PIG MUSCLE FLAP VASCULAR RESISTANCE

by Hubert YM Chao

BSc, The University of British Columbia, 1990
MD, The University of Western Ontario, 1995

A THESIS SUBMITTED IN PARTIAL FULFILMENT OF
THE REQUIREMENTS FOR THE DEGREE OF

MASTER OF SCIENCE

in THE FACULTY OF GRADUATE STUDIES

DEPARTMENT OF SURGERY

We accept this thesis as conforming
to the required standard

THE UNIVERSITY OF BRITISH COLUMBIA

VANCOUVER, BRITISH COLUMBIA, CANADA

APRIL 1998

In presenting this thesis in partial fulfilment of the requirements for an advanced degree at the University of British Columbia, I agree that the Library shall make it freely available for reference and study. I further agree that permission for extensive copying of this thesis for scholarly purposes may be granted by the head of my department or by his or her representatives. It is understood that copying or publication of this thesis for financial gain shall not be allowed without my written permission.

Department of Surgery

The University of British Columbia
Vancouver, Canada

Date 12 April '98

DE-6 (2/88)

ABSTRACT

Free muscle transfer is a surgical procedure used for treating wounds that may result from trauma, cancer excision, radiation necrosis, or defects due to congenital anomaly. It is often a salvage procedure and failure results in significant morbidity. Choke vessels may have a role in such failures. These vessels interconnect vascular territories which each have associated vascular pedicles. Choke vessels are believed to have high vascular resistance and are implicated in thrombosis leading to flap failure.

The vascular resistance of choke vessels has not previously been studied. The objective of this study was to determine the relationship between muscle flap vascular resistance and the presence or absence of choke vessels in the intraoperative period following muscle flap elevation in quantifiable terms. The alternative hypothesis is that the surgical resection of the zone of choke vessels from a muscle flap results in a statistically significant decrease in muscle flap vascular resistance per unit weight. Under isoflurane anaesthesia, 8 pig rectus abdominis flaps were elevated on the superior epigastric pedicle. The pedicles were sympathectomized. The flaps were serially resected towards the pedicle, resecting the zone of choke vessels in the process, with vascular resistance and flap weight determined concurrently. As the choke vessels were resected, no decrease in vascular resistance per unit weight was observed. Control flaps were included in the study, as well gracilis flaps were studied for comparison. The power of this study was 0.98. The role of choke vessels in failure of muscle flaps remains unclear. Vascular resistance per unit weight was seen to increase as muscle flaps were serially resected. A possible mechanism for this is the myogenic response. The myogenic response although well described has not previously been associated with muscle flaps.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1: INTRODUCTION AND BACKGROUND

1.1 INTRODUCTION

Free muscle transfer is one of many procedures performed by the reconstructive surgeon treating wounds that may result from trauma, cancer excision, radiation necrosis, or defects due to congenital anomaly. The use of muscle as a free tissue transfer is indicated for large, complex, poorly vascularized wounds. Free muscle transfer is often a salvage procedure and failure results in significant morbidity.

This thesis project evaluates the role of choke vessels as a possible factor in muscle flap failure. Choke vessels interconnect vascular territories within a muscle flap. Experimental and clinical evidence has verified that choke vessels, between adjacent vascular territories, dilate over time and result in survival of that vascular territory which has had its own original arterial blood supply ligated. The inference is that the choke vessels are of high vascular resistance, and that their dilation over time decreases vascular resistance and thereby improves blood flow between the adjacent vascular territories. The vascular resistance across choke vessels, however, has not previously been studied.

The objective of this thesis project was to quantify the relationship between muscle flap vascular resistance and the presence or absence of choke vessels in the intraoperative period immediately following elevation of muscle flaps.

1.2 RECONSTRUCTIVE SURGERY

The reconstructive surgeon is faced with the problem of treating wounds that may result from trauma, cancer excision, radiation necrosis, or defects due to congenital anomaly. A fundamental concept for the reconstructive surgeon is the "reconstructive ladder." The concept is a rank ordering of surgical techniques from simple to complex. From simple to complex, these techniques are direct local closure (primary, secondary, delayed primary), grafts (split thickness skin graft, full thickness skin graft, composite graft), flaps (local, regional, distant and free flaps). The most simple surgical technique that meets the requirements of the defect should be used. These requirements include achieving wound closure with viable coverage, and also achieving adequate form and function.

Skin grafts are the transplantation of skin tissue from a donor site to a recipient site. The graft is separate from its blood supply and thus depends on the local ingrowth of a blood supply at the recipient site for its survival. Local flaps involve the movement of tissue from the donor site to the recipient site with intact blood supply. Thus, the anatomy of the blood supply limits the distance and direction that these tissues may be moved. Distant flaps are those where tissue is moved from the donor site to a recipient site that is remote from the donor site. Initially the original blood supply to the distant flap is from the donor site, but subsequently the blood supply comes from vascular ingrowth at the recipient site. Free flaps are those where tissue is transplanted from the donor site to a recipient site with the blood supply sectioned at the donor site and then surgically anastomosed to blood vessels in proximity to the recipient site. Various

tissues can be used as flaps, and are classified according to their composition.

Muscle flaps (see Figure 1) and musculocutaneous flaps are particularly useful because their bulk can fill three dimensional defects to achieve adequate form. These flaps have a predictable vascular anatomy that allows for safe elevation. With predictable blood supply, these flaps achieve wound healing and resist infection.

Free flaps are advantageous over local flaps at times because they allow for the selection of specific donor characteristics to fit the requirements of the recipient site. These characteristics include donor tissue size and vascular anatomy, as well as function in some cases. However, local flaps tend to be more robust than free flaps when all factors are equal. Shaw reported on the advantages and disadvantages of free flap surgery (Shaw, 1984). Table 1 summarizes the advantages and disadvantages of free flaps.

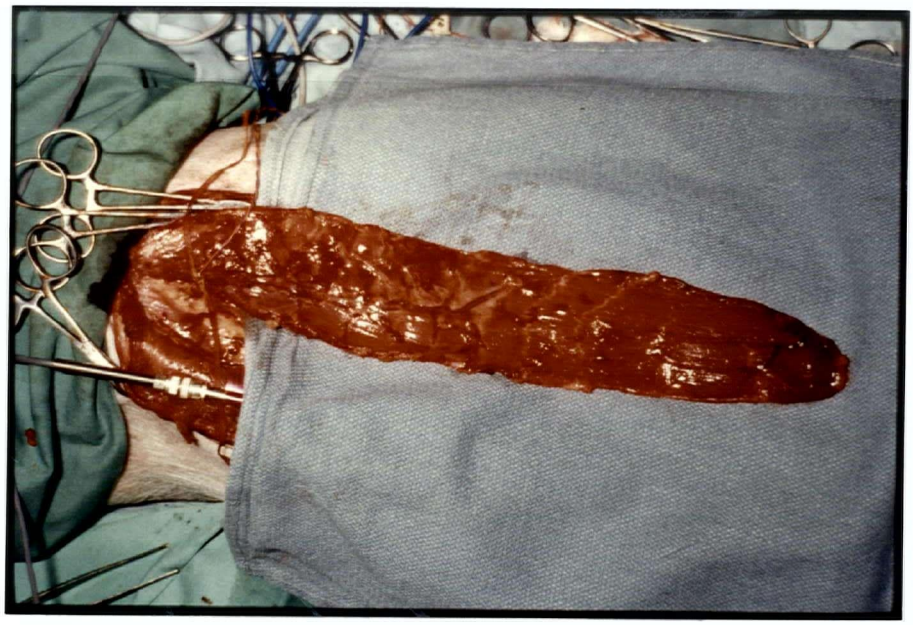


Figure 1. Pig rectus abdominis flap. The blood supply is from the superior epigastric pedicle at the proximal end (left). The inferior epigastric pedicle at the distal end (right) has been ligated.

Table 1. Advantages and disadvantages to free flaps.

Advantages:

i. One-stage surgery. The microvascular anastomosis immediately restores viability to the flap thereby avoiding delays of multistaged procedures.

ii. Distant donor tissue. The use of distant donor tissue spares limited resources nearby the recipient site. Often trauma or radiation makes tissue nearby the recipient site unsuitable for use.

iii. Independent blood supply. Free flaps have a well defined blood supply of their own and are not dependent on the recipient bed for support.

iv. Specialized tissue. Specialized tissue can be used as free flaps to restore sensation, muscle power, bone, nerve, or gastrointestinal tract.

v. Freedom of design. Free flaps gives the reconstructive surgeon the freedom to choose the type and size of tissue from an appropriate donor site, and set it in appropriate orientation at the recipient site to achieve optimal aesthetic and functional result.

Disadvantages:

i. Special equipment and training. The necessary operating equipment is not available at non-specialized centres. Free flap surgery requires considerable specialized training.

ii. Need for recipient artery and vein. Situations do occur in which no suitable recipient artery and vein occur near the recipient site, despite possible use of intervening vein grafts.

iii. Specific donor site problems. Transplant of tissue from donor sites leaves defects which need to be weighed against the benefit to the recipient site.

iv. Long operating time. Free flap surgery adds approximately two hours operating time over simpler surgical procedures.

v. Risk of failure. Success of free flaps is less than that of simpler procedures.

Shaw reported a survey of microsurgery experts in 1984 and reported a free flap success rate of 94% and a thrombosis rate of 10% (Shaw, 1984). In 1992, Khouri reported on a similar survey to that made by Shaw in 1984. He reported a success rate of 98.8% and a thrombosis rate of 3.7% (Khouri, 1992). Despite such a success rate, Khouri states that "It is the spectre of failure and disaster, however, that still stands as a major deterrent to their [free flaps] wider acceptance." The survey by Khouri reports a consensus among experts that include the following:

- Free flaps can fail despite what appeared to be a perfect anastomosis between what were assessed to be healthy vessels.
- Free flaps fail for a variety of reasons. Although a single major factor alone may be sufficient, often a few minor factors may add up to ultimately cause the demise of the flap.
- A single event that may finally seal the fate of every failed flap is an occlusive thrombus at the arterial and/or venous anastomosis. Formation of the thrombus though, may have been a late event in a complex sequence of adverse changes.
- The acquisition of the central skill of microvascular anastomosis is absolutely critical, but it cannot by itself ensure success. Most failures that occur in clinical practice are caused by reasons other than the surgeon's innate inability to perform a good anastomosis.
- Preventing thrombus formation through routine systemic anticoagulation is counterproductive and not desirable.

Free flap series with assessment of complications are shown in Table 2.

Table 2. Free flap series

AUTHOR & YEAR	NUMBER	ASSESSMENT OF COMPLICATIONS	
Irons et.al.	100	18	vascular
1987		7	post-operative bleeding
		4	post-operative infection
		5	recurrent osteomyelitis
Harashina	200	4	failure of anastomosis
1988		4	inappropriate recipient vessels
		2	vessel kinking
		2	spasm
		2	failure of monitoring
		1	rupture of pseudoaneurysm
		1	congestion of skin island
		1	clamp left in place

Tsai et.al.	182	28	vascular
1988		3	vessel kinking
		5	compression from tight closure
		3	haematoma
		1	leaking vein
		1	hypecoagulable
		1	infection
Hidalgo & Jones	150	5	vascular
1990		2	compression from tight closure
		1	obstruction by drain
		1	inadequate recipient vessels
		1	spasm
		1	vessel kinking
Whitney et.al.	100	6	demarcation, skin edge loss
1990		4	partial flap loss
		3	recipient infection
		3	haematoma, bleeding
Urken et.al.	200	18	total flap ischemia
1994		5	infection

The "complex sequence of adverse changes" that leads to an occlusive thrombus is as yet unclear. The clinical experience at this centre, the University of British Columbia, may give direction to the etiology of flap failures.

The clinical experience with macro-micro free tissue transfer at the University of British Columbia was reviewed. Macro-micro free tissue transfer is a procedure in which a tissue's pedicle is microanastomosed to a large calibre vein graft which is then macroanastomosed to high flow vessels remote from the wound/zone of injury. The gracilis macro-micro transfers failed at a higher rate (3 of 4) than the latissimus dorsi macro-micro transfers (4 of 27) (Williamson, 1996). One rectus abdominis and one iliac crest macro-micro free tissue transfer both survived. The latissimus dorsi failures were explainable (blood vessel compression, septic wound, small recipient vessels, long vein graft), but the gracilis failures were not readily explainable. The gracilis failures evaluated in the operating room had low venous outflow. This suggested that possibly, vascular resistance to blood flow may have been a factor. In a further study (Clinical Screening Committee for Research Involving Human Subjects certificate #C95-0319), vascular resistance per unit weight of muscle in a local flap configuration was determined for gracilis flaps, rectus abdominis flaps, and a latissimus dorsi flap. The vascular resistance per unit weight of the five gracilis flaps (2.6 +/- 1.5 (mmHg/cc/min)/100gm) was higher than that for the three rectus abdominis flaps (1.3 +/- 1.2 (mmHg/cc/min)/100gm) and for the latissimus dorsi flap (0.9 (mmHg/cc/min)/100gm). The vascular resistance per unit weight appeared to be inversely proportional to the muscle's original weight prior to surgical resection. This relationship may be due to the presence of choke vessels. These are believed to be high resistance vessels that

interconnect vascular territories (each territory is a zone of tissue supplied by a vascular pedicle)(discussed below). The gracilis, rectus abdominis, and latissimus dorsi muscles have anatomically different vascular arrangement as determined by Mathes and Nahai in 1981 (discussed below). Possibly, this difference in vascular arrangement and difference in the arrangement of choke vessels is related to the predisposition of a muscle flap to fail. Choke vessels with their presumed high vascular resistance may be involved in the sequence of events that lead to occlusive thrombus formation and flap failure. The vascular resistance of choke vessels has not been experimentally evaluated before.

1.3 MUSCLE VASCULAR ANATOMY

Successful muscle flap surgery requires precise knowledge of vascular anatomy. Knowledge of the location of a vascular pedicle is essential, since muscle flaps require a reliable blood supply. A muscle's vascular pedicle plays a role in determining the usefulness of that muscle in flap surgery. The vascular pedicle must be the appropriate length and diameter, and must be free from pathology. Furthermore, the intramuscular vascular pattern determines the extent of muscle that a vascular pedicle can support. Using coloured latex vascular injections, and latex barium mixture vascular injections, Mathes and Nahai have determined and classified muscle and musculocutaneous flaps (Mathes and Nahai, 1981). Their classification describes five patterns of muscle vascular anatomy (See Figure 2). Type I (one vascular pedicle) muscles are defined as those muscles with only one vascular pedicle entering the muscle, e.g. gastrocnemius, rectus femoris and tensor fascia lata. Type II (dominant vascular pedicles plus minor pedicles)

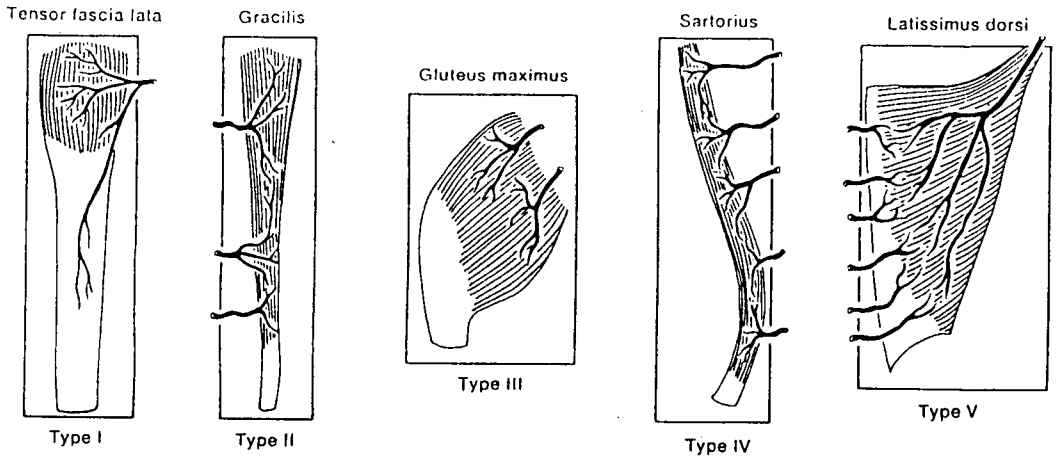


Figure 2. Patterns of vascular anatomy of muscle: Type I, one vascular pedicle; Type II, dominant pedicle pedicle(s) and minor pedicle(s); Type III, two dominant pedicles; Type IV, segmental vascular pedicles; Type V, one dominant pedicle and secondary segmental pedicles. From Mathes SJ and Nahai F. Classification of the vascular anatomy of muscles: experimental and clinical correlation. Plast Reconst Surg 67:177;1981.

muscles are defined as those muscles with one or more large vascular pedicles entering in either close proximity to muscle origin or insertion, and with small vascular pedicles entering the muscle belly, e.g. gracilis, biceps femoris, and semitendinosus. Type III (two dominant pedicles) muscles are defined as muscles with two large vascular pedicles, each arising from a separate regional artery, e.g. rectus abdominis, semimembranosus, gluteus maximus and serratus anterior. Type IV (segmental vascular pedicles) are defined as muscles with multiple pedicles entering the muscle between its origin and insertion, e.g. extensor digitorum longus, sartorius and tibialis anterior. The pedicles are similar in size and have a segmental distribution. Type V (one dominant vascular pedicle and secondary segmental vascular pedicles) are defined as those muscles with a single large vascular pedicle close to the muscle insertion and segmental pedicles entering the muscle close to its origin, e.g. latissimus dorsi and pectoralis major.

Dominant vascular pedicles are those which are required to ensure flap viability following surgical elevation. Sectioning a dominant vascular pedicle usually leads to avascular necrosis. Type I, II, III and V flaps will usually survive when supplied by their dominant vascular pedicle. Type IV flaps require several pedicles for survival. Thus, type IV flaps have a limited "arc of rotation" (the extent that a flap can be moved about its pedicle at the donor site without devascularization). The vascular territory supplied by each pedicle must be known. Vascular territories are interconnected by choke vessels (Taylor and Minabe, 1992).

1.4 CHOKE VESSELS AND ANGIOSOMES

The concept of vascular territories is not new. Salmon described cutaneous territories in *Arteres de la Peau* (1937) and arterial territories of muscles in *Les Arteres des Muscles des Membres et du Tronc* (1933, coauthored by J Dor) and *Arteres des Muscles de la Tete et du Cou* (1936). His radiographic studies demonstrated an artery entering skin or muscle and dividing into branches defining a vascular territory. He recognized the previously described types of arterial anastomoses joining these territories: simple anastomoses (true) and reticular (choke) anastomoses. The simple anastomosis occurs when an artery connects with another without change in calibre. Salmon describes the reticular anastomoses as being "constituted by the union of fine rami that are the terminal branches of the collateral arteries" (Taylor and Razaboni, 1994). He published an in depth anatomic study of anastomoses in the extremities, *Les Voies Anastomotiques Arterielles des Membres* (1939). Taylor and Palmer described the angiosome concept resulting from their study of the human body (Taylor and Palmer, 1987). The angiosome is a three dimensional anatomic territory supplied by a source artery with its accompanying vein(s) that span between skin and bone. Each angiosome consists of a matching arteriosome and venosome. Each angiosome is linked to another by either true (simple) anastomoses or by choke (retiform) anastomoses (See Figure 3). Taylor and Minabe performed an anatomic study on the pig, rat, rabbit, duck, and toad, and compared these to previous human studies (Taylor and Minabe, 1992). Again, true and choke anastomoses were demonstrated; the usual being the choke anastomoses. Their study demonstrated marked dissimilarity of the integument vascularity among the species and marked similarity of the deep-tissue vascularity among the species. They

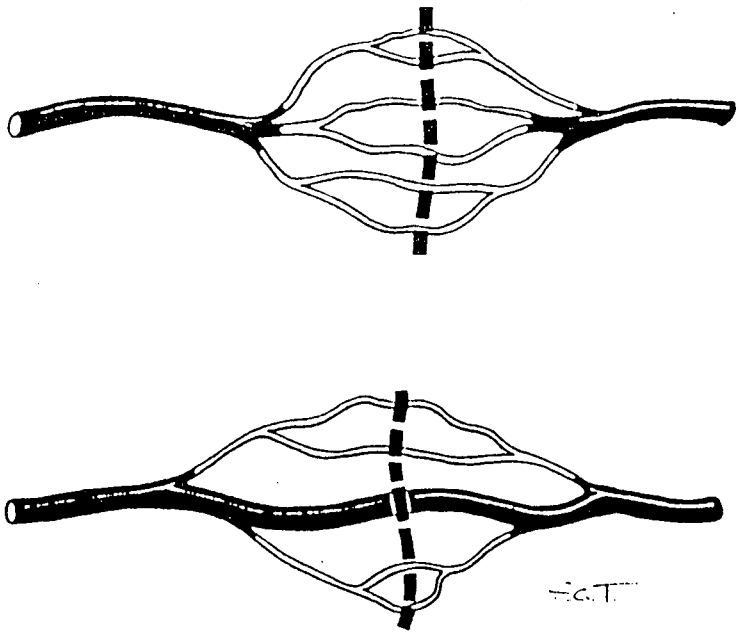


Figure 3. Diagrams of a choke anastomosis (above) and a true anastomosis (below). The broken line defines the anatomic boundary between adjacent vascular territories. From Taylor GI and Minabe T. The angiosomes of the mammals and other vertebrates. Plast Reconst Surg 89:193;1992.

noted that although the morphology of a particular muscle varied between each animal and the human, a distinct resemblance in origin, site, size, orientation, and number of the supplying arterial pedicles existed. Their study provided useful information in the selection of an animal model that would possibly have clinical correlation with humans.

1.5 DELAY PHENOMENON IN SKIN

Understanding of choke vessel physiology has stemmed from delay phenomenon experimentation. This experimentation has in large part been performed on skin tissue. One method of delay is accomplished by surgically incising a flap's margins at a preliminary procedure. The definitive flap procedure is performed at a later time. This preliminary procedure results in changes in the flap that allow the delayed portion to survive at the definitive procedure, where it would not have survived had the preliminary procedure of delay not been performed. In 1933, German et.al. determined that a delay procedure increased the number and size of small arteries in tubed skin flaps (German, 1933). Another method of delay is to ligate a vascular pedicle supplying a flap at a preliminary procedure such that it survives on an alternative pedicle. The definitive flap procedure is performed at a later time. Again, the delayed portion survives at the definitive procedure, where it would not have survived had the preliminary procedure not been performed. In 1992, Callegari et.al. showed that ligation of a dominant vascular pedicle will cause dilation of choke vessels between the "ligated" vascular territory and the adjacent vascular territory. This results in "capture" of the "ligated" vascular territory to the to the blood supply of the adjacent vascular territory and pedicle (Callegari, 1992). They performed a total body

arterial injection with lead oxide mixture in the dog. Radiographs demonstrated that in the skin, an adjacent vascular territory could be captured across choke vessels without delay. However, necrosis occurred, at the zone of choke vessels of further adjoining adjacent vascular territories. They demonstrated that delay results in dilation of existing vessels with maximal effect at the zone of choke vessels. With delay, survival of a third vascular territory adjacent to the second vascular territory was possible.

The time sequence of the delay phenomenon was studied by Morris and Taylor (Morris and Taylor, 1995). Choke vessels dilate rapidly between 48 and 72 hours following flap elevation. Rabbit two-territory osteocutaneous flaps were elevated in delay fashion, with the animals then sacrificed at various post-operative periods (1,2,3,4,6,8,12,24,48 and 72 hours, and 7 days). The vasculature was evaluated by a lead oxide arteriographic technique. Sequential dilatation of vessel diameter (arteries and veins) particularly in the zone of choke vessels was seen with time with rapid increase in dilatation seen between 48 and 72 hours. Control vessels on the contralateral side were studied for comparison. Histologic analysis showed that the choke vessels had enlarged to the order of true anastomotic vessels with associated thinning of the vessels walls. More recently, the delay phenomenon was studied by Cederna et.al. in rabbit abdominal cutaneous island flaps (Cederna a, 1997). The purpose of this study was to quantify the vascular changes occurring with delay. Rabbit abdominal cutaneous flaps were delayed on the right superficial inferior epigastric pedicle. Animals were sacrificed at 0, 5, 10, 15, 21 and 27 days, and the vasculature studied by methylene blue and lead oxide angiography. An increase in the number of vessels was seen from day 0 to day 27 in both delayed side and the contralateral side. A

statistically significant increase was seen at 21 days. These vessels consisted of "choke" vessels (> 5 mm) crossing the midline, total number of vessels (all sizes) crossing the midline, total number of vessels (all sizes) at the medial aspect of the superficial inferior epigastric artery angiosome. The effect of the delay phenomenon was greater on the delayed side compared to the non-delayed side.

1.6 DELAY PHENOMENON IN MUSCLE

Cederna et.al. also performed study on delay of the rabbit rectus abdominis muscle (Cederna b, 1997). Rabbit rectus abdominis muscles were delayed by ligation of the superficial and deep inferior epigastric artery and vein. An increase in the number of vessels was seen from day 0 to day 27 in both delayed side and the contralateral side. A statistically significant increase was seen at 21 days. These vessels consisted of "choke" vessels (> 5 mm) and the total number of vessels (all sizes) crossing the abdominal wall midline, total number of vessels (all sizes) at the medial, superior, and lateral aspects of the deep inferior epigastric artery angiosome. The effect of the delay phenomenon was greater on the delayed side compared to the non-delayed side.

The understanding of delay in muscle flaps was derived from the above findings involving skin. In the non-delay elevation of muscle flaps, common belief holds that choke vessels act as high resistance units which compromise blood flow and thus lead to flap failure. Presumably, the choke vessel dilatation seen with delay phenomenon results in decrease vascular resistance, improved blood flow, and improved flap survival. The vascular resistance across

choke vessels, in muscle and in skin, has not yet formally been determined by experimental methods.

1.7 PIG MODEL

The pig is an ideal animal for muscle flap experimentation. The size of this animal is comparable to that of humans, making them easy to work with. This animal is easy to obtain and to house. This animal is commonly used for flap experimentation and so its anatomy is well known (Guba, 1980)(Heden a, 1989)(Heden b, 1989)(Hjortdal, 1991)(Kerrigan, 1984). Kerrigan et.al. has described a number of skin, myocutaneous, and fasciocutaneous flaps in pigs (Kerrigan, 1996). Millican and Poole described the elevation of the latissimus dorsi flap in pigs (Millican and Poole, 1985). A comparative study of vascular anatomy of mammals was specifically studied in by Taylor and Minabe (Taylor and Minabe, 1992). Angiosomes could be defined in each of the mammals studied with intervening choke vessels. A close resemblance between the pig torso and that of the human was found. In regards to the rectus abdominis vasculature, the deep superior epigastric artery is larger compared to the deep inferior epigastric artery in the pig. The converse is true in the human. The integument is firmly adherent over the entire body of the pig where it is supplied by numerous small perforators. However, in the region of the shoulder, hip, and adjacent to the dorsal and ventral midline, the skin in mobile. The cutaneous vascular anatomy in the pig is dissimilar to that of the human. The delay phenomenon has been demonstrated in the pig (Pang, 1986)(Boyd, 1990). Blood flow studies in pig flaps have been performed (Pang, 1984)(Hjortdal, 1992). Pharmacologic studies have been performed on pig

flaps (Pang, 1985). In addition, the effect of anaesthetics on pig muscle is known (Lundeen, 1983)(Sigurdsson, 1994).

CHAPTER 2: EXPERIMENTAL

2.1 OBJECTIVE

The objective of this thesis project was to determine the relationship between muscle flap vascular resistance and the presence or absence of choke vessels in the intraoperative period following elevation of muscle flaps in quantifiable terms.

2.2 HYPOTHESIS

The null hypothesis was that the surgical resection of choke vessels does not result in a statistically significant decrease in muscle flap vascular resistance per unit weight. According to the null hypothesis, the slope of vascular resistance per unit weight plotted against muscle flap weight in the region of the choke vessels should be less than or equal to zero (See Figure 4a). Thus, according to the alternative hypothesis, the slope of vascular resistance per unit weight plotted against muscle flap weight in the region of the choke vessels is statistically different from a slope less than or equal to zero (See Figure 4b).

Figure 4a.

