# ADRENERGIC REGULATION OF LIPID MOBILIZATION IN OVINE ADIPOSE TISSUE: REGIONAL VARIATION IN SENSITIVITY AND RESPONSIVENESS

BY

TRACY L. GILSON

A Thesis Submitted to the Faculty
of Graduate Studies in Partial Fulfillment
of the Requirements for the Degree of

DOCTOR OF PHILOSOPHY

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# ABSTRACT

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Investigations have been conducted to examine the interplay of α- and ß-adrenergic effects on ovine adipose tissue from subcutaneous (SC) and omental depots from Outaouais (OTA) and SC, omental and tail depots from the Karakul fat-tailed breed. Lipolytic properties of the adipose tissue were examined *in vitro* with isoproterenol (ß agonist), clonidine (α agonist), epinephrine (α and ß agonist), and epinephrine plus propranolol (ß-adrenergic blocker). All treatments contained adenosine deaminase (ADA) to prevent adenosine accumulation during incubation. Maximal lipolytic response ($\mu$mol/$10^6$cells/3 h) to isoproterenol stimulation was greatest in the Karakul omental (11.1) and SC (10.2), intermediate in OTA omental (8.8) and SC (8.3), and lowest in the tail (5.5) tissue (S.E.=0.64). A similar comparison with epinephrine was Karakul omental (11.9) > Karakul SC (9.4) = OTA omental (9.8) > OTA SC (7.1) > Karakul tail (5.2) tissue (S.E.=0.64). Depot-specific differences in maximal response

within a breed that were found with epinephrine, but which were not detected with isoproterenol, were not due to an increased α-mediated effect in the SC tissue. No clonidine-induced antilipolytic effect was found in any depot and the α-mediated response to epinephrine plus propranolol was not detected in the OTA omental and SC depots. A 52 and 77% α-induced inhibition was detected in the Karakul omental tissue ($p=0.05$) and tail ($p=0.1$) tissue, respectively. The magnitude of inhibition in the Karakul SC tissue was similar to that found in the Karakul omental tissue, but the effect was not significant. Karakul adipose tissue was more sensitive to ß-adrenergic stimulation than OTA SC tissue ($p<0.05$) and OTA omental ($p<0.1$) tissue. Tail adipose tissue displayed the lowest lipolytic response to ß-adrenergic stimulation and the greatest lipolytic response to adenosine removal. Isolated perfusion studies of the tail adipose mass also revealed its reduced lipolytic potential and suggested that endogenous antilipolytic agents play an important role in the regulation of lipid metabolism in this depot. The vasoconstrictive properties of the tail were more responsive to norepinephrine than were the lipolytic properties, and the vasoconstrictive response was desensitized by prolonged exposure to clonidine. The Karakul sheep displays a dramatic heterogeneity in adipose tissue response to adrenergic stimulation compared to a non-fat-tail breed.

# FOREWARD

This thesis is written in manuscript style. The first manuscript will be submitted to the "Journal of Physiology". The second manuscript will be submitted to "Comparative Biochemistry and Physiology" and the third manuscript will be submitted to the "International Journal of Biochemistry".

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# GENERAL INTRODUCTION

Adipose tissue, once thought to be static or inert, is now recognized as a highly dynamic tissue that is subject to hormonal regulation (Wertherimer and Shapiro, 1948). Adipose tissue is comprised of clusters of adipocytes, whose role is to store excess energy in the form of triglycerides and to release the stored lipid into the circulation in the form of free fatty acids and glycerol.

Although adipose tissue serves a necessary function, excessive fat accretion is of interest and concern in the production of domestic meat animals. The goal of the producer is to generate a product that is high in protein while reducing the fat proportion without impairing the quality and consumer acceptabilty of the commodity (Reeds, 1991). Recently, attempts to achieve this goal have involved the use of $\beta$-adrenergic agonists or repartitioning agents. These synthetic agents are designed to simulate the activities of the endogenous catecholamines, epinephrine and norepinephrine, on adipose tissue. The success of these agents has been variable and their use has been accepted in varying degrees by consumers.

To more effectively accomplish the goal of reduced fat accretion more information is needed with respect to adipose

tissue function and the role that catecholamines play. The endogenous catecholamines have a broad spectrum of physiological actions both in the regulation of cellular mechanisms and blood flow to organs and tissues, and are thought to be the primary regulators of adipose tissue metabolism (Arner, 1992; Fain & Garcia-Sainz, 1983).

The purpose of this study was to examine the effects of catecholamines on adipose tissue, including the variation in response depending on the anatomical location of the adipose mass. The greater our knowledge of adipose tissue function from the major depots of the animal the greater the number of options available in our attempts to manipulate the composition of the final product.

# LITERATURE REVIEW

## 1.0 Adipose Tissue

Adipose tissue serves as an energy storage organ. The high energetic efficiency of fat as a storage form makes it the preferred source over carbohydrate or protein (van Es, 1977). White adipose tissue is comprised of clusters of specialized spherical cells which contain lipid in the form of triglycerides. Clusters of adipocytes, held together by a collagen matrix, form entire adipose depots, which are associated with muscles, major nerves, blood vessels and organs. The size of an adipose mass is dependent on the number and size of the adipocytes within the depot. During conditions of positive energy the volume of the adipocyte will increase. The size or volume of an individual adipocyte is able to fluctuate more than the actual number of adipocytes within a depot (Hirsch et al., 1989). The cellular contents form a droplet of lipid, which restricts the cytoplasm containing the nucleus and organelles to the peripheral portion of the cell. A mature adipocyte does not replicate, but new adipocytes are formed from precursor cells called preadipocytes or adipoblasts (Van, 1985). In cases of prolonged overfeeding or extended periods of positive energy balance, new adipocytes will be formed from

preadipocyte proliferation (Hirsch et al., 1989; Sypniewska & Bjorntorp, 1987).

The adipocytes possess a supply of nerves and a capillary network, which provide the service to regulate the functions of the cell (Fredholm, 1985; Rosell & Belfrage, 1979; Weiss & Maickel, 1965). The net effect of the specialized functions of lipid synthesis, storage and mobilization are responsible for the resulting size of each adipose mass. To better understand the factors that regulate lipid metabolism it is necessary to have an appreciation of the role adipose tissue and the precursors of fatty acid synthesis have in lipid metabolism.

## 1.1 Methods of Investigation

The desired result of investigations is to propose the mechanisms that function *in vivo*; however, it is often technically difficult or the degree of control is not met by using an *in vivo* preparation. In attempts to accomplish the desired goals it is often necessary to test hypotheses in a situation removed from the live animal. Over the past decades methods have been established that provide the environment for testing hypotheses while allowing guarded extrapolation to the live animal.

The perfusion system is the most technically difficult and requires an adipose mass that lends itself to circulatory isolation. For example, the rat parametrial (Robert & Scow, 1963) or epididymal (Ho & Meng, 1964) fat

pads and the fat-tail depot of the Karakul sheep (Gooden et al., 1986; Khachadurian et al., 1966) have been employed. The data acquired from the perfusion techniques are limited but a major finding was the role of blood flow in the regulation of adipose tissue metabolism.

A second technique that closely imitates the perfusion system is the perifusion system (Allen et al., 1979). Collagenase-isolated adipocytes are bathed in a column with a continuous flow of perfusate containing specified drugs or hormones while monitoring rates of substrate utilization or metabolite release. Like the perfusion system it prevents the build up of metabolic end products.

Slices or pieces of adipose tissue are more extensively used than the previous methods, but unlike the previous methods it is necessary to control or account for the build up of metabolites. For example, adenosine deaminase is used to control the accumulation of adenosine (Schwabe et al., 1973) and albumin is included in the medium to bind released fatty acids (Allen, 1979). In addition, it is necessary to ensure adipose pieces or slices are cut thin enough to provide adequate oxygen and nutrient exchange.

Isolated adipocytes obtained by collagenase digestion (Rodbell, 1964) are also extensively used. This system provides for good exchange of nutrients and oxygen, but caution must be observed. Collagenase contaminants, such as proteases, can affect the responses of cells (Belluco et

al., 1991). Also, there is little data available on the breakage or preferential breakage of adipocytes during collagenase treatment. Larger adipocytes are believed to be at higher risk unless special care is taken with preparation. Lipolysis rates in perfusion preparations and tissue slices are similar to rates found *in vivo*; however, rates displayed in isolated cells have a tendency to be elevated compared to rates found *in vivo* (Rudman & Del Rio, 1969; Vernon & Clegg, 1985). The increased lipolytic response in isolated cells may be due to a collagenase-induced reduction in phosphodiesterase activity (Engfeldt et al., 1980) or possibly an alteration in the adenlyate cyclase cascade (De Mazancourt et al., 1991). However, the free adipocytes possess the same pattern of responsiveness or unresponsiveness as tissue slices (Rudman & Del Rio, 1969).

Tissue culture preparations have been used to examine adipose metabolism over time. However, it is difficult to maintain the adipocytes without some alteration in function (Bernstein, 1979; Robertson et al., 1980). The difficulty is then assigning the source of the alteration. Others suggest that the cultured tissue remains metabolically active for several days and retains sensitivity to hormonal stimulation (Vernon, 1979, 1982). Weber et al (1992) suggest preparations of preadipocytes induced to differentiate to mature adipocytes may be the preferred

procedure for culturing cells.

Finally, microdialysis, an *in vivo* or *in situ* procedure, provides a localized measure of lipolytic activity. A hormone or substance is dispersed into a small mass of adipose tissue and simultaneous samples of the interstitial fluid are obtained (Arner & Bolinder, 1991; Watford & Fried, 1991). However, sensitive analytical methods are required because only microsamples are obtained.

The accurate expression of data in a form that reflects the activity of the tissue and the depot as a whole is often difficult. For example, data may be expressed as activity per cell number, cell size, cell volume, cell surface area, g tissue or g lipid. In rapidly changing tissue (e.g., growth) or tissue containing a range of cells sizes it is best to make the comparisons on an equal number of cells (Hartman et al., 1971; Hood & Allen, 1973). It may also be necessary to use data transformations or express proportional changes in discerning patterns of activity in changing tissue or tissue from different depots (Mersmann & Brown, 1973). Regardless of the form of data expression discretion must be employed when extrapolating from the *in vitro* preparation to the *in vivo* situation.

