

# Increasing Perfusion Pressure Does Not Distend Perforators or Anastomoses but Reveals Arteriovenous Shuntings

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**Background:** It has been proposed that hyperperfusion of perforators and distension of anastomotic vessels may be a mechanism by which large perforator flaps are perfused. This study investigates whether increasing perfusion pressure of radiographic contrast in cadaveric studies altered the radiographic appearance of vessels, particularly by distending their anastomotic connections.

**Methods:** From 10 fresh cadavers, bilateral upper limbs above the elbow were removed. Three cadavers were excluded. Seven pairs of limbs were injected with lead oxide solutions via the brachial artery while distally monitoring intravascular pressure in the radial artery using a pressure transducer. One limb was injected slowly (0.5 mL/s) and the other rapidly (1.5 mL/s) to produce low and high perfusion pressures, respectively. Skin and subcutaneous tissue were then removed and radiographed.

**Results:** The filling of perforators and their larger caliber branches appeared unchanged between low- and high-pressure injections, with *no significant increase in true anastomoses* ( $P = 0.32$ ) and *no association between maximum perfusion pressure and number* ( $P = 0.94$ ) or *caliber* ( $P = 0.10$ ). However, high-pressure injections revealed arteriovenous shunting with filling of the tributaries of the major veins.

**Conclusions:** This study demonstrated that increased perfusion pressure of the cutaneous arteries (1) did not change the caliber of vessels; (2) did not convert choke to true anastomoses; and (3) revealed arteriovenous shunting between major vessels with retrograde filling of venous tributaries as pressure increased. This suggests that it is not possible to distend anastomotic connections between vascular territories by increasing perfusion alone. (*Plast Reconstr Surg Glob Open* 2020;8:e2857; doi: 10.1097/GOX.0000000000002857; Published online 24 June 2020.)

## INTRODUCTION

Cadaver studies of the vasculature using an injection of radiographic contrast solutions have a long history, from the seminal works of Manchot,<sup>1,2</sup> Spalteholz,<sup>3</sup> and Salmon<sup>4,5</sup> to modern studies culminating in the development of the angiosome concept.<sup>6,7</sup>

Taylor<sup>8</sup> and Chubb et al<sup>9</sup> and Sur et al<sup>10</sup> have highlighted the importance of “true” versus “choke” anastomoses

between angiosomes as a major determinant of tissue perfusion. Furthermore, the identification of true anastomoses has been shown to be a valuable preoperative adjunct in the optimal design of large fasciocutaneous flaps. True anastomoses differ from choke anastomoses in their vessel diameter and function.<sup>11</sup> True anastomoses link adjacent perforators without a reduction in vessel caliber, allowing an unimpeded flow between territories, whereas choke anastomoses are of reduced caliber, are capable of spasm, and regulate flow between adjacent territories.

To date, the effect of varying perfusion pressure on the diameter of these vessels between perforators has not been investigated. Of particular interest is whether it is possible to distend cutaneous perforating arteries and their anastomotic connections in fresh cadaveric studies, resulting in the conversion of small-caliber choke anastomoses to large caliber true anastomoses.<sup>8,9</sup> This study is aimed at answering this question. This is particularly relevant given recent theories

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regarding perfusion of perforator flaps, such as “perforator hyperperfusion,” and in particular the suggestion that increasing the flow within, and hence the perfusion pressure of a perforator, may enable dilatation of anastomotic vessels and “capture” of additional territories.<sup>12–15</sup>

To achieve this, we investigated the effect of increasing perfusion pressure by comparing the radiographic appearance of paired upper limbs injected at the same site with those injected with identical solutions but at different rates, while monitoring pressure in a distal artery.

## METHODS

Ethics approval for the project was obtained from our institution’s Human Ethics Advisory Group. Ten fresh cadavers were acquired (5 men and 5 women, age range, 63–94 years). Specimens with severe peripheral vascular disease or operative scars (eg, from radial artery harvest) were avoided. Limbs from 3 cadavers were excluded from analysis due to technical difficulties during injection. Seven pairs of upper limbs were ultimately included in the study, and 6 were analyzed for results. The aim was to control all variables except for pressure, including the site of injection, temperature, and concentration of injection solution, and the method of assessment.