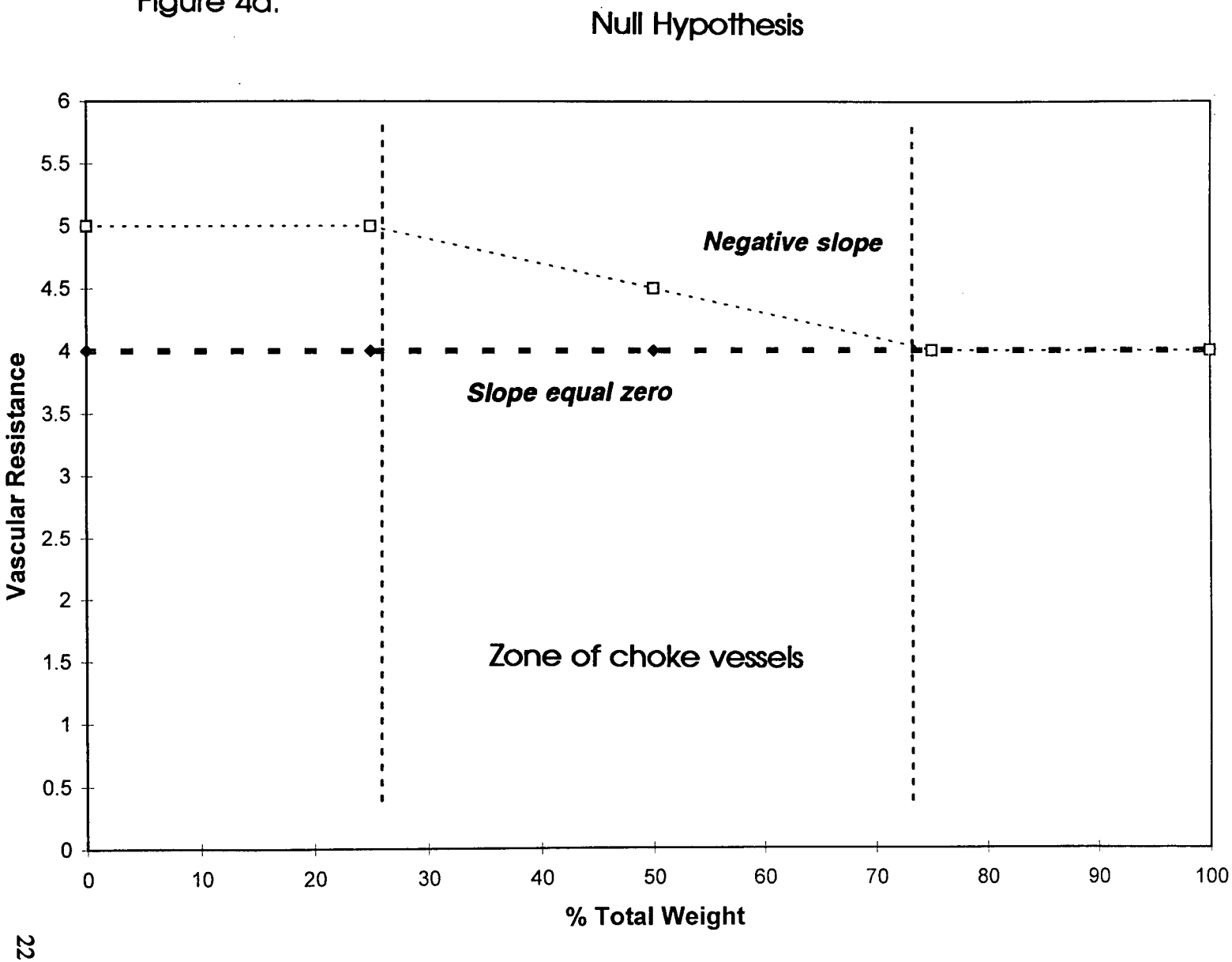
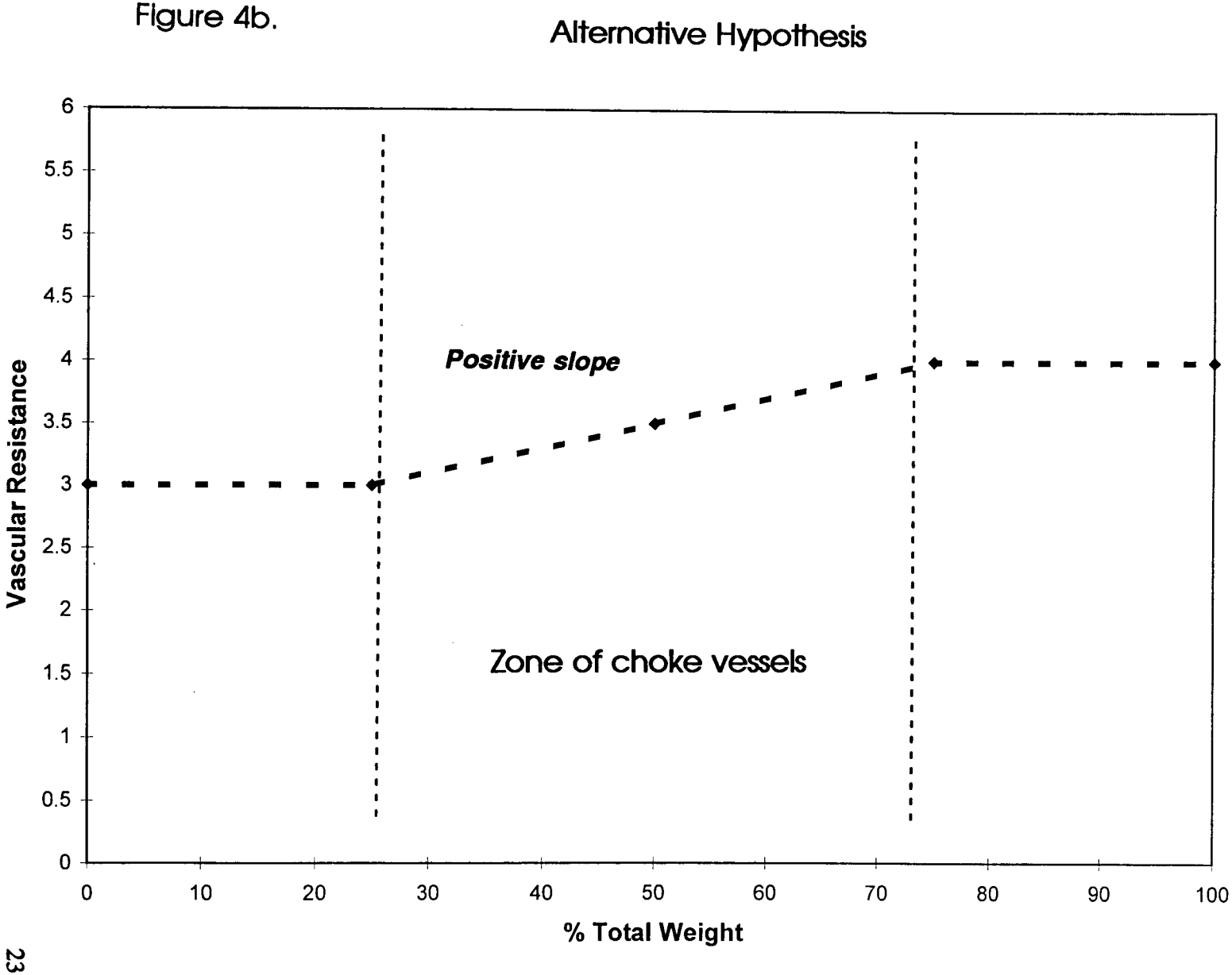

Null Hypothesis
Negative slope
Slope equal zero
Zone of choke vessels
Vascular Resistance
% Total Weight
0
0.5
1
1.5
2
2.5
3
3.5
4
4.5
5
5.5
6
0
10
20
30
40
50
60
70
80
90
100

Figure 4b.


Alternative Hypothesis
Vascular Resistance
% Total Weight
Positive slope
Zone of choke vessels
0
0.5
1
1.5
2
2.5
3
3.5
4
4.5
5
5.5
6
0
10
20
30
40
50
60
70
80
90
100

2.3 MATERIALS AND METHODS

2.3.1 PART I - ASCERTAINMENT OF INTRAMUSCULAR VASCULAR ANATOMY

The location of choke vessels within the rectus abdominis muscle and the gracilis were determined radiographically. A barium sulfate injection into pigs previously euthanased for other approved experimentation were used. The technique used was modified from a previously published technique (Pounder, 1982).

2.3.1.1 Barium sulfate preparation - 240 mg of barium sulfate (E-Z-HD Inc, Montreal, Canada) was mixed with 14 grams of Knox unflavoured gelatine (Thomas J. Lipton Inc, Toronto, Canada) in 2 litres D5W/0.45 NS at 55 degree Celsius.

2.3.1.2 Animal preparation and injection - Cadaveric pigs heparinized prior to euthanasia were used. Two litres of normal saline was heated to 50 degree Celsius and infused through the common iliac artery to displace clots and warm the muscles. The muscles were subsequently massaged to discharge blood and saline. The barium sulfate-gelatine mixture was then injected through the common iliac artery. Distal arteries in the extremities were nicked in order to observe extravasation of mixture. The mixture was allowed to gel for over 1 hour. The rectus abdominis and gracilis muscles were then dissected carefully identifying the vascular pedicles.

2.3.1.3 Radiography - The dissected muscles were rinsed superficially and radiographed (50 mA, 64 kV, 0.3 sec at 140 cm).

2.3.2 PART II - VASCULAR RESISTANCE IN PIG MUSCLE FLAPS

Approval for animal experimentation was obtained from the University of British Columbia Committee for Animal Care Committee. Eight Yorkshire-white cross pigs (22.6 - 33.8 kg) were used.

2.3.2.1 Anaesthesia and monitoring - Each animal was induced with ketamine 20 mg/kg IM and placed supine on the operating table covered with a heating pad adjusted to maintain body temperature at 37 degrees celsius. A well, the animal was covered with a towel on its ventral surface whenever possible to maintain body temperature. Anaesthesia was maintained with 1.5 - 2.0 % isoflurane, and 50 % air/50 % oxygen by endotracheal intubation in circuit with a volume ventilator. The ventilator was adjusted to maintain arterial pH normal (7.35 - 7.45) and arterial oxygen saturation $> 92\%$. Neck dissection was performed to place a line in the left common carotid, and a line in the left internal jugular. These lines were in continuity with standard pressure transducers for monitoring mean arterial pressure and central venous pressure, respectively. Peripheral intravenous access was achieved through an ear vein with 0.9 % normal saline at 1 cc/kg/hr.

2.3.2.2 Experimental - Eight experimental rectus abdominis flaps - Type III (pigs # 1,2,3,4,5 (2),7,8) and two control rectus abdominis flaps (pigs # 1,2) were raised on the superior epigastric pedicle. Also, four experimental gracilis flaps - Type II (pigs # 2,6,7,8) and two control gracilis flaps (pigs # 2 & 6) were raised on the major branch from the deep femoral artery. The gracilis muscles were studied for comparative purposes.

Rectus abdominis elevation - A long midline skin incision was made from the xiphoid to the pubis. The skin was then elevated from the rectus sheath. The rectus sheath was then dissected away from the rectus abdominis anteriorly and posteriorly. The intercostal and segmental vessels and nerves were divided in the process. The deep superior epigastric vessels and deep inferior epigastric vessels were identified on the posterior aspect of the rectus abdominis. The inferior vessels were ligated thereby isolating the flap on the deep superior epigastric vessels. The insertion of the rectus abdominis on the pubis was then detached. The rectus abdominis origin was then detached from the sternum and lowest rib with care to preserve the deep superior epigastric vessels in the sternocostal triangle.

Gracilis elevation - The gracilis was identified as a muscle bulge at the medial aspect of the thigh. A U-shaped skin incision is made from the medial cephalic aspect of the thigh to the cephalic region of the tibia, caudally along the line of the tibia, and then medially to the medial caudal aspect of the thigh. This developed a skin flap which was elevated with care directed towards ligating perforating arteries. The gracilis muscle arises from the pubic arch and inserts via broad aponeurosis into the upper tibia. Its blood supply is from one dominant pedicle and one

minor pedicle. The dominant arterial pedicle arises from the femoral artery and is located at the cephalic border of the muscle origin. The minor pedicle may be located following the cephalic border of the muscle towards the tibia. The minor pedicle is close to the aponeurosis. The muscle is elevated by incising the aponeurosis and bluntly dissecting the muscle from the underlying adductor and semimembranosus muscle towards the origin. The minor pedicle was ligated. The gracilis was detached from its pubic origin with care to preserve the major pedicle.

Sympathectomy - Vascular pedicles were sympathectomized by dividing the nerves accompanying the vascular pedicle as well as resecting the adventitia surrounding the vascular structures.

Flow measurement - Doppler flow probes (Crystal Biotech, Mass, U.S.A.) were placed around each arterial pedicle with conducting gel. 2.0 mm probes placed around the deep superior epigastric artery for the rectus abdominis studies. 1.0 or 1.5 mm probes were placed around the femoral artery branch supplying the gracilis muscles. A twenty MHz doppler flow signal transducer with a signal output via Dataflow software (Crystal Biotech, Mass, U.S.A.) was used.

Other variables - Muscle flap temperature was monitored using a needle probe inserted into the muscle substance. Hematocrit and arterial pH were determined intraoperatively. Blood flow, mean arterial pressure, central venous pressure, and temperature were recorded. See Table 3.

Table 3. Experimental Variables

Dependent Variable:	
	Pedicle blood flow via non-invasive doppler flow probe
Independent Variables:	
	Muscle flap weight
	Mean arterial pressure
	Central venous pressure
	Hematocrit
	Muscle flap temperature
	Arterial pH

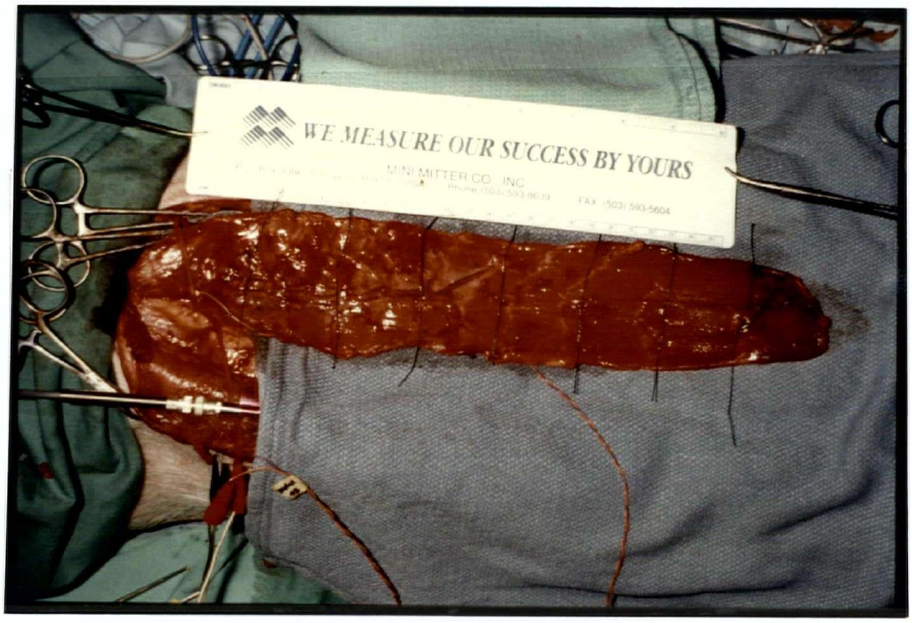
Experimental procedure - Large vascular clamps were placed across each experimental rectus abdominis flap perpendicular to the vascular pedicle, initially distally, and the muscle resected distal to the clamp. The flap was allowed to equilibrate for 10 minutes and then the above variables were recorded along with the weight of the excised muscle. This was done sequentially, from distal to proximal. Serial muscle flap weight was determined by subtracting each excised muscle flap weight from the calculated total muscle flap weight. See Figure 5.

Large vascular clamps were placed across each experimental gracilis flap, initially distally, and then sequentially proximally as for the rectus abdominis flap. See Figure 6. Control gracilis flaps followed the above procedure, however, were not serially resected.

Control procedure - Control rectus abdominis and gracilis flaps were raised as for the experimental flaps. Doppler flow probes were placed around the arterial pedicle. Control blood flow was measured at the same time that experimental blood flow was measured. No clamping or resection of the control flaps was done. The weight of the control flaps was measured when the experimental procedure was completed.

Euthanasia - Following the experimental procedure, the animals were euthanazed with an overdose of pentobarbital.

a.



b.

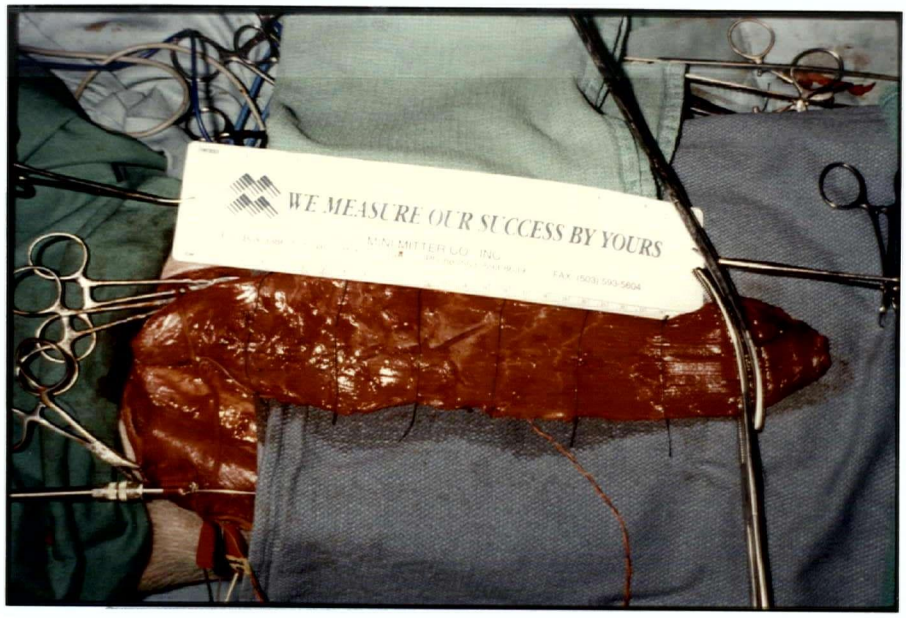
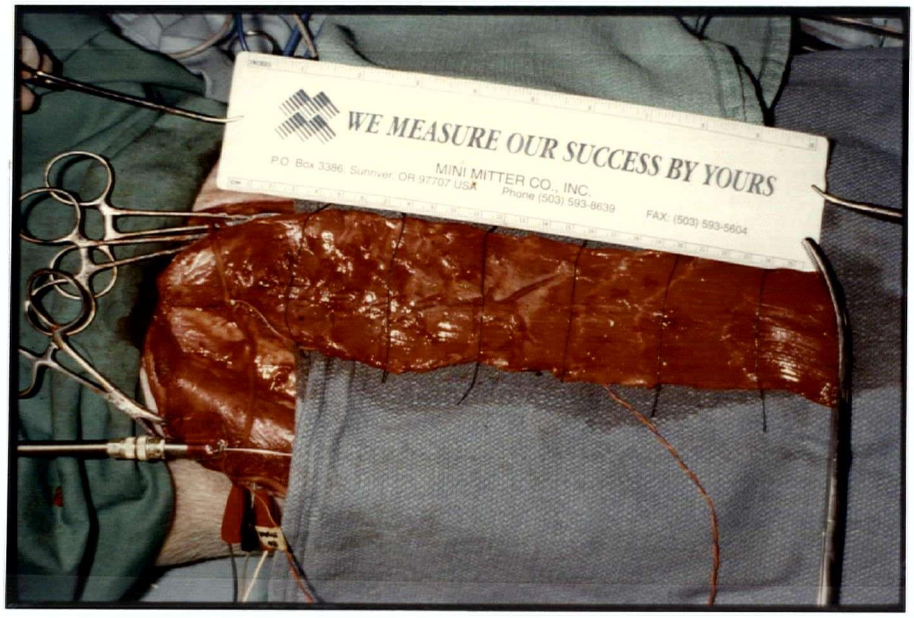
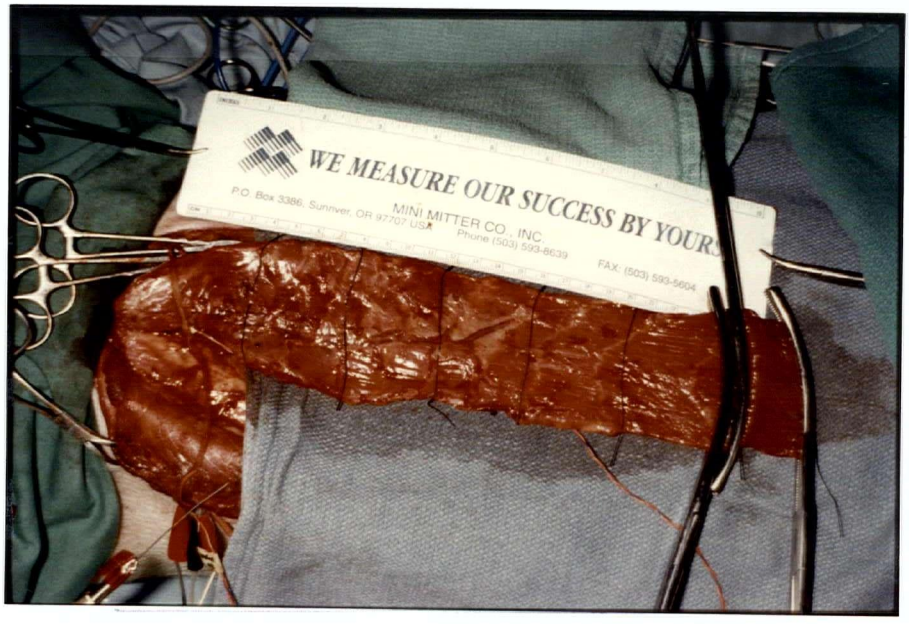


Figure 5. Serial resection of rectus abdominis flap. a. Flap at 100 % size raised on the superior epigastric pedicle proximally (left), blood flow probe place around the superior epigastric artery, variables are measured. b. Vascular clamps placed at the distal end of flap. c. Distal portion of flap is resected, variables measured 10 minutes later. d. Vascular clamps placed more proximally. e. Distal portion of flap resected further, variable measured 10 minutes later. Process is repeated serially towards the superior epigastric pedicle proximally.

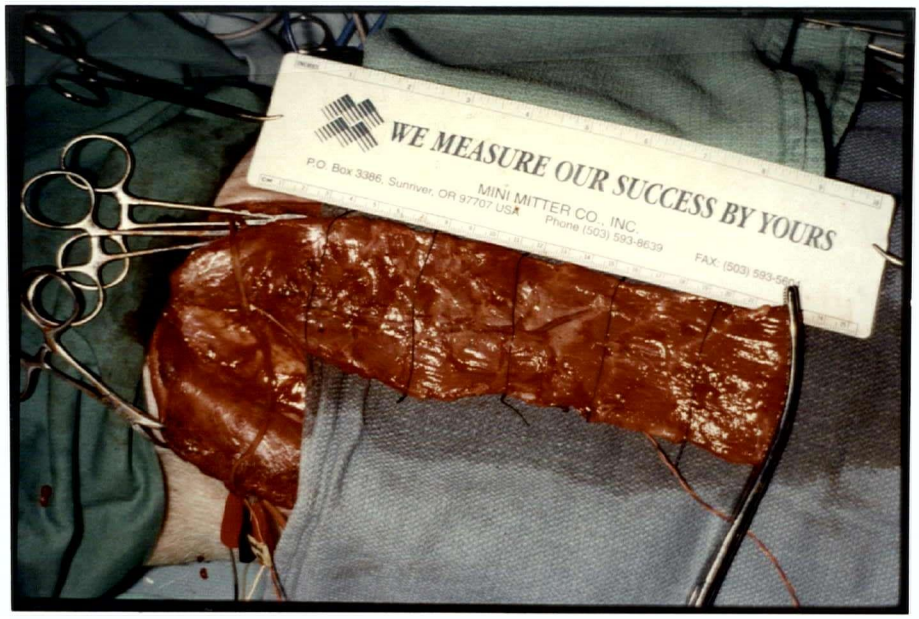
c.


WE MEASURE OUR SUCCESS BY YOURS
MINI MITTER CO., INC.
P.O. Box 3386, Sunriver, OR 97707 USA
Phone (503) 593-8639
FAX: (503) 593-5604

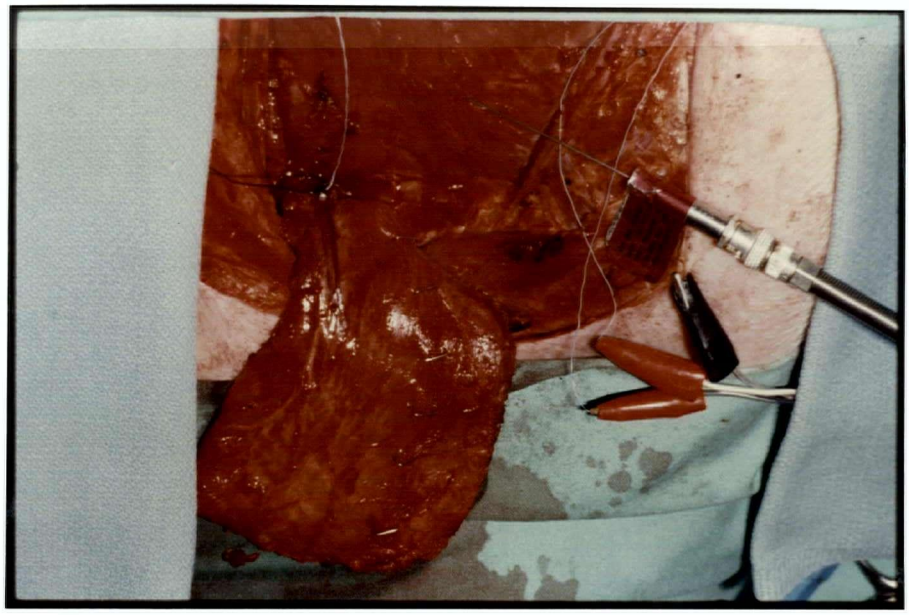
d.


WE MEASURE OUR SUCCESS BY YOURS
MINI MITTER CO., INC.
P.O. Box 3386, Sunriver, OR 97707 USA
Phone (503) 593-8639
FAX: (503) 593-5604

e.


WE MEASURE OUR SUCCESS BY YOURS
MINI MITTER CO., INC.
P.O. Box 3386, Sunriver, OR 97707 USA
Phone (503) 593-8639

a.



b.

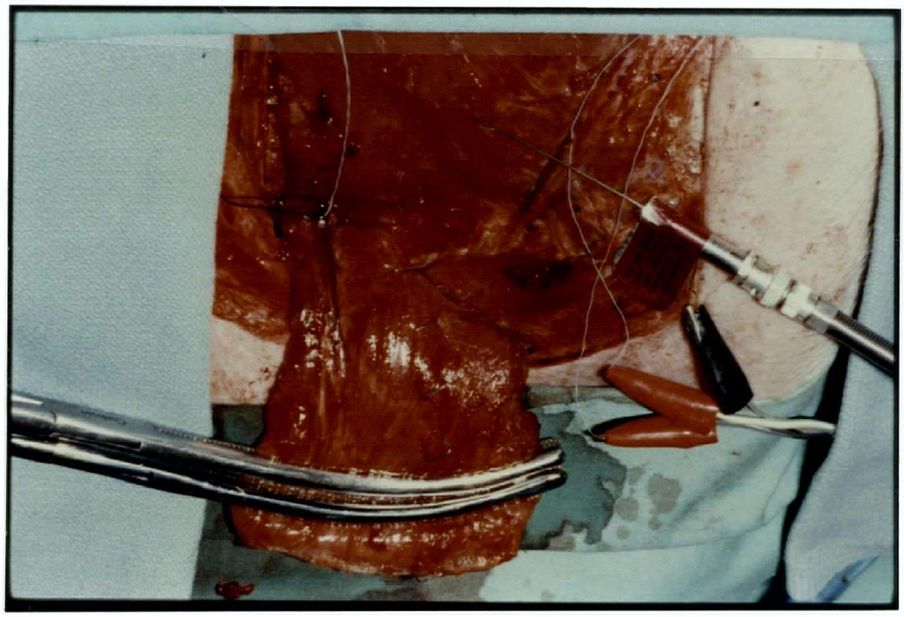
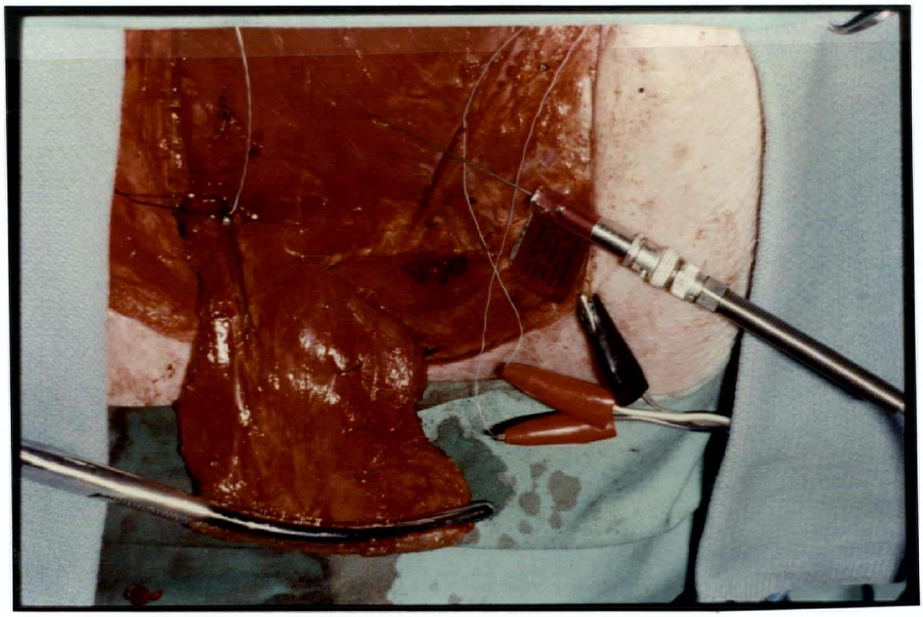


Figure 6. Serial resection of gracilis flap. a. Flap at 100 % size raised on branch of femoral proximally (top and left), blood flow probe place around the major pedicle artery, variables are measured. b. Vascular clamps placed at the distal (bottom) end of flap. c. Distal portion of flap is resected, variables measured 10 minutes later. Process is repeated serially towards the major pedicle proximally.

c.



2.3.2.3 Calculations - Equations used to determine vascular resistance are shown in Table 4. All measurements are corrected for change in temperature and hematocrit over the course of the experiment. Vascular resistance was calculated for each muscle flap as it was diminished by serial resection.

2.2.2.4 Data analysis - The power of this study was determined by calculating the beta value using the central limit theorem comparing the slope of resistance per unit weight vs percent total flap weight with a slope equal to zero. Minitab software was used for linear regression analysis of resistance per unit weight vs percent total flap weight and for linear regression analysis of blood flow per unit weight vs percent total flap weight.