Comparisons between control and treated groups are often made with respect to responsiveness and sensitivity of the adipose tissue to hormone or drug stimulation. Responsiveness, also called efficacy or intrinsic activity

is the ability of the drug or hormone to elicit the measured response, for example lipolysis (Bentley, 1980). Maximal responsiveness is the ceiling effect that the drug can produce. Sensitivity is a measure of the affinity of the hormone-receptor interaction and is often expressed as the concentration of drug or hormone required to produce a response equivalent to 50% of the maximal response ($ED_{50}$) (Bentley, 1980).

## 2.0 Fatty Acid Synthesis and Storage

Triglycerides are energetically rich and a highly efficient form of energy storage. The free fatty acids that are esterified to the glycerol backbone forming triglycerides originate from either the diet, previously stored triglycerides or *de novo* synthesis from available precursors, primarily glucose and acetate. In mammals, adipose tissue is the major organ of lipid storage, but both the liver and adipose tissue are responsible for fatty acid synthesis; however, the extent of contribution from each is dependent on the species and the availability of major precursors.

### 2.1 Fatty Acid Synthesis in the Liver and Adipose Tissue

In monogastrics and ruminants exogenous fatty acids are those obtained in the diet. In monogastrics, dietary fat is hydrolyzed in the duodenum by pancreatic lipase. In ruminants, the small intestine receives mainly saturated free fatty acids because of the lipolysis and hydrogenation

in the rumen (Bartley, 1989). The available or released monoglycerides and free fatty acids form micelles, which adhere to the intestinal epithelial cells and are absorbed in their free form (Tennant & Hornbuckle, 1989). Once in the epithelial cells the free fatty acids are reformed into triglycerides and packaged in a phospholipid and protein coat and are now called chylomicrons. The chylomicra enter the lymph system, which accounts for 90% of fat absorption, and enter the plasma via the subclavian vein. The packaged lipids then enter adipose tissue through the action of lipoprotein lipase, an enzyme that is located in the endothelial cells but synthesized in adipocytes (Taskinen, 1988). This enzyme is responsilbe for the cleavage and subsequent release of free fatty acids making them available for uptake into the adipocytes.

The major distinction between monogastrics, notably humans and rodents, and that of ruminants and swine is the primary organ responsible for fatty acid synthesis. The major site of fatty acid synthesis in the rat is the liver, which contributes approximately 50% of newly synthesized fatty acids (Grandemer, et al., 1983). The rate of fatty acid synthesis in the adipose tissue of the rat is 2.5 times lower than that of an equal weight of liver tissue (Grandermer et al., 1983). In the non-lactating ruminant adipose tissue contributes over 90% of newly synthesized fatty acids, whereas, the liver, rumen, abomasum and small

intestine account for 8% (Ingle et al., 1972a). In the pig 99% of fatty acid synthesis occurs in its adipose tissue (O'Hea & Leveille, 1969). The adult rabbit has a similar pattern to that of the ruminant as the majority of fatty acids are derived from adipose tissue synthesis (Leung & Bauman, 1976; Smith, S., 1975).

In the ruminant dietary carbohydrate is fermented to volatile fatty acids; acetic, propionic and butyric and they are absorbed into the circulation as such, not as hexoses as is the case with monogastrics. Fermentation products serving as a major energy source in the ruminant are associated with a different role for the liver and adipose tissue. Approximately 50-80% of a ruminants energy requirements are met with acetate utilization (Bergman et al., 1965); whereas, in the monogastric only 5% is met by volatile fatty acid use (Yang et al, 1970). In the ruminant the liver serves primarily for gluconeogenesis while the adipose tissue is responsible for fatty acid synthesis (Ballard et al., 1969). The liver has adapted to a low glucose diet through the loss of the most active enzyme for glucose utilization, glucokinase. Liver glucokinase activity in the rat is 1000-fold greater than that found in the adult ruminant liver (Ballard et al., 1969). The reduced reliance on glucose in the ruminant liver is substantiated by a lower glucose oxidation rate and a lower incorporation of glucose into fatty acids (Ballard et al.,

1969; Hanson & Ballard, 1967). Allocating the metabolic load between the liver and adipose tissue reduces the competition for carbon, reducing equivalents and energy, while sparing glucose for obligatory processes (Bauman, 1976). Adipose tissue contributes to glucose sparing through reduced fatty acid synthesis from glucose. The rejection of glucose as a primary precursor is attributed to low levels of ATP-citrate lyase and NADP-malate dehdrogenase, which are the two enzymes necessary for transport of acyl units out of the mitochondria in the incorporation of glucose into fatty acids (Hanson & Ballard, 1968; Ingle et al., 1972b). Pyruvate kinase and pyruvate dehydrogenase activities may also be responsible for channelling glucose away from fatty acid synthesis (Robertson et al., 1982). Conversely, acetyl-CoA carboxylase, the regulatory enzyme in fatty acid synthesis, (Ingle et al., 1973; O'Hea & Leveille, 1969a) has a much higher activity in bovine and ovine adipose tissue than it does in rat adipose tissue (Hanson & Ballard, 1967).

## 2.2 Regulation of Fatty Acid Synthesis

### 2.2.1 Precursors

In ruminants of various ages, approximately 30-40% of all fatty acids esterified are newly synthesized, the remaining portion resulting from lipolysis or exogenous origin (see Vernon, 1980). Acetate is the primary precursor for fatty acid synthesis *de novo* in the ruminant (Bartos &

Skarda, 1970; Hanson & Ballard, 1967; Hood et al., 1972; Ingle et al., 1972a). Acetate incorporation into fatty acids displays anatomical variation in both ovine (Ingle et al, 1972b) and bovine adipose tissue (Hood & Allen, 1975; Hood et al., 1972). Glucose contributes 10-20% of the acyl units compared to 70-80% derived from acetate in subcutaneous adipose tissue, and conversely, in intramuscular adipose tissue glucose contributes 50-70%, while acetate contributes only 10-25% of the acyl units (Smith & Crouse, 1984). Variations of acetate or glucose concentration or availability can alter the rate of fatty acid synthesis through competition between precursors. Increased acetate concentration reduces fatty acid synthesis from other precursors (Bartos & Skarda, 1970), while increased glucose concentration increases acetate incorporation into fatty acids (Hanson & Ballard, 1967; Ingle et al., 1972a; Yang & Baldwin, 1973). However, the effect of increased acetate concentration may be limited to the caprine model (Ingle et al., 1972a).

In ruminant adipose tissue acetate serves as the major precursor for fatty acid synthesis; however, glucose is required to support *de novo* synthesis and for production of glycerol-3-phosphate which is necessary for fatty acid esterification (see Vernon, 1980).

**2.2.2 Insulin**

Insulin's effects are not as dramatic in ruminants

compared to monogastrics possibly due to 1) the limited capacity of adipose tissue to utilize glucose and 2) rumen fermentation ensures a slow continual influx of nutrients to tissues, thus the need to regulate sudden changes in nutrient availability is reduced. Although insulin increases acetate incorporation into fatty acids (Baldwin & Smith, 1971; Vernon & Finley, 1988) there is no evidence to suggest that it increases acetate uptake (Baldwin et al., 1973; Vernon, 1979). Insulin stimulates glucose conversion to glycerol *in vitro* (Bartos & Skarda, 1970; Vernon, 1976, 1979; Yang & Baldwin, 1973a) and has been shown to stimulate glucose uptake in adipose tissue *in vitro* (Khachadurian et al., 1966; Vernon & Taylor, 1984) and *in vivo* (Khachadurian et al., 1967). However there are studies which suggest there is no stimulatory effect (Baldwin et al., 1973;). Regarding insulin's actions on fatty acid synthesis, it appears to promote the availability of glucose, thus supporting fatty acid synthesis from acetate.

**2.2.3 Growth Hormone**

Growth hormone has a variety of actions in adipose tissue which reduce fat accretion (see Etherton & Smith, 1991). Growth hormone-treated adipocytes display reduced basal and insulin-stimulated lipogenesis in pigs (Magrai et al., 1987) and sheep (Peterla et al., 1987). Similar effects were seen in cultured ovine adipocytes (Vernon, 1982) and in adipocytes isolated from growth hormone-treated

lambs (Sinnett-Smith & Woolliams, 1989). Growth hormone acts as an antilipogenic agent primarily through reducing the effects of insulin on glucose uptake and a reduction of lipogenic enzyme activity (Etherton & Smith, 1991).

### 2.2.4 Catecholamines

The major adrenergic effect is increased lipolysis, which then reduces the lipogenic rate through a feedback inhibition delivered by a build up of released fatty acids. There is limited evidence that suggests the catecholamines affect lipogenesis directly. Epinephrine increased glucose uptake in the fat-tail depot (Khachadurian et al., 1966) and reduced fatty acid synthesis from acetate (Yang & Baldwin, 1973a), but these effects could be the result of increased fatty acid availability and increased re-utilization. The effects of catecholamines on blood flow regulation may also contribute to lipogenesis by affecting the availability of substrates (see Section 3.5).

## 3.0 Regulation of Lipid Mobilization

The adipose mass is capable of storing a large supply of energy, as 10-20% of body weight or greater can be adipose tissue (Vernon & Clegg, 1985). Under normal intake conditions a net mobilization of lipid will not occur; however, vast quantities of lipid can be mobilized in response to stresses of either psychological (fear or anxiety) or physiological (temperature, exercise, fasting or lactation) nature. For example, a 50-kg fasted sheep or 70-

kg man may mobilize 100-150 g lipid/day to meet energy demands (Belfrage, 1985; Vernon, 1980).

The accretion of adipose mass is the net effect of lipid synthesis and lipid mobilization. The two processes function simultaneously in the form of a triglyceride/fatty acid cycle, sometimes labelled a futile cycle similar to that of the fructose-6-phosphate/fructose diphosphate cycle in glycolysis (Brooks et al., 1982, 1983; Newsholme & Start, 1973). Although it may appear to be an awkward design it serves to enhance the sensitivity of control.

Lipolysis, the hydrolysis of stored triglycerides to free fatty acids and glycerol, is accomplished in a stepwise fashion by the action of mono-, di- and triglyceride lipases, collectively called hormone-sensitive lipases (see Figure 1). The conversion of the hormone-sensitive lipases from the inactive to the active form is hormonally regulated. The rate of lipolysis directly reflects the activity of the rate-limiting enzyme, triglyceride lipase, in the stepwise degradation of triglycerides. The phosphorylation-induced activation of the enzyme is catalyzed by cAMP-dependent protein kinase. Initiation of the phosphorylation-induced activation rests in the hormone-receptor interaction (see Belfrage, 1985 and Cordle et al., 1986), which through G- proteins stimulates adenlyate cyclase activity that is translated into an increase in cAMP accumulation (Levitzki & Bar-Sinai, 1991 and Jackson, 1991).