### Preparation

A standardized contrast solution composed of lead oxide and milk powder, based on that originally described by Rees and Taylor<sup>16</sup> and modified by Suami et al,<sup>17,18</sup> was prepared. Similarly to Tang et al,<sup>19</sup> we found that a dilute solution was ideal (1L tap water, 100g lead oxide, and 10g milk powder), particularly for studies of small vessels such as a perforator’s anastomotic network. A satisfactory mixing of components was achieved by adding a small amount of boiling water to the lead oxide and milk powder to create a paste, then adding the remaining water gradually while mixing the solution. The preparation was maintained in solution by a magnetic stirrer while the injection was in progress.

Upper limbs were removed above the elbow from each cadaver. The brachial and radial arteries were cannulated separately using 16G cannulas. The radial artery cannula was attached to a pressure transducer (Hospira Transpac IV; ICU Medical Inc., San Clemente, Calif.) (Fig. 1). The pressure transducer was calibrated using a mercury sphygmomanometer before each experiment. Data acquisition was performed using ADInstruments PowerLab 7 software. Intravascular pressure was monitored throughout the injection, and the maximum perfusion pressure achieved for each study was recorded.

### Injection

Injection of radiographic contrast was performed via the brachial artery cannula. A proximal tourniquet was used to limit loss of contrast from the cut edge, and leaks were rigorously controlled using surgical clips, clamps, ties, thermoplastic adhesive, and direct pressure. In each pair of limbs, 1 was injected slowly and the other was injected rapidly to produce low and



**Fig. 1.** Cadaveric forearm with brachial artery cannulated proximally (blue arrow) and radial artery cannulated distally (yellow arrow). Note the blood pressure transducer attached to the radial artery cannula. Lead oxide contrast solution was injected via the brachial artery cannula.

high pressures, respectively. Each pair of limbs was injected with an equal volume of the contrast solution. Following injection, limbs were stored at 4°C for 24 hours to delay putrefaction and allow the lead oxide solution to set.

### Manual Injection Studies

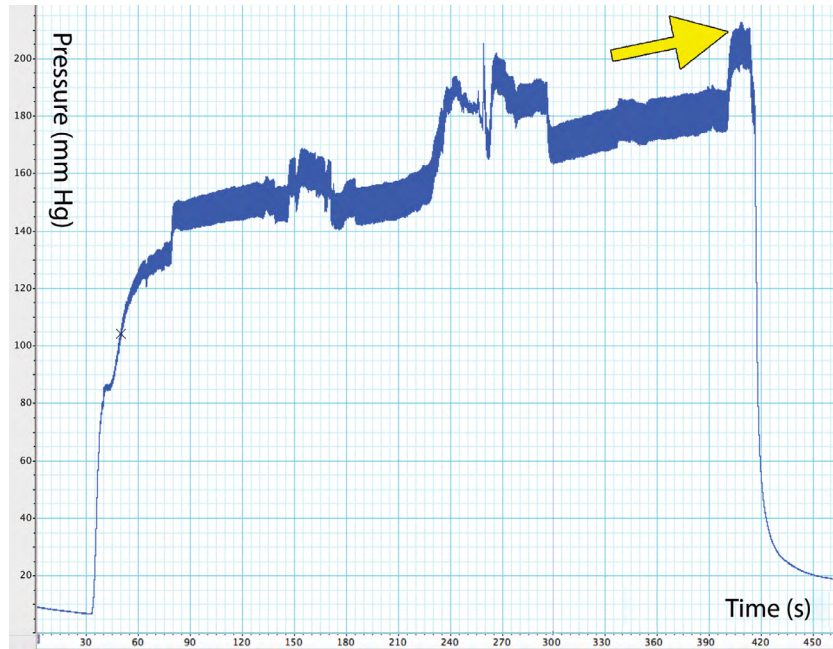
Initially, the solution was injected manually using a syringe as has been the Unit’s practice in the past. However, when attempts were made to increase the perfusion pressure, there was leakage of contrast solution from the cut edges of the specimen, reducing the maximum achievable perfusion pressure, or alternatively vessel blowout, resulting in a radiographic “whiteout” and failure to fully perfuse the specimen. To avoid this, attempts were made at pulsing the injection to achieve a more consistent perfusion pressure; however, this was technically difficult and had only minimal effect on the pressure curve.

