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Mini-review

Does perivascular fat influence neural control of the saphenous vein? Implications in coronary artery bypass surgery (CABG).

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Abstract.

The saphenous vein (SV) is the most commonly used conduit for CABG but its patency rate is poor compared with the internal mammary artery (IMA). In conventional harvesting the saphenous vein's outer layer, the adventitia, is damaged and this affects the perivascular nerves. In addition the cushion of perivascular fat (PVF) surrounding the vein is removed. The performance of SV grafts removed with surrounding cushion of fat intact, using a no-touch technique, is markedly improved. This finding suggests that the outermost layers of the SV play a potentially important role in the success of this technique. In recent years there has been increasing interest in the potential 'protective' role of the PVF surrounding blood vessels. In this mini-review we discuss evidence for the beneficial role of PVF in the improved performance of SV grafts prepared using a 'no-touch' technique and put forward the hypothesis that PVF may affect the neural control of grafts and perhaps *vice versa*.

Perivascular fat.

Perhaps the original suggestion for a beneficial role of perivascular fat (PVF) is based on a study in 1991 by Soltis and Cassis [1] who showed that perivascular adipose tissue altered the responsiveness of rat aortic rings *in vitro*. These authors compared the effects of various manipulations on aortic segments with PVF intact and PVF removed. There was a decreased sensitivity to the constrictor effect of norepinephrine in those aortic segments with PVF intact compared with those where PVF had been removed. In addition, electrical field stimulation caused a frequency-dependent contraction of intact aortic rings but had no response in 'stripped' segments. Interestingly, phentolamine blocked the contractile effects where PVF was intact, suggesting that PVF exerts its effect via the sympathetic neuroeffector system. More recently, Lohn et al [2] compared the constrictor effects of various compounds on rat aortic rings with and without PVF as well as demonstrating the existence of a transferable relaxing factor that was later named 'adipocyte-derived relaxing factor' (ADRF) [3]. Subsequently many studies have provided supporting evidence for the relaxant, or anti-contractile, effects of PVF.

Apart from beneficial effects of PVF there is recent evidence that perivascular adipose tissue surrounding coronary arteries stimulates the release of pro-inflammatory cytokines and adipokines involved in the atherosclerotic process [4] with a recent review suggesting that crosstalk between PVF and blood vessels occurs involving three key aspects of vessel pathology: inflammation, vasoreactivity and smooth muscle cell proliferation [5].

Fat and the autonomic nervous system.

The influence of the central nervous system (CNS) on fat has been discussed in a recent review in some detail [6]. In this context, white adipose tissue (WAT) is said to receive a noradrenergic innervation that is closely associated with blood vessels with noradrenaline implicated in the control of WAT blood flow. Neural tracing techniques have shown that CNS 'control' of WAT is from the paraventricular hypothalamus, noradrenergic tegmental system and caudal raphe nucleus, all part of sympathetic outflow [7, 8]. Over the last two decades WAT has been suggested as a source of factors involved in physiological function with adipose tissue being considered an endocrine organ. The synthesis and secretion of these adipocyte-derived factors is under control of the sympathetic nervous system with most studies factors being leptin and

adiponection, both being negatively regulated by ß-adrenoceptors. There is also some suggestion that the parasympathetic system is involved, via the nicotinic receptor [6].

CNS and leptin.

Shortly after its discovery leptin was hailed as the key to understanding obesity via its hypothalamic satiety action although it is now recognised to influence many other physiologic processes including food intake, thermoregulation, fertility, sympathetic nerve activation, blood vessel tone and blood pressure (see the review by Richards et al [9]). In the CNS, central administration of leptin 'modulates' noradrenaline release in the paraventricular nucleus of female rats [10].

Leptin receptors are located in the hypothalamus, hippocampus and cerebral cortex where they regulate neural development. Leptin is also suggested to be a 'neuroprotective' agent since leptin administration protects in animal models of cerebral ischemic injury and hemiparkinsonism via pathways that are downstream of leptin receptor signalling all of which are pro-survival and anti-apoptotic [11]. In terms of its 'neural' effects on blood vessels, Fruhbeck [12] showed that leptin administration increases sympathetic activity to kidney, adrenals and lumber regions but this not always accompanied by increased blood pressure. This author tested the hypothesis that leptin-induced nitric oxide (NO) release opposes the pressor effect of sympathoexcitation. Intravenous leptin caused a dose-dependent increase in serum NO concentration and when NO synthase (NOS) was inhibited by L-NAME, leptin produced increased blood pressure (BP) and heart rate leading Fruhbeck to suggest there was a pivotal role of NO in the control of blood pressure after leptin administration [12]. This is supported by data generated by Mitchell et al [13] who studied the effect of intravenous leptin on arterial BP and blood flow in various vascular beds in conscious rats. Although intravenous leptin administration caused an increase in lumbar sympathetic activity there was no effect on BP or blood flow. However, administration of L-NAME increased arterial BP and caused vasoconstriction whereas leptin had no effect on arterial pressure or blood flow in the presence of sympathetic blockade. Taken together the authors conclude that the chronic haemodynamic actions of leptin are related to sympathetic activation.