Lipid Mobilization

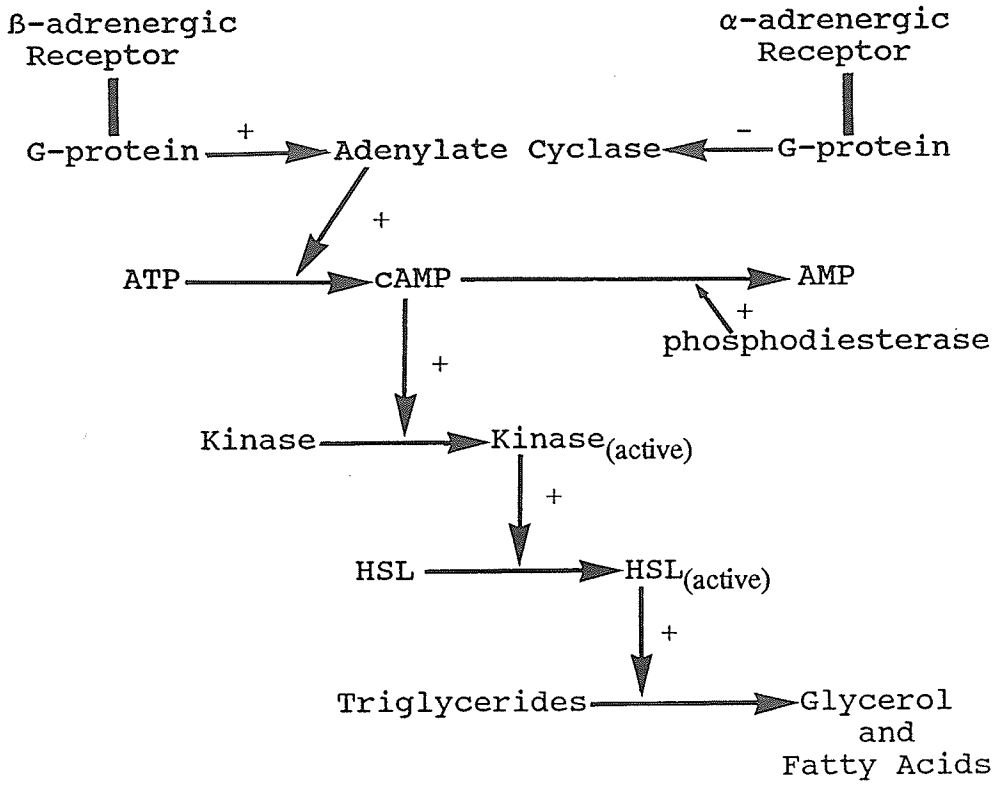


Figure 1. The sequence of events in the adipocyte resulting in the hydrolysis of triglycerides to yield glycerol and free fatty acids. Stimulation or inhibition of the mechanism is dependent on the hormone receptor interaction. Catecholamine stimulation of the ß-adrenergic receptor results in an accumulation of cAMP and activation of HSL producing and increase in lipolysis. Interaction with the α-adrenergic receptor inhibits cAMP accumulation and, thus, glycerol release. HSL, hormone sensitive lipase. (See text for discussion.)

The G-proteins, however, provide dual regulation of adenlyate cyclase activation by allowing either stimulation or inhibition depending on the hormone-receptor interaction (Gilman, 1984). The system is such that increases in cAMP and lipolysis are not linear, whereby, only a small increase in cAMP produces lipolysis while the majority of cAMP accumulation becomes "physiologically unnecessary" (Fredholm, 1978; Schwabe et al., 1973; Schimmel, 1974).

A reduction in lipolysis is accomplished by a reduction in cAMP accumulation, which is initiated by the hormonally regulated enzyme cAMP phosphodiesterase. Some antilipolytic hormones or agents reduce the rate of lipolysis through a decrease in cAMP accumulation by an increase phosphodiesterase activity (see Figure 1). For example, one of the mechanisms by which adrenocorticotropin hormone (Pawlson et al., 1974; Solomon et al., 1991) and insulin (Solomon, 1975) reduce the lipolytic rate is via an increase in phosphodiesterase activity. The accumulation of cAMP can also be reduced by inhibiting the adenylate cyclase cascade (see Figure 1). The $\alpha$-mediated response to catecholamines inhibits adenylate cyclase production through interaction with the $\alpha$-receptor and stimulating inhibitory G-proteins.

Hormone-sensitive lipase activation results in the release of free fatty acids and glycerol into the cytoplasm. The free fatty acids leave the adipocyte by diffusion and the rate of diffusion is proportional to a concentration

gradient. Once released into the plasma, because of their hydrophobic nature, they are bound and carried by albumin to other tissues for utilization. They are either re-esterified, oxidized, or converted to ketone bodies in the liver. The released glycerol is restricted to recycling by the liver as adipocytes do not contain glycerokinase, the enzyme necessary for recycling.

The plasma free fatty acid level varies with physiological and nutritional status and the addition of hormones influence the circulating levels. To examine the role of hormones and adipose tissue in energy balance the rates of fatty acid and glycerol release are studied in a variety of situations. Measurement of glycerol release is considered the best indicator of triglyceride hydrolysis as opposed to measuring free fatty acid levels. Firstly, glycerol is released into circulation to be recaptured by the liver as adipocytes cannot utilize or recycle it because of low or negligible glycerokinase activity (Hood et al., 1972; Khachadurian et al., 1967). Although there is the possibility of partial hydrolysis, it is not usually observed under moderate levels of stimulation (Bergman, 1968; Etherton et al., 1977; Metz et al., 1973, 1974). Secondly, re-esterification or re-uptake of the released fatty acids by adjacent adipocytes may account for a reduction in monitored levels by 30-60% in stimulated tissue (Vernon & Clegg, 1985). Thus, without accounting for re-

utilization, monitoring fatty acid levels may not reflect hormone-sensitive lipase activity.

### 3.1 Insulin and Regulation of Lipid Mobilization

Insulin is generally thought of as an anabolic hormone, because it stimulates anabolic processes or it inhibits the catabolic processes (Prior & Smith, 1982). The actions of insulin in ruminants are similar to actions in monogastrics with respect to glucose uptake and utilization. However, in human and rat adipose tissue insulin may have actions independent of glucose metabolism as concentrations necessary to inhibit lipolysis are lower than those needed to stimulate glucose uptake (Jacobsson et al., 1976), and adipocytes displaying insulin resistance for glucose can respond by decreasing ongoing lipolysis (Koopmans et al., 1989).

Ruminant metabolism displays a sensitivity and necessity for insulin as diabetic conditions in sheep (increased plasma glucose and free fatty acids) are alleviated by insulin supplementation (Jarett et al., 1974). Also, infusion of insulin in sheep results in a decrease in plasma free fatty acids and glycerol *in vivo* (Bergman, 1968; Radolff & Schultz, 1966) or in an isolated adipose depot (Khachadurian et al., 1966). However, the effects are not always produced (Yang & Baldwin, 1973b; Di Marco et al., 1991) or are of limited duration (Randolff & Scholtz, 1966). Similar equivocal results are found in adipocytes maintained

in culture including antilipolytic effects (Bernstein, 1979), no effect (Vernon, 1979) or a lipolytic effect (Watt et al., 1991). The discrepancies in these results could be due to the energy balance (fed or fasted) of the animals prior to infusion or biopsy. In human adipocytes a prior exposure of the subject to a fat-rich diet reduces insulin's antilipolytic actions (Kather et al., 1987).

The antilipolytic effects of insulin are believed to be mediated through a reduction in cAMP accumulation by activation of phosphodiesterase (Lonnqvist et al., 1984; Zinman & Hollenberg, 1974). However, there is evidence to suggest that other mechanisms independent of cAMP may be involved (Londos, et al., 1985).

**3.2 Growth Hormone and Regulation of Lipid Mobilization**

Growth hormone's effects of partitioning nutrients away from fat toward accretion of protein have been known for many years (see Bauman et al., 1982 and Vernon & Flint, 1989). The biological effects of growth hormone are such that it promotes the mobilization of lipids and reduces the lipogenic capacity of adipose tissue. For example, insulin-stimulated glucose metabolism is reduced, while catecholamine-stimulated lipolysis is increased (Etherton & Smith, 1991).

It is difficult to compare and contrast the actions of growth hormone on lipolysis either *in vivo* or *in vitro* because of the different feeding regimens or methods of

tissue preparation. However, Duquette et al (1984) has provided insight by treating both rat and sheep adipose tissue by the same procedures. Rat adipose tissue, either as free adipocytes or tissue pieces, displayed a 200% increase in lipolysis with exposure to growth hormone, whereas, sheep adipose tissue displayed no significant increase regardless of cell preparation (Duquette et al., 1984). Also, biopsied tissue from sheep treated with growth hormone for 6 weeks failed to displayed an enhanced response to norepinephrine compared to adipose tissue from a saline-treated control group (Sinnett-Smith & Woolliams, 1989).

To observe the lipolytic effects of growth hormone *in vitro*, a prolonged exposure (48 h) is required, which has been shown to increase the ß-adrenergic receptor number on cultured ovine adipocytes (Watt et al., 1991). Direct effects of growth hormone on ß-adrenergic receptor number are thought to be responsible for the enhancement of norepinephrine-induced lipolysis (Watt et al., 1991).

In lactating cows growth hormone treatment either chronically elevated plasma free fatty acid levels (Sechen et al., 1989, 1990) or in similar studies had no effect on plasma fatty acid levels in lactating cows (Michel et al., 1991) or three breeds of sheep (Sinnett-Smith & Woolliams, 1989). The ability of growth hormone to increase plasma free fatty acids is increased if the animal is in a negative energy balance (Etherton & Smith, 1991). Growth hormone

treatment did increase the lipolytic response to norepinephrine infusion in lactating cows (Michel et al., 1991; Sechen et al., 1990).

The variations in response to growth hormone treatment suggests that other factors need to be taken into consideration. The physiological status (lactation) appears to promote the effect of growth hormone on catecholamine-induced lipolysis *in vivo*. Also, the duration of exposure during incubation of adipose tissue required for *in vitro* response suggests that the actions of growth hormone are not acute.