### Peristaltic Pump Studies

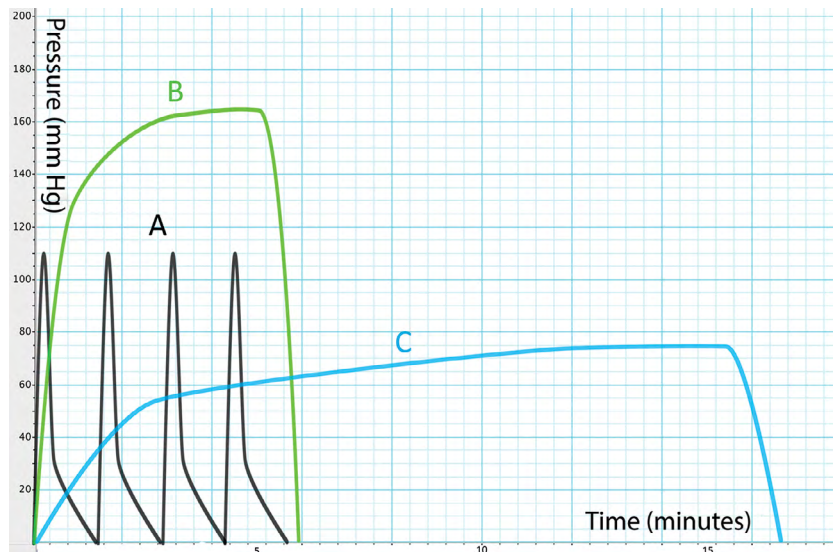
Because of these unsatisfactory results, the experimental technique was modified by introducing a peristaltic pump (Masterflex peristaltic pump; Cole-Parmer, Vernon Hills, Ill.) for injection of contrast solution in the remaining pairs of limbs. This allowed more accurate regulation of rate of injection, leaving the investigator free to control the leakage of contrast solution and to monitor perfusion pressure. Using the peristaltic pump, high and low-rate injection rates corresponded to an average of 1.5 and 0.5 mL/s, respectively. This resulted in a more even, consistent perfusion pressure, with a gradual increase in pressure over time and without vessel blowout (Figs. 2, 3).

### Dissection and Radiography

The skin and subcutaneous tissue were dissected free of the underlying muscle and bone at the subfascial plane and were laid flat. Significant perforators (>0.5mm diameter) were identified and marked during dissection. The specimens were radiographed (MC-150 Linear Collimator; EUREKA Progeny Inc., Buffalo Grove, Ill.) with digital x-ray acquisition (FCR CapsulaX; Fujifilm Australia, Macquarie Park, Australia.).



**Fig. 2.** One of the recorded high-pressure curves for fast pump injection (No. 9). Note the rapid increase in pressure at the commencement of injection, followed by a gradual increase with pressure spikes when contrast leakage is controlled. Recorded maximum perfusion pressure (211 mm Hg) is indicated via a yellow arrow.



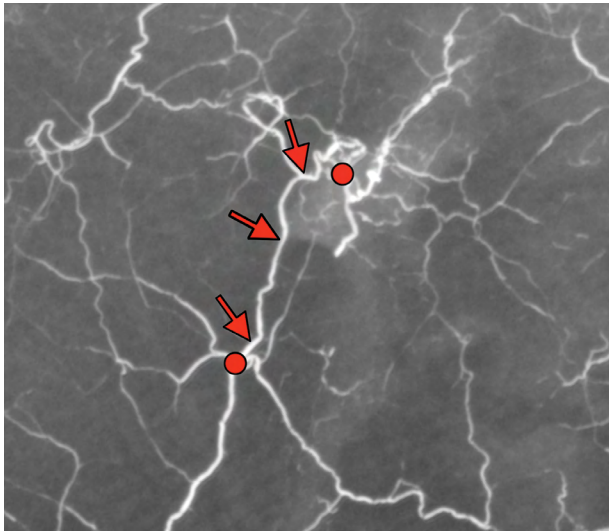
**Fig. 3.** Graphic representation of average pressure curves for (A) manual, (B) fast pump, and (C) slow pump injections. Note the rapid peak and dropoff in pressure during manual injections, compared to a gradual increase in pressure using a peristaltic pump. Injecting at a faster rate resulted in a higher peak perfusion pressure. Identical volumes were injected in each specimen. Only 4 manual injections are shown because many injections were required to perfuse the specimen with a similar volume of contrast solution.