Table 4. Equations for calculating resistance

$$R_{uncorrected} = (MAP - CVP) / Q$$

$$R_{corrected} = R_{uncorrected} \times (1.025)\exp(T-37) \times (1 + 0.025xHct1 + 0.000735x(Hct1\ exp2))/(1 + 0.025xHct2 + 0.000735x(Hct2\ exp2))$$

R = resistance (mmHg/cc/min)
MAP = mean arterial pressure (mmHg)
CVP = central venous pressure (mmHg)
Q = blood flow (cc/min)
T = temperature (degrees Celsius)
Hct1 = initial hematocrit (%)
Hct2 = experimental hematocrit (%)

CHAPTER 3: RESULTS AND DATA ANALYSIS

3.1 PART I - ASCERTAINMENT OF INTRAMUSCULAR VASCULAR ANATOMY

The intramuscular vascular pattern of the rectus abdominis and the gracilis is shown radiographically in Figure 7. The choke vessel region in the rectus abdominis radiographically correlate with that region seen visually between the two vascular territories supplied by the deep superior epigastric artery and the deep inferior epigastric artery (Compare with Figure 5). This correlation indicates that the region seen between the course of the two arteries when intramuscular is the zone of choke vessels. The choke vessels occur at approximately 60% to 90% of the linear flap length with 0% being at the proximal end and 100% at the distal end. These vessels run longitudinally along the length of the rectus abdominis.

The choke vessel region in the gracilis radiographically correlate with that region seen visually between the two vascular between the major and minor vascular pedicles. (Compare with Figure 6). The choke vessels run in the medial-lateral direction perpendicular to the length of the gracilis.

The radiographic studies of the rectus abdominis and gracilis were merely preliminary studies. These studies were intended to ascertain the location and orientation of choke vessel region such that the application of the vascular clamps could be correlated with the underlying vascular anatomy. Statistical analysis was not intended.

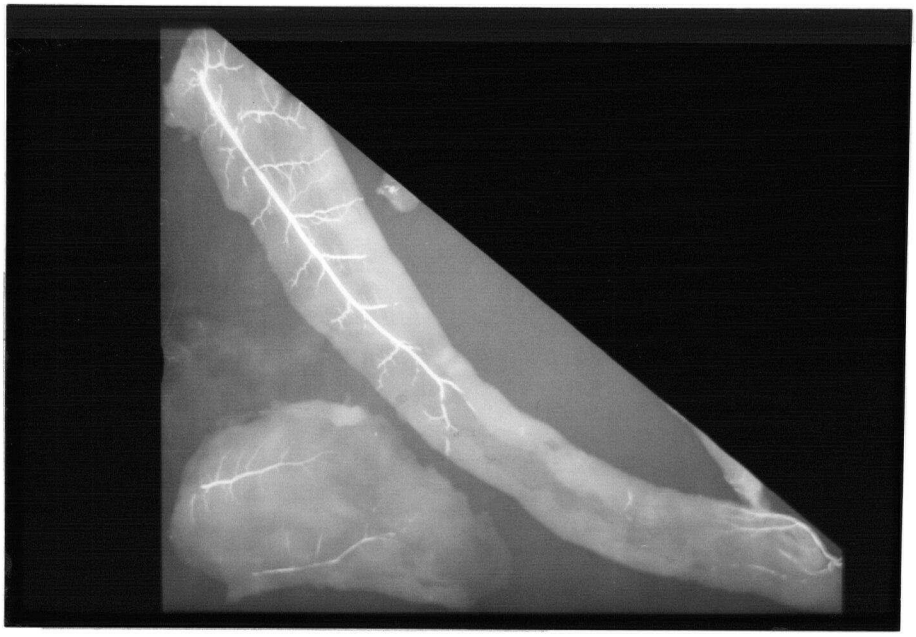


Figure 7. Rectus abdominis and gracilis angiography. The rectus abdominis is oriented diagonally. The superior epigastric pedicle is at the top and the inferior epigastric pedicle is at the bottom. The zone of choke vessels is between the two pedicles. The gracilis is at the bottom left. It is oriented with proximal being left and medial being at the bottom. The major vascular pedicle is at the bottom and the minor pedicle is at the top. The zone of choke vessels is between the two pedicles.

3.2 PART II - VASCULAR RESISTANCE IN PIG MUSCLE FLAPS

3.2.1 Experimental - Rectus abdominis - The total vascular resistance and total blood flow is shown in Figure 8. This shows the total vascular resistance increases for each of the flaps as the flaps are resected from distal (100%) towards proximal (0%). Notably, there is no decrease in vascular resistance as the zone of choke vessels is resected for any of the flaps. The total blood flow tends to decrease as the flaps are resected from distal (100%) towards proximal (0%).

The vascular resistance per unit weight and blood flow per unit weight is shown in Figure 9. Vascular resistance and blood flow are calculated on a unit weight basis for comparative purposes. For example, the vascular resistance of a 50% total weight rectus abdominis flap can then be compared to a 50% total weight gracilis flap even though the absolute weight of these 50% flaps could be different. Additionally, the vascular resistance of a 25% rectus abdominis flap could be compared to a 75% rectus abdominis flap. Differences in vascular resistance would then be due to differences within each unit of tissue rather than merely differences in total amount of tissue (with equal resistance for each unit of tissue). The vascular resistance per unit weight is seen to increase as the flap is resected from distal (100%) towards proximal (0%). The vascular resistance per unit weight does not decrease for any of the flaps as the choke vessels are resected. For each flap, blood flow per unit weight is seen to increase as the flap is resected from distal (100%) towards proximal (0%).

Figure 8a.

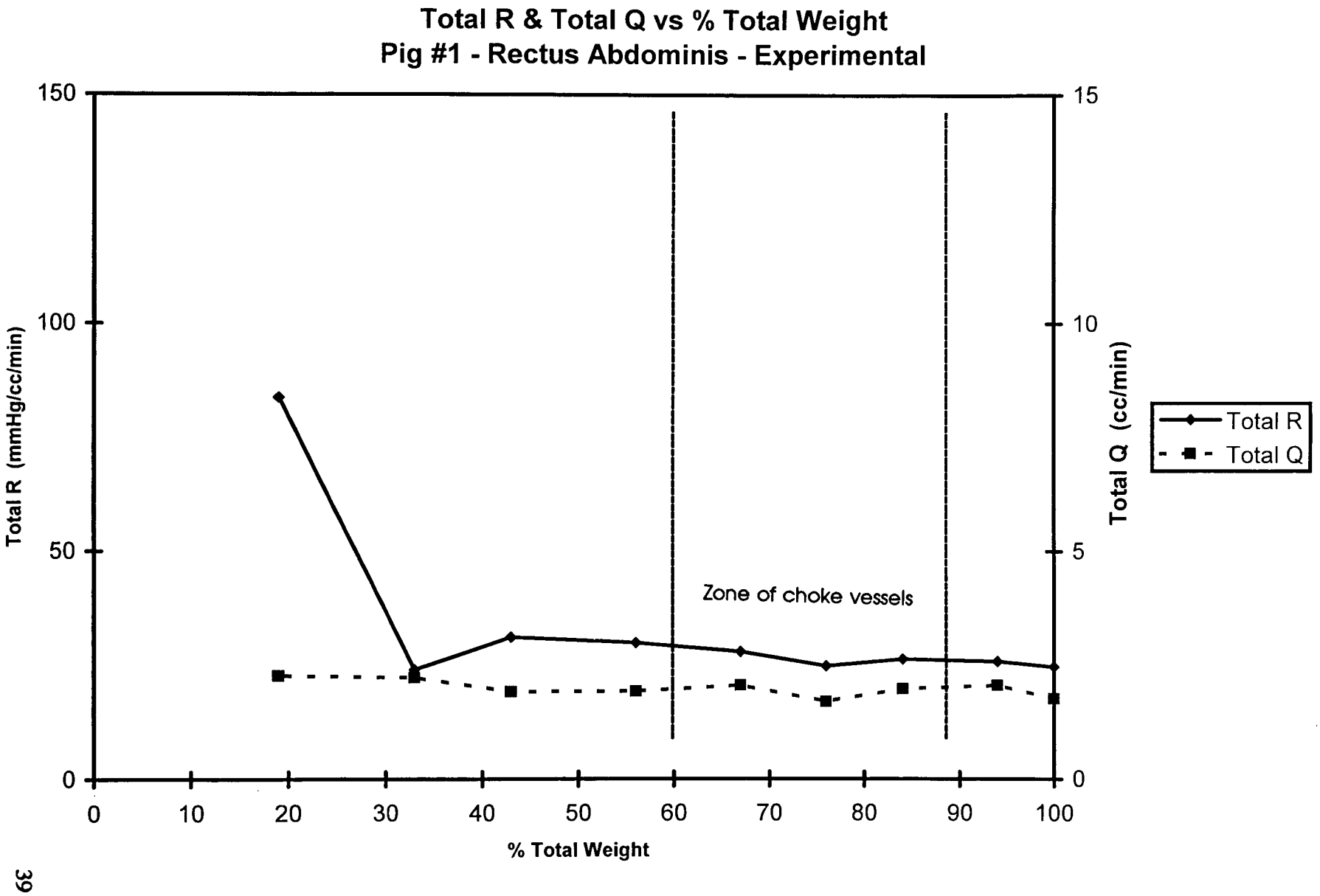

Total R & Total Q vs % Total Weight
Pig #1 - Rectus Abdominis - Experimental
150
100
50
0
15
10
5
0
Total R (mmHg/cc/min)
Total Q (cc/min)
Zone of choke vessels
Total R
Total Q
0
10
20
30
40
50
60
70
80
90
100
% Total Weight

Figure 8b.

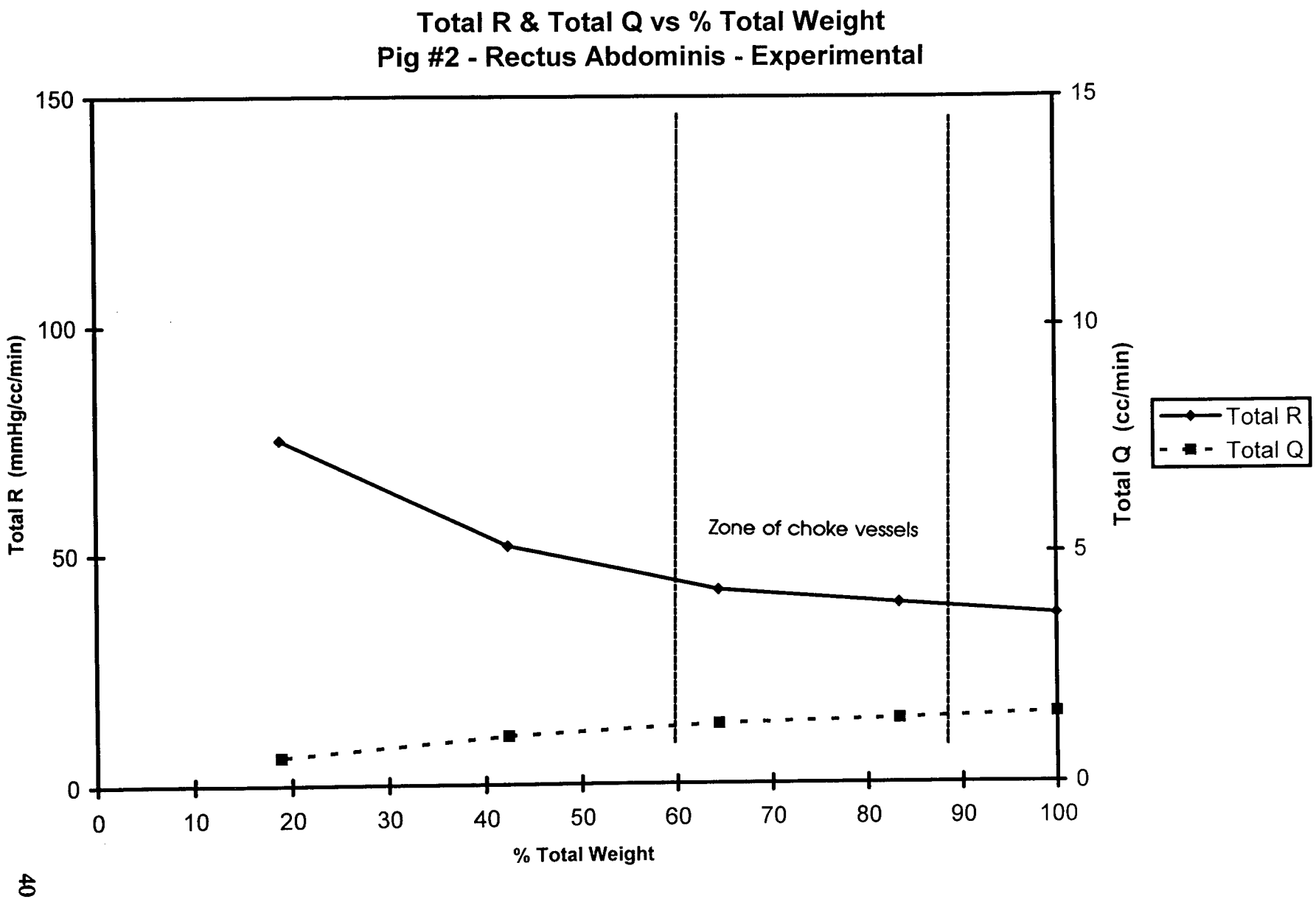

Total R & Total Q vs % Total Weight
Pig #2 - Rectus Abdominis - Experimental
150
100
50
0
15
10
5
0
Total R (mmHg/cc/min)
Total Q (cc/min)
Zone of choke vessels
Total R
Total Q
0
10
20
30
40
50
60
70
80
90
100
% Total Weight

Figure 8c.

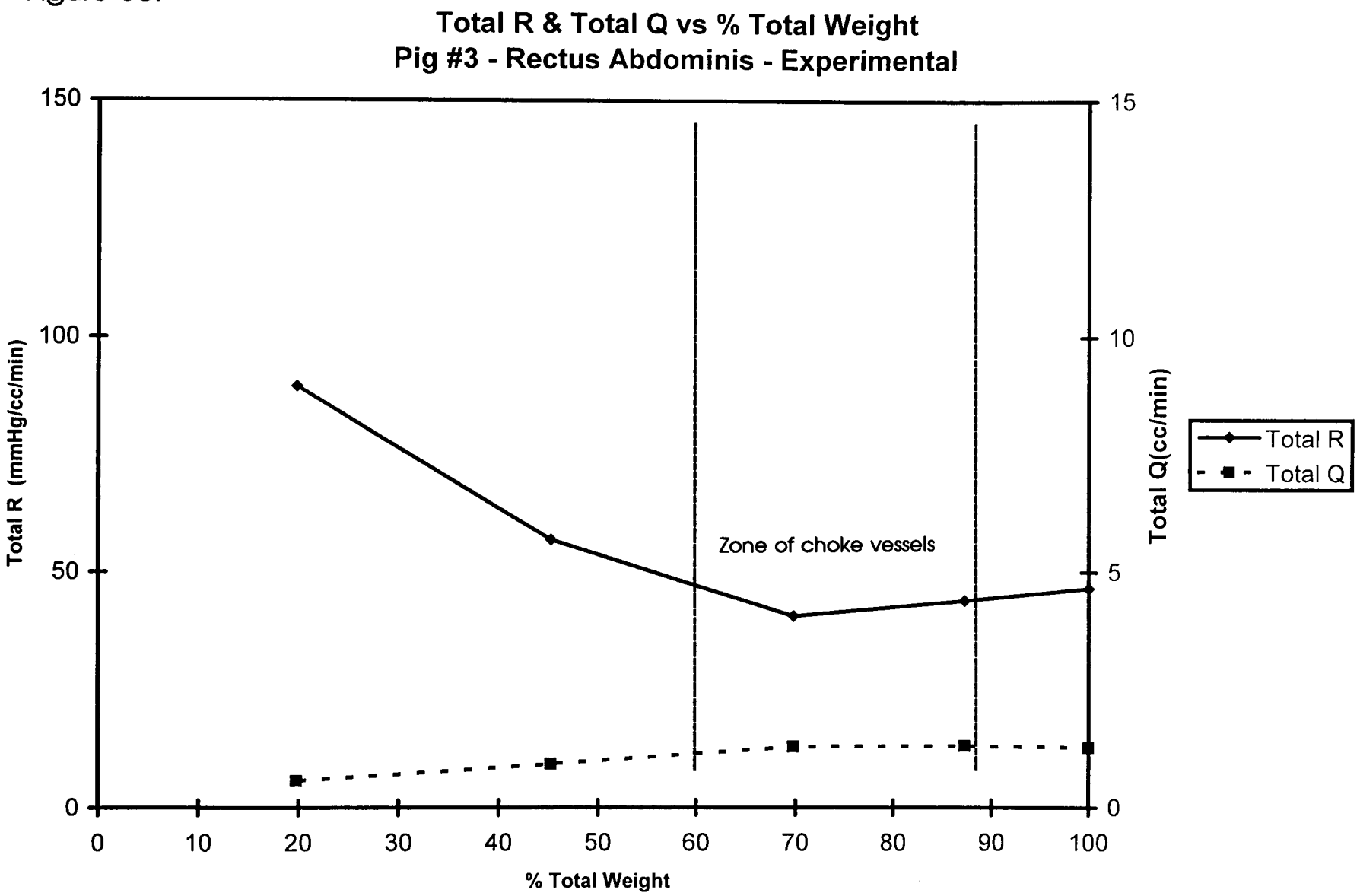

Total R & Total Q vs % Total Weight
Pig #3 - Rectus Abdominis - Experimental
150
100
50
0
15
10
5
0
Total R (mmHg/cc/min)
Total Q(cc/min)
Zone of choke vessels
Total R
Total Q
0
10
20
30
40
50
60
70
80
90
100
% Total Weight

Figure 8d.

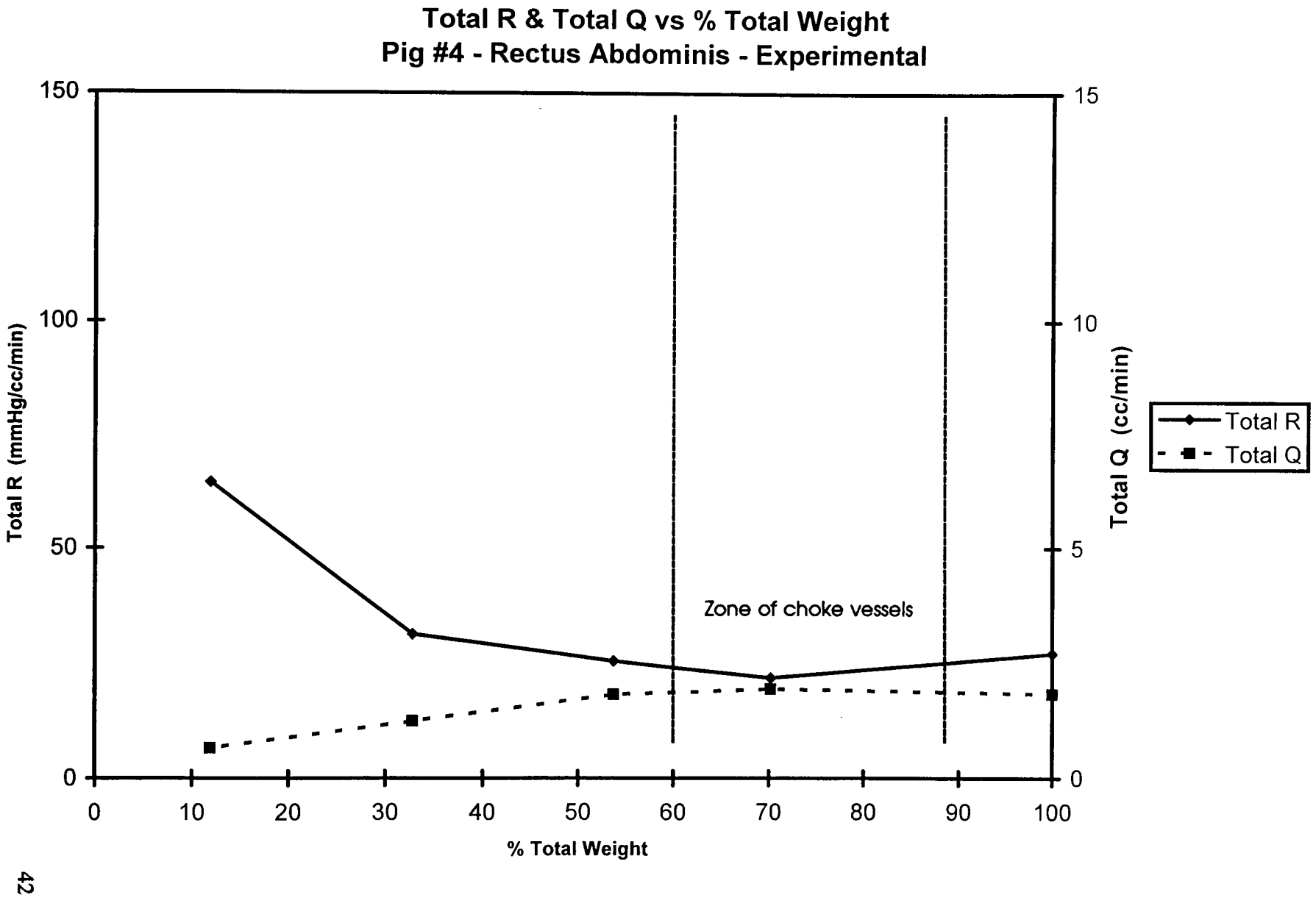

Total R & Total Q vs % Total Weight
Pig #4 - Rectus Abdominis - Experimental
150
100
50
0
15
10
5
0
Total R (mmHg/cc/min)
Total Q (cc/min)
Zone of choke vessels
Total R
Total Q
0
10
20
30
40
50
60
70
80
90
100
% Total Weight

Figure 8e.

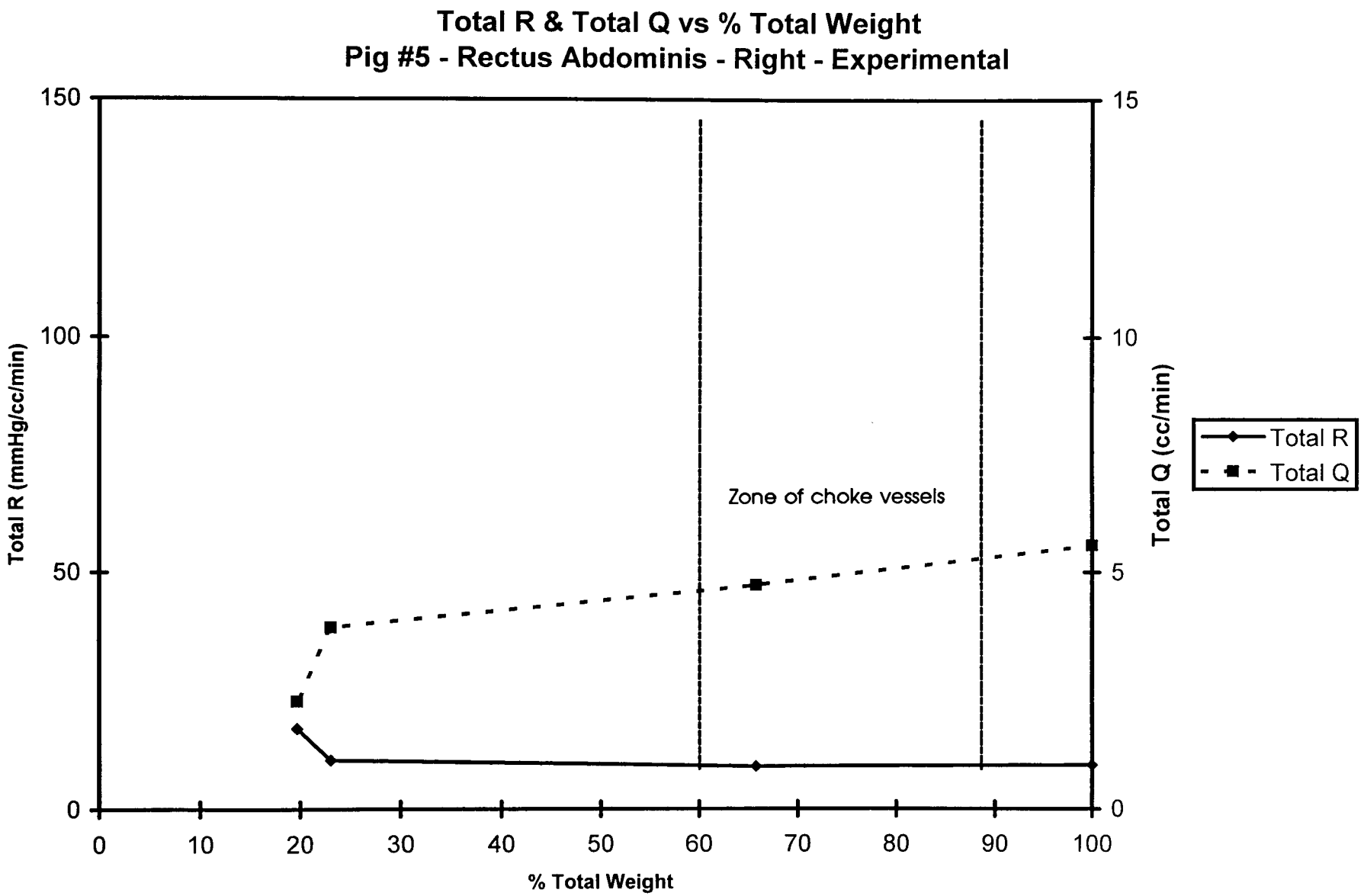

Total R & Total Q vs % Total Weight
Pig #5 - Rectus Abdominis - Right - Experimental
150
100
50
0
15
10
5
0
Total R (mmHg/cc/min)
Total Q (cc/min)
Zone of choke vessels
Total R
Total Q
0
10
20
30
40
50
60
70
80
90
100
% Total Weight

Figure 8f.

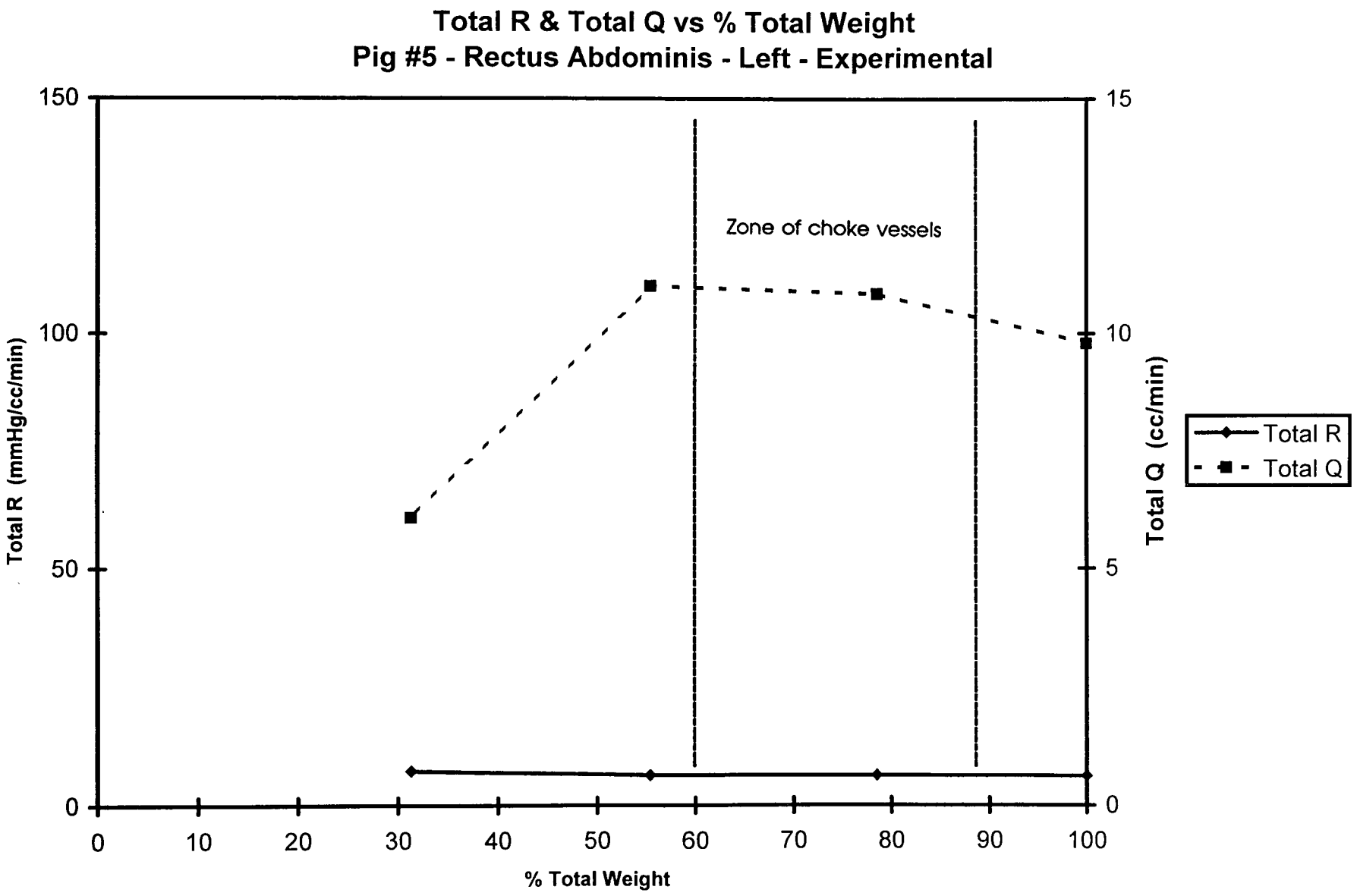

Total R & Total Q vs % Total Weight
Pig #5 - Rectus Abdominis - Left - Experimental
Zone of choke vessels
Total R (mmHg/cc/min)
Total Q (cc/min)
% Total Weight
Total R
Total Q
150
100
50
0
15
10
5
0
0
10
20
30
40
50
60
70
80
90
100

Figure 8g.