**3.3 Adenosine and Regulation of Lipid Mobilization**

Adenosine has been suggested to be a local regulator of cellular metabolism. Adenosine is released and metabolized by most animal tissues (see Arch & Newsholme, 1978a, 1978b) including adipose tissue (Fain, 1979; Schwabe et al., 1973; Vernon et al., 1991). It is produced by the hydrolysis of AMP by the action of 5'nucleotidase. Adenosine exerts its effects through modulation of adenylate cyclase activity by adenosine-specific membrane receptors (see Vernon et al., 1991).

Schwabe et al (1973) demonstated that adenosine accumulated in the medium of isolated adipocytes and the accumulation resulted in a reduction of catecholamine-induced lipolysis. This inhibition could be eliminated by adding adenosine deaminase to the incubation medium, which

promoted the deamination of adenosine to inosine (for example, Honnor et al., 1985; Shechter, 1982). The addition of adenosine deaminase increased cAMP accumulation in norepinephrine- and glucagon-stimulated adipocytes (Fain & Wieser, 1975; Honnor & Saggerson, 1980; Shechter, 1982). Adenosine may provide modulation of the response to lipolytic stimuli under various conditions. Increased adenosine levels or adenosine sensitivity may account for fat accretion and inhibited mobilization. The adipose tissue of the obese Zucker rat (*fa/fa*) displayed an increased sensitivity to adenosine compared to lean (*Fa/fa*) control rats (Vannucci et al., 1989). Adenosine levels are also higher in omental adipose tissue than subcutaneous adipose tissue in obese humans. However, an isoproterenol-induced lipolysis displayed a reduced sensitivity to adenosine inhibition in the omental tissue *in vitro* (Vikman et al., 1991). Adipocytes obtained from the abdominal subcutaneous depot of these obese patients had higher levels of adenosine than did tissue obtained from a similar depot in lean patients (Kaartinen et al., 1991). Again, the adenosine inhibitory effect *in vitro* was less in the obese patients and more adenosine receptors with reduced affinity were found on the adipocytes from the obese patients (Kaartinen et al., 1991). These data suggest that adenosine modulates lipid mobilization through high adenosine content, however, a down regulation of receptors may limit the

effectiveness of adenosine in promoting fat accretion.

Although the general practice is to include adenosine deaminase in incubation preparations and to interpret its effects as a reflection of adenosine's role in adipose metabolism, this procedure may require additional examination. Adenosine deaminase effects were more pronounced in isolated cells than in tissue slices (Shechter, 1982). In addition, an event may be masked by including adenosine deaminase in the preparation, for example, the antilipolytic effect of insulin on norepinephrine-stimulated lipolysis (Fain & Wieser, 1975; Koopmans et al., 1989).

**3.4 Repartitioning Agents and Lipid Mobilization**

In the growing animal energy in excess of maintenance is deposited into muscle and adipose tissue (see van Es, 1977). As mentioned earlier, the challenge now is to reduce economic loss by restricting the amount of energy deposited as fat and increasing protein deposition without decreasing the quality of the product (Berg & Walters, 1983; Reeds, 1991).

Repartitioning agents or $\beta$-adrenergic agonists are substances that shift energy from fat deposition to protein accretion. The most often used and tested are cimaterol, clenbuterol and ractopamine. Their repartitioning effects have been displayed in laboratory rodents (for review see Yang & McElligott, 1989). However, more important

consequences are the similar effects found in meat producing animals. Increases of greater than 10% in daily gain or increased feed efficiency have been observed in lambs (Kim et al., 1987b; Shackelford et al., 1992) steers (Moloney et al., 1990; Wheeler & Koohmaraie, 1992) and pigs (Bark et al., 1992; Watkins et al., 1990). In each of the above examples protein accretion was gained at the expense of fat. Although this may seem like a miracle drug allowing producers to attain production goals, considerations are necessary. There is often an increased toughness in the meat product with the use of $\beta$ agonists (Koohmaraie et al., 1990, 1991; Shiavetta et al., 1990). In addition, the doses used have to be in a range that produce the desired reponse but do not evoke unwanted side effects, such as reduced intake (Moloney et al., 1990; Ricks et al., 1984). Also, because the treatments have to meet consumer demands, a withdrawal period may be necessary, which could reduce the effectiveness of the treatment (Schiavetta et al., 1990).

$\beta$-adrenergic agonists express their repartitioning effects by interacting with the $\beta$-adrenergic receptors; however, examination of the specificity or preference for a specific subtype has not been clearly established (Liu et al., 1989; Mersmann, 1984a, 1984b; Mersmann & McNeil, 1992). The focus of this section is to consider the mechanisms by which $\beta$-adrenergic agonists reduce the mass of adipose tissue in the treated animal.

Broadly, the reduction in adipose mass can result from either a net increase in lipid mobilization or a net decrease in lipid synthesis. Basically there are two methods employed for examining the effects of agonists on adipose tissue, either 1) adipose tissue is removed from agonist-fed animals or 2) exposure of adipose tissue or adipocytes to incubation medium containing the agonist in question. A third method of infusing the agonist into an anesthetized model (pig) has also been used (see Mersmann, 1989b).

The first event that is obvious is that those agonists used as repartitioning agents are weak lipolytic agents when compared to effects elicited by isoproterenol or epinephrine (Etherton & Smith, 1991; Liu et al., 1989; Peterla & Scanes, 1990). For example, clenbuterol produced a two-fold increase in glycerol release compared to a five-fold increase produced by epinephrine under the same conditions (Liu et al., 1989), or it produced no increase (Mersmann, 1987; Peterla & Scanes, 1990; Rule et al., 1987). Cimaterol and ractopamine produced a 2.7-fold and a 2.9-fold increase, respectively, compared to a 5.5-fold increase with isoproterenol treatment (Peterla & Scanes, 1990). Ractopamine's modest lipolytic effects can be enhanced by including theophylline, a potent phosphodiesterase inhibitor, or adenosine deaminase in the medium (Liu et al., 1989). In contrast to its effects *in vitro*, clenbuterol

does induce lipolysis *in vivo* (Mersmann, 1987); however, its lipolytic activity *in vivo* may be through stimulation of either norepinephrine or epinephrine release (Mersmann, 1989b).

Because the repartitioning agents are not lipolytically potent their actions are probably exerted through other routes, for example, physiological modulation of insulin. Ractopamine and clenbuterol have been shown to reduce insulin binding in isolated porcine adipocytes through $\beta$-adrenergic receptor interaction (Liu & Mills, 1990). Furthermore, adding insulin to the incubation medium reduced the lipolytic effects of both isoproterenol and ractopamine, and completely eliminated the lipolytic effects of cimaterol (Peterla & Scanes, 1990). Adipose tissue biopsied from cimaterol-fed lambs lacked the antilipolytic effects of insulin (Hu et al., 1988). These results obtained from *in vitro* studies would suggest that cimaterol and ractopamine are moderately lipolytic but it is likely that the majority of their effects are through a reduction in the antilipolytic effects of insulin.

The $\beta$ agonists also possess antilipogenic effects (Merkel et al., 1991; Miller et al., 1988; Peterla & Scanes, 1990; Schiavetta et al., 1991; Smith et al., 1991). This action is also indirect through inhibition of insulin effects. It is likely that the majority of fat-reducing effects exhibited by the $\beta$ agonists are produced through

insulin either by reducing its antilipolytic or lipogenic potential or increasing the the release of endogenous catecholamines, which are more resistant to insulin action than are the synthetic $\beta$ agonists.

### 3.5 Blood Flow and Regulation of Lipid Mobilization

The regulation of substrate availability and metabolite removal is necessary for any metabolic process to function at optimum rates. Blood flow is a key factor in this regulatory process. In addition to the catecholamine's role as regulators of lipolysis they also have control over vascular response in adipose tissue. However, the technical difficulties associated with monitoring blood flow through adipose tissue have restricted wide-spread studies of this action of catecholamines. The isolated perfusion of mesenteric, subcutaneous or omental adipose depot of the dog (Ballard & Rosell, 1969, 1971; Belfrage et al., 1979; Oro et al., 1965), the epididymal or parametrial fat pad of the rat (Ho & Meng, 1964; Robert & Scow, 1963) and the fat-tail depot of sheep (Gooden et al., 1986) are the limited examples of monitoring blood flow through an isolated fat mass. Isotope dilution techniques have been used in humans (Hjemdahl & Linde, 1983; Silverberg et al., 1978), sheep (Barnes et al., 1983; Gregory et al., 1986), cattle (Lomax & Baird, 1983), pigs (Mersmann, 1989a) and rats (West et al., 1987) to monitor blood flow, however, it has not always been restricted to adipose tissue. Depending on the procedure

and the extra-adipose tissue included values range from 2-8 ml/min/100 g tissue in man, 3-13 in dogs, and 5-6 in sheep (see Vernon & Clegg, 1985).

Although blood flow does not appear to be a major contributor to fat accretion it is reduced in adipose depots of obese animals and there are interdepot differences within a species (Gregory et al., 1986; West et al., 1987). Also, changes in blood flow may reflect the activity of a tissue, for example, blood flow to the liver of lactating cows increased 52% with feeding (Lomax & Baird, 1983). Similarly, during and after feeding blood flow decreased in ovine omental and mesenteric tissue while it increased in the rumen and liver (Barnes et al., 1983). These studies demonstrate that increased blood flow reflects the location of metabolic prioity.

The regulation of blood flow partitioning throughout the body is a complex process that involves both the central and peripheral nervous systems (see Rosell & Belfrage, 1979 and Shimazu, 1981). Moreover, evidence suggests that the catecholamines provide a coordinated regulation of lipid metabolism and blood flow. Nerve stimulation of isolated adipose depots results in a release of glycerol and free fatty acids in addition to a constriction of vessels (Belfrage et al., 1979; Ngai et al., 1966; Oro et al., 1965; Weiss & Maickel, 1965). Similar results are seen with tracer isotopes in humans where norepinephrine increased

vascular resistance and increased glycerol release (Hjemdahl & Linde, 1983; Silverberg et al., 1978). The catecholamine-induced vascular effects are mediated through the $\alpha$- and $\beta$-adrenergic receptor complement; vasocontriction resulting from interaction with the $\alpha$ receptors and vasodilation from stimulation of the $\beta$ receptors (see Ballard & Rosell, 1971; Hjemdahl et al., 1983; Ngai et al., 1966).