### Image and Statistical Analysis

Image analysis and processing was performed in a DICOM viewer (OsiriX 64-bit; Pixmeo SARL, Bernex, Switzerland). Overall and selected areas were compared with horizontal mirroring of 1 image from each pair of limbs for ease of comparison. These images were reviewed by 3 of the

authors (G.I. Taylor, R.J. Corlett, M.W. Ashton), comparing vessel caliber and the number of true anastomoses in each limb, and were blinded as to whether individual images represented low- or high-pressure injections. True anastomoses were defined as connections between adjacent perforators that maintained the same caliber between their primary

branches. Following identification, the average caliber of the true anastomoses was measured at 3 points: at each end and in the center (Fig. 4). The mean average was then calculated for the true anastomoses in each specimen. Statistical



**Fig. 4.** Method for approximating average caliber of true anastomoses. Two perforators are shown (red dots) with their primary branches connected by a true anastomosis. This has been measured at each end and in the center (arrows).

**Table 1. Recorded Pressure Data**

Cadaver	Low	High	Volume, mL	Method
1	Discarded: proximal vessel blowout			Manual
2	51	99	125	Manual
3	77	137	250	Manual
4	60	101	500	Manual
5	Discarded: poor perfusion due to atheroma			Manual
6	Discarded: proximal vessel blowout			Manual
7	75	155	250	Pump
8	53	115	250	Pump
9	71	211	250	Pump
10	103	170	250	Pump
Average	70	141		

Data showing the maximum low and high pressure recordings (mm Hg) in each pair of limbs, corresponding to low and high rates of injection, respectively. Three studies were discarded for the reasons indicated.

analysis (Wilcoxon signed-rank test and linear regression analysis) was performed in IBM SPSS Statistics Version 24 (IBM Corp., Armonk, N.Y.).

## RESULTS

Recorded pressure data are shown in Table 1, and outcome measures are shown in Table 2. On performing a paired Wilcoxon signed-rank test, it was found that regardless of the rate of the injection, there was no alteration in the structural architecture of the vasculature, including the number of true anastomoses ( $P = 0.32$ ) and their caliber ( $P = 0.46$ ). Linear regression analysis revealed no association between maximum perfusion pressure and number ( $P = 0.94$ ) or caliber ( $P = 0.10$ ) of true anastomoses.

Instead of distending anastomotic vessels, high-pressure injections resulted in more distal filling of each perforator's anastomotic network and overflow into veins. Comparisons of the radiographic appearance of these injections are shown in Figure 5. Slow, low-pressure injections resulted in filling of perforators and their large caliber branches, including true anastomoses, but with limited filling of the smaller caliber choke anastomotic vessels (Fig. 5A, C, E). Rapid, high-pressure injections resulted in (1) better filling of perforators; (2) more distal filling of small-caliber choke anastomotic vessels; (3) significant filling of the major superficial veins with retrograde filling of their tributaries as the pressure increased; but (4) no increase in vessel caliber (Fig. 5B, D, F). Notably in paired specimens, large caliber true anastomotic connections were found in comparable locations (Fig. 5C, D) and with no greater frequency in number in higher pressure studies. In 1 specimen (No. 9), attentive control of leaks and fast-rate injection resulted in extreme (211 mm Hg) perfusion pressure, with excessive filling of smaller branches of the arterial perforators and more extensive retrograde filling of the superficial veins, rendering vascular territories indistinct (Figs. 6, 7).