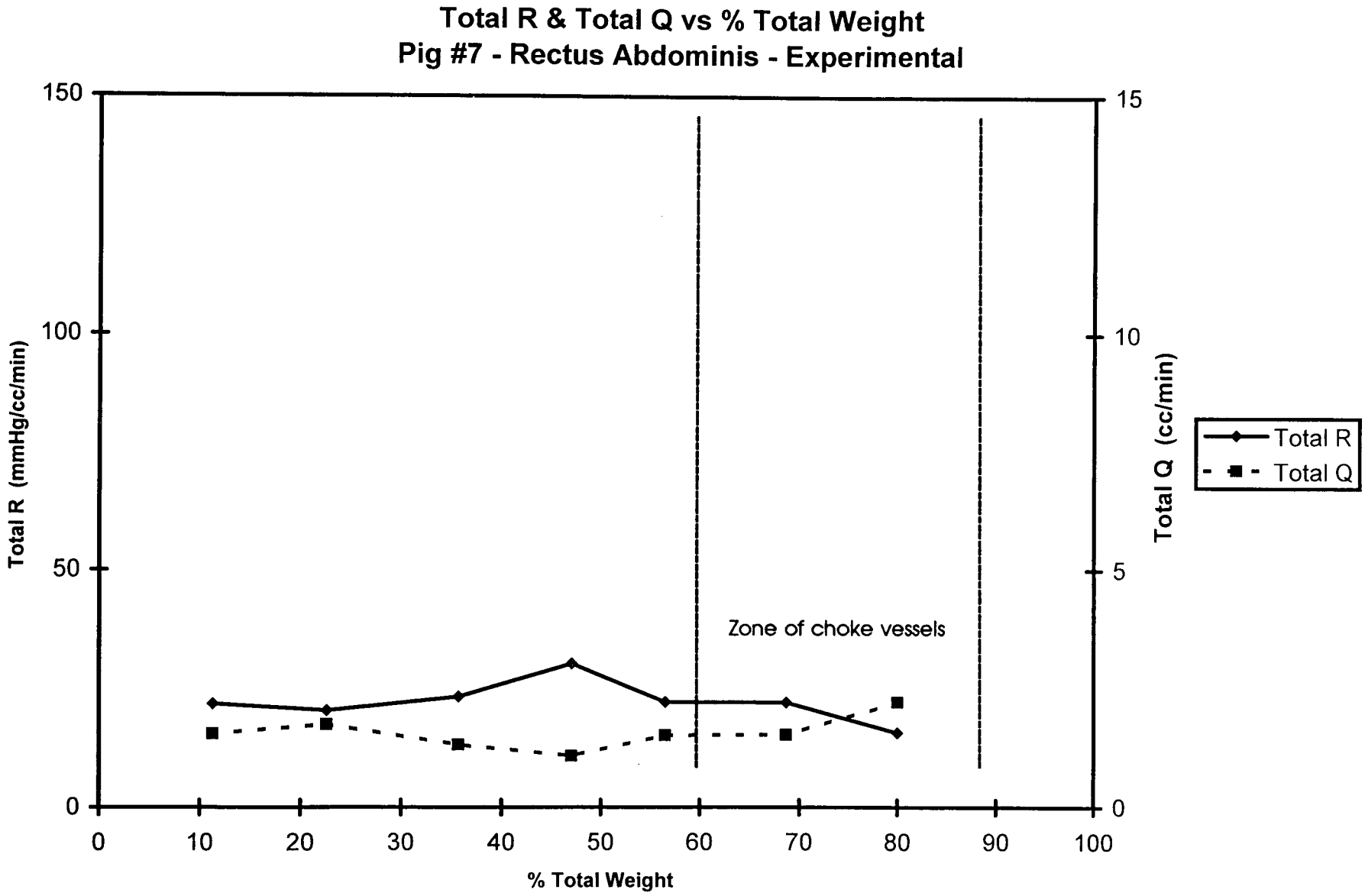

Total R & Total Q vs % Total Weight
Pig #7 - Rectus Abdominis - Experimental
150
100
50
0
15
10
5
0
Total R (mmHg/cc/min)
Total Q (cc/min)
Zone of choke vessels
Total R
Total Q
0
10
20
30
40
50
60
70
80
90
100
% Total Weight

Figure 8h.

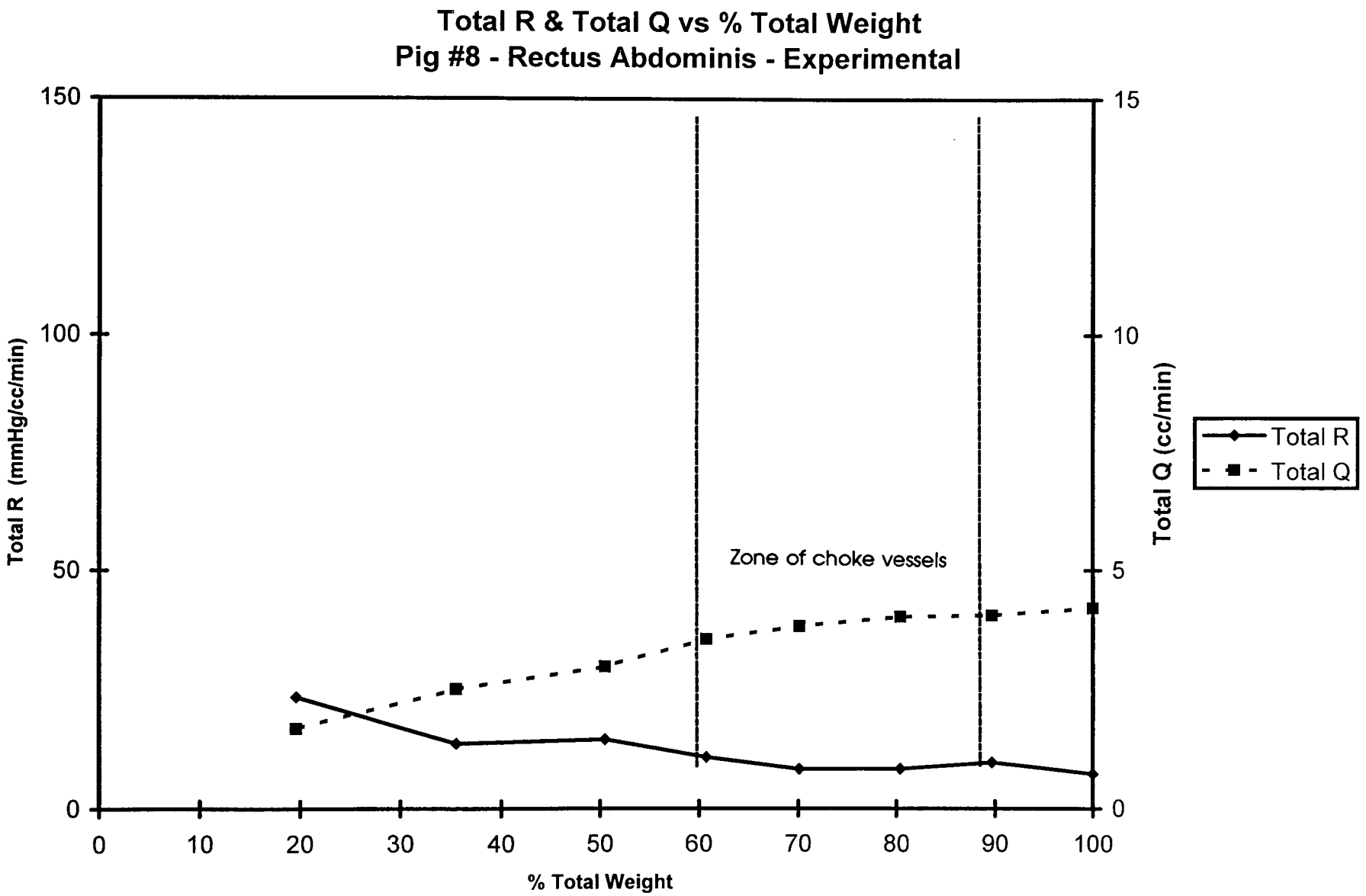

Total R & Total Q vs % Total Weight
Pig #8 - Rectus Abdominis - Experimental
150
100
50
0
15
10
5
0
Total R (mmHg/cc/min)
Total Q (cc/min)
Zone of choke vessels
Total R
Total Q
0
10
20
30
40
50
60
70
80
90
100
% Total Weight

Figure 9a.

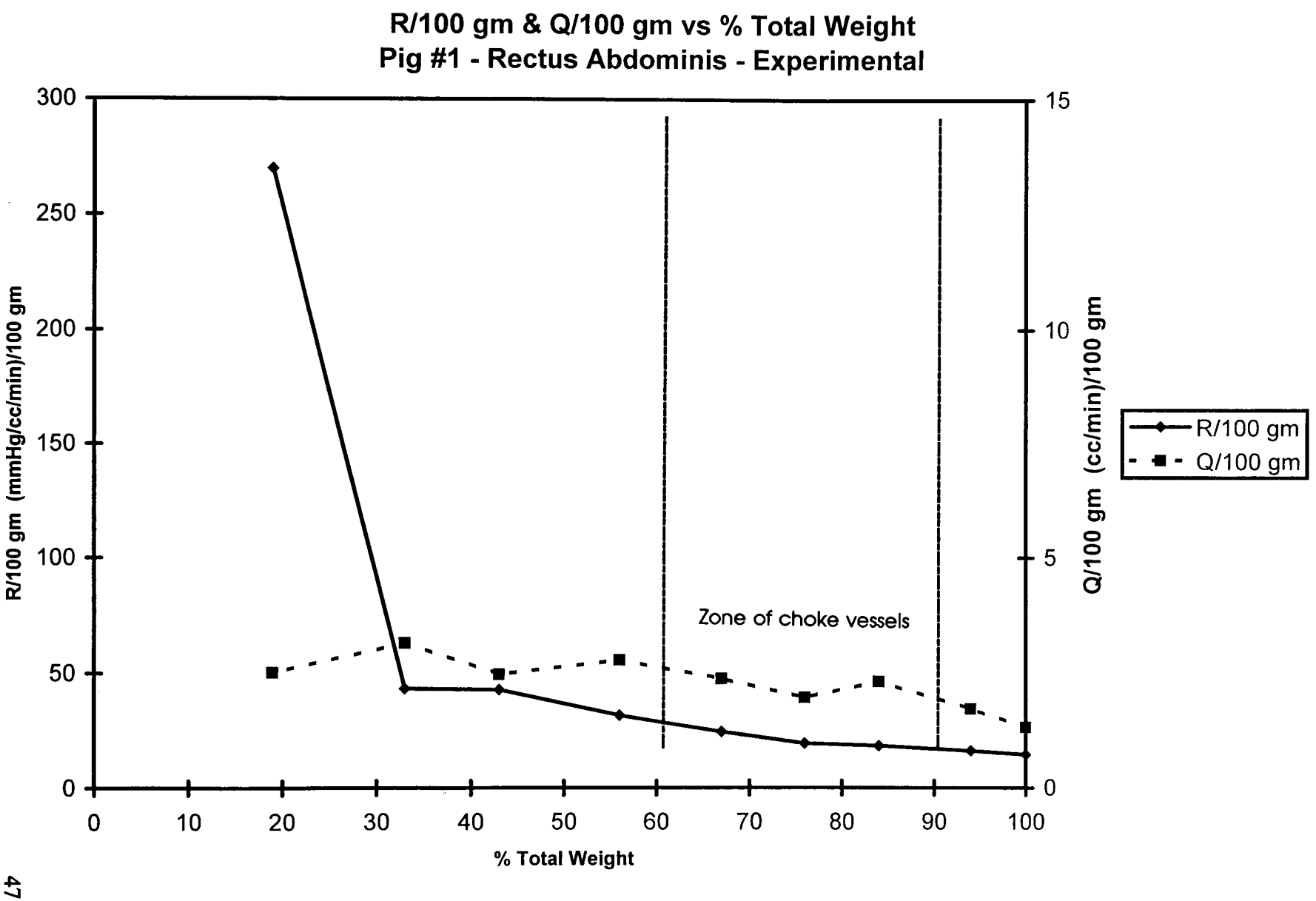

R/100 gm & Q/100 gm vs % Total Weight
Pig #1 - Rectus Abdominis - Experimental
R/100 gm (mmHg/cc/min)/100 gm
Q/100 gm (cc/min)/100 gm
% Total Weight
Zone of choke vessels
R/100 gm
Q/100 gm
300
250
200
150
100
50
0
15
10
5
0
0
10
20
30
40
50
60
70
80
90
100

Figure 9b.

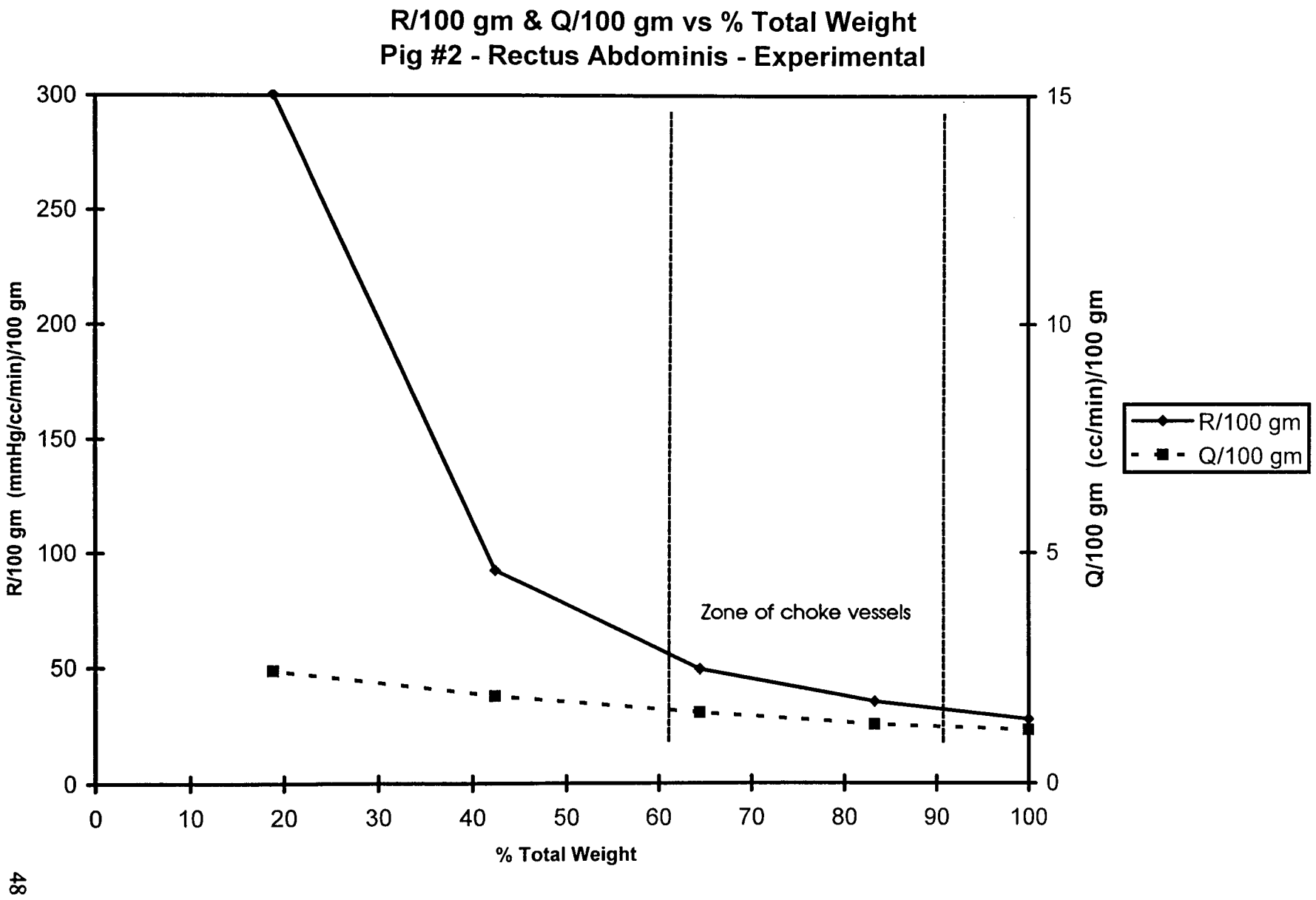

R/100 gm & Q/100 gm vs % Total Weight
Pig #2 - Rectus Abdominis - Experimental
R/100 gm (mmHg/cc/min)/100 gm
Q/100 gm (cc/min)/100 gm
% Total Weight
Zone of choke vessels
R/100 gm
Q/100 gm
300
250
200
150
100
50
0
15
10
5
0
0
10
20
30
40
50
60
70
80
90
100

Figure 9c.

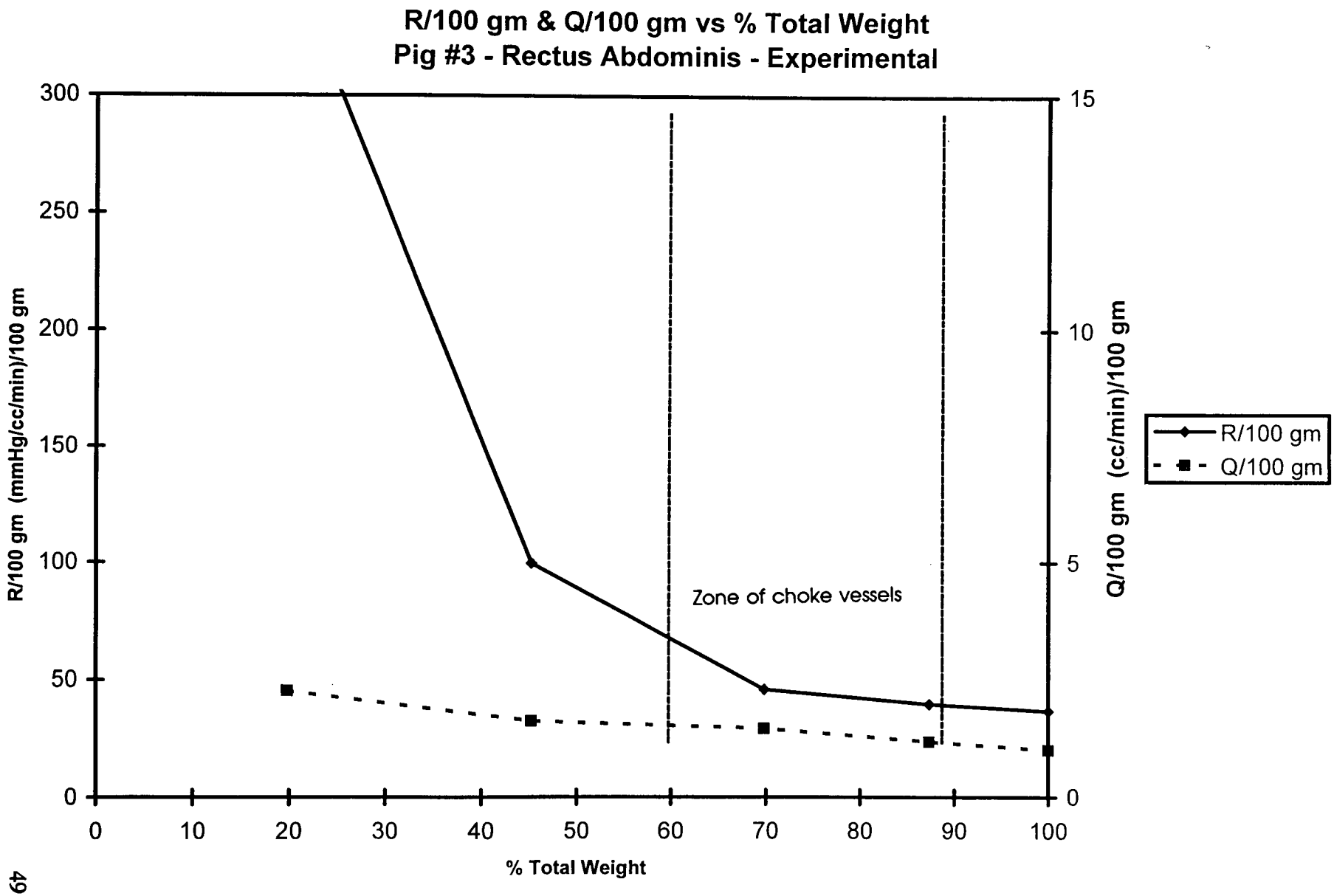
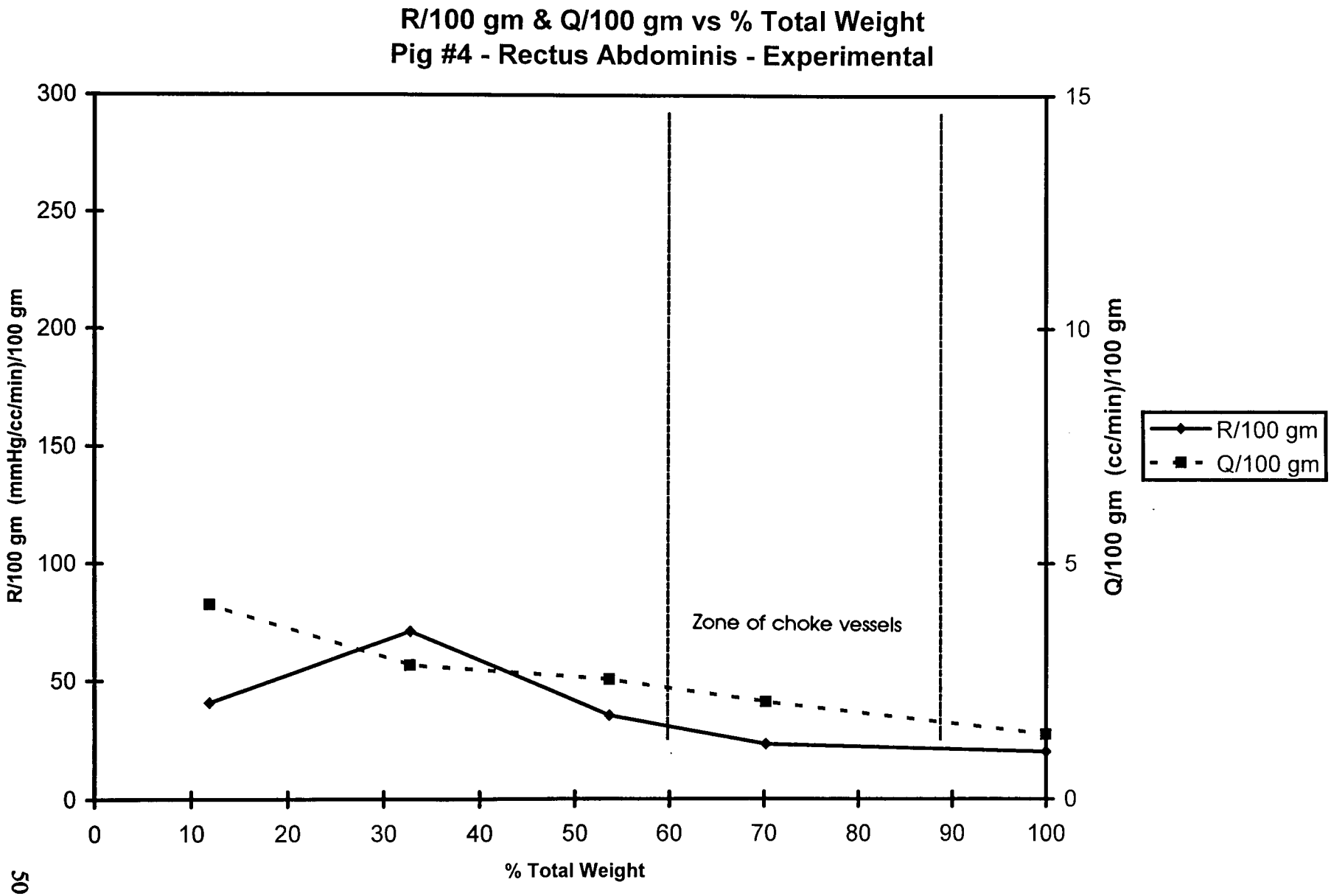

R/100 gm & Q/100 gm vs % Total Weight
Pig #3 - Rectus Abdominis - Experimental
R/100 gm (mmHg/cc/min)/100 gm
Q/100 gm (cc/min)/100 gm
% Total Weight
Zone of choke vessels
R/100 gm
Q/100 gm
300
250
200
150
100
50
0
15
10
5
0
0
10
20
30
40
50
60
70
80
90
100

Figure 9d.


R/100 gm & Q/100 gm vs % Total Weight
Pig #4 - Rectus Abdominis - Experimental
R/100 gm (mmHg/cc/min)/100 gm
Q/100 gm (cc/min)/100 gm
% Total Weight
Zone of choke vessels
R/100 gm
Q/100 gm
300
250
200
150
100
50
0
15
10
5
0
0
10
20
30
40
50
60
70
80
90
100

50

Figure 9e.

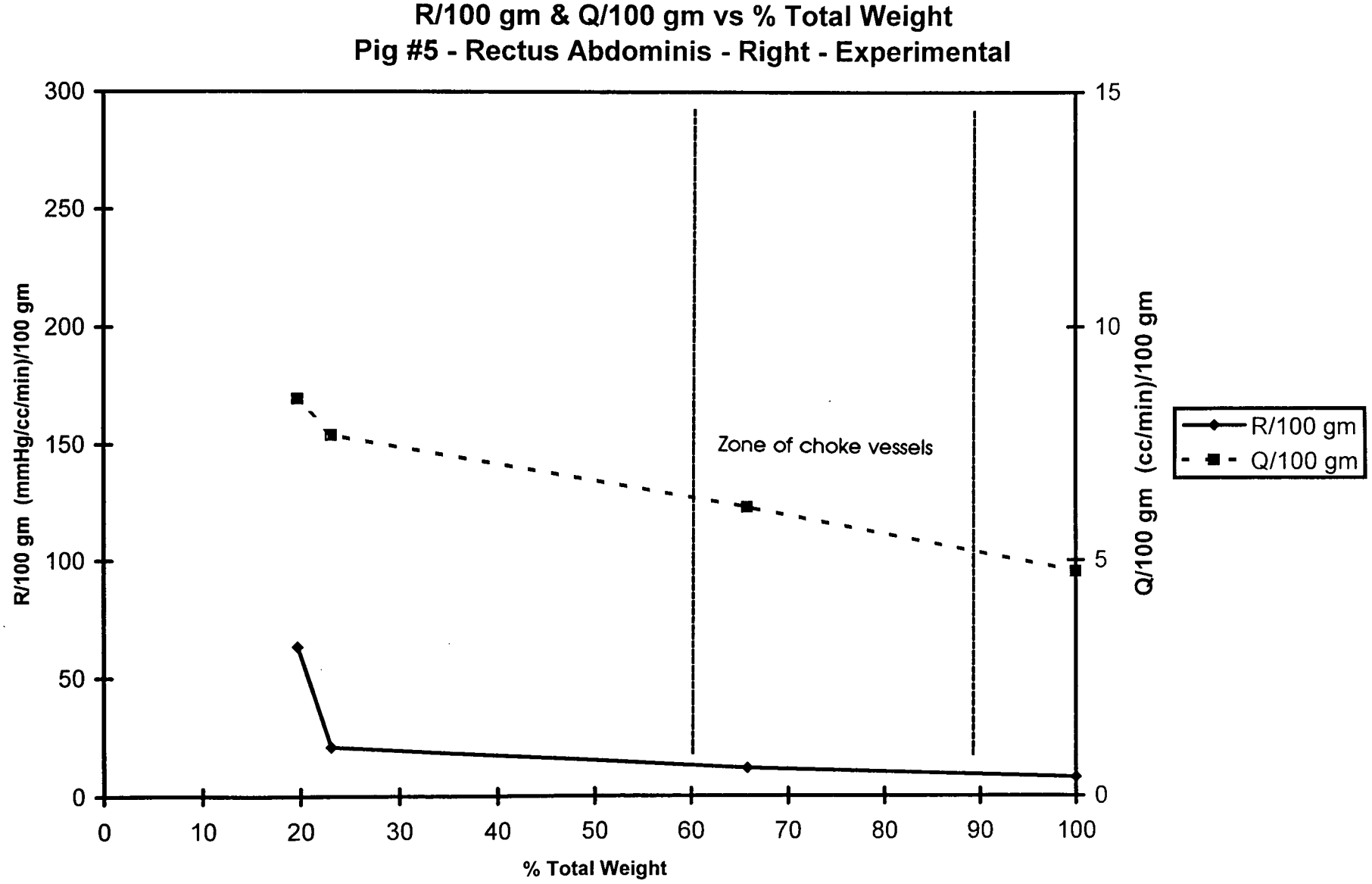

R/100 gm & Q/100 gm vs % Total Weight
Pig #5 - Rectus Abdominis - Right - Experimental
R/100 gm (mmHg/cc/min)/100 gm
Q/100 gm (cc/min)/100 gm
% Total Weight
Zone of choke vessels
R/100 gm
Q/100 gm
300
250
200
150
100
50
0
15
10
5
0
0
10
20
30
40
50
60
70
80
90
100

Figure 9f.