In vitro effects of leptin.

Most early studies on the 'relaxant' effects of PVF were performed on arterial vessel segments. Using rat arterial rings, human leptin caused vasorelaxation that was blocked by L-NAME as well as removal of endothelium showing leptin's action to be NO-mediated [14]. Subsequently, vasodilator effects on canine mesenteric vessels have been described where leptin caused dose-dependent dilatation in both arterial and venous segments that were abolished by L-NAME or endothelial removal. This is therefore a mechanism involving endothelial NO release that counteracts neurally-mediated vasoconstriction [15]. A recent study has shown the anticontractile effect of PVF surrounding the rat vena cava, 'relaxant' effects that were mediated via endothelial NO release and activation of $K_{(V)}$ channels [16]. The relaxant effects of leptin on the saphenous vein and internal mammary artery, both vessels used as bypass grafts in CABG patients, have been described *in vitro* by Momin et al 2006 [17] that suggest that leptin may have a beneficial influence on grafts when the PVF is left intact.

Studies from our group suggest that the surgical trauma inflicted on the SV and subsequent vascular damage caused when using conventional harvesting methods contribute to this vessel's poor performance when used as a bypass conduit. Firstly, the adventitia is a potentially rich source of NO and regions within this outermost vessel layer exhibit immunostaining for NOS (the enzymes responsible for NO production) associated with the perivascular nerves [18 - 21]. We have also shown that the PVF surrounding no-touch SV segments used as bypass grafts not only exhibits positive eNOS immunostaining and protein but that PVF extracts also possess NOS enzymatic activity [22]. These observations are taken as evidence for a 'vasculoprotective' role of PVF in the SV used in CABG patients that contributes to its improved performance. Very recently the presence of immunopositive staining for leptin within the PVF of no-touch vein grafts has been described [23]. This raises the possibility that, as an ADRF, leptin may play an important 'anticontractile' role in saphenous veins prepared by the no-touch technique, particularly as relaxant levels of leptin were identified in PVF extracts.

Based on the data described above it is clear that PVF sources of NO have the potential to improve vein graft performance in addition to a number of adipokines contained within the cushion of fat surrounding no-touch vein grafts. This perivascular cushion contains a dense capillary network that is suggested to transport PVF-derived factors not only to the graft wall but potentially to the graft lumen via the vasa vasorum [24]. We have preliminary immunohistochemical results showing a dense innervation of the PVF surrounding no-touch vein grafts with electron microscopic evidence of tyrosine hydroxylase containing neurones in this region (**Figures 1 and 2**). Given the well established interaction between leptin and the sympathetic system these observations suggest that leptin may have a (beneficial) modulatory affect on the neural control of no-touch vein grafts.