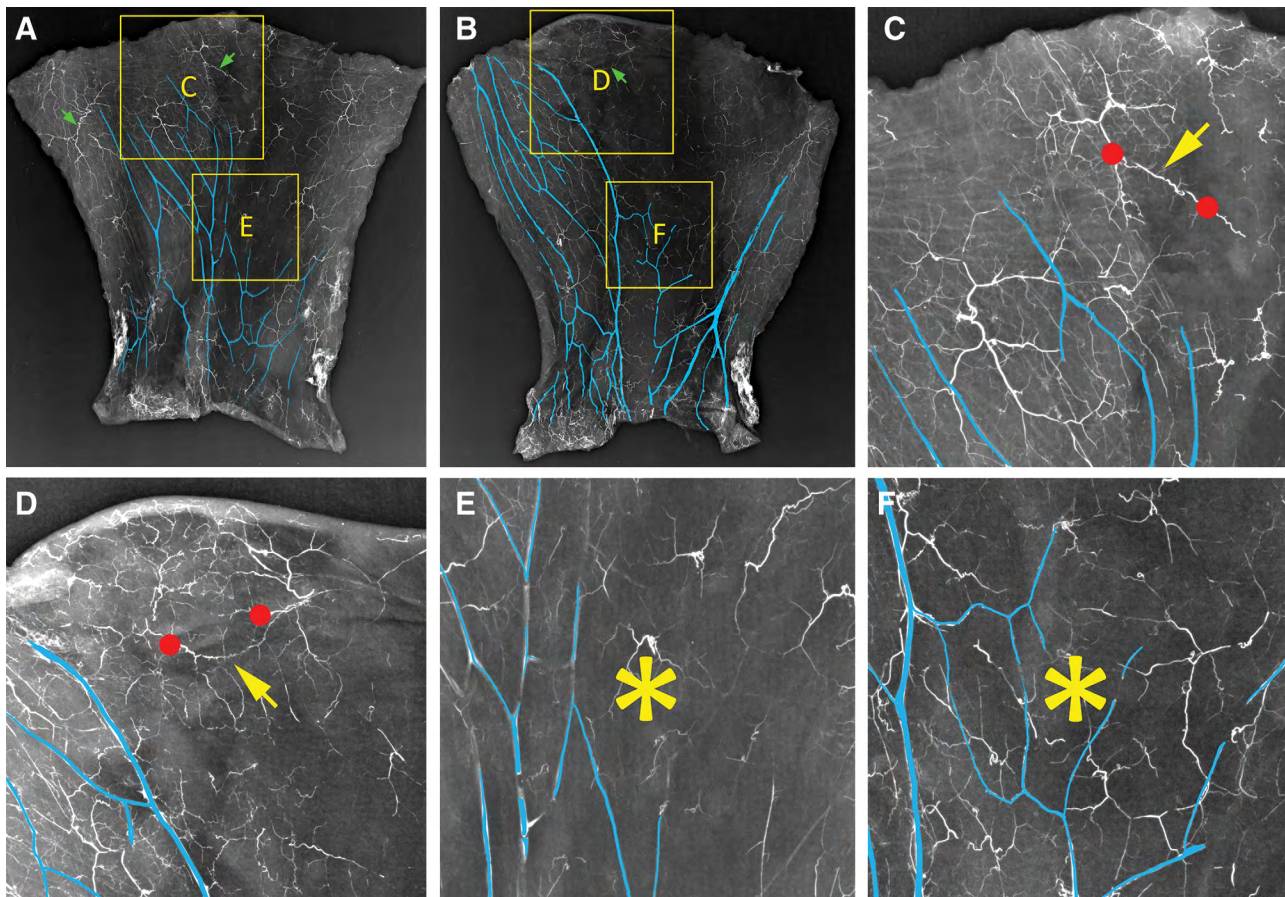
Unsurprisingly, there was a significant association between rate of injection and recorded maximum perfusion pressure ( $P = 0.018$ ). It is worth nothing that optimal perfusion of perforators and their anastomotic network was obtained with the peristaltic pump at a high injection rate (155–211 mm Hg perfusion pressure). However, even following low-rate injection (51–103 mm Hg), perforators and their large caliber branches demonstrated good

**Table 2. The Number of TA Observed in Each Study and the Average Diameter Thereof**

Cadaver	Low Pressure		High Pressure	
	TA Number	Average Diameter, mm	TA Number	Average Diameter, mm
1	Discarded: proximal vessel blowout			
2	2	0.39	3	0.35
3	4	0.39	5	0.44
4	3	0.40	2	0.39
5	Discarded: poor perfusion due to atheroma			
6	Discarded: proximal vessel blowout			
7	2	0.49	1	0.51
8	2	0.38	2	0.43
9	Excluded from analysis: uninterpretable			
10	4	0.45	3	0.43
Average	2.8	0.42	2.6	0.43

Note that although the diameter varied moderately between cadavers, their caliber was comparable in each pair of limbs. Four studies were not included in the statistical analysis for the reasons indicated.

TA, true anastomoses.



**Fig. 5.** Montage of radiographs of paired forearms from same cadaver (No. 7), showing low-pressure injection (75 mm Hg, A, C, and E) and high-pressure injection (155 mm Hg, B, D, and F). Note that the images on the right have been reversed so that direct comparisons could be made between the 2 specimens. Overview radiographs (A, B) showing areas of interest, with veins highlighted in blue. In the magnified panels note (1) the large caliber true anastomotic vessels between perforators highlighted in both low- and high-pressure injections (yellow arrows, C and D) and (2) increased filling of small-caliber choke anastomotic vessels (asterisks, E and F) at a high pressure. Note that there has been no change in the overall architecture or in the caliber of either true or choke anastomoses in the limb perfused at a high pressure when the studies are compared and backfilling of venous tributaries which is enhanced by high-pressure injection.