The general conclusions are that catecholamines coordinate lipid mobilization through interaction with the adrenergic receptors located in the vascular component as well as those associated with the adipocyte. It is believed that the receptor subtype localization on the vessel plays a major role in the process. That is, neuronally released norepinephrine would activate $\alpha$ receptors located in close proximity to the vessel-neuron synapse thereby inducing vasoconstriction (Belfrage, 1978; Fredholm, 1985; Hjemdahl et al., 1983). In contrast, circulating catecholamines would activate the $\beta$ receptors situated on the lumen of the vessels producing vasodilation (Belfrage, 1978; Fredholm, 1985; Hjemdahl et al., 1983). Thus, norepinephrine-stimulated lipolysis will cause the fatty acids and glycerol to build up in the tissue and restricted vessel, but prolonged stimulation would assist in the movement of fatty acids and glycerol away from the cell through vasodilation (Ballard & Rosell, 1971; Oro et al., 1965).

The enhanced removal of metabolites is accomplished by

a unique physiological design. In the extensive capillary network that surrounds the adipocytes the major portion is the venous segment of the network (Ballard et al., 1974). As a result of adrenergic stimulation the capillary permeability is higher in the venous segment, which assists in the diffusion of fatty acids and glycerol from the adipocyte (Ballard et al., 1974; Belfrage, et al., 1979; Fredholm, 1985; Rosell & Belfrage, 1979).

Other mechanisms are also in place to regulate lipolysis. Glycerol may be allowed to build up in the tissue to be released at a later time (Belfrage et al., 1979); however, the accumulation of fatty acids serves as a feedback inhibitor to ongoing lipolysis (Fain, 1980; Fain & Shepard, 1975; Metz et al., 1973). The rate of fatty acid intracellular accumulation and re-esterification is then dependent on the blood flow to the active tissue (Belfrage et al., 1979; Edens et al., 1990b; Fain, 1980).

Sollevi & Fredhom (1981) suggested that adenosine acts as a local regulator of adipose tissue by increasing blood flow and hence, contributing to the regulation of lipolysis in addition to its direct effects (see Section 3.3).

**3.6 Catecholamines and Regulation of Lipid Mobilization**

The catecholamines reach the adipose mass either through stimulation of sympathetic adrenergic fibers, which results in postsynaptic norepinephrine release or stimulation of the adrenal medulla, which results in

epinephrine release into the circulation (see Fredholm, 1985; Rebuffe-Scrive, 1991; Rosell & Belfrage, 1979; Vernon & Clegg, 1985). The response elicited depends on the specific receptor-hormone interaction and the potency of the catecholamine at that particular receptor (see Figure 1). Generally, the receptor complement is divided into two classes and each class is then further subdivided. The $\beta_1$ and $\beta_2$ isoreceptors are responsible for adenlyate cyclase stimulation while the $\alpha_2$ receptor is responsible for inhibition of the cascade (Fain & Garcia-Sainz, 1983; Garcia-Sainz & Fain, 1982). The potency at the $\beta_1$-adrenergic receptor is in the order of isoproterenol > norepinephrine $\simeq$ epinephrine, and at the $\beta_2$-adrenergic receptor is isoproterenol > epinephrine >> norepinephrine. The potency at $\alpha_2$-adrenergic receptor is epinephrine $\geq$ norepinephrine > isoproterenol (Lefkowitz et al., 1983). Both the $\alpha_2$- and $\beta$-adrenergic receptors are present in excess, that is, there are spare receptors of which only a small fraction would be considered silent, or irresponsive to hormone occupation (Arner et al., 1988; Arner, 1992). Interaction with the functional spare receptors results in an increase in cAMP production, but the additional cAMP does not lead to further lipolytic response. In fact, only 50% of the total complement of ß-adrenergic receptors need be occupied to obtain a full response. Thus, the availability of spare receptors provides an another mechanism for

regulating adipocyte function. (Arner et al., 1988; Arner, 1992).

**β-Adrenergic Receptors**

Stimulation of the β-adrenergic receptors results in an increase in glycerol and free fatty acid release (see Figure 1). This effect is readily observed in several species, for example, humans (Mauriege et al., 1988), dogs (Belfrage, 1978; Valet et al., 1989), rats (Aitchison et al., 1982), guinea pigs (Pond & Mattacks, 1991), sheep (Guesnet et al., 1987; Iliou & Demarne, 1987; Vernon & Finley, 1985), pigs ( Mersmann, 1984a) and cattle (Etherton et al., 1977; Rule et al., 1992; Sidhu & Emery, 1973). The receptor subtype responsible for the stimulation of lipolysis has yet to be determined. The use of new highly selective β-adrenergic ligands has brought about a re-evaluation the role of different subtypes in lipid mobilization. Furthermore, the radioligands chosen to determine the subtype and the conditions employed can influence the binding kinetics and specificity of the receptors (Mersmann, 1984a; Mersmann & McNeel, 1992). In the human (Emorine et al., 1992) rat (Granneman & Lahners, 1992; Hollenga et al., 1991; van Liefde et al., 1992) and rabbit (Langin et al., 1992) the new "atypical" $\beta_3$-adrenergic receptor is believed to be responsible for regulation of lipid mobilization. In the sheep, Bowen et al (1992) recently provided evidence for a single $\beta_2$ receptor subtype, which is predominantly

responsible for lipid mobilization and the affinity of the receptor varies depending on the anatomical location of the depot. In the pig a conclusive subtype classification has not been made (Mersmann, 1984b, 1992).

Alterations in $\beta$ receptor number (Wahrenberg et al., 1989) and receptor sensitivity (Hellmer et al., 1992) can account for an increase in lipolytic response to endogenous catecholamines or adrenergic agents. Even an alteration in the ratio of $\beta_1$ to $\beta_2$ (Valet et al., 1989) or varying sensitivity of the $\beta$ subtypes to desensitization (Arner et al., 1991) can prove to be methods of regulating the adipose tissue responsiveness to adrenergic stimulation. The adipocyte $\beta$-adrenergic receptor is subject to desensitization, or catecholamine tachyphlaxia (see Arner, 1992) after prolonged exposure of the receptor to stimulation. There are three processes associated with desensitization: 1) rapid uncoupling of the receptor from stimulatory G-proteins (Hausdorff et al., 1990), 2) sequestration of the receptor (Lefkowitz et al., 1983; Liggett, 1991; Roth et al., 1991), and 3) down regulation (Collins et al., 1991; Hadcock & Malbon, 1988). Although each of the processes result in reduced responsiveness to adrenergic stimulation, the time frame varies from minutes in the first two, to hours in the third case. Also, the quantitative contribution from each process to the reduced responsiveness may differ (Hadcock & Malbon, 1988; Lohse et

al., 1990; Roth et al., 1991). Although desensitization has been demonstrated in adipose tissue (Arner et al., 1991; Balkin & Sonenberg, 1981; Burns et al., 1982; Pecquery et al., 1984; Valet et al., 1989), much of the information regarding underlying mechanisms has been collected from cells other than adipocytes (for example, Human Epidermoid Carcinoma A431 cells), thus the mechanisms involved in adipocyte catecholamine desensitization have yet to be clarified.

Interestingly, exercise-induced periods of elevated sympathetic nervous activity do not result in a desensitization of adrenergic receptor reponsiveness (Crampes et al., 1988). Similarly, young developing animals appear to be less susceptible to adrenergic desensitization, suggesting that receptor regulation may be fundamentally different in growing animals (Stein et al., 1992). Finally, the adipocyte $\alpha_2$-adrenergic receptor in adipose tissue appears to be exempt from desensitization (Arner et al., 1990c; Burns et al., 1982; Pecquery et al., 1984; Villeneuve et al., 1985); however, the $\alpha_2$-adrenergic receptor of human blood platelets (Insel & Motulsky, 1988) and the rat tail artery and saphenous vein (Cheung, 1986) display diminished adrenergic responsiveness upon continual or cummulative $\alpha_2$ agonist exposure.

**α-Adrenergic Receptors**

The role of the $\alpha_2$-adrenergic receptors with respect to

lipid metabolism is to provide inhibition of lipolysis (see Figure 1). It has been suggested that inhibition of a system is needed before stimulation can occur (Kather, 1988; Kather et al., 1985a,b). Lipid mobilization is kept in check through modulation of the $\alpha_2$ receptor until lipolysis is stimulated by increased levels of norepinephrine (Berlan & Lafontan, 1985; Lafontan et al., 1979). At rest it is the $\alpha_2$ receptor interaction that modifies lipolysis, while during exercise the $\beta$ response is induced (Arner et al., 1990b, 1990c).

The $\alpha_2$-adrenergic receptors of adipocytes have been found in a variety of species including humans (Berlan & Lafontan, 1980, 1985; Engfeldt & Arner, 1988; Wright & Simpson, 1982; Zahorska-Markiewicz & Piskorska, 1986), rabbits (Lafontan, 1979, 1981), dogs (Berlan et al., 1982; Taouis et al., 1988), hamsters (Carpene et al., 1983, 1990b) and sheep (Finley et al., 1990; Watt et al., 1991). The $\alpha_2$ antilipolytic effects are not displayed in guinea pig (Maroto et al., 1992) or the pig (Mersmann, 1984a). Defining $\alpha_2$-antilipolytic effects in rat adipocytes have proven most controversial ranging from a lack of functional $\alpha_2$ receptors (Carpene et al., 1983) to an increase in $\alpha_2$ response in genetically obese rats (Carpene et al., 1990c) to the discovery of functional $\alpha_2$ receptors using a new highly selective agonist (Rebourcet et al., 1988). It has been suggested that the reasons behind the equivocal results

regarding the $\alpha_2$ receptors in the rat is that they may be of a slightly different nature than the $\alpha_2$ receptors found in other species (Carpene et al., 1990a). Also, as suggested earlier, the radioligands selected and the presence of receptor reserves (spare receptors) may influence the identity of the specific subtypes or binding characteristics of the receptor (Galitzky et al., 1989a, 1989b, 1990; Insel & Motulsky, 1988). The $\alpha_2$-adrenergic receptor has been even further subcategorized, which may explain the different conclusions reached with regard to full and partial agonists and interspecies differences (see Lafontan et al., 1992).

Variations in the $\alpha_2$ receptor density or affinity account for depot differences in ability to mobilize fat (Arner et al., 1987; Engfeldt & Arner, 1988; Wahrenberg et al., 1989). Also, in obese models there is an increase in $\alpha_2$ antilipolytic response, for example, in dogs (Berlan et al., 1982; Taouis et al., 1989), rabbits (Lafontan, 1981) and rats (Carpene et al., 1990c) which may partially explain the increased adiposity. Blocking of the antilipolytic response results in an enhanced mobilization of lipid and has proven to be useful in treating obesity because of the desired effects are not complicated with adverse side effects (Berlan et al., 1991; Saulnier-Blanche et al., 1989; Zahorka-Martiewicz & Piskorska, 1986).

