Enhanced peripheral neovascularization of musculocutaneous flaps following pedicle ligation in the pig

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HM Clarke, G Chen. Enhanced peripheral neovascularization of musculocutaneous flaps following pedicle ligation in the pig. Can J Plast Surg 1994:2(4):159-163. The augmentation of blood flow from the periphery following dominant pedicle ligation was studied in island latissimus dorsi muscle and musculocutaneous flaps in the pig. In the first study, a musculocutaneous flap and a muscle flap covered with a skin graft were raised on two sides of the same pig (n=25). Silicone rubber sheeting inhibited revascularization from the base. From one to seven days later the dominant vascular pedicles were ligated and blood flow from neovascularization to the flap was assessed immediately using radiolabelled 15 µm microspheres. The peripheral neovascularization of muscle and musculocutaneous flaps was not different when assessed at the time of pedicle ligation. Comparison with a previous study of flap survival, however, revealed that increased blood flow was seen in flaps in which the pedicle had been ligated two days prior to rather than at the time of assessment. Therefore, in a second study, musculocutaneous flaps were raised bilaterally and from one to eight days later the pedicle was ligated on one side only (n=29). After two days of further neovascularization the blood flow to the components of the flaps was assessed using 15 µm microspheres. Significant enhancement of peripheral neovascularization was seen in all animals in which two days had elapsed between pedicle ligation and the assessment of neovascularization ($P<0.05$). In addition, the flows seen in the skin component of the flaps were substantially greater than normal, suggesting a delay-like phenomenon.

Key Words: *Blood flow, Microspheres, Musculocutaneous flaps, Neovascularization, Pedicle ligation, Pigs*

Néovascularisation périphérique améliorée au niveau de lambeaux musculocutanés après ligature du pédicule chez le porc

RÉSUMÉ : L'augmentation du débit sanguin périphérique après une ligature du pédicule a été étudiée au niveau du grand muscle dorsal et de lambeaux musculocutanés chez le porc. Dans la première étude, un lambeau musculocutané et un lambeau musculaire recouvert d'un greffon cutané ont été soulevés des deux côtés d'un même animal (n=25). Une pellicule de silicone a empêché la revascularisation à partir de la base. De un à sept jours après, les pédicules vasculaires dominants ont été ligaturés et le débit sanguin provenant de la néovascularisation du lambeau a été évalué immédiatement au moyen de microsphères de 15 µm radiomarquées. La néovascularisation périphérique du muscle et des lambeaux n'était pas différente au moment de la ligature des pédicules. La comparaison avec une étude précédente sur la survie des lambeaux a toutefois révélé un débit sanguin accru dans les lambeaux où le pédicule avait été ligaturé deux jours avant le moment de l'évaluation. Dans une deuxième étude, les lambeaux musculocutanés ont été soulevés bilatéralement et de un à huit à jours après, le pédicule a été ligaturé, mais d'un côté seulement (n=29). Après deux jours de néovascularisation, le débit sanguin des éléments du lambeau a été évalué au moyen de microsphères de 15 µm. Un rehaussement significatif de la néovascularisation a pu être observé chez tous les animaux pour lesquels un intervalle de deux jours avait séparé la ligature des pédicules et l'évaluation de la néovascularisation ($P<0,05$). En outre, les débits observés dans la composante cutanée des lambeaux étaient substantiellement plus grands que la normale, ce qui suggère l'existence d'un phénomène qui agirait à retardement.

Many factors determine the quantity of neovascularization of a flap that has been transferred. These factors include the constituent tissues in the flap, the nature of the bed, and the added insults of previous trauma or irradiation. In free tissue transfers for reconstruction following tumour or trauma, the transferred flap's immediate survival is based on the blood flow restored through its dominant vascular pedicle at the time of surgery. These flaps become independent of their original dominant pedicle after a variable period of time which has not been elucidated in the human. In experimental animals, however, this period of time is very short (1-4).

The dependence of a transferred flap upon its dominant blood supply has been studied by a number of authors (1-6). The sufficiency of the dominant blood supply may in fact impede peripheral neovascularization.

The following study was designed to evaluate the effect of the loss of the dominant vascular pedicle in flaps during the early period of neovascularization.