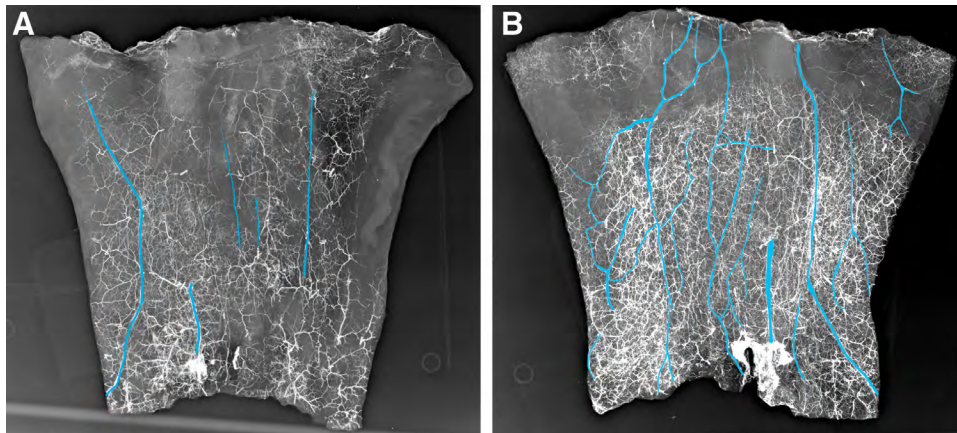
filling. Smaller caliber anastomotic vessels, by comparison, only filled at a higher pressure (Fig. 5E, F).

## DISCUSSION

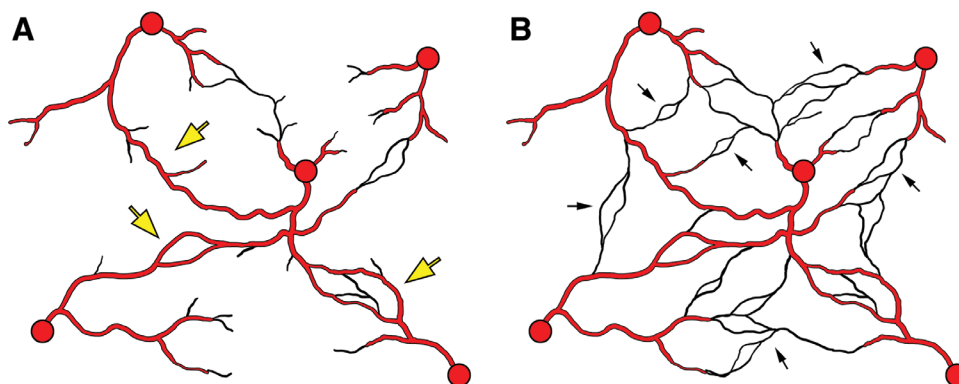
Though contrast studies of the cadaveric vasculature have a long history and continue to be widely used, the effect of perfusion pressure has not been studied previously. In the past, most of the cadaver injection studies by the unit had been done manually,<sup>6-9</sup> pulsing the injectant into arteries to simulate the heart beat and to avoid vessel rupture. This has been the case especially when injecting the thin-walled lymphatics.<sup>17,18</sup> In most cases of arterial injection, the rate of injection has been low, and the perfusion pressure has also been presumably low. During this study, when the rate of injection was increased, it was noted that vessel rupture occurred more frequently, resulting in 2 studies being discarded. These blowouts tended to occur in the larger vessels, consistent with the protective effect of small vessel diameter because wall tension increases with vessel radius for a given internal pressure as expressed by the Law of Laplace ( $T = P \cdot r$ ).<sup>20</sup> Vessel rupture did not

occur when high-rate injections were performed with a peristaltic pump. Fortunately, although not the aim of this study, we found that the best results were obtained using the peristaltic pump with a high-rate injection.