The dynamic nature and the energetic necessity of adipose tissue and the role catecholamines play in this

tissue were discussed earlier. The major focus of the following section is the variation among adipose depots within the same animal and the sensitivity and responsiveness of individual depots to hormonal regulation and physiological challenges, such as, pregnancy, lactation, temperature, diet and exercise.

### 3.6.1 Influence of Anatomical Location

Pond (1986) dissected and categorized adipose tissue depots from 200 specimens representing over 20 species. Possibly the most arresting conclusion was that extrapolating adipose tissue function from one species to another or even from one depot to another within a species was a precarious proposal. Although the anatomical location of adipose depots is highly conserved across mammals (Pond, 1986; Pond & Mattacks, 1989), the response to hormonal intervention may vary. This variation may not limit extrapolation from one species to another, but it may require comparisons to be made between appropriate depots. That is, extrapolating metabolic events of the epididymal fat pad of the rat may not be appropriate for human omental tissue, but rather comparisons between omental tissue of both species may be more appropriate.

It has been known for several years that in meat producing animals there are species and breed differences in rate of fattening and distribution of fat (Berg & Walters, 1983; Kempster & Evans, 1979; Trenkle & Marple, 1983;

Vezinhet & Prud'hon, 1975). However, to date, the majority of information regarding depot-specific variations in adipose tissue responsiveness to hormonal regulation has been gleaned from human subjects (see Arner, 1992). Without doubt the major impetus for these investigations has been the high correlation between depot-specific fat accretion, notably the abdomen, and the increased risk of heart disease, diabetes and mortality (Bjorntorp, 1988; Kissebah et al., 1982; Krotkiewski et al., 1983).

One of the leading hormonal influences on depot-specific lipid metabolism is the catecholamines. The catecholamines are the major fast-acting regulators of lipid mobilization, and because of this, research has focused on their role in variations of depot-specific responsiveness. This is not to suggest that catecholamines are solely responsible for the preferential fat accretion in certain depots. Sex differences (Richelsen, 1986) in body fat distribution and the lack of site-specific differences in responsiveness in prepubertal children (Rosenbaum et al., 1991) would suggest that sex hormones are also involved, at least in the initial phases of fat accretion. However, the contribution that each depot has in energy metabolism and in the increased risk of pathological conditions of obesity is strongly influenced by the response to adrenergic stimulation. It has been repeatedly demonstrated in humans that abdominal adipose tissue expresses a greater lipolytic

response to adrenergic stimulation than does subcutaneous tissue from the gluteal/femoral region (see Arner, 1992). This regional variation in response is independent of sex (Leibel & Hirsch, 1987) and obesity (Mauriege et al., 1991). Although there are sex (Richelsen, 1986) and obesity (Mauriege et al., 1991) differences found within a depot.

Catecholamines possess both $\beta$-mediated lipolytic actions as well as $\alpha_2$-mediated antilipolytic effects; therefore, it has been suggested that the lipid mobilizing effects can be altered through differences (number and affinity) in receptor populations within each depot. However, caution must be used when transferring receptor number and affinity differences to functional significance as the catecholamines have different potencies depending on the receptor population (Lafontan et al., 1983).

The ability of an adipose tissue mass to mobilize lipids is dependent on the cell's ability to activate the adenylate cyclase cascade. When stimulation of glycerol release bypasses, or is independent from the receptor (for example, with forskolin or dibutyrl-cAMP) the depot differences in mobilization were removed, indicating depot differences in response are a receptor-mediated effect (Hellmer et al., 1992). There is evidence to suggest that post-receptor mechanisms may also be involved in depot differences in response to catecholamine stimulation (Dieudonne et al., 1992).

There is evidence that depot differences in mobilization rates may be due to an increase in $\beta$ receptor density in the abdominal sites (Arner et al., 1990a; Wahrenberg et al., 1989). However, these data were collected from healthy, nonobese males and it may be limited to that category only (Mauriege et al., 1991). A greater collection of evidence would suggest that increased number and affinity of $\alpha_2$-adrenergic receptors accounts for the reduced ability of the subcutaneous depots to mobilize lipids after adrenergic stimulation (Berlan & Lafontan, 1985; Lafontan et al., 1979; Mauriege et al., 1987; Richelsen, 1986). Similar nonhomogeneous characteristics can also be found in depots of rats (Hartman & Christ, 1978) and guinea-pigs (Pond & Mattacks, 1991). However, more pertinent to the focus of this review are depot-specific variations in the domestic meat-producing animals.

In sheep, subcutaneous and perirenal adipose tissue had similar basal lipolytic rates and both responded with a significant 2-fold increase after epinephrine stimulation (Etherton et al., 1977; Sinnett-Smith & Woolliams, 1987). A similar comparison in steers showed the subcutaneous tissue had a higher basal lipolytic rate and was less responsive to adrenergic stimulation than was the perirenal tissue (Etherton et al., 1977). In a later study with steers, Rule et al (1992) found an epinephrine-induced increase in lipolytic rate in perirenal tissue, however, direct

comparisons are difficult because the lipolytic rates were expressed per mg of protein not adipocyte number.

Moderate depot differences in fatty acid release have been shown among the fat-tail, mesenteric and abdominal subcutaneous depots of the Karakul sheep, but because glycerol release was not included these values may not represent lipolysis (Khachadurian et al., 1967). Also, there is recent evidence supporting differences in $\beta_2$-receptor affinity among the omental subcutaneous and popliteal depots of sheep (Bowen et al., 1992). The predominant receptor subtype was found to be of the $\beta_2$ variety possessing a greater affinity in the subcutaneous depot compared to the omental depot.

Porcine adipose tissue also displayed a nonhomogeneous nature in response to growth hormone exposure, that is, the subcutaneous backfat was more affected compared to other depots (Clark et al., 1992) Furthermore, within the subcutaneous depot, reduced caloric intake affects the middle layer in preference to the inner or outer layer (Leymaster & Mersmann, 1991).

### 3.6.2 Influence of Pregnancy and Lactation

During pregnancy and subsequent lactation shifts in adipose tissue from lipid accumulation to that of lipid mobilization occur. This event is accomplished through shifts in enzyme activites and hormonal sensitivity and responsiveness (Guesnet et al., 1987; Iliou & Demarne, 1987;

Vernon et al., 1987). With lactation there is an increase in the number of $\beta$ receptors per cell (Jaster & Wegner, 1981), and an increase in hormone-sensitive lipase activity (McNamara et al., 1987; Sidhu & Emery, 1972). Also, there is an increase in adenosine sensitivity and receptor number, which may affect adrenergic responsiveness of the adipose tissue (Vernon et al., 1991; Vernon & Finley, 1985).

The increase in adrenergic-stimulated lipolysis occurs in late pregnancy in the rat (Aitchison et al., 1982) and in the omental tissue of sheep (Guesnet et al., 1987; Iliou & Demarne, 1987) or during lactation in the subcutaneous tissue of sheep (Vernon & Finley, 1985). The later onset of lipid mobilization in subcutaneous tissue compared to omental tissue, found in the Vernon and Finley study (1985) may be a result of the adrenergic agonist employed. In the omental tissue isoproterenol was used to stimulate lipolysis, thus the $\alpha_2$ antilipolytic effect would not be detected (Guesnet et al., 1987; Iliou & Demarne, 1987). In the Vernon & Finley (1985) study when response to theophylline was examined the greater lipolytic effect was in late pregnancy as well. These events provide evidence that either both receptor populations must be examined or conclusions must be limited to the receptor population studied.

### 3.6.3 Influence of Dietary Manipulations

There is limited information on dietary manipulations

and lipid mobilization in ruminants and the differences in protocol make comparisons tenuous. Feeding a high concentrate diet to lactating cows resulted in an increase in lipolytic rate in response to norepinephrine infusion (Sidhu & Emery, 1973) or a decrease *in vitro* in response to epinephrine (Yang & Baldwin, 1973a). Sheep fed a high energy diet had reduced norepinephrine-stimulated lipolytic response *in vitro*. However, response to isoproterenol and dibutryl-cAMP effects was similar to controls suggesting an increased $\alpha_2$-mediated effect (Bouyekhf et al., 1991).

Increased caloric energy derived from fat in the diet decreased adrenergic-stimulated lipolysis in rats (Smith et al., 1974); however, with a similar dietary manipulation in ruminants the lipogenic capacity was affected (Yang et al., 1978). Glycerol release was not measured but epinephrine-induced free fatty acid release was increased in isolated tissue from high fat-fed steers but only in soybean diets not tallow diets (Yang et al., 1978) It is possible that the increase seen in epinephrine-stimulated fatty acid release was a reflection of re-esterification and not lipolysis (Vernon, 1976; Yang et al., 1978).

Fasting increases lipolysis in rats (Dax et al., 1981; Lacasa et al., 1986) and humans (Jensen et al., 1987) and pigs (Grisdale-Helland et al., 1985). Restricted feeding in steers reduced basal lipolysis in backfat and omental tissue but epinephrine-stimulated lipolysis was either similar

(omental) or reduced (backfat) compared to full feeding (Pothoven et al., 1975). The lack of enhanced lipolytic response to stimulation may be because the feeding regimen was restricted and not a true fast.

### 3.6.4 Influence of Temperature

Environmental or ambient temperature are important factors in energy regulation of the animal (see van Es, 1977). Cold stress in the form of winter shearing resulted in a significant change in the rate of lipid mobilization. Norepinephrine infusions resulted in similar increases in glycerol release, however, fatty acid release was higher in the unshorn group (Symonds & Lomax, 1989). The inconsistency between glycerol and free fatty acid release could be due to a temperature-induced increase in fatty acid utilization or partial hydrolysis of the triglycerides (Symonds et al., 1989).

The immediate fast-acting response to cold stress are likely mediated through the catecholamines (Thompson et al., 1978); whereas, long-term metabolic adaptation to cold stress is probably through the thyroid hormones (Christopherson et al., 1978) and insulin (Sano et al., 1992).