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TABLE 1: Peripheral neovascularization at the time of pedicle ligation

Days from flap creation to pedicle division	n	Blood flow (mL/min/100 g) Musculocutaneous flaps Skin	Musculocutaneous flaps Muscle	Muscle flaps Muscle
1	3	0.005 ± 0.003	0.001 ± 0.001	0.008 ± 0.003
2	5	0.02 ± 0.00	0.02 ± 0.01	0.02 ± 0.01
3	5	0.2 ± 0.0	0.3 ± 0.0	0.4 ± 0.1
4	3	0.4 ± 0.2	0.9 ± 0.4	1.1 ± 0.5
5	3	0.8 ± 0.0	2.0 ± 0.1	1.8 ± 0.2
6	3	1.4 ± 0.3	2.9 ± 0.5	2.9 ± 0.2
7	3	2.3 ± 1.0	3.0 ± 1.0	3.7 ± 0.6
Total	25			($\bar{X}$ ± SEM)

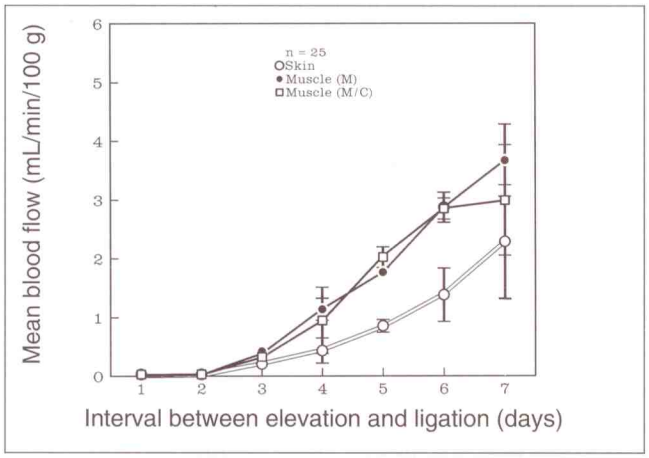

Figure 1) *Results of experiment 1 in which increasing intervals are allowed between flap elevation and pedicle ligation in muscle and musculocutaneous flaps. Blood flow, measured immediately after pedicle ligation, is from peripheral neovascularization alone. Blood flow is not different in the muscle components of muscle and musculocutaneous flaps (M - Muscle flaps; M/C - Musculocutaneous flaps*

METHODS

Fifty-four castrated male Yorkshire pigs (mean ± SEM 10.2 ± 0.2 kg) were housed separately in an environmentally controlled facility and fed commercial pig feed and water ad libitum. Benzathine penicillin (50,000 units/kg) and procaine penicillin G (50,000 units/kg) were given intramuscularly during surgery.

Experiment 1

Twenty-five pigs were used to study the increasing peripheral neovascularization over time in the first week following flap elevation. Anaesthesia was induced with ketamine hydrochloride (40 mg/kg) and maintained with sodium pentobarbital (20 mg/kg loading dose). Endotracheal intubation and positive pressure ventilation with oxygen and nitrous oxide was used. Core temperature was maintained with a heating blanket. On one randomly assigned side, a 9x12 cm denervated island latissimus dorsi musculocutaneous flap was raised as previously described (5,7,8). Silastic silicone rubber sheeting (0.20 inch) (Dow Corning Corp, Midland, MI) was placed beneath the flap and sutured to the base. The flap was then inset into its original position with 3-0 silk sutures. On the opposite side a similar flap was raised but the skin and subcutaneous tissue was discarded after harvesting a partial thickness skin graft from it. Silicone rubber sheeting was placed, the muscle was sutured in position and the skin graft was applied to its surface. A bolster dressing was applied and later removed under anaesthesia at the next operation.

After a variable period of time from one to seven days the pig was anaesthetized and the thoracodorsal artery and vein supplying the flap as its dominant vascular pedicle were ligated and divided through a small incision at the base of the flap on each side. Microspheres were given and the flaps were harvested and cut into one centimetre slices which were submitted for counting.

Experiment 2

Twenty-nine pigs were used in the second study to determine the effect of earlier pedicle ligation. At the first operation, bilateral denervated island latissimus dorsi musculocutaneous flaps were created on both sides of the same pig with silicon rubber sheeting interposed between the flap and the base. After waiting between one and eight days, the dominant vascular pedicle was ligated and divided under anaesthesia on one randomized side of the pig. The vessels in the pedicle on the opposite side were exposed but not ligated.

After an additional two days of peripheral neovascularization the animals were anaesthetized again. The previously sham-ligated side had its dominant pedicle ligated, microspheres were given and the tissues were submitted for counting.