In addition to rate and volume of injection, the maximum perfusion pressure achievable in each study was largely dependent on the degree of leakage control. To achieve sufficient perfusion pressure and to perfuse the specimen satisfactorily, it was necessary to secure the injection cannula firmly and control assiduously any leakage of contrast solution. Constant injection using a peristaltic pump resulted in a smoother pressure curve with a very gradual increase in overall pressure and perfusion of the vasculature without rupture. According to Poiseuille's equation ( $F \propto \frac{\Delta P \cdot r}{\eta \cdot L}$ ), flow is a function of pressure, vessel radius and length, and viscosity.<sup>21</sup> Accordingly, increasing resistance (by reducing radius or increasing length) or viscosity diminishes flow, while increasing pressure enhances it. In the current study, variables other than injection rate, and hence perfusion pressure, were controlled. Composition, temperature, and preparation of the lead



**Fig. 6.** Comparison of radiographs of (A) low- and (B) very high-pressure injection studies from the same cadaver (No. 9). In the very high-pressure study (B, 211 mm Hg), note the diffuse filling of perforators, their small and large caliber branches, and significant venous filling. This “snowstorm” renders the study difficult to interpret as overlapping arterial and venous territories are virtually indistinguishable. Significantly, however, the perforators and their branches are not increased in size by comparison with low-pressure injection in the contralateral limb (A, 71 mm Hg), and there is again more extensive filling of venous tributaries at a high pressure.



**Fig. 7.** Schematic diagram of (A) low- and (B) high-pressure filling of the same perforator's anastomotic network. Note that there is increased perfusion of the finer network at a high pressure, but the diameter of the perforators branches and their true (large arrows) and choke (small arrows) anastomoses are unchanged.

oxide solution was identical for each injection, and the same volume was injected in each limb at the same site and under the same conditions. The anatomical characteristics of the vessels were similar, as left and right upper limbs from the same cadaver were used for comparison. Hence, we consider that the appearance of the vasculature between low- and high-pressure injection studies can be attributed to the difference in perfusion pressure.

Limitations of the study included the relatively small sample size, as well as the use of a cadaveric model, findings from which may not translate directly into clinical surgery. Nevertheless, by controlling as much as possible all variables aside from perfusion pressure on the structure of the cutaneous vasculature, the below conclusions are supported by our findings.

The major findings in this study were (1) the inability to distend the vasculature at a higher pressures and convert small-caliber choke anastomoses to large caliber true

anastomoses and (2) demonstration of widespread arteriovenous shunting into major superficial veins.

#### Effect of Perfusion Pressure on Anastomotic Vessels

Perforators and their larger caliber branches have relatively low resistance and fill at low pressure, while smaller caliber branches and anastomotic vessels, by comparison, exhibited greater (more distal) filling with higher pressures (Fig. 8). However, although the extent of filling varied, their relative size remained unchanged despite a range of perfusion pressures.

These findings are consistent with previous work done in this laboratory that has focused on true and choke anastomoses and the delay phenomenon.<sup>22-25</sup> It should be emphasized that though it is impossible to distend small-caliber choke connections through direct application of pressure, it is possible for this conversion to take place in vivo over time using surgical delay procedures. This leads

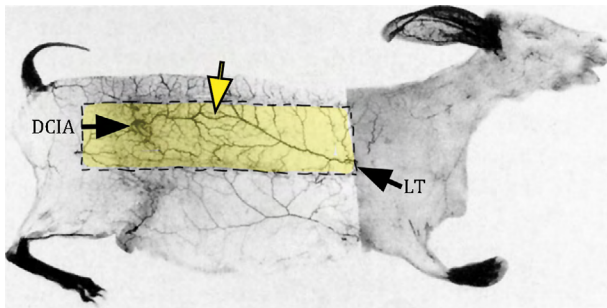
to remodeling and enlargement of the vessels within a flap, with conversion of choke to true anastomoses. Previous research has demonstrated that these changes do not take place until 48–72 hours postsurgery (see Figs. 8, 9).<sup>24</sup> They involve hypertrophy and hyperplasia of the endothelial and smooth muscle cells in the vessel wall and are likely to be a physiological response to ischemia<sup>26</sup> mediated by cytokines such as vascular endothelium growth factor and nitric oxide.<sup>27–29</sup>