### 3.6.5 Influence of Exercise

In humans, microdialysis measurements of glycerol release increased with exercise in both abdominal and gluteal regions and can be blocked by propranolol, a $\beta$-

adrenergic blocker (Arner et al., 1990b). The effect was more prominent in the abdominal compared to the gluteal region and the effect was significantly less in the gluteal tissue of women compared to men (Arner et al., 1990b; Wahrenberg et al., 1991). This *in situ* study suggested that exercise-induced lipolysis is primarily a ß-adrenergic response. Exercise reduced subcutaneous fat but not intermuscular fat in sheep (Aalhus & Price, 1984). Also, plasma free fatty acid levels were reduced in exercised calves, which was enhanced by clenbuterol treatment (Bruckmaier & Blum, 1992). Plasma glycerol levels increased in exercised lambs but hepatic extraction of lactate and glycerol also increased (Brockman, 1987). The reduction of fatty acids seen in exercised calves may be the result of increased extraction; therefore, because the glycerol response was not measured a rate of lipolysis is not available.

**4.0 Summary**

Catecholamines provide fast-acting regulation of lipid mobilization through interaction with the adrenergic receptor either at the adipocyte or the vascular network surrounding the adipocyte. The response of the adipose mass to adrenergic stimulation is dependent on the receptor complement within the adipose depot, and it may vary depending on the anatomical location. The majority of information regarding depot-specific differences in response

to adrenergic stimulation has been acquired through human subjects, while the information in domestic meat animals is limited. Increasing our understanding of depot-specific responses of adipose tissue will provide options in the process of altering the lean to fat ratio or the partitioning of fat in the carcass that provides a consumer acceptable product.

It is proposed that in the nonpregnant, nonlactating sheep depot-specific differences in responsiveness and sensitivity to catecholamines may be regulated through mechanisms similar to those in humans. That is, an interplay between α- and ß-mediated effects will influence the ability of a depot to respond to adrenergic stimulation. Moreover, it is expected that breed differences will also influence the lipolytic capacity of a depot.

# MANUSCRIPT I

## Lipid Metabolism in the Perfused Isolated Fat-Tail Depot of the Karakul Ewe

ABSTRACT

The metabolic and vascular responses to adrenergic stimulation were simultaneously measured in isolated perfused adipose tissue of the Karakul tail. The initial exposure to isoproterenol (0.1 and .425 μg/min/kg tail) increased lipolysis 55 and 51%, respectively, but a subsequent exposure did not and no effects were found on blood flow. Norepinephrine (1.0 and 4.25 μg/min/kg tail) significantly decreased flowrate by 7.1 and 15.4%, respectively, but had no lipolytic action. The mean glucose uptake over a minimum perfusion duration of seven hours was 10.9 ± 1.5 nmol/min/g perfused adipose tissue. Acetate uptake declined with time from an initial 12.2 to a final 1.3 (±1.7) nmol/min/g perfused adipose tissue. These data indicated that the perfused isolated adipose depot remained metabolically active, but displayed limited lipolytic capacity. The vasoconstrictive properties of norepinephrine, however, were exhibited.

## INTRODUCTION

Regulation of adipose tissue metabolism is a dynamic process involving both neural and endocrine factors. The catecholamines are among the major regulators of adipose tissue metabolic function, as demonstrated in a variety of species (Fain & Garcia-Sainz, 1983 and Vernon, 1980). However, little is known about the functional relationship between the metabolic and vascular effects of catecholamines in adipose tissue.

The procedure presented utilized the large, white adipose tissue mass of the fat-tailed, Karakul sheep. This depot has a vascular arrangement that lends itself to circulatory isolation (Khachadurian et al., 1966). The objective was to measure metabolic and vascular responses to adrenergic stimulation simultaneously in a non-recirculating perfusion system of the isolated tail adipose tissue. Glycerol release from the perfused adipose tissue was monitored as an index of the lipolytic response to adrenergic agonist infusion. The rate of glucose and acetate uptake from the perfusate was monitored as indicators of adipose tissue function during perfusion.

## MATERIALS AND METHODS

### *Animals*

Four adult ewes (53-56 kg) of the Karakul breed were used. The nonpregnant, nonlactating ewes were fed at maintenance on alfalfa hay and were provided free access to

water and cobalt-iodinized salt (NRC, 1985).

*Materials*

Chemicals were purchased from Sigma, St. Louis, MO unless otherwise indicated and all solutions were prepared with deionized water.

*Surgical procedure*

Feed was withheld for 16 h prior to surgery and the sheep underwent a 5 min transport to the surgical facility. The tail was sheared and tail size (for drug dose calculation) was visually estimated. A 25 ml blood sample was taken via jugular venous puncture and through the same puncture, 26 mg/kg sodium pentobarbitone (Somnotol, MTC Pharmaceuticals, Cambridge, Canada) was administered as initial anaesthetic. The sheep was intubated and maintained on halothane surgical anaesthetic for appoximately 2.5 h during surgical isolation of the tail circulation.

A 20-30 cm incision was made along the right abdominal surface to expose the posterior portion of the aorta and vena cava and the iliac vessels (see Figure 2). Major vessels supplying the hindlimbs, reproductive tract, mammary gland and urinary bladder were ligatured to isolate circulation to the tail-adipose depot. In addition, a tourniquet was secured around each hindlimb to further ensure isolated tail circulation. Polypropylene catheters (4 mm I.D./5 mm O.D.) attached to Tygon® tubing were positioned in the posterior end of the aorta and vena cava

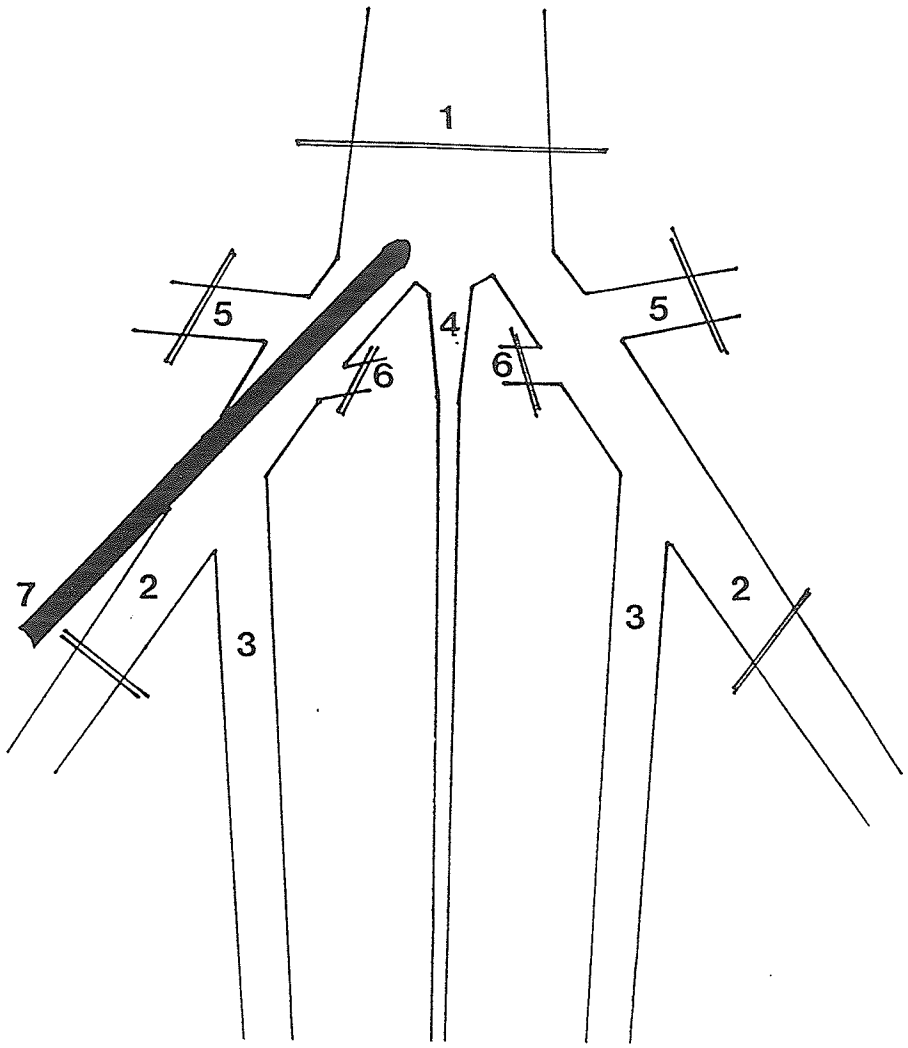


Figure 2. The circulation to the tail adipose depot was isolated under general halothane anesthetic. Double lines indicate ligatured vessels. 1=aorta, 2=external iliac, 3=internal iliac, 4=medial sacral, 5=deep circumflex iliac, 6=mammary artery, 7=catheter. Corresponding veins were treated in a similar manner.

by introduction via the right external iliac artery and vein, respectively. Ligatures distal to the internal iliac artery and vein were used to secure the catheters and to occlude the right external iliac vessels. Similarly, the left external iliac vessels were ligatured distal to the internal iliac branches. The ligatures and catheter placements isolated circulation to that of the internal iliac vessels and the caudal sacral vessels, which supply the tail-adipose depot.

Perfusion of the tail-adipose depot was initiated and the ewe was euthanized upon ligature of the aorta and vena cava cranial to the catheter tips. The tail (approximately 1-3 kg) was surrounded by a network of Tygon® tubing (7mm ID/9mm OD) through which water (38 °C) was circulated to prevent tissue cooling.

*Tissue perfusion and drug administration*

The perfusate was a mixture (1:1) of previously frozen bovine plasma (1 mg/ml EDTA) and M199 tissue culture medium with final concentrations of other components as shown in Table 1. Bovine plasma was prepared from blood collected at a local abattoir, centrifuged at 1500 · *g* and stored as 2 L aliquots at -20°C until preparation of the perfusate. Approximately 24-36 L of perfusate were prepared fresh during the course of each perfusion (3 L/h). Immediately before infusion the perfusate was brought to 39°C, the pH adjusted to 7.4 and continuously aerated with 95% $O_2$/5% $CO_2$

Table 1. Components and final concentrations of the perfusate mixture

| Component | Concentration |
|---|---|
| Bovine plasma | 50% v/v |
| M199 solution[1] | 50% v/v |
| EDTA[2] | 0.5 mg/ml |
| HEPES[3] | 12.5 mM |
| Sodium Bicarbonate | 13.2 mM |
| Glucose | 3.43 ± 0.01 mM |
| Acetate | 3.59 ± 0.23 mM |
| Glycerol | 0.03 ± 0.01 mM |

[1]M199 Tissue culture media with Earle's salts obtained from Sigma (St. Louis, MO; catalog number M2520).
[2]EDTA (ethylenediaminetetraacetate) used as an anticoagulant in blood collection from the rural abattoir.
[3]HEPES N-2Hydroxyethylpiperazine-N'2-ethanesulfonic acid

(see Appendix I). The perfusate was administered at a constant rate of 54 ml/min via the aorta-placed catheter using a Masterflex peristaltic pump (Cole-Parmer, Chicago IL). Drugs and saline were administered at a rate of 1 ml/min into the aorta-placed catheter with a proportioning pump (Technicon Corp, Tarrytown, NY).

