the case, lymphangiogenic treatments may indeed prove beneficial for the treatment of obesity. It seems plausible that the improvement of lymph flow by increased physical activity, compression/massage therapy and weight loss regimens, might prove beneficial for the management of obesity and associated complications.

CP: Rodent studies show that the interchelated lymphoid cells in adipose tissue are different in obesity (Caspar-Bauguil et al, 2006). Reducing the inflammatory processes associated with chronic obesity would probably improve lymphatic drainage and thus avoid many of these problems. But it would be unrealistic to suggest that such measures could substitute for the traditional prevention and cure for obesity, i.e. improved diet and more exercise.

Are the fatty deposits due to one gene that is mutated in multiple ways, or is there a molecular pathway which can be interrupted in multiple places? Will this have a bearing on finding a responsible gene(s) and, ultimately, molecular correction of the defects in the lymphatic system?

RD: Obesity in general is the result of both genetic and environmental factors. Some estimate the environmental factor to be 60–70% and the genetic 30–40% (Pi-Sunyer et al, 2002). Although the vast majority of cases of obesity are the result of a complex polygenic disease involving interactions between multiple genes and the environment, some researchers identified monogenic forms of obesity resulting from mutations in genes involved in central pathways of food intake regulation (Snyder et al, 2004; Canello et al, 2004). Two genes, leptin and proopiomelanocortin (POMC), are known to have a direct effect on the skin (Yosipovitch et al, 2007). Loffler et al (2002) showed that increased BMI leads to significant increased cutaneous bloodflow. Perhaps this, in combination with dependency factors, can contribute to oedema formation in patients with lipoedema as well.

IFC: There is probably a multifactorial mechanism that involves a genetic cause and some ambient factors that trigger and maintain the pathogenic way to provoke lymphoedema.

NH: In the case of Prox1 heterozygous mice, reduction in the expression level of a single gene, Prox1, is sufficient to result in adult onset obesity. Prox1 is a master gene required to specify lymphatic endothelial cell fate, therefore, the effects of Prox1 gene deletion affect the entire molecular pathway responsible for lymphatic endothelial cell identity. In Prox1 heterozygous mice, both the visceral and superficial lymphatic vessels are affected, resulting in the accumulation of both subcutaneous and intra-abdominal adipose tissue.

In Chy mice that have a heterozygous inactivating mutation in VEGFR3, only superficial lymphatic vessels are affected, resulting in lymphoedema and subcutaneous adipose tissue accumulation without visceral lymphatic vascular abnormalities and visceral adipose tissue deposition (Karkkainen et al, 2001). Differences between these two mouse models suggest that it will be important to establish the underlying cause of obesity onset, whether it be lymphatic mediated or not, prior to any corrective treatment regimen being undertaken. Obviously it will be critical to know whether lymphatic vascular abnormalities are causative of obesity in the human population and, if so, which genes are responsible. Advances in our understanding of the molecular mechanisms that regulate lymphangiogenesis should greatly aid our knowledge of the genetic events involved in human lymphatic vascular diseases, which may turn out to include obesity.

CP: Genes are not my area of expertise so I offer these general comments. The functioning of the many different proteins in, on and through membranes is modulated by the lipid composition of the membranes, though exactly how remains to be clarified. Anomalous fat deposition may arise from the cellular environment in which the proteins operate, rather than defects in the proteins themselves. The origins of defects in the supply, utilisation and storage of lipids are difficult to find, as there are no genes for lipids, only genes for proteins that manipulate lipids, and many of them are important for basic cell structure and metabolism and enzymes and transporters are quite non-specific. The lipid composition of membranes is at least partially determined by diet and, therefore, could be modulated by dietary change. Unfortunately, any benefits are likely to be vague and very slow to appear, as except in drastic circumstances like starvation, lipid turnover takes many months. **JL**

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- NH:** ... it will be critical to know whether lymphatic vascular abnormalities are causative of obesity in the human population and, if so, which genes are responsible.
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