**Arteriovenous Shunting**

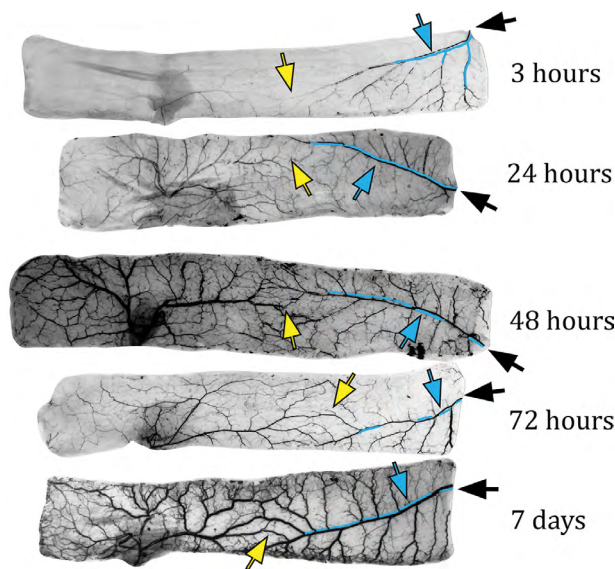
The revelation of lead oxide appearing in the cephalic and other major veins, especially with back filling of their tributaries as the pressure increased, would strongly

support the presence of arteriovenous shunts in the cutaneous circulation. Though lead oxide particles are similar in diameter to red blood cells (4–10 μm; c.f. 6.2–8.2 μm),<sup>30</sup> they usually form aggregates within the radiographic contrast solution. In addition, the fact that the veins filled in a retrograde manner with smaller tributaries only appearing with very high pressure of 211 mm Hg demonstrates that this filling is taking place through proximal arteriovenous shunting in the vascular network rather than across the capillary bed itself. Though not highlighted before, we have observed this phenomenon of arteriovenous shunting in vivo in animal studies. In many postmortem angiographic studies of delayed flaps in dogs and rabbits, we observed lead oxide in the venae comitantes of the flap artery but not in their tributaries (see Figs. 8, 9).

It is interesting to speculate about the clinical and pathological implications of this observation. The superficial veins and venae comitantes being anatomically in juxtaposition to the arteries throughout the body are ideally situated for arteriovenous shunting. This may well be a protective mechanism against (1) sudden raised intra-arterial pressure and major vessel rupture and (2) a bypass pathway to divert a toxin away from the capillary bed, especially if combined with choke vessel spasm to limit the area of impact.<sup>11,31</sup> This has been demonstrated recently in an animal model in a specific study of embolization of hyaluronic acid (HA) injected as a cosmetic filler. HA was injected into the central auricular artery of a rabbit and was found on histology to be present also within the accompanying vein.<sup>32</sup> Though the authors hypothesize that these particles have been forced through the capillary bed, given the very large size of the HA particles, it seems more likely that they passed via arteriovenous shunts, as demonstrated in the current study. Similar shunting phenomena have previously been shown in vivo in studies of the coronary circulation<sup>33</sup> and also in the microcirculation of patients with septic shock.<sup>34</sup>



**Fig. 8.** Overview of a rabbit with delayed thoraco-groin flap at day 7. Note true anastomoses (yellow arrow) connecting the LTA and DCIA territories (labelled black arrows). DCIA indicates deep circumflex iliac artery; LTA, lateral thoracic artery.



**Fig. 9.** Five different delayed thoraco-groin flaps at different times based on the lateral thoracic artery (black arrow) in the rabbit demonstrating arteriovenous shunting between the flap artery and its venae comitantes (blue arrows). Note also the choke vessel communications between the LTA and DCIA territories that show progression to true anastomoses after 48 hours (yellow arrows). DCIA indicates deep circumflex iliac artery; LTA, lateral thoracic artery. Reproduced with permission from Taylor GI, Pan WR. *The Angiosome Concept and Tissue Transfer*. NY: Thieme; 2014:441.

**CONCLUSIONS**

This study has demonstrated that the effect of high perfusion pressure on the radiographic appearance of the vessels in cadaveric studies is to increase the distal filling of small-caliber, high resistance, choke anastomotic vessels, without affecting their relative caliber or distorting the vascular architecture. The appearance of perforators and their large caliber, low resistance, true anastomotic vessels was unchanged because these vessels fill at lower injection pressures. Most notably, the number and caliber of true anastomoses observed in specimens perfused at a high pressure were not increased, demonstrating that high perfusion pressure does not convert choke to true anastomoses in the cadaver. These findings suggest that it is not possible to alter the structural architecture of the vasculature by application of high perfusion pressure alone. Though it is certainly possible to cause remodeling through use of a surgical delay, it is likely that large perforator flaps are otherwise dependent on the presence of preexisting large caliber true anastomotic vessels. In addition, significant filling of large superficial veins was

observed, a phenomenon that was enhanced by a high-pressure injection, likely due to arteriovenous shunting in the cutaneous circulation.

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