Measurement of capillary blood flow

The well established technique of capillary blood flow measurement using radiolabelled microspheres has been used (9,10). In brief, 15 μm microspheres are injected into the left ventricle and are distributed evenly throughout the body. Blood collected at a calibrated rate in a withdrawal pump (Harvard apparatus model 907) allows standardization of measurements. Microspheres are counted in an LKB Wallak 1202 Compu Gamma CS gamma counter.

Statistics

Means, standard deviation and standard errors as well as paired *t* testing were undertaken using StatView 512+ software. Analysis of variance was used to compare base line physiologic parameters between the two groups.

RESULTS

Pigs were monitored to ensure stable hemodynamics throughout the experiments. Mean arterial blood pressure was 111±2 mmHg, cardiac output was 187±8 mm Hg and temperature was 37.6±0.1°C. The difference in core temperature between the two groups was smaller than the measurement error of the technique, and the difference in weights was 0.8 kg. No other significant differences were found between the groups.

TABLE 2: Peripheral neovascularization two days following pedicle ligation

Days from flap creation to microsphere injection	n	Blood flow (mL/min/100g)		
		Musculocutaneous flaps		Muscle flaps
		Skin	Muscle	Muscle
3	3	0.6±0.2	0.7±0.3	0.5±0.2
4	5	1.9±0.9	2.5±1.0	2.0±0.7
5	5	2.8±0.6	4.7±0.7	4.6±0.8
6	3	4.6±2.5	6.4±2.1	6.3±1.9
7	2	3.2±1.1	5.7±1.6	6.5±0.5
Total	18			($\bar{X}$±SEM)

Data excerpted and adapted from a previously published study

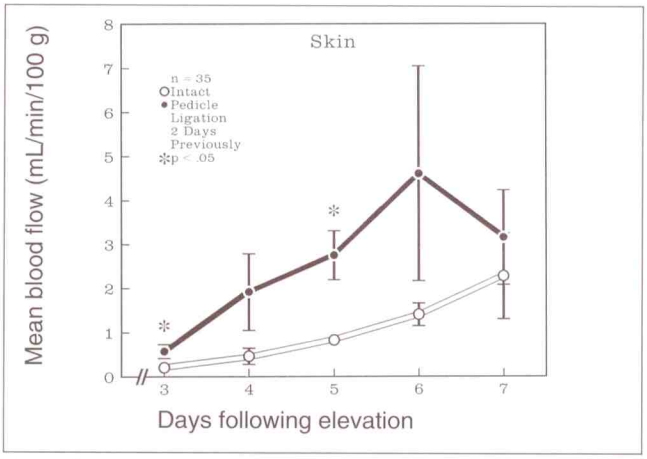

Figure 2) *Skin blood flow from neovascularization measured immediately after pedicle ligation in musculocutaneous flaps is compared with flow in flaps in which the pedicle had been ligated two days earlier allowing further neovascularization. Total time from original flap elevation is matched in both groups. *Differences are significant at some intervals*

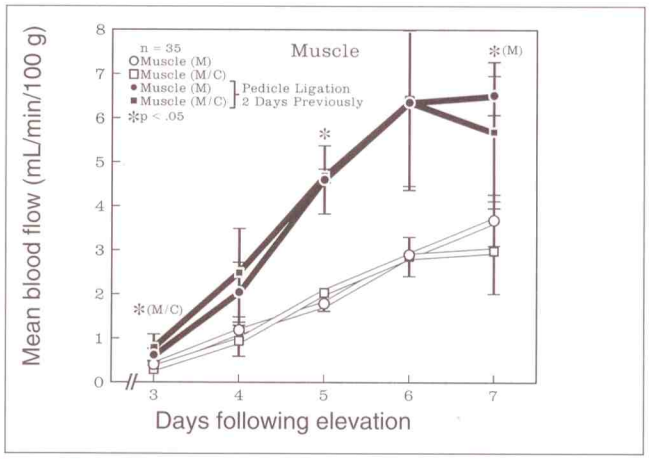

Figure 3) *Muscle blood flow from neovascularization measured immediately after pedicle ligation in muscle and musculocutaneous flaps is compared to flow in flaps in which the pedicle had been ligated two days earlier. Total time from original flap elevation is matched in both groups. *Differences are significant at some intervals. (M - Muscle flaps; M/C - Musculocutaneous flaps)*

Experiment 1

A progressive increase in peripheral neovascularization was found in muscle and musculocutaneous flaps through the first week following flap elevation (Table 1, Figure 1). These flows through the flap from neovascularization had not reached normal values for intact tissue, as determined in a previous study (5). No significant difference was seen, however, between the blood flow in the muscle components of the muscle and musculocutaneous flaps in these pigs.