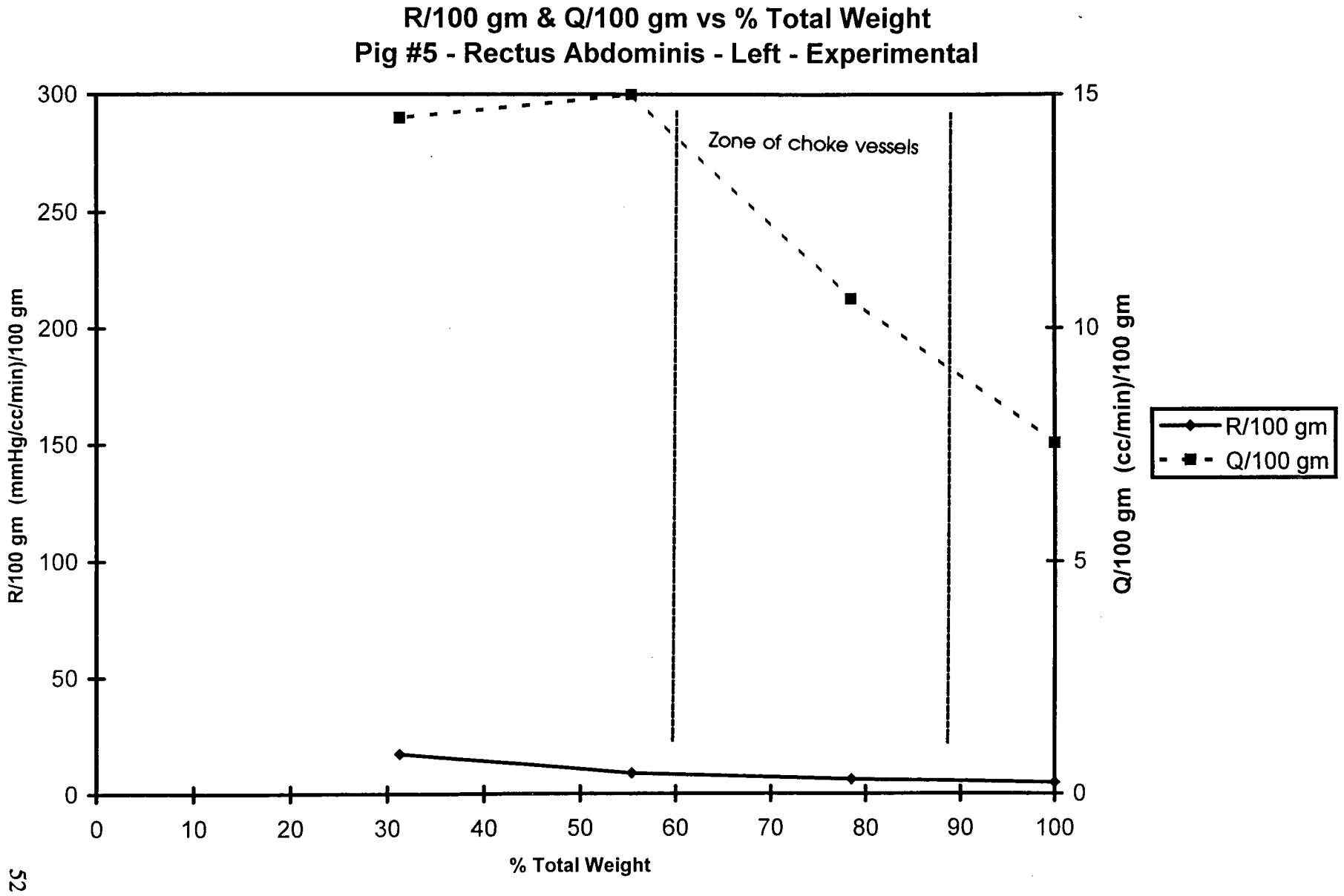

R/100 gm & Q/100 gm vs % Total Weight
Pig #5 - Rectus Abdominis - Left - Experimental
Zone of choke vessels
R/100 gm (mmHg/cc/min)/100 gm
Q/100 gm (cc/min)/100 gm
% Total Weight
R/100 gm
Q/100 gm
300
250
200
150
100
50
0
15
10
5
0
0
10
20
30
40
50
60
70
80
90
100

Figure 9g.

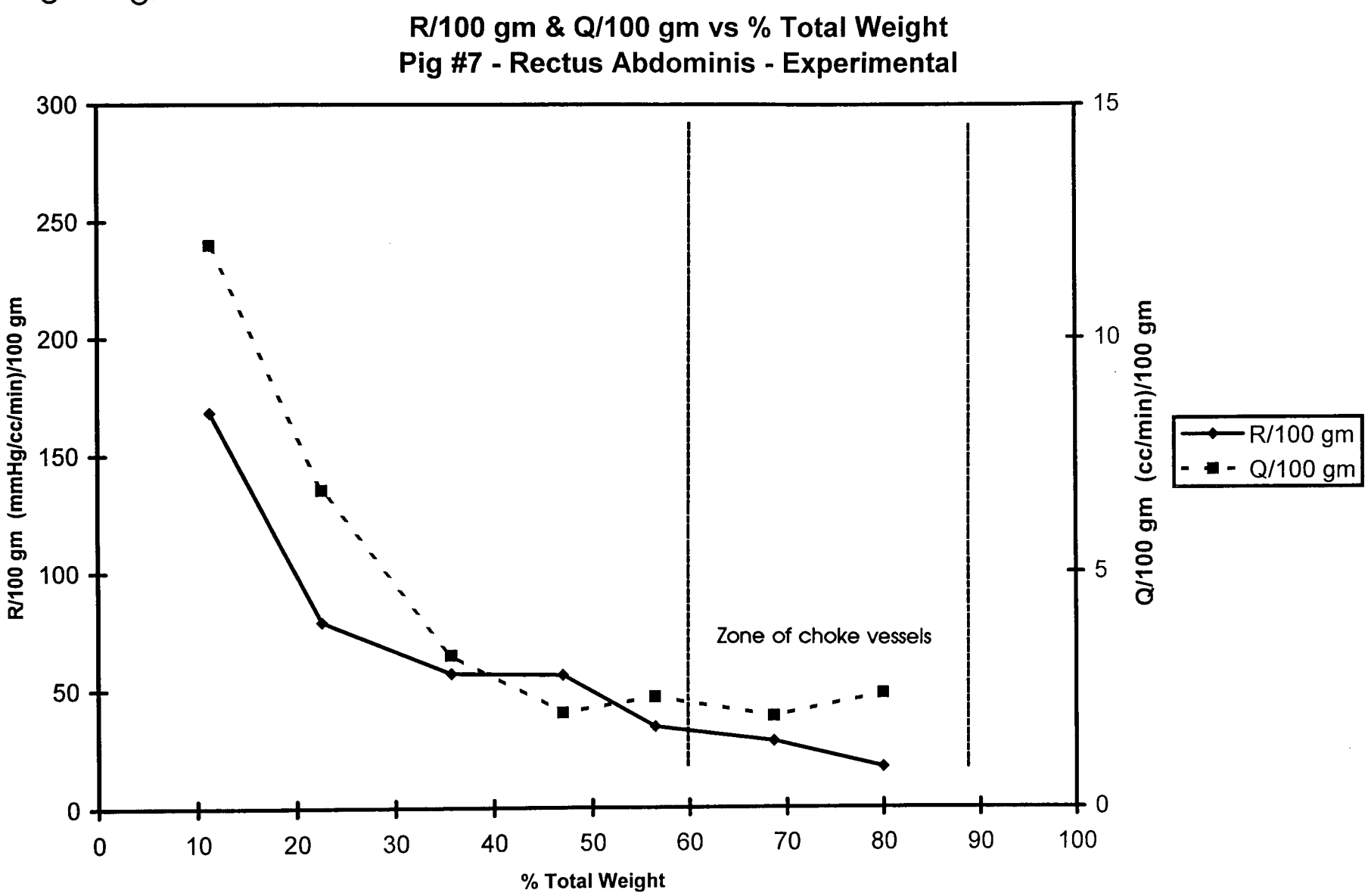
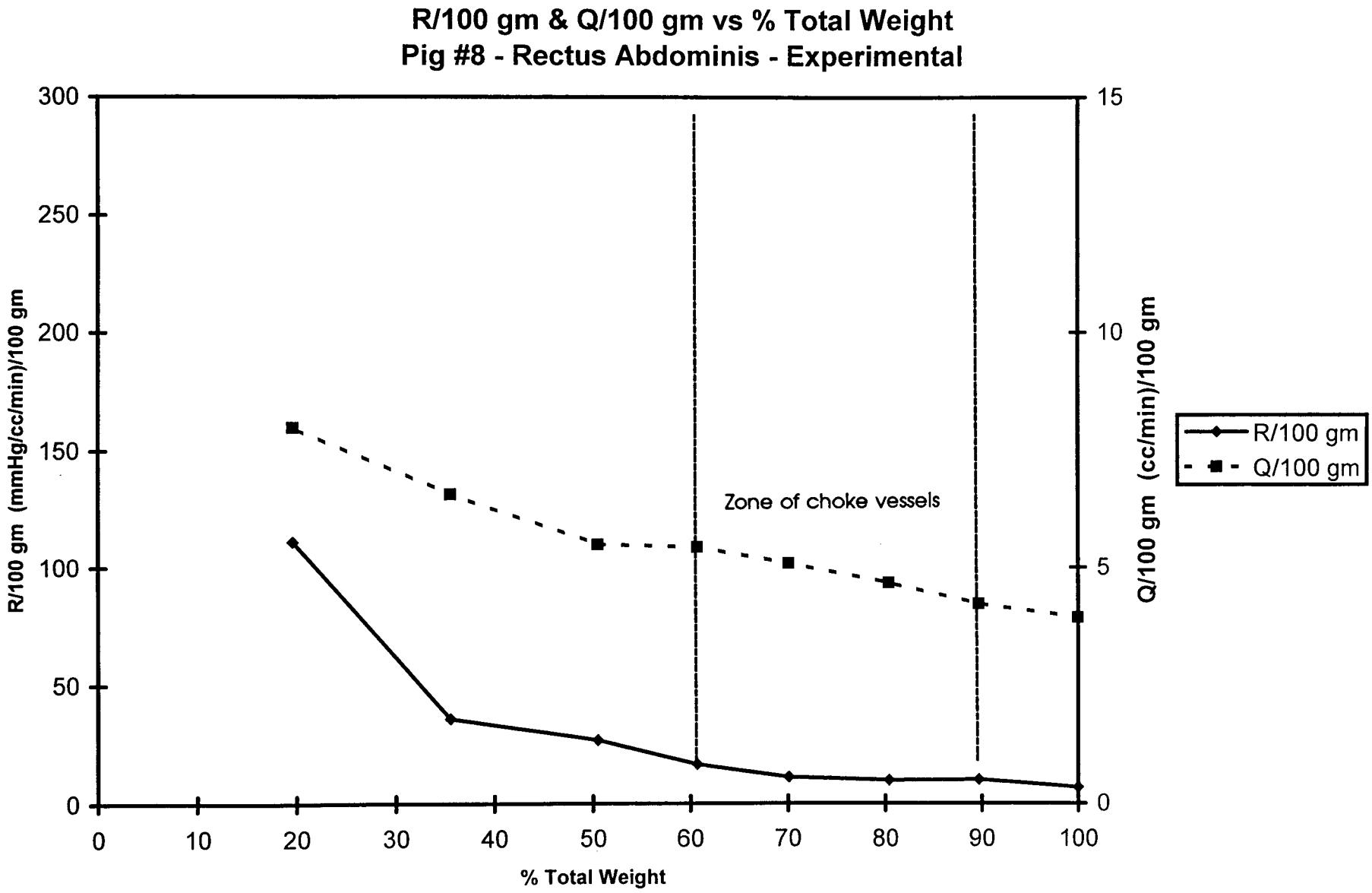

R/100 gm & Q/100 gm vs % Total Weight
Pig #7 - Rectus Abdominis - Experimental
R/100 gm (mmHg/cc/min)/100 gm
Q/100 gm (cc/min)/100 gm
% Total Weight
Zone of choke vessels
R/100 gm
Q/100 gm
300
250
200
150
100
50
0
15
10
5
0
0
10
20
30
40
50
60
70
80
90
100

Figure 9h.


R/100 gm & Q/100 gm vs % Total Weight
Pig #8 - Rectus Abdominis - Experimental
R/100 gm (mmHg/cc/min)/100 gm
Q/100 gm (cc/min)/100 gm
% Total Weight
Zone of choke vessels
R/100 gm
Q/100 gm
300
250
200
150
100
50
0
15
10
5
0
0
10
20
30
40
50
60
70
80
90
100

For each flap, the slope across the choke vessels is calculated and presented in Table 5. From the radiographic portion of this study, the choke vessels occurred from 60% to 90 % of the flap linear length with 0% being proximal and 100% being distal. The average slope calculated across the zone of choke vessels (from 60% to 90%) of flap linear length) is negative 0.60 +/- 0.44 (mmHg/cc/min)/100gm/%flap. The null hypothesis is that the surgical resection of choke vessels does not result in a statistically significant decrease in muscle flap resistance per unit weight. According to the null hypothesis, the slope of vascular resistance per unit weight in the region of the choke vessels should be less than or equal to zero. Thus, the null hypothesis cannot be rejected.

The beta error of this study was calculated using the central limit theorem. u1 is - 0.60, u2 is 0, sd is 0.44, and n = 8. Beta is calculated to be 0.014. Thus, the power of the study, 1 -beta, is 0.98.

Table 5. Slope of R/Wt vs % Total Flap Weight across choke zone of rectus abdominis flaps.

Pig #1	- 0.50
Pig #2	- 1.12
Pig #3	- 1.15
Pig #4	- 0.32
Pig #5 - right	- 0.17
Pig #5 - left	- 0.09
Pig #7	- 1.02
Pig #8	- 0.40
Average	- 0.60
Std	0.44

3.2.2 Control - Rectus abdominis - The control vascular resistance per unit weight and blood flow per unit weight is shown in Figure 10. These values were determined at the same time as the values for experimental flaps were determined. The control flaps were not serially resected. The control vascular resistance per unit weight and blood flow per unit weight are relatively constant.

3.2.3 Experimental - Gracilis - The total vascular resistance and total blood flow is shown in Figure 11. This shows the total vascular resistance increases for each of the flaps as the flaps are resected from distal (100%) towards proximal (0%). The total blood flow tends to decrease as the flaps are resected from distal (100%) towards proximal (0%).

The vascular resistance unit weight and blood flow per unit weight is shown in Figure 12. The vascular resistance per unit weight is seen to increase as the flap is resected from distal (100%) towards proximal (0%). Blood flow per unit weight tends to decrease as the flap is resected from distal (100%) towards proximal (0%). However, in Pig #7 blood flow per unit weight does not show a decrease as the flap is resected from distal (100%) towards proximal (0%). Review of the experimental data does not indicate a reason for this finding.

Figure 10a.

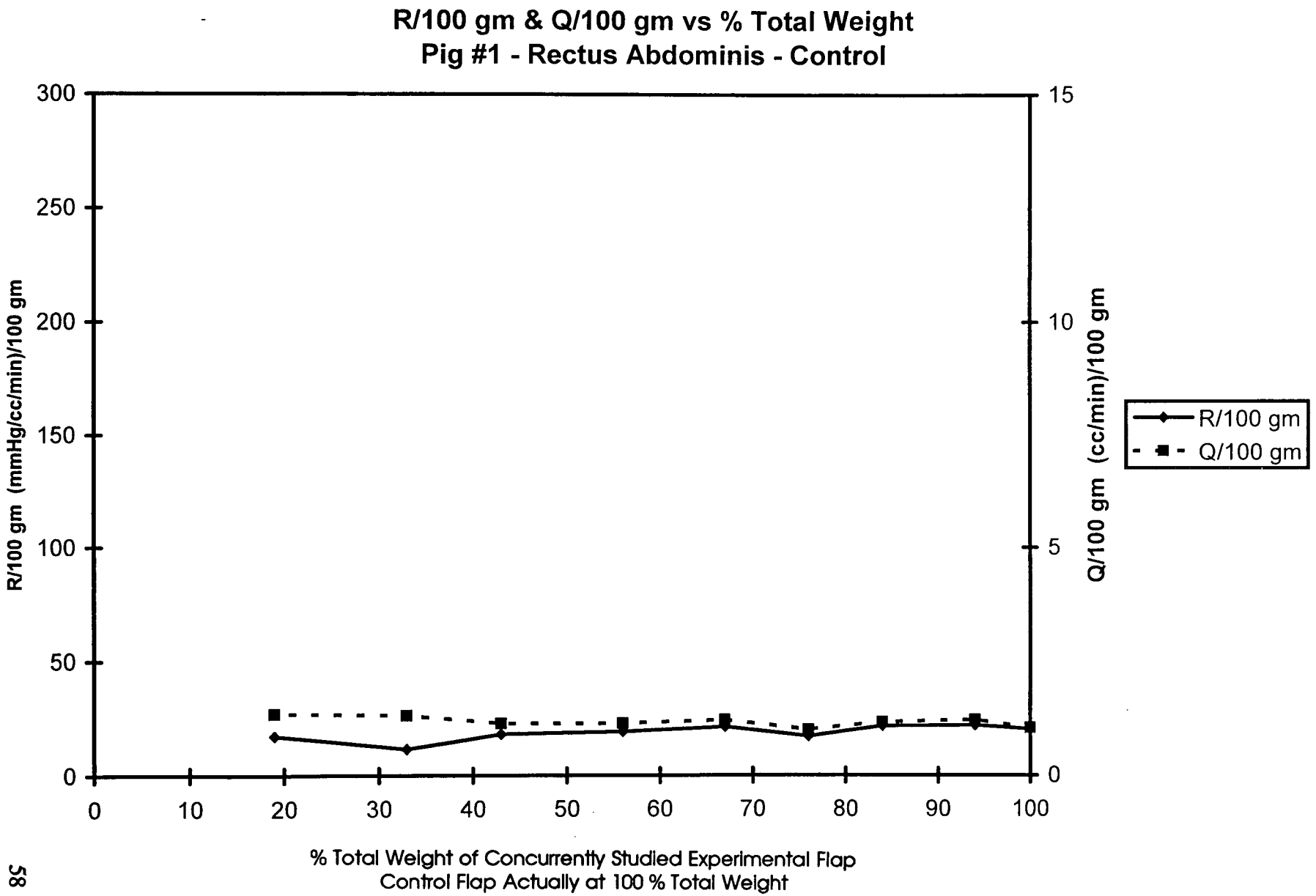

R/100 gm & Q/100 gm vs % Total Weight
Pig #1 - Rectus Abdominis - Control
R/100 gm (mmHg/cc/min)/100 gm
Q/100 gm (cc/min)/100 gm
300
250
200
150
100
50
0
15
10
5
0
0
10
20
30
40
50
60
70
80
90
100
R/100 gm
Q/100 gm
% Total Weight of Concurrently Studied Experimental Flap
Control Flap Actually at 100 % Total Weight

Figure 10b.

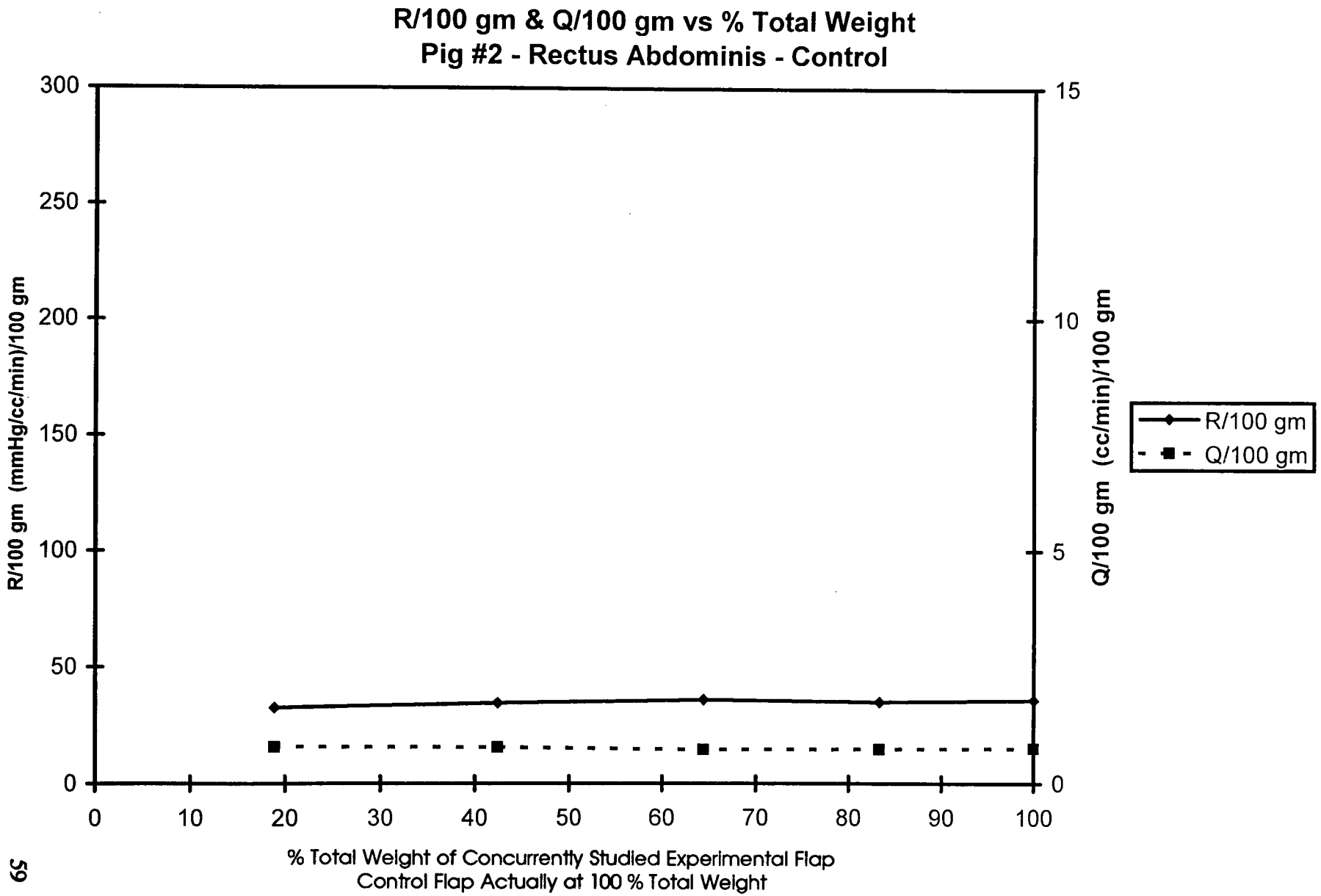

R/100 gm & Q/100 gm vs % Total Weight
Pig #2 - Rectus Abdominis - Control
R/100 gm (mmHg/cc/min)/100 gm
Q/100 gm (cc/min)/100 gm
R/100 gm
Q/100 gm
300
250
200
150
100
50
0
15
10
5
0
0
10
20
30
40
50
60
70
80
90
100
% Total Weight of Concurrently Studied Experimental Flap
Control Flap Actually at 100 % Total Weight

Figure 11a.

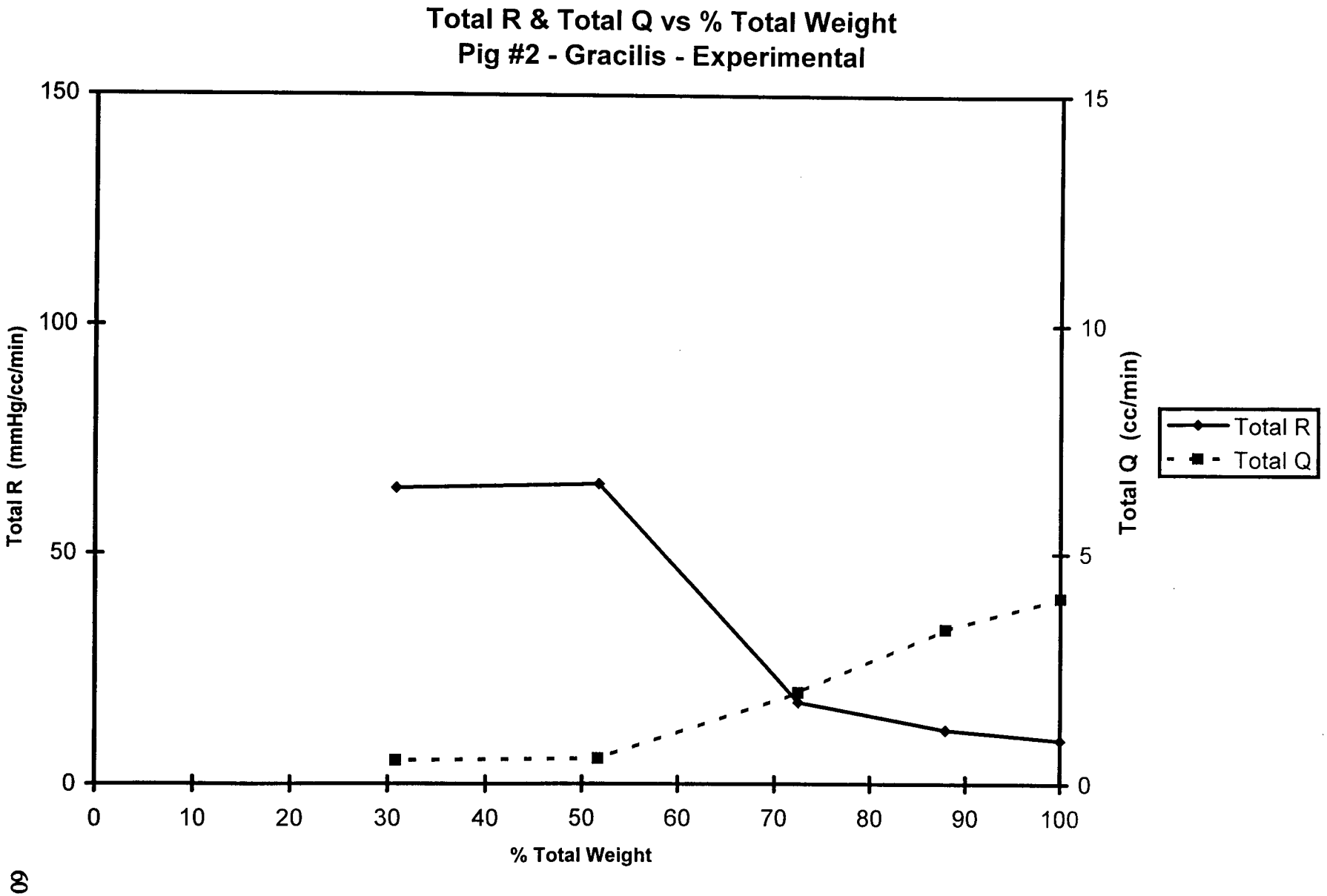

Total R & Total Q vs % Total Weight
Pig #2 - Gracilis - Experimental
150
100
50
0
15
10
5
0
Total R (mmHg/cc/min)
Total Q (cc/min)
0
10
20
30
40
50
60
70
80
90
100
% Total Weight
Total R
Total Q

Figure 11b.

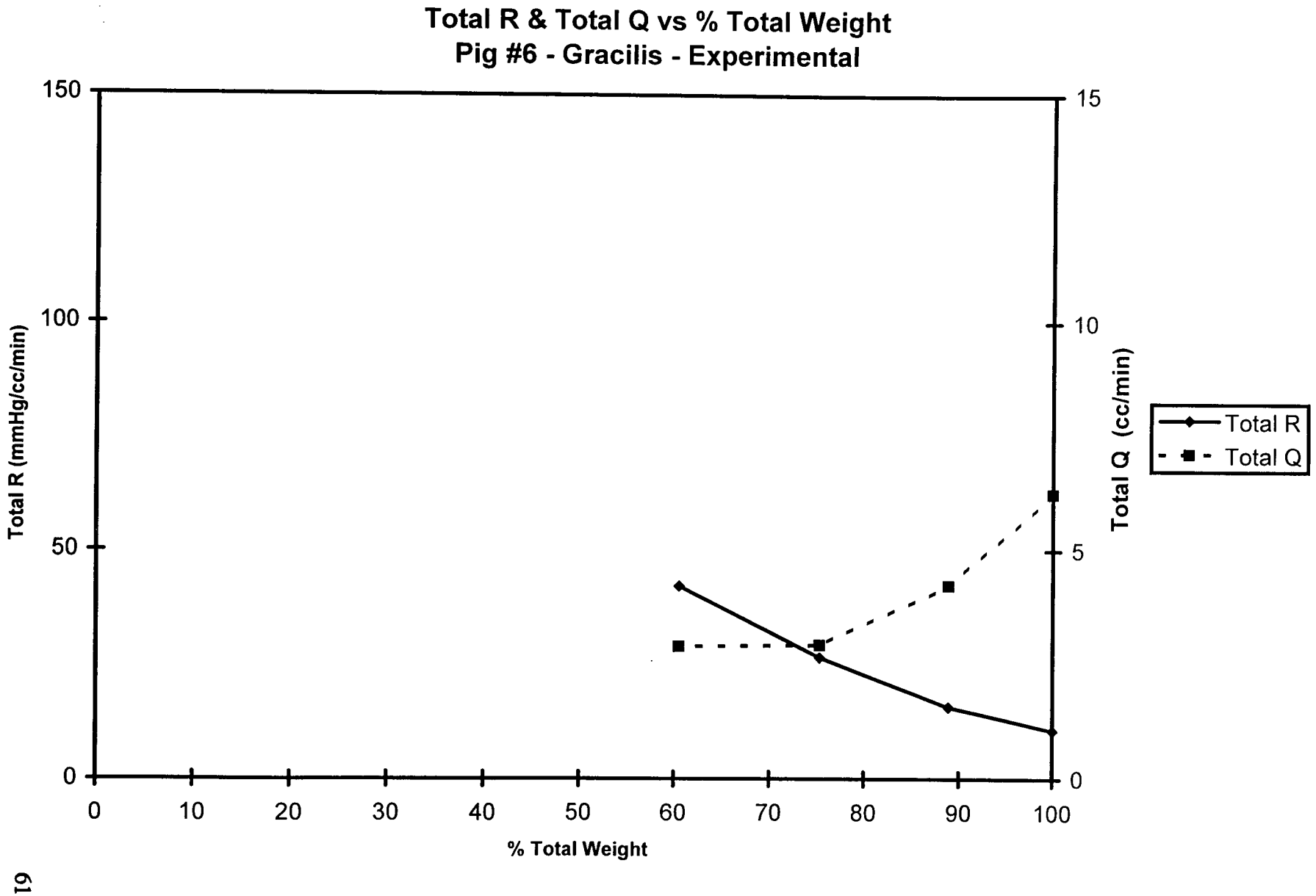

Total R & Total Q vs % Total Weight
Pig #6 - Gracilis - Experimental
150
100
50
0
15
10
5
0
0
10
20
30
40
50
60
70
80
90
100
% Total Weight
Total R (mmHg/cc/min)
Total Q (cc/min)
Total R
Total Q

Figure 11c.