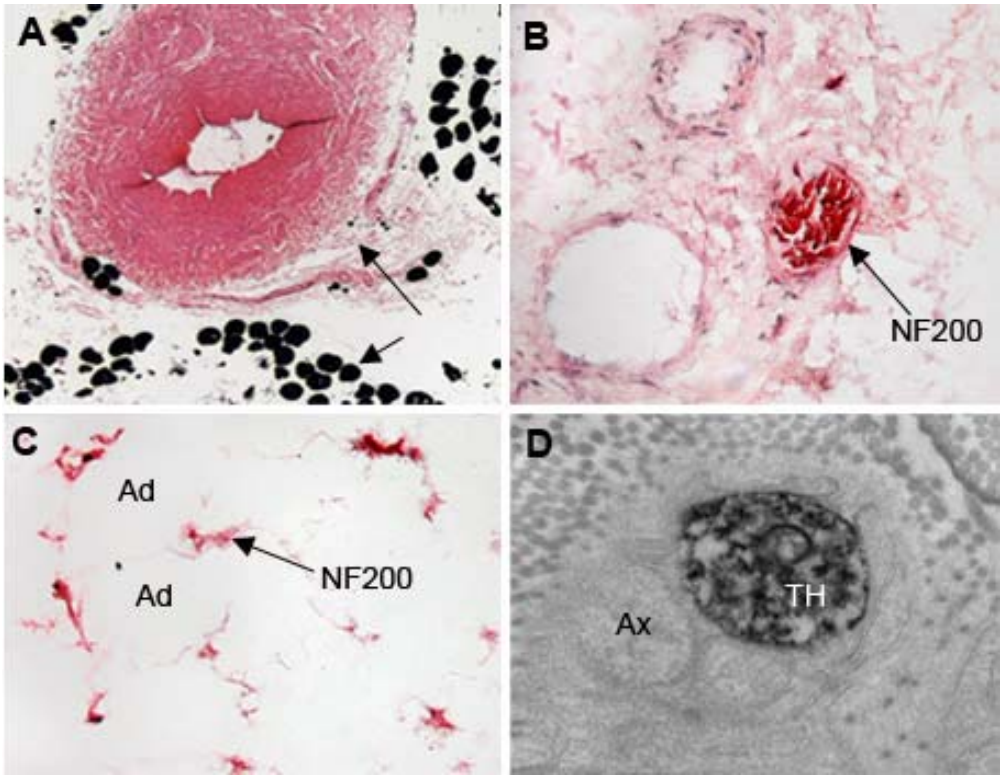

Figure 1. Nerves within perivascular fat surrounding SV. *Image **A** shows a transverse section of SV harvested by the no-touch technique with surrounding tissue for use as a coronary artery bypass graft. Much of the surrounding tissue is perivascular fat, as shown by the Marchi technique where fat stains black (short arrow). Long arrow points to the region containing perivascular nerves; these nerves and their bundle are identified using NF200 as shown at higher magnification in image **B** (x 12.5 and x 60 original magnifications for **A** and **B**, respectively). Image **C** shows NF200-positive nerve fibres (arrow) with the perivascular adipocytes (Ad) (x 120 original magnification). Image **D** is an electron micrograph showing tyrosine hydroxylase (TH) immunoreactivity (black stain) in sympathetic nerve fibre/axon within the connective tissue associated with perivascular fat; an axon profile (Ax) negative for TH can also be seen (x 10,000 original magnification).*

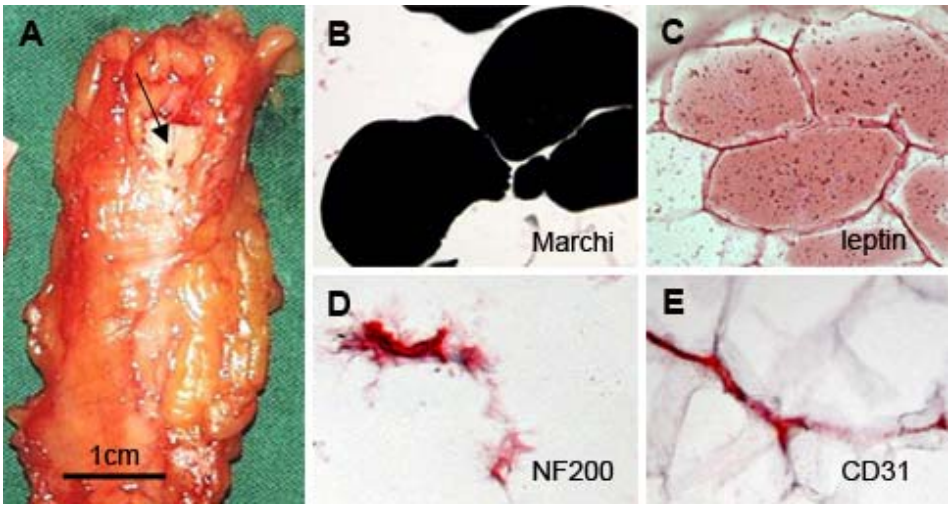

Figure 2. No-touch SV. *Image **A** shows SV harvested for coronary artery bypass surgery. The vein (distal part above the knee) is surrounded by a cushion of perivascular fat and the vessel lumen is indicated by the arrow. Image **B** shows perivascular fat staining (black) using the Marchi technique. In **C** the adipocytes exhibit positive leptin immunostaining (dark pink), and within this cushion of fat are both nerves (NF200 immunostaining; image **D**), and endothelial cells (CD31 immunostaining; image **E**) of the capillary network (x 120 original magnification of images **B-E**).*

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