Data from a previous and comparable experiment (5) in which pedicle ligation was undertaken two days before final determinations of survival and blood flow in identical porcine flaps (Table 2) were then compared to these new results (Figures 2 and 3). Animals with equal total time between flap elevation and evaluation of blood flow from neovascularization were compared in groups. While the statistical evaluation was made less rigorous by the fact that these flaps are not raised in the same animals, the blood flow in the animals in which pedicle had been ligated two days previous to the end of the total time from original elevation was greater. Unpaired *t* testing between these overall groups showed significant differences at three and five days following flap creation in the skin blood flows and at three, five and seven days following ligation for some muscle blood flows ($P<0.05$).

Experiment 2

In order to elucidate this phenomenon a further study was undertaken in which earlier pedicle ligation was accomplished in one of two musculocutaneous flaps in the same pig. Musculocutaneous flaps were raised on both sides of the same animal and the dominant vascular pedicle was ligated on one side two days before the assessment of peripheral neovascularization. Again a progressive increase of blood flow to the flap provided by peripheral neovascularization was seen over the intervals from three to 10 days studied (Table 3). There was a significant enhancement, however, of blood flow in those flaps in which the pedicle had been ligated two days previously (Figures 4 and 5). Paired *t* testing between the two flaps on either side of the same animal was undertaken for both skin and muscle and was significantly different at all time intervals studied ($P<0.05$).

DISCUSSION

Substantial blood flow resulting from peripheral neovascularization was seen in both muscle and musculocutaneous flaps even a few days after initial flap elevation. Other studies have also demonstrated the rapidity of neovascularization seen in island (2,3) and free skin flaps (1) in the rat and in musculocutaneous flaps in the pig (4). Similar rapid neovascularization may also occur in the human given the reports of free flap survival after early vascular pedicle loss (11-13).

That there is no significant difference in blood flow from peripheral neovascularization seen in muscle and musculocutaneous flaps at this early stage can be correlated with the survival data from our previous study (5) which showed no difference in survival patterns between these two types of flaps. Millican and Poole (6) offer conflicting evidence that

TABLE 3: Peripheral neovascularization in musculocutaneous flaps

Days from flap creation to microsphere injection	n	Blood flow (mL/min/100 g)			
		Control		Pedicle ligation 2 days previously	
		Skin	Muscle	Skin	Muscle
3	5	0.09 ± 0.02	0.2 ± 0.0	0.2 ± 0.1	0.3 ± 0.1
4	3	0.5 ± 0.3	0.5 ± 0.2	1.4 ± 0.3	1.5 ± 0.5
5	3	1.0 ± 0.2	1.7 ± 0.3	3.3 ± 0.5	4.7 ± 0.6
6	3	1.4 ± 0.2	2.4 ± 0.2	5.7 ± 1.3	6.7 ± 0.8
7	4	1.6 ± 0.2	3.1 ± 0.3	5.1 ± 1.2	6.7 ± 0.7
8	4	1.7 ± 0.3	2.4 ± 0.2	5.3 ± 1.8	5.1 ± 0.8
9	4	2.5 ± 0.7	4.4 ± 0.8	7.8 ± 2.1	6.4 ± 0.7
10	3	2.3 ± 0.7	3.0 ± 0.5	5.9 ± 1.3	7.2 ± 1.1
Total	29				($\overline{X}$ ± SEM)

the revascularization of musculocutaneous flaps progresses more rapidly than that of muscle flaps. In their study, which also used silicon rubber sheeting beneath musculocutaneous flaps in pigs, filling with disulphine blue dye after occlusion of the original vascular pedicle did not occur for three to four weeks following flap elevation. This qualitative technique for the assessment of blood flow does not appear to have the sensitivity of the microsphere technique. The revascularization of the base by the flap, the reverse of the situation we are studying, has been examined in cutaneous and musculocutaneous flaps in the pig by Fisher and Wood (14). They have shown that more substantial revascularization of bone grafts occurs under the muscle component of a musculocutaneous flap than under a cutaneous flap.

The second study of prior pedicle ligation reported here has been undertaken to evaluate further the observation made by comparing the results of immediate pedicle ligation cited above with those of a previously reported group of pigs (5) in which prior ligation had occurred. Given the variability seen both in normal blood flow in intact tissues and the variability in peripheral neovascularization between animals, an experiment with comparisons made within the same animal was necessary. Two days was selected as the time interval to be studied since our preliminary observations indicated an effect at this interval. A more or less substantial effect may have been seen had a different interval been selected.