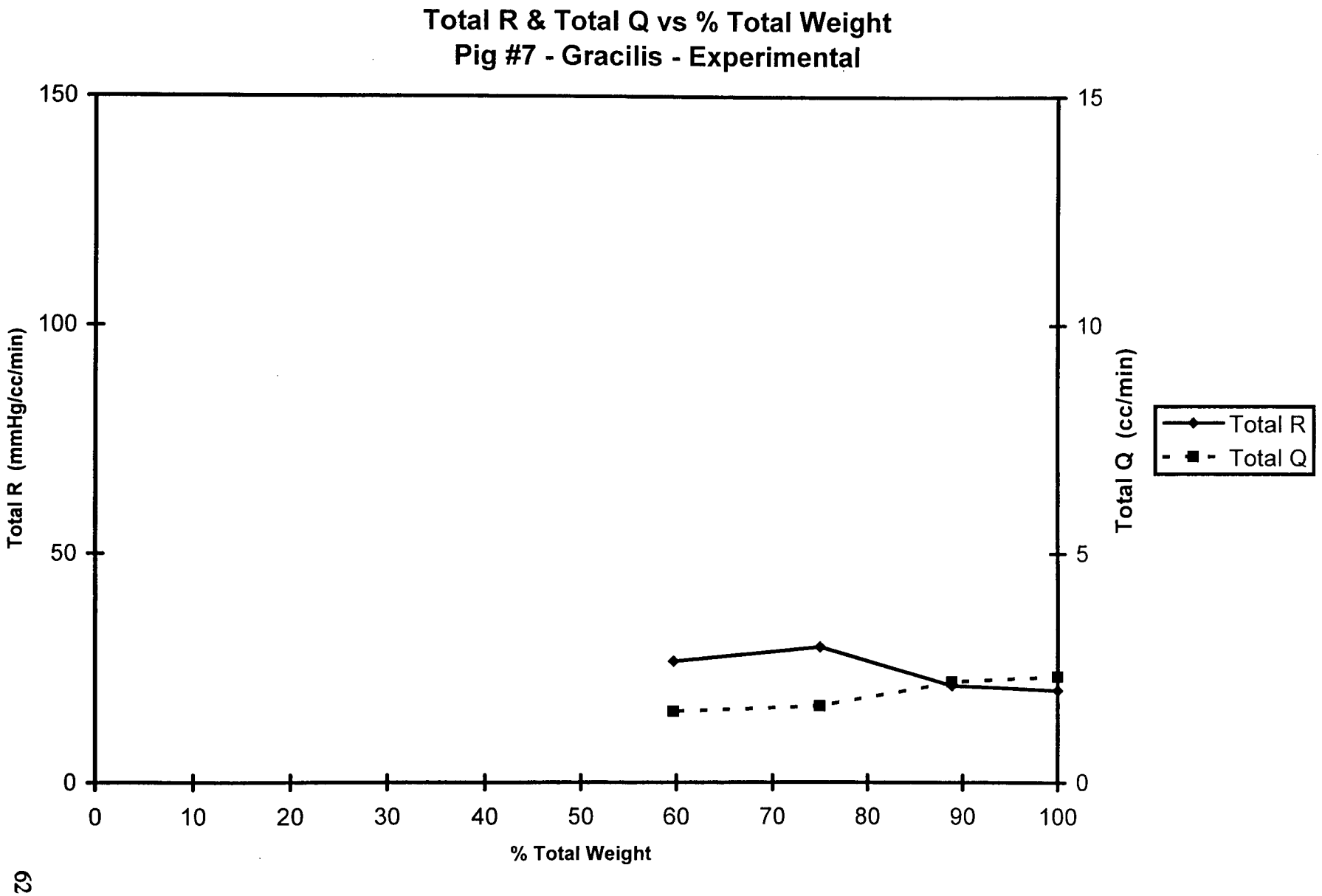

Total R & Total Q vs % Total Weight
Pig #7 - Gracilis - Experimental
150
100
50
0
15
10
5
0
Total R (mmHg/cc/min)
Total Q (cc/min)
0
10
20
30
40
50
60
70
80
90
100
% Total Weight
Total R
Total Q

Figure 11d.

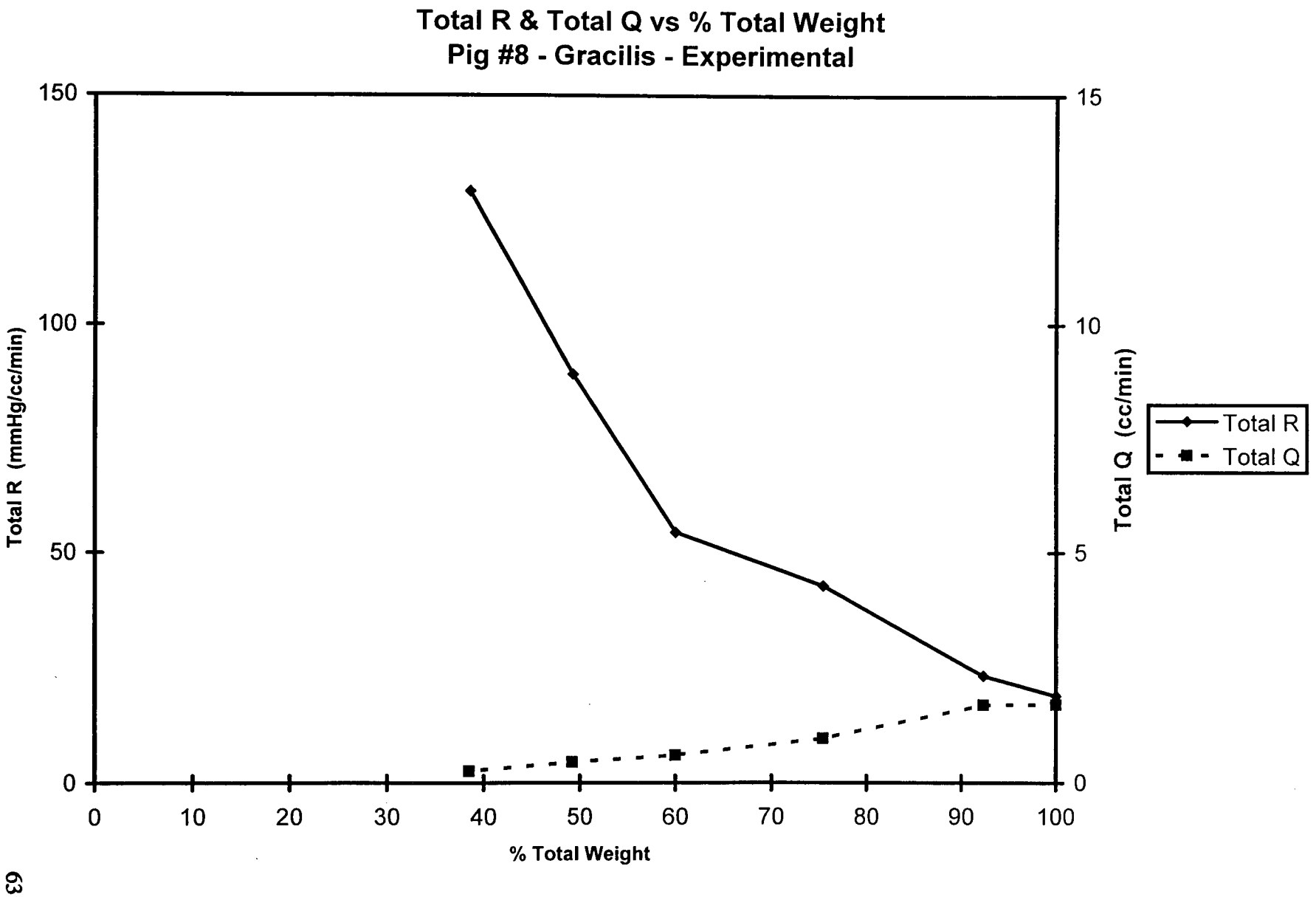

Total R & Total Q vs % Total Weight
Pig #8 - Gracilis - Experimental
150
100
50
0
15
10
5
0
Total R (mmHg/cc/min)
Total Q (cc/min)
0
10
20
30
40
50
60
70
80
90
100
% Total Weight
Total R
Total Q

Figure 12a.

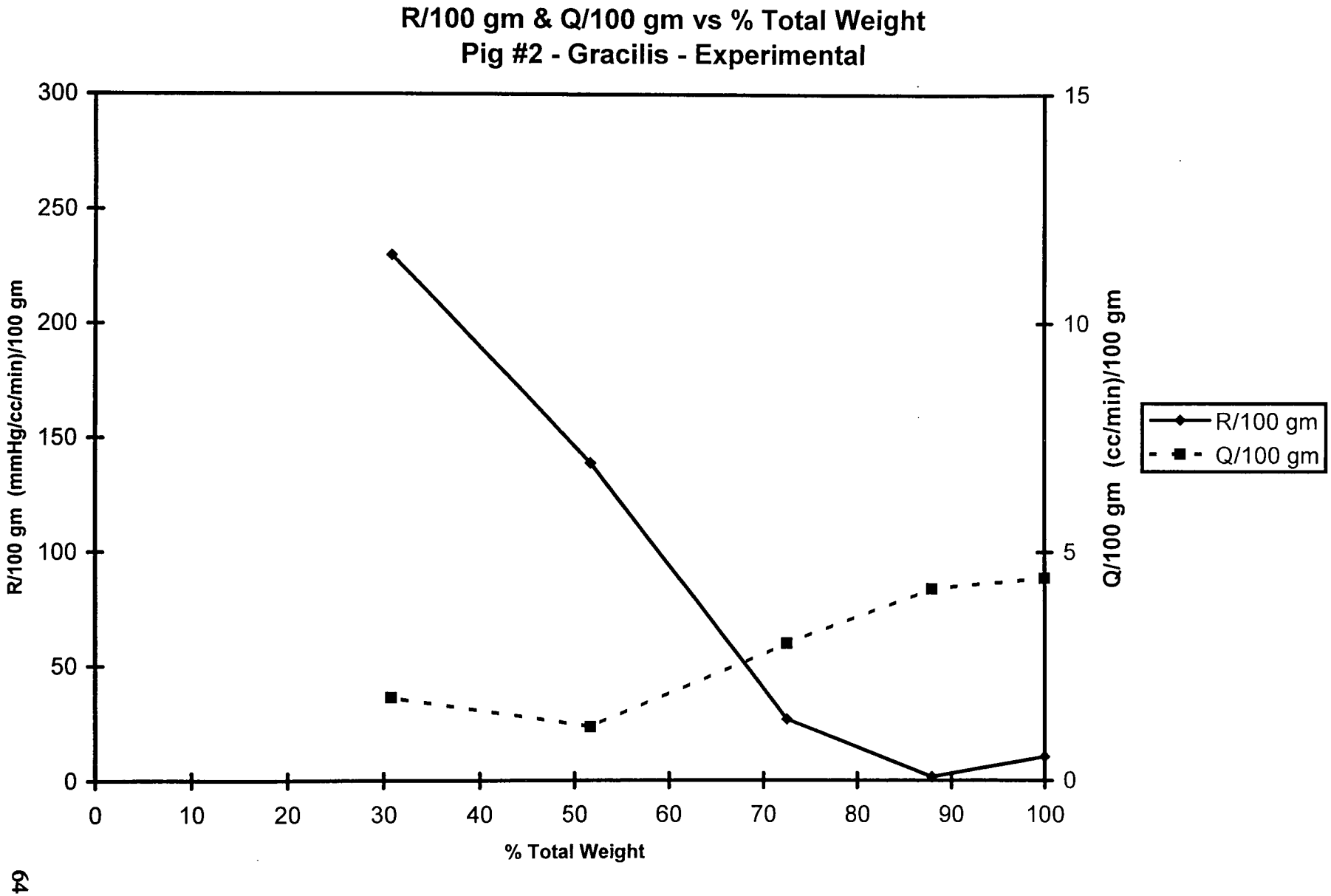

R/100 gm & Q/100 gm vs % Total Weight
Pig #2 - Gracilis - Experimental
R/100 gm (mmHg/cc/min)/100 gm
Q/100 gm (cc/min)/100 gm
% Total Weight
R/100 gm
Q/100 gm
300
250
200
150
100
50
0
15
10
5
0
0
10
20
30
40
50
60
70
80
90
100

Figure 12b.

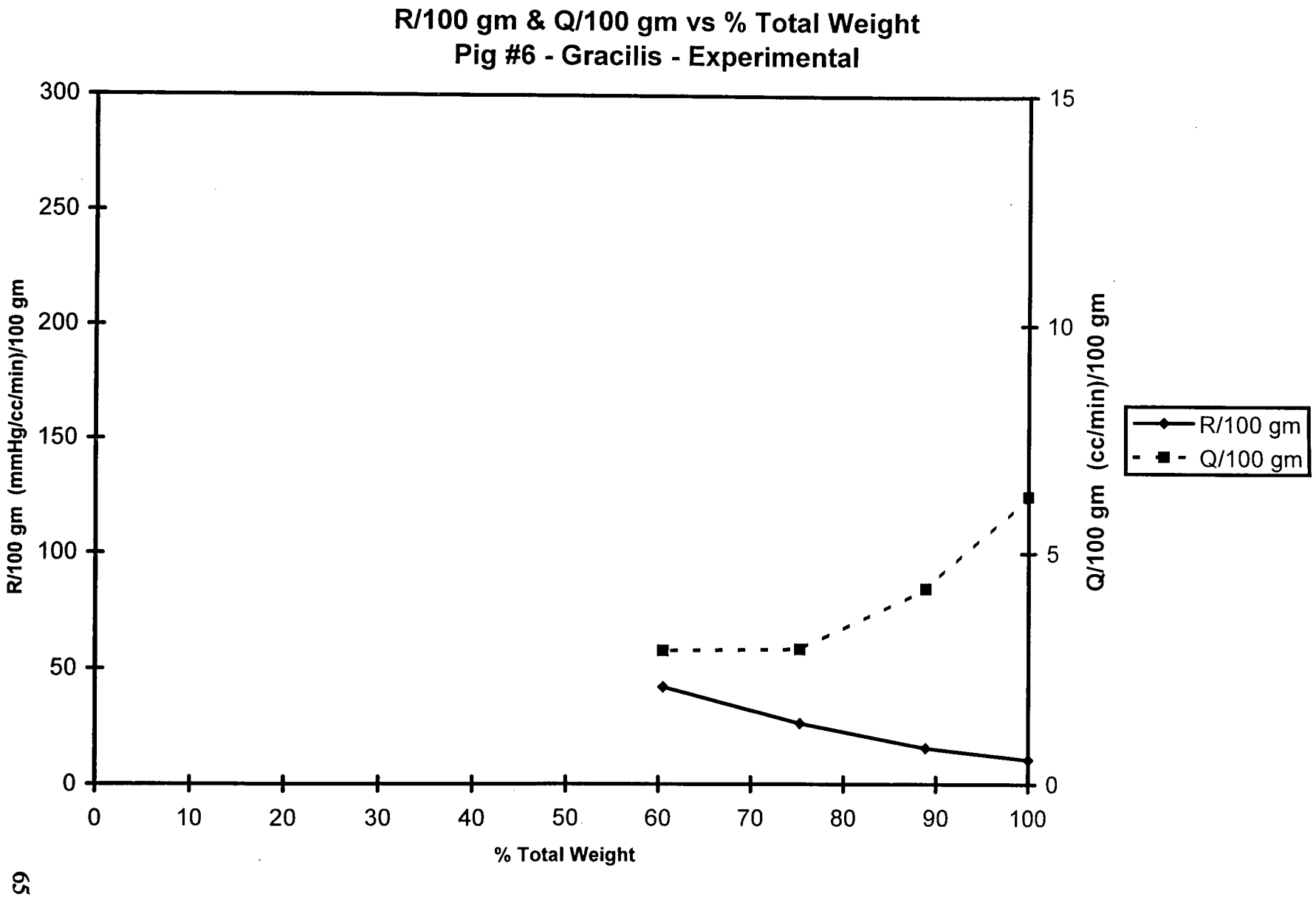

R/100 gm & Q/100 gm vs % Total Weight
Pig #6 - Gracilis - Experimental
R/100 gm (mmHg/cc/min)/100 gm
Q/100 gm (cc/min)/100 gm
% Total Weight
R/100 gm
Q/100 gm
300
250
200
150
100
50
0
15
10
5
0
0
10
20
30
40
50
60
70
80
90
100

Figure 12c.

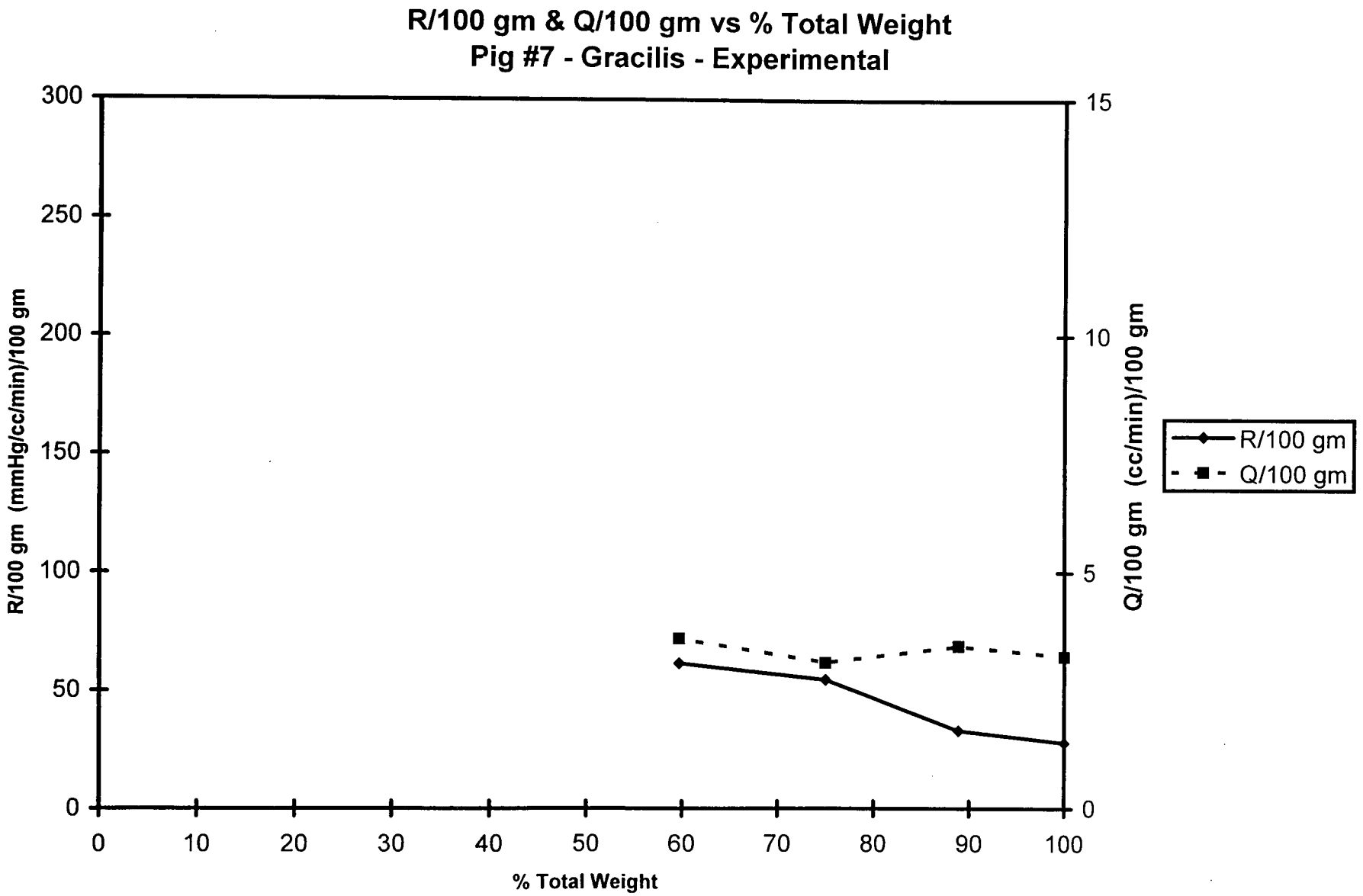
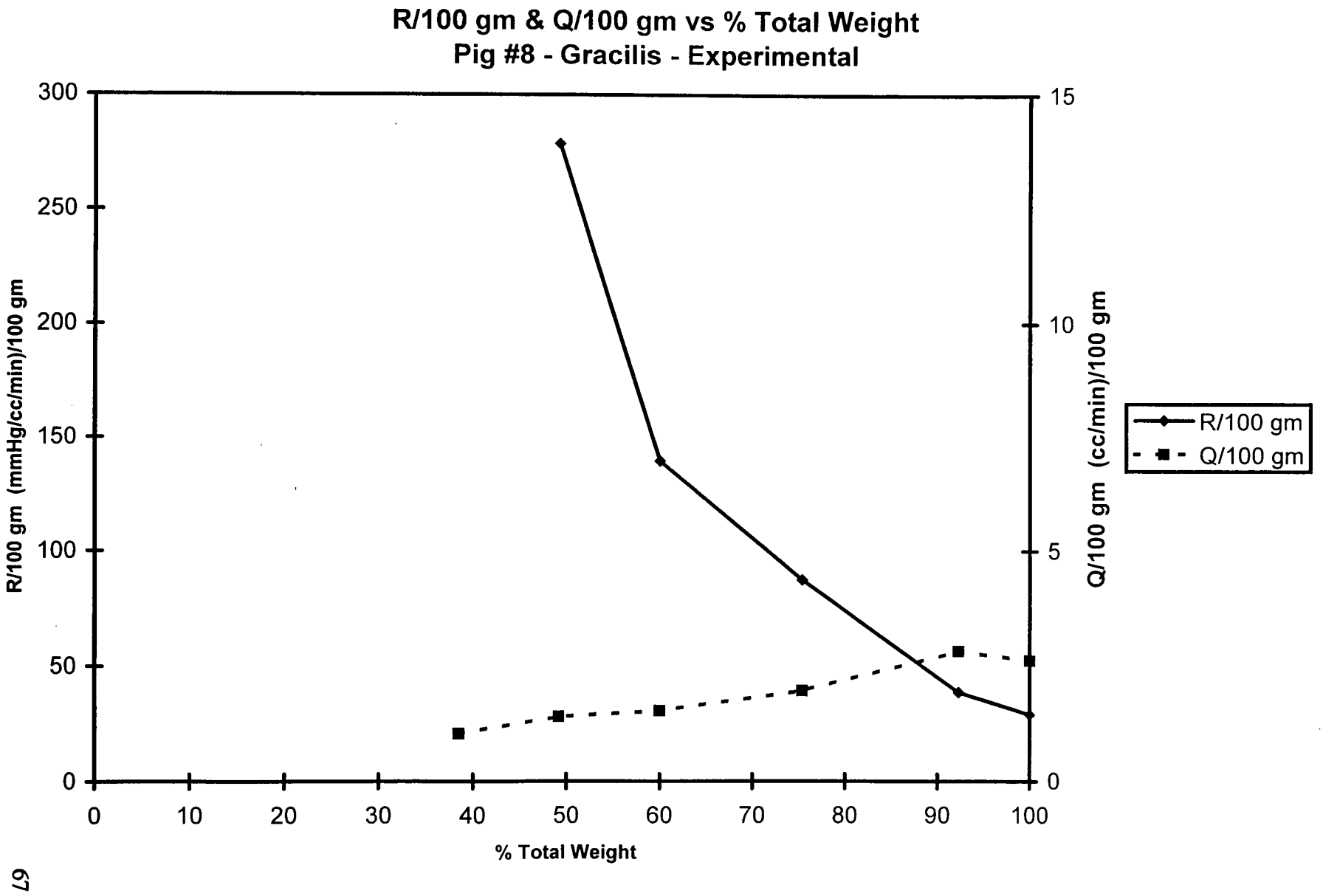

R/100 gm & Q/100 gm vs % Total Weight
Pig #7 - Gracilis - Experimental
R/100 gm (mmHg/cc/min)/100 gm
Q/100 gm (cc/min)/100 gm
% Total Weight
300
250
200
150
100
50
0
15
10
5
0
0
10
20
30
40
50
60
70
80
90
100
R/100 gm
Q/100 gm

Figure 12d.


R/100 gm & Q/100 gm vs % Total Weight
Pig #8 - Gracilis - Experimental
R/100 gm (mmHg/cc/min)/100 gm
Q/100 gm (cc/min)/100 gm
% Total Weight
R/100 gm
Q/100 gm
300
250
200
150
100
50
0
15
10
5
0
0
10
20
30
40
50
60
70
80
90
100

3.2.4 Control - Gracilis - The control vascular resistance per unit weight and blood flow per unit weight is shown in Figure 13. These values were determined at the same time as the values for experimental flaps were determined. The control flaps were not serially resected. The control vascular resistance per unit weight and blood flow per unit weight are relatively constant compared to the experimental flaps.

3.2.5 Data analysis - rectus abdominis - Each rectus abdominis flap in Figure 9 shows a similar plot for the resistance per unit weight vs % total weight, as well a similar plot for the blood flow per unit weight vs % total weight is seen. Linear regression analysis was performed. An equation relating Log resistance per unit weight vs % total weight was developed (see Table 6). The Log (R/wt) vs % total weight is plotted in Figure 14. The summary equation is Log (R/wt) = 2.20 - 0.0103 (% total wt), with st dev b = 0.42 and st dev m = 0.0048. The coefficient of determination (R square) ranges from 75.5 to 95.3 with a mean of 87.8. An equation relating blood flow per unit weight vs % total weight was developed (see Table 7). The summary equation is Q/wt = 7.36 - 0.050 (% total wt), with st dev b = 5.75 and st dev m = 0.045. The R square ranges from 60.5 to 97.5 with a mean of 86.1. The summary equations for the rectus abdominis are plotted in Figure 15. The summary equations demonstrate the trend in resistance per unit weight vs % total weight, and the trend in blood flow per unit weight vs % total weight. One should recognize that each linear regression equation, from which the summary linear regression equation was derived, is in itself also an estimate of the true relationship.

Figure 13a.

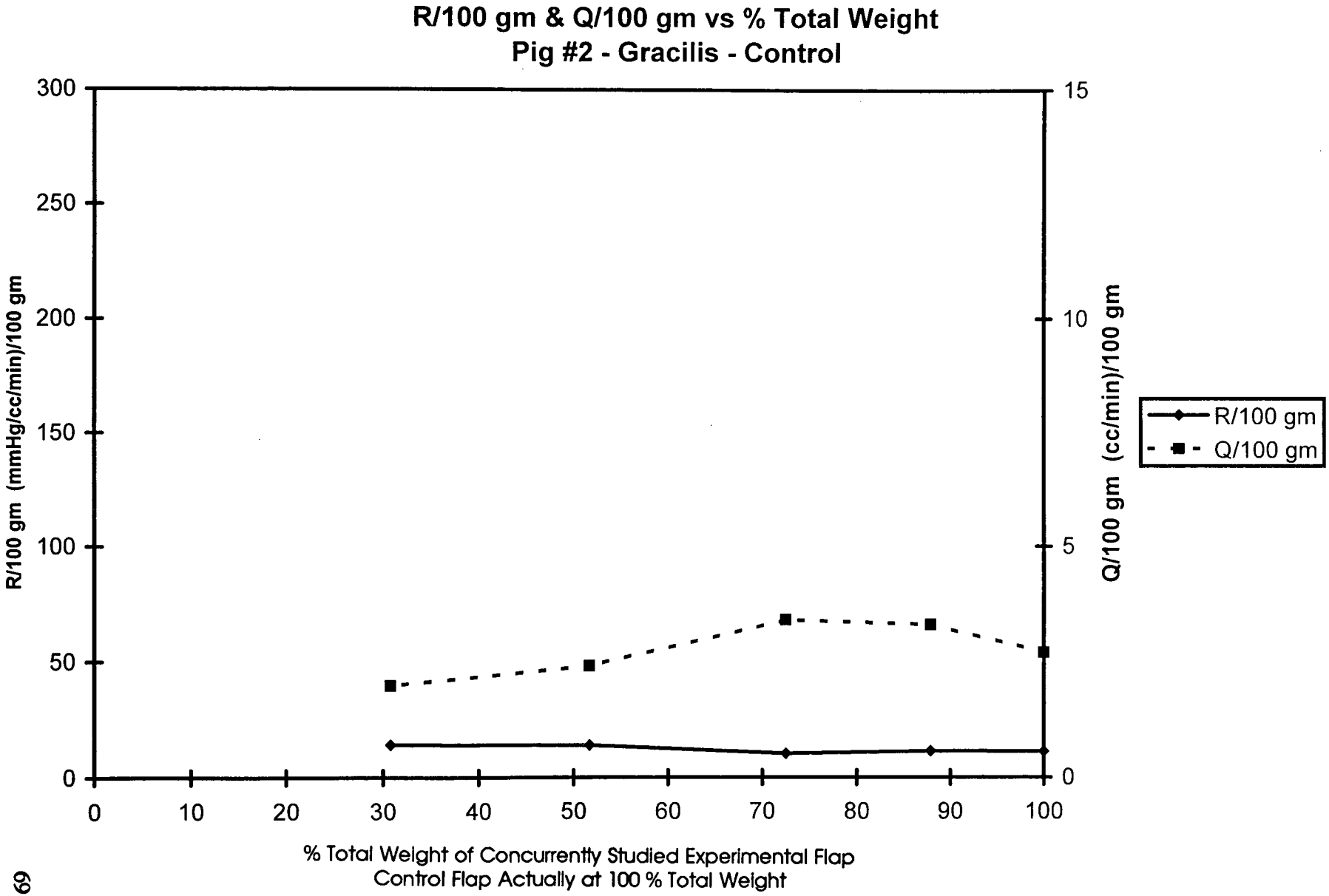

R/100 gm & Q/100 gm vs % Total Weight
Pig #2 - Gracilis - Control
R/100 gm (mmHg/cc/min)/100 gm
Q/100 gm (cc/min)/100 gm
300
250
200
150
100
50
0
15
10
5
0
0
10
20
30
40
50
60
70
80
90
100
R/100 gm
Q/100 gm
% Total Weight of Concurrently Studied Experimental Flap
Control Flap Actually at 100 % Total Weight

Figure 13b.

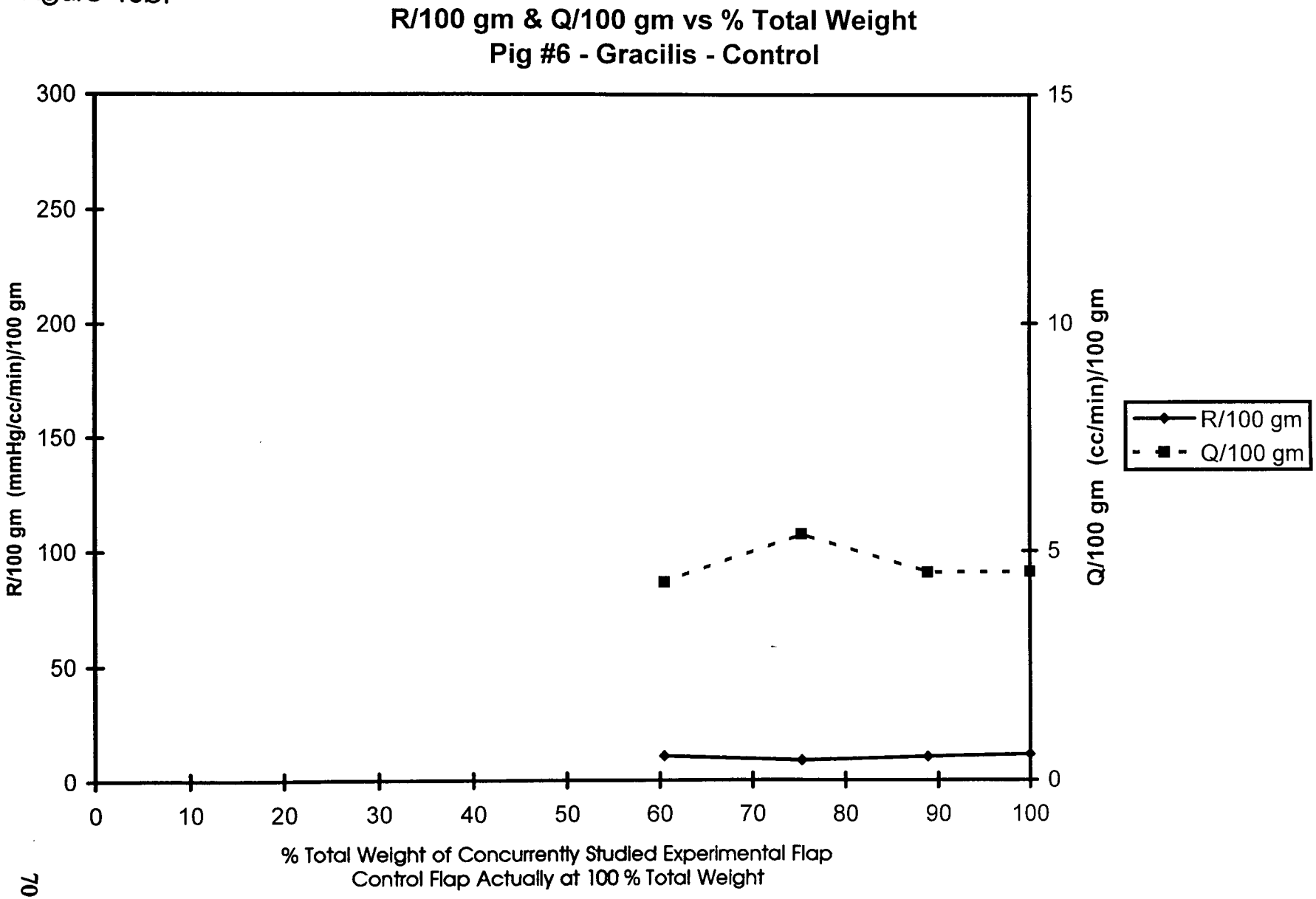

R/100 gm & Q/100 gm vs % Total Weight
Pig #6 - Gracilis - Control
R/100 gm (mmHg/cc/min)/100 gm
Q/100 gm (cc/min)/100 gm
300
250
200
150
100
50
0
15
10
5
0
0
10
20
30
40
50
60
70
80
90
100
% Total Weight of Concurrently Studied Experimental Flap
Control Flap Actually at 100 % Total Weight
R/100 gm
Q/100 gm

Table 6. Linear regression equations for rectus abdominis R/wt vs % Total Weight

$$\text{Log (R/wt)} = b + m \times (\% \text{ Total Weight})$$

Pig #	b	m	R square
1	2.15	- 0.014	92.6
2	2.28	- 0.013	95.3
3	1.74	- 0.009	75.5
4	1.44	- 0.008	96.3
5 Right	2.49	- 0.014	81.0
5 Left	2.30	- 0.008	88.6
7	2.60	- 0.013	93.8
8	2.63	- 0.017	79.1

Figure 14.

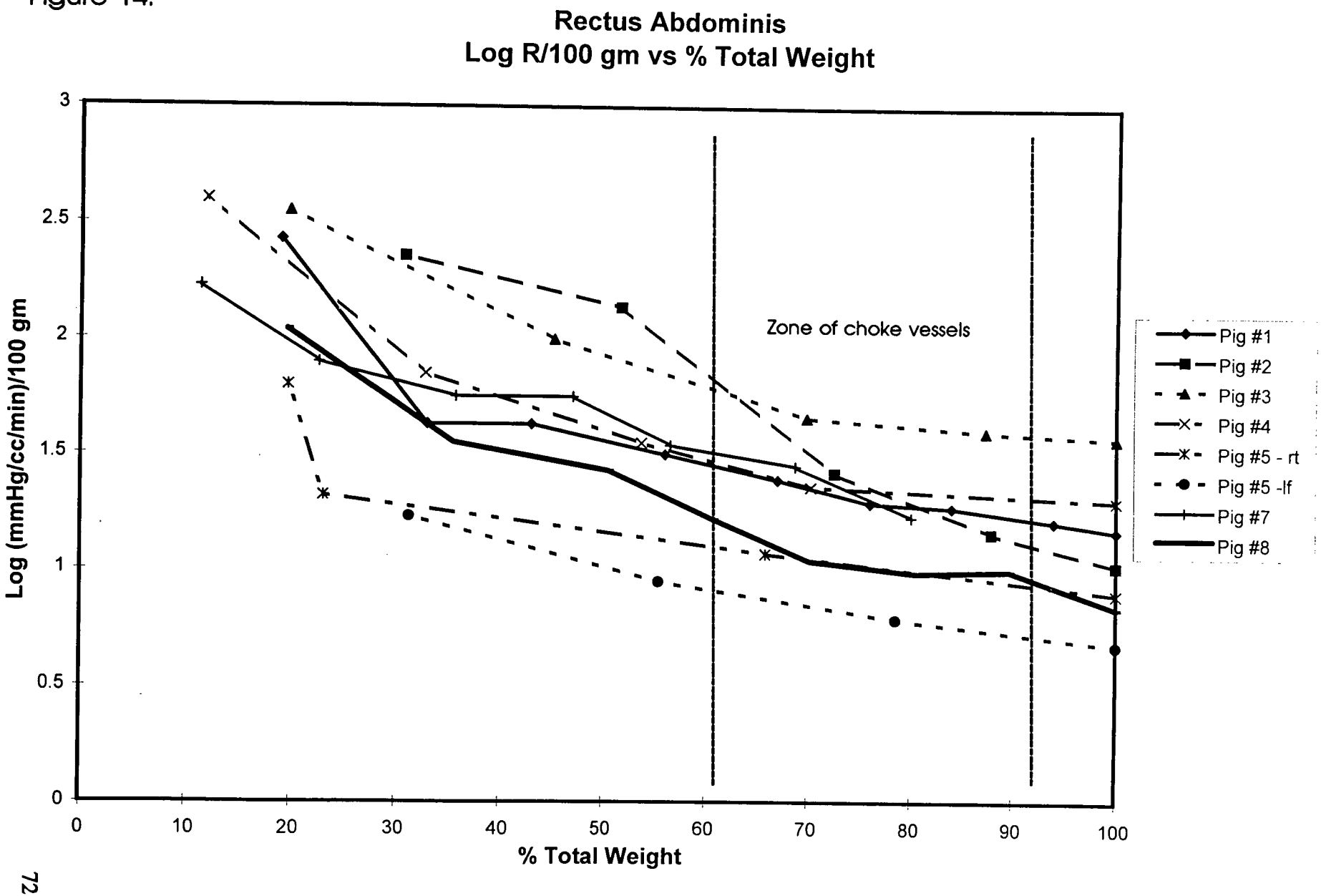

Rectus Abdominis
Log R/100 gm vs % Total Weight
Log (mmHg/cc/min)/100 gm
% Total Weight
Zone of choke vessels
Pig #1
Pig #2
Pig #3
Pig #4
Pig #5 - rt
Pig #5 -lf
Pig #7
Pig #8
0
0.5
1
1.5
2
2.5
3
0
10
20
30
40
50
60
70
80
90
100

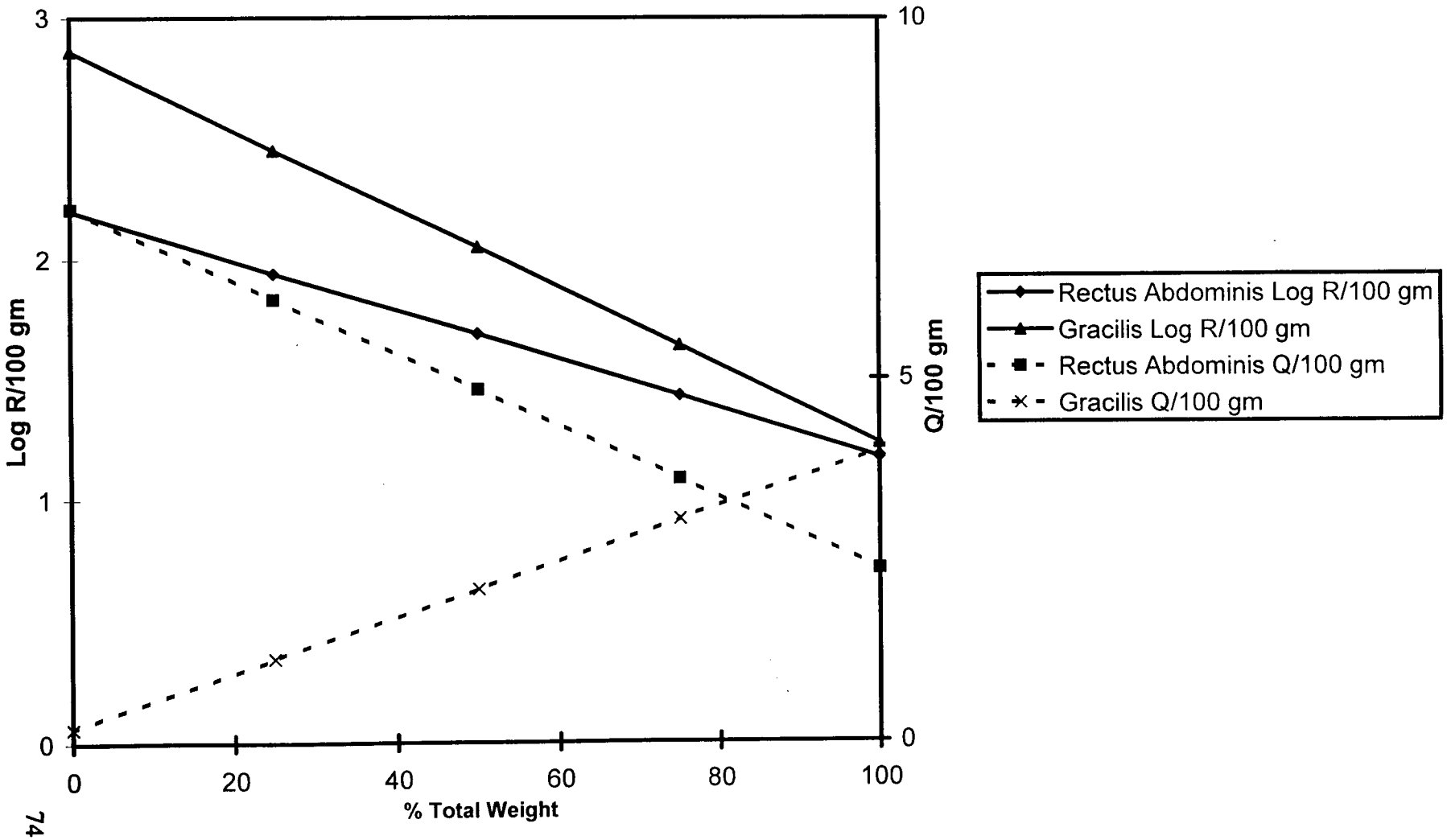
Table 7. Linear regression equations for rectus abdominis Q/wt vs % Total Weight

$$Q/wt = b + m \times (\% \text{ Total Weight})$$

Pig #	b	m	R square
1	3.10	- 0.013	60.5
2	2.64	- 0.016	96.8
3	2.19	- 0.011	95.5
4	4.16	- 0.029	93.9
5 Right	19.3	- 0.111	97.5
5 Left	9.00	- 0.043	82.6
7	10.1	- 0.125	66.9
8	8.42	- 0.047	94.8

Figure 15.

Summary Log R/100 gm vs % Total Weight
& Summary Q/100 gm vs % Total Weight
for Rectus Abdominis and Gracilis



3.2.6 Data analysis - gracilis - Each flap in Figure 12 shows a similar plot for the resistance per unit weight vs % total weight, as well a similar plot for the blood flow per unit weight vs % total weight is seen with the exception of Pig #7. The blood flow per unit weight in Pig #7 does not show the decrease with decreasing % total weight seen with other flaps. Linear regression analysis is performed. An equation relating Log resistance per unit weight vs % total weight was developed (see Table 8). The Log (R/wt) vs % total weight is plotted in Figure 16. The summary equation is Log (R/wt) = 2.86 - 0.0163 (% total weight), with st dev b = 0.48 and st dev m = 0.0054. The R square ranges from 93.4 to 99.8 with a mean of 97.3. An equation relating blood flow per unit weight vs % total weight was developed (see Table 9). The summary equation is Q/wt = 0.20 + 0.038 (% total weight), with st dev b = 2.70 and st dev m = 0.037. The R square ranges from 22.4 to 95.2 with a mean of 70.3. The summary equations for the gracilis are plotted in Figure 15.

Table 8. Linear regression equations for gracilis R/wt vs % Total Weight

$$\text{Log (R/wt)} = b + m \times (\% \text{ Total Weight})$$

Pig #	b	m	R square
2	3.07	- 0.021	96.8
6	2.56	- 0.015	99.8
7	2.37	- 0.009	93.4
8	3.42	- 0.019	99.0

Figure 16.

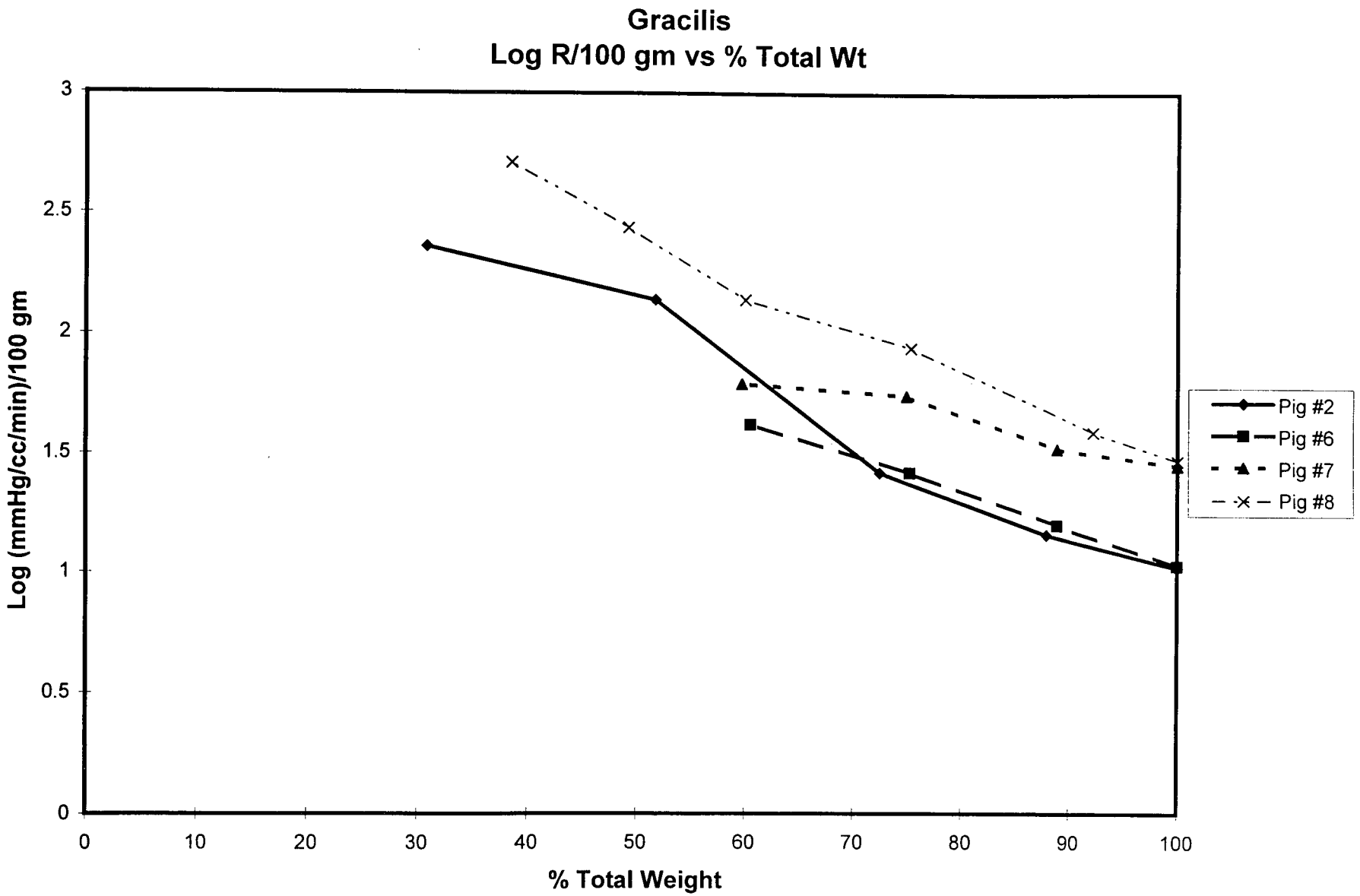

Gracilis
Log R/100 gm vs % Total Wt
Log (mmHg/cc/min)/100 gm
% Total Weight
0
0.5
1
1.5
2
2.5
3
0
10
20
30
40
50
60
70
80
90
100
Pig #2
Pig #6
Pig #7
Pig #8

Table 9. Linear regression equations for rectus abdominis Q/wt vs % Total Weight

$$Q/wt = b + m \times (\% \text{ Total Weight})$$

Pig #	b	m	R square
2	- 0.26	+ 0.046	81.5
6	- 2.69	+ 0.083	82.1
7	+3.84	- 0.006	22.4
8	- 0.07	+ 0.029	95.2

CHAPTER 4: DISCUSSION

4.1 EVALUATION OF HYPOTHESIS

The findings of this study do not allow rejection of the null hypothesis. The surgical resection of choke vessels from the rectus abdominis flap did not result in a statistically significant decrease in muscle flap vascular resistance per unit weight. The power of this study was 0.98, and so this finding does not appear to be false negative. Indeed, the vascular resistance per unit weight in the rectus abdominis is seen to increase with decreasing % total weight over the entire muscle flap length, and linear regression analysis supports this with equations demonstrating a coefficient of determination, R square, value ranging from 75.5 to 95.3 and a mean of 87.8. Furthermore, this relationship of increasing vascular resistance per unit weight with decreasing % total weight is also seen in the gracilis flaps. In the rectus abdominis, the orientation of choke vessels was perpendicular to the placement of the vascular clamps and also the line of surgical resection. In the gracilis, the orientation of choke vessels is actually parallel to the placement of the vascular clamps and also the line of surgical resection. The relationship of the increasing vascular resistance per unit weight with decreasing % total weight appears to be independent of choke vessels.

4.2 NEGATIVE RESULT

The results of this study are negative in that the vascular resistance per unit weight did not decrease as the choke vessels were resected. One consideration for this negative result is the sample size of this study, n = 8. A sample size could not be calculated for this study as no previous experimentation of this type was performed at this centre or was published in the literature. The sample size of 8 was determined on the basis of discussion with experienced animal experimenters at this centre. Despite not having an evidence based sample size, inadequate sample size does not appear to be a factor in this negative result. A beta error could be calculated and was 0.014. Thus, the result does not appear to be a false negative result.

The presumption that choke vessels are high resistance is based delay phenomenon studies with subjects recovering from the operation for a period of time. This study only considers the possible role of choke vessels in muscle flaps in the intraoperative period which have not undergone delay. Intraoperative factors may be masking effect of the possibly high resistance choke vessels. One such factor may be that of aneasthetics. Ketamine and isoflurane were used and are discussed in detail below. Ketamine is not expected have an effect, whereas isoflurane is expected to have a vasoconstrictive effect in skeletal muscle. Isoflurane then would be expected to possibly potentiate the possibly high resistance of choke vessels. The effect of isoflurane, however, is not known specifically in regards to choke vessels. Possibly, non-choke vessels vasoconstrict to a greater extent relative to choke vessels when under the effect of isoflurane such that the possibly high resistance choke vessels would now be relatively low resistance. The effect

of isoflurane specifically on choke vessels requires further experimental evaluation.

The vascular resistance per unit weight increases with serial resection of muscle flaps in both the region of choke vessels and non-choke vessels. Possible mechanisms (epinephrine, arrangement of resistance units, autoregulation) are discussed below. This relationship is opposite to that expected as described in the alternative hypothesis. This relationship occurs for both the rectus abdominis and the gracilis and for each of these flaps studied. Thus, choke vessels not being high resistance vessels appears to have face validity. Further studies examining local blood flow distribution with specific delineation of blood flow at the zone of choke vessels are necessary. Preliminary studies using radioactive microspheres and fluorescent microspheres are ongoing at this centre. The results of these studies appear to support the finding that in the operative setting, choke vessels are not high resistance vessels.

4.3 CONTROLS

Control muscle flaps were performed for the rectus abdominis pigs number 1 & 2, and for the gracilis in pigs number 2 & 6. The vascular resistance per unit weight and blood flow per unit weight for these control flaps (Figures 8 & 11) was determined concurrently as the experimental flaps were serially resected. These control flaps demonstrate that in the absence of the experimental procedure, serial resection, vascular resistance per unit weight and blood flow per unit weight are relatively constant. The findings of the experimental flaps (Figures 8 & 11) are thus due to the experimental procedure.

Notably, a control flap is not performed for each experimental flap. The reason for this is that over time these study animals become haemodynamically unstable and would thus possibly lead to invalid findings. The surgical elevation of the muscle flaps is time consuming (up to two hours per flap), and the time required to raise a control flap following the experimental flap could be deleterious to haemodynamic stability. Control rectus abdominis flaps were raised in the first two study animals with careful adjustments of fluids, anaesthetic, and temperature maintenance. The control values for these flaps were found to be stable, and thus in subsequent animals, control flaps were not performed so that the experimental procedure could be performed expediently. The first gracilis study was performed in pig number two, and a control gracilis flap was performed here. As well, a control gracilis flap was performed in pig number 6 to confirm that the values were indeed stable for this muscle type.

The values for the control flaps are presented in graphical form and clearly do not show the trends of the experimental flaps. Statistical comparison of the control with the experimental values was not performed as this is not indicated by the hypothesis as stated. To perform statistical analysis would be an error of multiple comparisons.

4.4 THE ROLE OF CHOKE VESSELS IN DELAY PHENOMENON

The vascular resistance in this current study did not decrease as the choke vessels were resected. The presumption on the basis of delay studies in skin and in muscle (discussed in Background) is that choke vessel dilatation decreases vascular resistance. However, the current study does not support the extension that choke vessels are high resistance units prior to dilatation, at least not in muscle in the immediate period of time following flap elevation. Surgical delay is an important procedure and the current study does not contradict this. The role of choke vessels in the period of time immediately following flap elevation is as yet unclear; however, these vessels do not appear to be high resistance vessels.

4.5 THE ROLE OF NON-CHOKE VESSELS IN DELAY PHENOMENON

The current study suggests that possibly, a high vascular resistance phenomenon does occur; however, not at the zone of choke vessels, but across the whole mass of the muscle flap, such that vascular resistance per unit weight increases with decreasing flap weight. The delay phenomenon may involve dilatation of all flap vessels, non-choke vessels as well as choke vessels, and perhaps the dilatation of non-choke vessels may be the critical event. Perhaps the dilatation of non-choke vessels may be as important as dilatation of choke vessels. The study by Morris and Taylor did indicate that non-choke vessels dilated as well as choke vessels (Morris & Taylor, 1995). As well the studies by Cederna et.al. may support dilatation of non-choke vessels in a role in the delay phenomenon (Cederna et.al. a 1997, b 1997). Cederna et.al. suggest that the increase

in number of "choke vessels" may be due to dilatation such that non observable vessels become observable. Other studies implicate blood vessel dilatation rather than angiogenesis as the possible factor in increasing the capillary blood flow associated with the delay phenomenon.

Pang et.al. used radioactive microspheres to quantify blood flow in pig flank random skin flaps (Pang et.al. 1986). Fifteen animals were delayed for 2, 3, 4, 6 or 14 days. Blood flow increased by 100% between days 2 and 3, and then was unchanged from day 4 to 14. Blood vessel density (arteries/mm square) was unchanged over the 14 days suggesting that vasodilatation rather than angiogenesis lead to the increase blood flow.

Boyd et.al. drew similar conclusion in a radioactive microspheres study of blood flow in a pig musculocutaneous flap (Boyd et.al. 1990). The well known clinical and experimental finding of flap failure in the vascular territory distal to the vascular territory receiving the vascular pedicle in the non-delay setting may simply be due to high vascular resistance in the distal vascular territory, and not to presumed high vascular resistance at the zone of choke vessels. The delay procedure may be an effective surgical procedure due to dilating high vascular resistance vessels in the distal vascular territory rather than dilating choke vessels.

A relationship of increase vascular resistance per unit weight with decreasing flap weight in the intraoperative period is shown in this study. The increase vascular resistance in the non-choke vessels may be the deleterious factor in some cases of flap failure (occurring postoperatively). The delay phenomenon may be associated with vasodilatation of the non-choke

vessels decreasing their vascular resistance. The role of choke vessels remains unknown. Possible mechanisms whereby increase vascular resistance per unit weight with decreasing flap weight occurs are discussed below. These mechanisms involve the effect of norepinephrine, arrangement of resistance units, and autoregulatory process.

4.6 NOREPINEPHRINE

The relationship of increasing resistance per unit weight with decreasing flap weight may be due to norepinephrine. Hendel et.al. performed a series of experiments studying the effects of sympathetic and vasodilator drugs on rat acute skin flaps (Hendel a, 1983). Their findings regarding acute flaps included increased vessel tone, greater resistance to vasodilator drugs, and hypersensitivity to alpha-agonist vasoconstrictors. A naturally occurring vasoconstrictor confined to the flap was implicated. Hendel et.al performed a series of further experiments (Hendel b, 1983). These findings showed that preoperative chemical destruction of sympathetic terminals eliminated the hypersensitivity of acute flaps to alpha-agonist vasoconstrictors. Also, they showed by radioimmunoassay that the catecholamine contents of flaps falls dramatically during the delay period. They hypothesized that in the acute flaps, sympathetic nerve terminals are partially loaded, lowering the threshold to alpha-agonists and increasing vascular tone. Then with delay, the catecholamines decrease, decreasing the vascular tone.