The delay phenomenon as classically described and studied (15) is the enhancement of the surviving length of a flap by prior surgical manipulation, usually by incision of the sides and undermining the flap. Many mechanisms have been proposed as responsible for this effect including the re-orientation of vascular channels, the closure of arteriovenous anastomoses (16), sympathectomy leading to vasodilation (17), the conditioning of the tissues to withstand hypoxia (15) or ischemia, prolonged vascular smooth muscle relaxation in the precapillary arterioles caused by sympathectomy, ischemia and inflammation (18), and vasoconstrictive substances released by the trauma to the flap or a nonspecific inflammatory reaction (19).

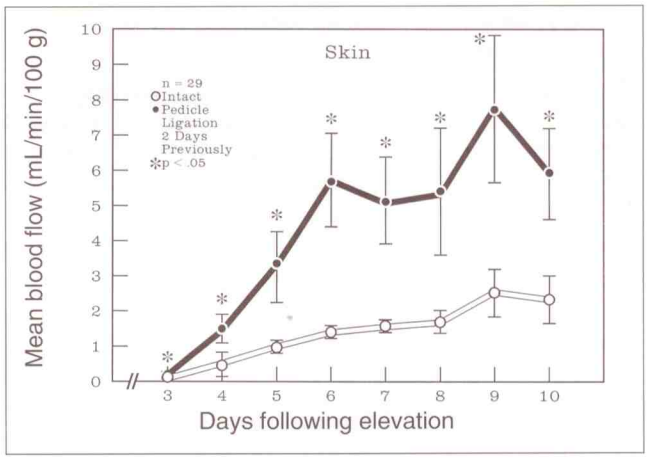

Figure 4) *Results from experiment 2 in which dominant pedicle ligation is undertaken two days earlier on one side of two musculocutaneous flaps in the same animal. At all intervals studied the skin blood flow from peripheral neovascularization for the prior ligation side shows greater blood flow compared with controls*

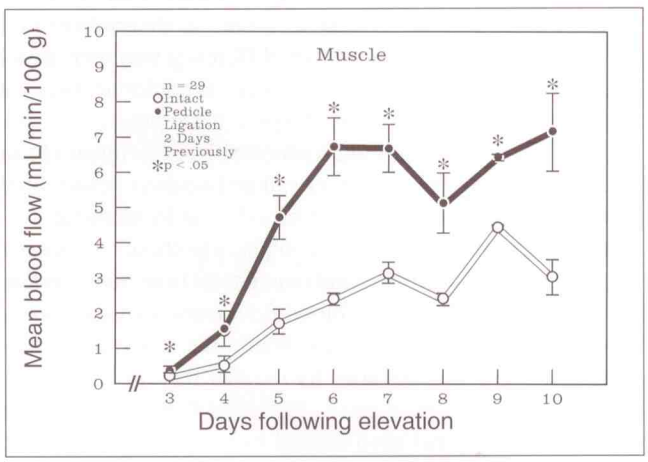

Figure 5) *Results from experiment 2 show muscle blood flow from peripheral neovascularization for the musculocutaneous flap with earlier pedicle ligation is greater at all intervals studied compared to flaps in which the dominant pedicle was ligated immediately before blood flow assessment*

Finseth and Cutting (20) have raised bilateral abdominal wall flaps in rats and found that earlier division of any or all of the elements of the ipsilateral vascular pedicle resulted in a statistically significant increase in surviving flap area on the ipsilateral side when the flap was raised on the contralateral vessels. No difference was demonstrated, however, between the different specific treatments. Boyd et al (21) used TRAM flaps in pigs based on the deep inferior epigastric vessels with transverse skin paddles at the superior end of the muscle. Ligation of the superior epigastric vessels at four to 28 days prior to flap creation significantly increased the length and area of viable skin. There was also a significant increase in skin and muscle capillary blood flow. In the present study, we have surgically manipulated the vessels directly supplying the flap rather than a secondary or alternate pedicle and have thereby enhanced neovascularization. Our concept of the delay phenomenon must, therefore, incorporate varied forms

of surgical manipulation of the macro and microcirculation of the flap.

In conclusion, the augmentation of flap blood flow seen in delay can be stimulated by surgery to the base or dominant pedicle as well as the periphery of a flap. This effect is observed as early as the first day following flap creation in the pig and increases with further time intervals.

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