Pearl in 1981 and Jurell in 1986 published studies supporting the role of norepinephrine in acute and delay flaps (Pearl, 1981)(Jurell, 1986). Their studies implicated release of

norepinephrine, from the sympathetic nerve destruction associated with surgical trauma, in the acute flap resulting in vasoconstriction. Also, depletion of norepinephrine occurs in the delay flap with small or nonexistent vasoconstriction.

The increase vascular resistance seen in this study may be due to norepinephrine. With serial resection of the flaps, an additive increase in norepinephrine may cause the increasing vascular resistance per unit weight with decreasing flap weight. In this study, surgical sympathectomy was carried out to reduce extrinsic sympathetic activity effects on the flaps. A potential study to test this hypothesis would be to preoperatively block sympathetic nerve terminal and perform the experiment as before.

4.7 ARRANGEMENT OF RESISTANCE UNITS

The relationship of increasing vascular resistance per unit weight with decreasing flap weight may conceptually be due to the organization of the sites of resistance, "resistance units," in the muscle flap. Bjornberg et.al. performed arterial and venous microcannulation studies in the cat gastrocnemius muscle (Bjornberg, 1988). Their studies allowed determination of segmental resistance in defined sections of the vascular bed. These sections were large arterial vessels (> 25 um), arterioles (< 25 um), and the venous side. The site of resistance was determined to be occurring at the arterioles. In fact the resistance at the arterioles was found to be dynamic and increased with increase in mean arterial pressure in an autoregulatory fashion. The possibility of an autoregulatory process is discussed below; however, for the purposes of this

current discussion we compare the concepts of resistance units arranged in series fashion versus parallel fashion.

If the arrangement of resistance units in a muscle flap were in series, sequential resection of muscle flap, and thus resistance units, would result in a decrease in total vascular resistance across the muscle flap. If the arrangement of resistance units in a muscle flap were in parallel, sequential resection of muscle flap, and thus resistance units, would result in an increase in total vascular resistance across the muscle flap. Thus, the concept of parallel arrangement of resistance units in a muscle flap would be in keeping with the finding of this study, increasing vascular resistance per unit weight with decreasing muscle flap weight.

In order to evaluate the hypothesis that resistance units are arranged in parallel as opposed to series, requires further study involving mathematical modelling. However, this hypothesis would rely on the "resistance units" being relatively static in nature, with a constant level of resistance. The study by Bjornberg et.al. has demonstrated that sites of resistance are actually dynamic, varying directly with mean arterial pressure. Also, Borgstrom and Gestrelius have successfully tested *in vivo* a mathematical model regarding vascular tone in skeletal muscle. Their mathematical model assumed that resistance in muscle was organized in series. The organization of "resistance units" may not be as simplistic as the concept of static "resistance units" in either series arrangement or parallel arrangement; however, this concept should not currently be discounted.

4.8 AUTOREGULATION

An autoregulatory process may explain the current study findings, increase vascular resistance per unit weight with decreasing flap weight. Autoregulation of blood flow in tissue is described in the basic science literature as a combination of myogenic response, flow-induced vasodilation, metabolic control, and neural control. Myogenic activity has been a clinical concept in hypertension, and in control of intracranial pressure. Autoregulation, to my knowledge, has not been attributed to having a clinical role in muscle flaps.

The goal of autoregulation is to optimize the factors in Starling's law of ultrafiltration governing fluid bulk flow movement. Starling's law of ultrafiltration is given by:

$$FM = K \times [(Pc + pii) - (Pi + pic)]$$

FM (ml/time/unit pressure) is the net fluid motion, K is the filtration coefficient of the capillary wall, Pc is the hydrostatic pressure in the capillary, pii is the oncotic pressure in the interstitial fluid, Pi is the hydrostatic pressure in the interstitial fluid, and pic is the oncotic pressure of the plasma in the capillary. Autoregulation is a normal physiological process and may be reasonably expected to occur in muscle flaps. This will be discussed below.

4.8.1 Myogenic response - The myogenic theory was originally described by Bayliss in 1902, and revived by Folkow in 1949 (Bayliss, 1902)(Folkow, 1949). The theory is that intravascular blood pressure, via distension of the blood vessel, results in contraction of vascular smooth muscle contributing to vascular tone and to autoregulatory phenomena. Basic science studies on the myogenic response are well reported in the literature (Grande a, 1979)(Borgstrom, 1987)(Mellander, 1989). Grande reported on the myogenic response in cat gastrocnemius muscle (Grande b, 1989). The muscle was sympathectomized and isolated from the body, placed in a hermetically sealed temperature-controlled plethysmograph, and perfused via a femoral to popliteal arterial shunt, and a popliteal to external jugular venous shunt. The vascular resistance was determined for three consecutive vascular segments (proximal arterial vessels ($>$ 25 um), microvessels ($<$ 25 um), and veins) with changes to transmural pressure. Vasoconstriction was induced by increase transmural pressure changes almost entirely at the level of the microvessels.

Current understanding of myogenic activity was reviewed by D'Angelo and Meininger (D'Angelo and Meininger, 1994). Multiple transduction pathways are implicated in myogenic activity including involvement of stretch activated channels, voltage-dependent calcium channels, second messengers. Stretch of smooth muscle cells results in cell membrane deformation activating stretch activated channels. These nonspecific cation channels in the cell membrane allow inward current of sodium eliciting membrane depolarization resulting in opening of voltage-dependent calcium channels in the cell membrane. The ensuing influx of calcium results in an enzymatic cascade activating myosin light chain kinase which phosphorylates myosin light chain

and accelerates myosin ATPase activity. This allows crossbridge cycling between myosin and actin resulting in muscle shortening. Calcium-activated potassium channels in the cell membrane appear to provide negative feedback. With the influx of calcium, calcium-activated potassium channels open and allow efflux of potassium. This hyperpolarizes the cell membrane and opposes the membrane depolarization and influx of calcium.

D'Angelo and Meininger also discussed the role of second messenger systems in myogenic activity. G protein activation (Gi and Gq) are implicated. Activation of Gi protein result in inhibition of cAMP production of adenylate cyclase. This removes the vasodilator response of this cyclic nucleotide. Gq protein activation is coupled to phospholipase C (PLC). Stimulation of PLC catalyses the hydrolysis of phosphatidylinositol 4,5-biphosphate (PIP2) into inositol 1,4,5-triphosphate (IP3) and diacylglycerol (DAG). IP3 causes the release of intracellular calcium stores into the cytoplasm and contributes to the increase calcium due to stretch activated channels and voltage-dependent calcium channels. DAG is an endogenous activator of protein kinase C (PKC). PKC may sensitize contractile proteins to calcium so that the myogenic response can be maintained at a lower calcium concentration.

The literature regarding flow-induced, metabolic, and neural control in autoregulation is vast and often contradictory. The salient features are described only briefly.

4.8.2 Flow-induced vasodilation - Flow-induced vasodilation was demonstrated by Smiesko et.al. (Smiesko, 1989). They reported dilation of rat mesentery arterioles when blood flow was

increased. The dilation was graded according to the magnitude of the flow increase. Flow-induced vasodilation has been demonstrated in skeletal muscle by Koller and Kaley (Koller and Kaley a, 1989). The mechanism of flow-induced vasodilation involves the endothelial cell monolayer that sits at the interface between the smooth muscle cells and the blood flowing through the vessel lumen. Koller et.al. impaired the endothelium by light treatment which resulted in inhibition of arteriolar dilation by an increase in blood flow (Koller b, 1989). Flow-induced vasodilation was reviewed by Bevan and by Segal (Bevan, 1991)(Segal, 1994). Shear stress by blood flow on endothelial cells results mechanical deformation of the endothelial cell, and then by a signal transduction mechanism results in release endothelium-derived relaxing factors. This transduction mechanism may involve distortion of glycocalyx or integrins within the cell membrane. Nitric oxide or related compounds, prostaglandins, and reactive oxygen species have been implicated as endothelium-derived relaxing factors.

4.8.3 Metabolic control - The mechanism of metabolic control in autoregulation of blood flow is as yet unclear. This mechanism couples tissue metabolism with blood flow. Mohrman reviewed the metabolic control and indicated that several metabolic factors within tissue act to produce metabolic vasodilation (Mohrman, 1991). PO2, PCO2 and/or pH, and adenosine are believed to be important factors. With increased metabolic activity, oxygen consumption increases resulting in increase oxygen extraction an low PO2. Low PO2 is felt to attenuate the vasoconstrictive effect of the myogenic response. Adenosine is released during periods of inadequate oxygen supply an is a potent vasodilator substance. CO2 has a major role in regulation of cerebral circulation an a less obvious role in other tissues. CO2 does have an effect in skeletal

muscle and cardiac tissue. Hypercarbia is associated with a dilator response. CO2 effects may be mediated by changes in pH.

4.8.4 Neural control - Neural control of blood flow was reviewed by Neild and Brayden, by Johnson and Smiesko, and by Segal (Neild and Brayden, 1991)(Johnson and Smiesko, 1995)(Segal, 1994). Vascular beds receive innervation from the sympathetic nervous system. Stimulation of sympathetic nerves releases norepinephrine and ATP which act on adrenoceptors and P2 purinoceptors of vascular smooth muscle to cause vasoconstriction. With high frequencies of sympathetic nerve stimulation (above 10 Hz), neuropeptide Y is released and enhances vasoconstriction. Activation of adrenergic receptors has been shown to enhance myogenic vasoconstriction. Catecholamines have been found to impair endothelium-derived relaxing factors. Neural control of blood flow has been demonstrated in muscle, for example rat cremaster and cat sartorius.

4.8.5 Integration of autoregulatory processes - An integration of autoregulatory factors has been proposed by Segal (Segal, 1994). Blood pressure and blood flow act as opposing stimuli in producing vasomotor tone. Increase in transmural pressure induces the myogenic response such that vasoconstriction occurs. Increase blood flow induces shear stress and the release of endothelium-derived relaxing factors leading to vasodilatation. Metabolic and neural factors modulate the balance between myogenic vasoconstriction and flow-induced vasodilatation.

As mentioned above, autoregulation may reasonably be expected to occur in muscle flaps.

The relationship of increasing vascular resistance per unit weight with decreasing flap weight could possibly be due to myogenic activity. With serial resection of muscle flap, the interrelated parameters of transmural pressure, local blood flow and segmental vascular resistance within the muscle bulk are altered. These parameters at the tissue level are not determined in this study. The progressive induction of the myogenic response would be consistent with the increasing vascular resistance per unit weight with serial resection of muscle flap. This myogenic response would have to predominate over the other autoregulatory processes, flow-induced dilation, metabolic control, neural control (muscle flaps were sympathectomized), in order to produce the observed response.

4.9 INTERPRETATION OF THE DIFFERENCE IN BLOOD FLOW BETWEEN THE RECTUS ABDOMINIS AND GRACILIS

The blood flow per unit weight is altered in both the rectus abdominis and the gracilis flaps in this current study. The blood flow per unit weight increases with serial resection in the rectus abdominis flaps and decreases with serial resection in the gracilis flaps. With the epinephrine mechanism discussed above, the blood flow per unit weight would be expected to decrease with serial resection for both the rectus abdominis and the gracilis. Thus, the epinephrine mechanism does not appear to have a role. Possibly, the arrangement of resistance units in either series or parallel may be a factor in the difference in blood flow per unit weight, taking into account that the rectus abdominis flaps were resected perpendicular to the line between vascular pedicles, and the gracilis flaps were resected parallel to the line between vascular pedicles. The concept of

resistance units arranged in series or in parallel is discussed above and is not further considered here.

The difference in blood flow per unit weight between the rectus abdominis and the gracilis flaps may be evidence of appropriate autoregulation on the basis of difference in muscle type. The rectus abdominis is a muscle of posture which generally requires a satisfactory blood supply for aerobic metabolism. The gracilis is a muscle of locomotion which has greater anaerobic capacity and require a lesser blood supply. Thus, if autoregulation is appropriate, the rectus abdominis should receive a greater blood supply and the gracilis should receive a lesser blood supply.

Possibly, the difference in blood flow per unit weight between the rectus abdominis and the gracilis reflects an inappropriate autoregulatory response. The normal physiological autoregulatory process may be unable to adapt in the non-physiological setting of muscle flaps. With each serial clamping of muscle in the current study, less muscle receives perfusion (less muscle weight receives blood flow). Initially, the quotient blood flow per unit weight increases. Autoregulatory should be such that blood flow per unit weight returns to the original level. Perhaps, the autoregulatory response in the gracilis is inappropriate by overcompensating and reduces the blood flow per unit weight excessively below the original blood flow per unit weight. Perhaps, the autoregulatory response in the rectus abdominis is inappropriate by undercompensating and leaves the blood flow per unit weight elevated above the original blood flow per unit weight.

Among the autoregulatory processes, the myogenic response appears to have a leading role in the muscle flaps of this current study as vasoconstriction appears to be occurring with serial resection of muscle. The myogenic response in muscle flaps almost certainly occurs as those basic science experiments previously performed, for example by Grande, actually resemble muscles in a flap situation (Grande b, 1989). Studies similar to that of Grande would be a logical next step in order to demonstrate that the myogenic response is the factor causing increase vascular resistance per unit weight with decreasing flap weight. Muscles would be sympathectomized and isolated from the body, placed in a hermetically sealed temperature-controlled plethysmograph, and perfused via arterial and venous shunts. The vascular resistance would then be determined by microcannulation for three consecutive vascular segments (proximal arterial vessels ($>$ 25 um), microvessels ($<$ 25 um), and veins) as the muscle was serially resected. The possibility of microcannulation is discussed below.

Possible mechanisms of the relationship of increasing vascular resistance per unit weight with decreasing flap weight is discussed above. These mechanisms involve the effect of norepinephrine, the arrangement of resistance units, and the autoregulatory process. Autoregulation by myogenic constriction has not previously been described in the clinical situation of muscle flaps. Each of the mechanisms deserves consideration. Potential studies were described above.

4.10 PRESSURE DETERMINATION

As indicated above a microcannulation technique is known. This current study cannulates the common carotid and the internal jugular. Microcannulation of blood vessels would have been the ideal technique. For several reasons, microcannulation was not performed. The expertise required for microcannulation of blood vessels was not available. The experimental procedure requires repeated gross manipulation of the muscle flap making the delicate microcannulation of vessels unlikely to be satisfactory. The pressure gradient across the muscle flap determined by large vessel cannulation is not the gradient that would have been determined by microcannulation. Fronek and Zweifach showed that microvascular pressure in arterioles 70 um or larger was proportional to systemic arterial pressure (Fronek and Zweifach, 1975). The pressure in vessels 70 - 100 um was 90 - 95 percent of systemic blood pressure. Venular pressure was also proportional to central vein pressure. Thus, for this current study, the pressure gradient used in calculations is greater than the true value so that the calculated resistance is also greater than the true value. The calculated resistance and its trend are still valid however, as the systemic pressures are proportional to the small vessel pressures.

4.11 ANAESTHESIA

This study takes place in a defined setting: the muscle flaps studied are acutely raised with the animal under the influence of general anaesthetics. This is a study of physiology in the acute surgical situation. The results of this study do not necessarily pertain to muscle flaps that have

recovered from operative manipulation.

Ketamine was used as an induction agent. This drug was studied in the dog by Haskins et.al. (Haskins, 1985). Cardiopulmonary parameters as well as muscle tone had returned to baseline values 60 minutes following ketamine administration. Data procurement in the current study occurred at least some 5 hours following ketamine induction, and thus ketamine is unlikely to have had an effect on data values.

Isoflurane was used as the maintenance agent. Sigurdsson et.al. compared the effect of halothane with isoflurane anaesthesia on musculocutaneous flaps in minipigs (Sigurdsson, 1994). Microcirculatory blood flow was well maintained in musculocutaneous flaps with both halothane and isoflurane anaesthesia during normovolemic conditions. During mild to moderate hypovolemia, microcirculatory blood flow decreased markedly with halothane anaesthesia while it remained unchanged with isoflurane. Thus, isoflurane anaesthesia is the preferable anaesthetic for flap studies. Lundeen et.al. studied the effect of isoflurane on various tissues (Lundeen, 1983). They found that for skeletal muscle the awake blood flow was 0.153 ml/min/g, and decreased to 0.120 ml/min/g under 1.0 MAC isoflurane and decreased to 0.066 ml/min/g under 1.5 MAC isoflurane anaesthesia. Isoflurane thus has an effect on the blood flow in muscle. The evaluation of the results of this study need to be considered in the light that the data was obtained under isoflurane anaesthetic. Further studies are necessary to determine if the results found in this study are maintained in the post-operative period.

4.12 CLINICAL CORRELATION AND IMPLICATIONS

The finding of increasing vascular resistance per unit weight with decreasing % total weight supports the clinical finding of the failure prone macro-micro gracilis flap. The clinical experience at the University of British Columbia in regards to the macro-micro free tissue transfer is that among 33 flaps (4 gracilis, 27 latissimus dorsi, 1 rectus abdominis, 1 iliac crest), 7 failures (3 (75%) gracilis and 4 (15%) latissimus dorsi flaps) occurred (Williamson, 1996). While the causes for the 4 latissimus dorsi flap failures was evident (blood vessel compression, septic wound, small recipient vessels, long vein graft), the causes for the gracilis flap failures was not. Evaluation by vascular surgery implicated inadequate blood flow to maintain the bridging grafts patent in the gracilis transfers. A further clinical study was performed to elucidate the high rate of failure of the macro-micro gracilis transfer. Intraoperative measurements were used to determine the vascular resistance of, to-date, 5 gracilis, 3 rectus abdominis, and 1 latissimus dorsi flaps prior to pedicle division for free transfer. The vascular resistance of the gracilis flap was 2.6 +/- 1.5 (mmHg/cc/min)/100gm, of the rectus abdominis flap was 1.3 +/- 1.2 (mmHg/cc/min)/100gm, and of the latissimus dorsi flap was 0.9 (mmHg/cc/min)/100gm. The human gracilis flap appears to have a higher vascular resistance per unit weight than the rectus abdominis muscle. The relationship demonstrated in this pig study, increasing vascular resistance per unit weight with decreasing % total weight, supports the previous clinical findings, as the gracilis muscle has a smaller weight than the rectus abdominis muscle (in both the human and the pig). The higher vascular resistance per unit weight in the gracilis flap may have a role in the cause of failures in the macro-micro gracilis free tissue transfer. Local flaps are expected to be

robust, and the usual micro-micro free flap less so. The macro-micro flaps may be expected to be the most tenuous of all with factors such as a poorer patient health, recipient site morbidity, and the intervening venous graft itself. While the phenomenon of vascular resistance per unit weight with decreasing % total weight was demonstrated in this study on a local flap; such local flaps are expected to be robust. When this phenomenon occurs in the tenuous macro-micro free flap, this increasing vascular resistance may be a crucial factor among events leading to thrombosis and flap failure.

CHAPTER 5: CONCLUSIONS

The findings of this study do not allow rejection of the null hypothesis. The surgical resection of choke vessels did not result in a statistically significant decrease in muscle flap vascular resistance per unit weight. The power of this study was 0.98, and so this finding does not appear to be false negative. Choke vessels do not appear to have high vascular resistance, at least not in the intraoperative period. The role of choke vessels in failure of muscle flaps remains unclear.

The presumption that choke vessels have high vascular resistance is based on delay studies in both skin and muscle. Choke vessels dilate over the period of the delay procedure resulting in increase blood flow and improved flap survival. The reasoning is that the choke vessels are of high resistance prior to their dilation. This current study raises the possibility that dilation of non-choke vessels may be critical in the success of a delay procedure.

Vascular resistance per unit weight was found to increase as muscle flaps were serially resected. Possible mechanisms for this include the effect of norepinephrine, the arrangement of units of vascular resistance, and autoregulation processes (myogenic response, flow-induced vasodilation, metabolic control, neural control). In particular, the myogenic response may be an important mechanism in the control of blood flow in muscle flaps. The myogenic response has not previously been described as pertaining to muscle flaps.

This study directs further study into the distribution of regional blood flow within muscle flaps in the intraoperative period and delay period. The role of choke and non-choke vessels in delay and in flap failure require consideration. The role of epinephrine, arrangement of vascular resistance units, and autoregulation processes require further study. The myogenic response in particular may have clinical implications in muscle flap failure and certainly warrants consideration.

REFERENCES

Bayliss WM. On the local reactions of the arterial wall to changes of internal pressure. J Physiol (Lond) 28:230-41;1902.

Bevan JA. Flow-dependent vascular tone. In The resistance vasculature. Bevan JA et.al. eds. Humana Press, New Jersey 169-191;1991.

Bjornberg J, Grande PO, Maspers M, Mellander S. Site of autoregulatory reactions in the vascular bed of cat skeletal muscle as determined with a new technique for segmental vascular resistance recordings. Acta Physiol Scand 133:199-210;1988.

Borgstrom P, Gestrelius S. Integrated myogenic and metabolic control of vascular tone in skeletal muscle during autoregulation of blood flow. Microvasc Res 33:353-76;1987.

Boyd JB, Markland B, Dorion D, Pang CY, Morris S. Surgical augmentation of skin blood flow and viability in a pig musculocutaneous flap model. Plast Reconst Surg 86:731-8;1990.

Callegari PR, Taylor GI, Caddy CM, Minabe T. An anatomic review of the delay phenomenon: I. Experimental studies. Plast Reconst Surg 89:397-407;1992.

a. Cederna PS, Chang P, Pittet-Cuenod BM, et.al. The effect of the delay phenomenon on the vascularity of rabbit abdominal cutaneous island flaps. Plast Reconst Surg 99:183-93;1997.

b. Cederna PS, Chang P, Pittet-Cuenod BM, et.al. The effect of the delay phenomenon on the vascularity of the rabbit rectus abdominis muscles. Plast Reconst Surg 99:194-205;1997.

D'Angelo GD, Meininger GA. Transduction mechanisms involved in the regulation of myogenic activity. Hypertension 23[part 2]:1096-1105;1994.

Folkow B. Intravascular pressure as a factor regulating the tone of the small vessels. Acta Physiol Scand 17:289-310;1949.

Fronek K, Zweifech BW. Microvascular pressure distribution in skeletal muscle and the effect of vasodilation. Am J Phsyiol 228:791-6:1975.

German W, Finesilver EM, Davis JS. Establishment of circulation in tubed skin flaps. Arch Surg 26:27,1933.

Grande PO, Borgstrom P, Mellander S. On the nature of basal vascular tone in cat skeletal muscle and its dependence on transmural pressure stimuli. Acta Physiol Scand 107:365-76:1979.

Grande PO. Myogenic mechanisms in the skeletal muscle circulation. J Hypertension 7(suppl 4):S47-S53;1989.

Guba AM, Jr. Arteriovenous shunting in the pig. Plast Reconst Surg 65(3):323-7;1980.

Harashina T. Analysis of 200 free flaps. Br J Plast Surg 41:33-6;1988.

Haskins SC, Farver TB, Patz JD. Ketamine in dogs. Am J Vet Res 46:185560;1985.

a. Heden P, Sollevi A, Hamberger B. Early circulatory and metabolic events in island skin flaps of the pig. Plast Reconst Surg 84(3):468-74;1989.

b. Heden P, Sollevi A. Circulatory and metabolic events in pig island skin flaps after arterial or venous occlusion. Plast Reconst Surg 84(3):475-83;1989.

a. Hendel PM, Lilien DL, Buncke HJ. A study of the pharmacologic control of blood flow to acute skin flaps using xenon washout. Part I. Plast Reconst Surg 71:387-98;1983.

b. Hendel PM, Lilien DL, Buncke HJ. A study of the pharmacologic control of blood flow to delayed skin flaps using xenon washout. Part II. Plast Reconst Surg 71:399-410;1983.

Hidalgo, DA, Jones, CS. The role of emergent exploration in free-tissue transfer: A review of 150 consecutive cases. Plast Reconst Surg 86:492-8;1990.

Hjortdal VE, Hansen ES, Kjolseth D, et.al. Arteriovenous shunting and regional blood flow in myocutaneous island flaps: An experimental study in pigs. Plast Reconst Surg 87(2):326-334;1991.

Hjortdal VE, Hansen ES, Henriksen TB, et.al. The microcirculation of myocutaneous island flaps in pigs studied with radioactive blood volume tracers and microspheres of different sizes. Plast Reconst Surg 89:116-22;1992.

Irons GB, Wood MB, Schmitt EH. Experience with one hundred consecutive free flaps. Ann Plast Surg 18:17-23;1987.

Johnson PC, Smiesko V. Overview of regulatory mechanisms in the microcirculation. In Flow-dependent regulation of vascular function. ed Bevan JA. Oxford University Press, New York 261-73;1995.

Jurell G. Adrenergic nerves and the delay phenomenon. Ann Plast Surg 17:493-6;1986.

Kerrigan CL, Zelt RG, Daniel RK. Secondary critical ischemia time of experimental skin flaps. Plast Reconst Surg 74(4):522-4;1984.

Kerrigan CL, Zelt RG, Thomson JG, Diano E. The pig as an experimental animal in plastic surgery research for the study of skin flaps, myocutaneous flaps and fasciocutaneous flaps. Lab An Sci 36(4):408-12;1996.

Khouri RK. Avoiding free flap failure. Clin Plast Surg 19:773-81,1992.

a. Koller A, Kaley G. Flow velocity-dependent regulation of microvascular resistance in vivo. Microcirc. Endothelium and Lymphatics 5:519-30;1989.

b. Koller A, Messina EJ, Wolin MS, Kaley G. Effects of endothelial impairment on arteriolar dilator responses in vivo. Am J Physiol 257:H1485-H1489;1989.

Lundeen G, Manohar M, Parks C. Systemic distribution of blood flow in swine while awake and during 1.0 and 1.5 MAC isoflurane anaesthesia with or without 50% nitrous oxide. Anest Analg 62:499-512;1983.

Mathes S, Nahai F. Classification of the vascular anatomy of muscles: Experimental and clinical correlation. Plast and Reconst Surg 67:177-87;1981.

Mellander S. Functional aspects of myogenic vascular control. J Hypertension 7(suppl 4):S21-S30;1989.

Millican PG, Poole MD. A pig model for investigation of muscle and musculocutaneous flaps. Br J Plast Surg 38:364-8;1985.

Mohrman DE. Local metabolic influences on resistance vessels. In The resistance vasculature. Bevan JA et.al. eds. Humana Press, New Jersey 241-249;1991.

Morris SF, Taylor GI. The time sequence of the delay phenomenon: When is a surgical delay effective? An experimental study. Plast Reconst Surg 95:526-33;1995.

Neild TO, Brayden JE. Neural control of resistance arteries. In The resistance vasculature. Bevan JA et.al. eds. Humana Press, New Jersey 217-240;1991.

Pang CY, Forrest CR, Neligan PC, Lindsay WK. Augmentation of blood flow in delayed random skin flaps in the pig: Effect of length of delay period and angiogenesis. Plast Reconst Surg 78:68-74;1986.

Pang CY, Neligan P, Nakatsuka T. Assessment of microsphere technique for measurement of capillary blood flow in random skin flaps in pigs. Plast Reconst Surg 74:513-21;1984.

Pang CY, Neligan PC, Nakatsuka T, Sasaki GH. Pharmacologic manipulation of the microcirculation in cutaneous and myocutaneous flaps in pigs. Clin Plast Surg 12:173-84;1985.

Pearl RM. A unifying theory of the delay phenomenon: Recovery from the hyperadrenergic stat. Ann Plast Surg 7:102-12;1981.

Pounder DJ, Rowland R, Pietrerse AS, et.al. Angiodysplasia of the colon. J Clin Pathol 35:824-9;1982.

Segal SS. Cell-to-cell communication coordinates blood flow control. Hypertension 23(part 2):1113-1120;1994.

Shaw WW. Microvascular free flaps: Survival, donor sites, and applications. In Buncke, HJ, Furna, D (eds): Symposium on clinical frontiers in reconstructive microsurgery. St. Louis, CV Mosby, 1984, 3-10.

Sigurdsson GH, Banic A, Wheatley AM, Mettler D. Effects of halothane and isoflurane anaesthesia on microcirculatory blood flow in musculocutaneous flaps. Br J Anaesth 73:826-32;1994.

Smiesko V, Lang DJ, Johnson PC. Dilator response of rat mesenteric arcading arterioles to increased blood flow velocity. Am J Phsyiol 257:H1958-65;1989.

Taylor GI, Razaboni RM. Michael Salmon - Anatomic Studies. Quality Medical Publishing, Inc. St. Louis, 1994.

Taylor GI, Minabe T. The angiosomes of the mammals and other vertebrates. Plast Reconst Surg 89(2):181-215;1992.

Taylor GI, Palmer JH. The vascular territories (angiosomes) of the body; Experimental study and clinical applications. Br J Plast Surg 40:113;1987.

Tsai TM, Bennet DL, Pederson WC, Maitko J. Complications and vascular salvage of free-tissue transfers to the extremities. Plast Reconst Surg 82:1022-6;1988.

Urken ML, Weinberg H, Buchbinder D, et.al.. Microvascular free flaps in head and neck reconstruction. Arch Otolaryngol Head Neck Surg 120:633-40;1994.

Whitney TM, Buncke HJ, Alpert BS, et.al.. The serratus anterior free-muscle flap: Experience with 100 consecutive cases. Plast Reconst Surg 86:481-90;1990.

Williamson JS, Carr NJ, Lennox PA, Chao HYM, Courtemanche DJ. Muscle flaps - Vascular types, territories and resistance. Is there a physiological and clinical correlation ? Abst. Can J Plast Surg 4(1):17,1996.