Initially, as tail blood was being replaced by perfusate (ca. 20 min), heparin (1.5 mg/ml 0.9% saline) was infused at 1 ml/min to prevent clotting. Subsequently, 0.9% saline replaced the heparin and the perfusate infused with saline was continued until the effluent flowrate was considered stable (at least 1 h). The drug regimen was identical for each of the four sheep. It began with isoproterenol being delivered consecutively at 0.026, 0.1 and 0.425 $\mu$g/min/kg tail (0.25, 0.95, 4.0 x $10^{-8}$ M determined by $\mu$g base infused per min assuming a 54 ml/min flowrate), each for 15 min. A 45-min washout period, which consisted of replacing the drug infusion into the perfusate with a saline infusion. Doses of norepinephrine at 0.26, 1.0 and 4.25 $\mu$g/min/kg tail (0.31, 1.2, 5.0 x $10^{-7}$ M) were given consecutively, each for 15 min (Exposure 1) following the saline-washout period. The entire isoproterenol-norepinephrine sequence was repeated (Exposure 2) with a 45-min saline washout period before each drug regimen. At the conclusion, 2 ml Evan's Blue dye (10 mg/ml) was injected into the system to confirm the location of the perfusion

area. The perfused tissue (tail and associated rump fat as determined by Evan's Blue dye distribution) was dissected from the skin and bone and weighed before and after drying at 60°C for 48-72 h.

Flowrate was measured by continuously weighing (corrected for sample removal) the effluent from the vena cava-placed catheter (outflow). Effluent aliquots of 15 ml were collected on ice at 5-min intervals, then frozen for later analysis of glycerol, glucose and acetate content. Perfusate aliquots were sampled on an hourly basis and similarly treated.

*Determination of metabolites*

*Glycerol*. Glycerol concentration was determined enzymatically using commercially available kits (Boehringer Mannheim, Canada). Samples were prepared as per assay instructions except 1.0 ml was diluted with 2.0 ml distilled water.

*Acetate*. Acetate concentration was determined by gas chromatography using Supelco and prepared standards. Samples were prepared for analysis by mixing 250 $\mu$l of sample and 50 $\mu$l of *meta*-phosphoric acid (25% w/v). The mixture was capped, left for 30 min at 25°C, and then frozen for later analysis (24 h). The mixture was vortexed and centrifuged for 2 min at 15,600 $\cdot$ *g* and 1 or 2 $\mu$l of the supernatant were injected onto the column for analysis. Volatile fatty acids were separated on glass columns packed

with Supelco GP 10% SP1200/1% $H_3PO_4$ on 80/100 chromosorb W AW packing at 25 ml/min He flowrate. An injection temperature of 170°C and a programmed column temperature range of 100-160°C at 10°C/min was used. The mean acetic acid retention time was 1.79 ± 0.03 min.

*Glucose*. Glucose content was determined with an AutoAnalyzer II (Technicon Corp, Tarrytown, NY) following the Technicon AutoAnalyzer methodology bulletin AA II-2.

*Calculations and statistics*

Rates of uptake or release were calculated as follows: [(inflow-outflow concentration, nmol/ml) x effluent flow rate ml/min]/[tail + rump fat (g dry weight)] = nmol/min/g perfused adipose tissue. Each drug x dose combination served as an individual treatment and the last 15 min of each 45-min washout period served as the zero dose for the subsequent drug. Thus, there were eight treatment groups in each exposure. Data were analysed using the General Linear Models (SAS, 1985) procedure with tail, exposure repetition, and treatment group as main effects. The tail X treatment and the tail X treatment X exposure interactions were combined to provide the error term. The pairwise comparisons were made using Least Squares Means and the level of significance was set at $p<0.05$. All data are expressed as the mean ± SEM. The $R^2$ of the model for each dependent variable was 0.96 (flowrate) and 0.75-0.79 for glycerol, acetate and glucose.

# RESULTS

## *Perfusion*

Plasma variables, body weights and perfused adipose mass are shown in Table 2. The entire perfusion period continued for an average of 9.7 h (8-10.5 h) but the data reported were collected during the initial 7 h. (The results collected beyond the initial 7 h are shown in Appendix II and Appendix III.) The tails showed moderate signs of edema although after complete drying the tissue was found to contain 19.5 ± 7.0% water, which is not excessive compared to the 13-14% water content of nonperfused tail adipose tissue (Gooden et al., 1986; Khachadurian et al., 1966). Visual estimation of the tail adipose tissue mass (for drug dose calculation) was inaccurate, mainly because a large portion of the adipose tissue perfused consisted of associated rump fat (similar experiments found this non-tail portion to contribute 61 ± 8% of the perfused mass; unpublished observation). Consequently, the doses administered were calculated for each tail and found to be 43% ± 5% of the intended doses.

## *Flowrate*

The initial individual flowrates stabilised at 38.4, 39.0, 44.6 and 47.7 ml/min after the removal of the blood was complete. The overall mean perfusion flowrate was 38.6 ± 2.9 ml/min which represents an overall mean specific flowrate of 1.1 ± 0.2 ml/min/100 g. The mean individual

Table 2. Plasma concentrations, body weights and mass of perfused adipose tissue of the Karakul ewe

| Animal | Variable | | | | |
|---|---|---|---|---|---|
| | Plasma (mM) | | | Body Wt. | Tail Wt. |
| | Glucose | Acetate | Glycerol | (kg) | (kg)[1] |
| 44 | 5.24 | 0.55 | 0.16 | 55.9 | 3.1 |
| 64 | 7.20 | 0.73 | ND | 53.2 | 4.1 |
| 94 | 6.39 | 0.92 | 0.31 | 54.1 | 2.7 |
| 26 | 12.46 | 0.53 | 0.35 | 54.5 | 1.9 |

[1]Tail represents the dried weight of perfused adipose mass including associated rump fat.
ND not determined

flowrates were significantly different from each other ($p<0.0001$) and the specific flowrates for the tail depots ranged from 2.5 ml/min/100 g in the smallest perfused mass to 0.8 ml/min/100 g in the largest perfused mass. Flowrate decreased 11% from the initial saline baseline to the final saline baseline infusion. Isoproterenol had no significant effect on flowrate (Figure 3) although there was a tendency for flowrate to increase with the two highest doses upon both the first and second exposure. Norepinephrine, over the two exposures, significantly reduced the perfusate flowrate an average of 2.5, 7.1 and 15.4% with the low, medium, and high doses, respectively. However, norepinephrine was more potent ($p<0.001$) during the second exposure (Figure 3). The second highest dose of norepinephrine reduced flowrate 5.3% in the first exposure compared to 9.3% in the second exposure and the high dose reduced flowrate 10.6% in the first exposure compared to 20.7% in the second exposure. Flowrate partially recovered subsequent to the cessation of norepinephrine infusion.

*Glycerol release*

The initial exposure to the medium and high doses of isoproterenol increased glycerol release 55 and 51% (0.83 and 0.76 $\mu$mol/min/g, respectively) compared to the initial baseline glycerol release ($p<0.1$; Figure 4). This initial increase was followed by a gradual and general elevation in glycerol release for the duration of the perfusion period.

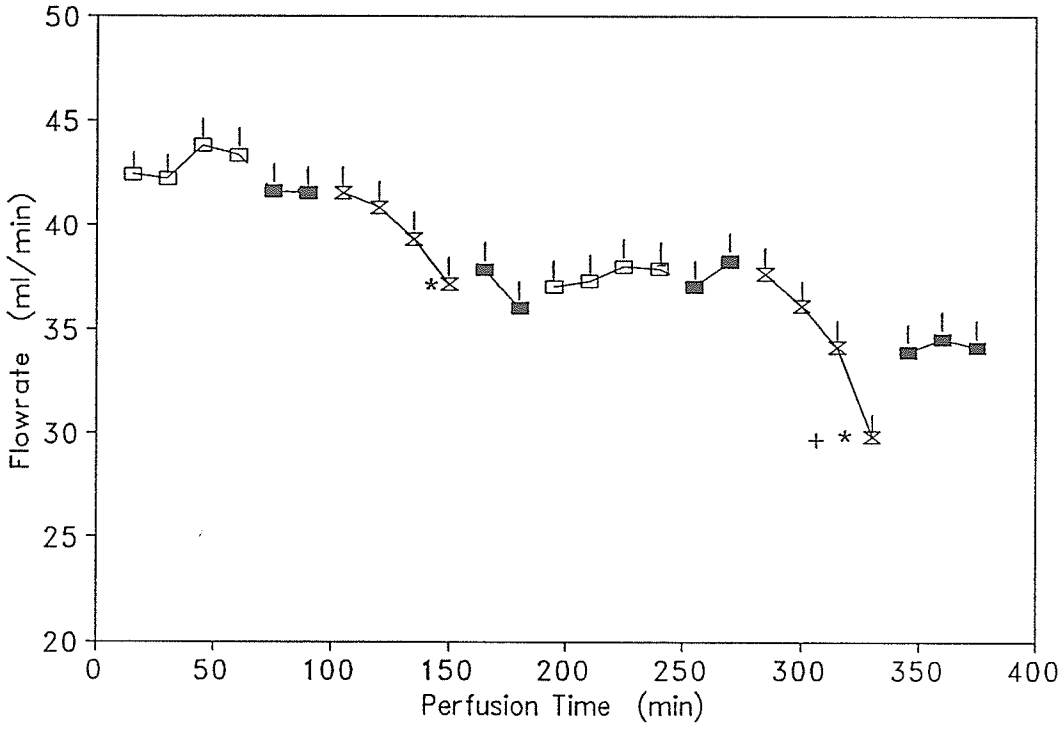


Figure 3. Effects of adrenergic stimulation on flowrate in the isolated fat-tail depot. Four doses of isoproterenol (□-□) or norepinephrine (X-X) were given consecutively for 15 min (see text for doses) followed by a 30 or 45-min washout period (■-■) between each drug sequence. Mean ± S.E. of 4 sheep. * $p<0.001$ vs zero dose norepinephrine; + $p<0.0001$ vs comparable dose first exposure.

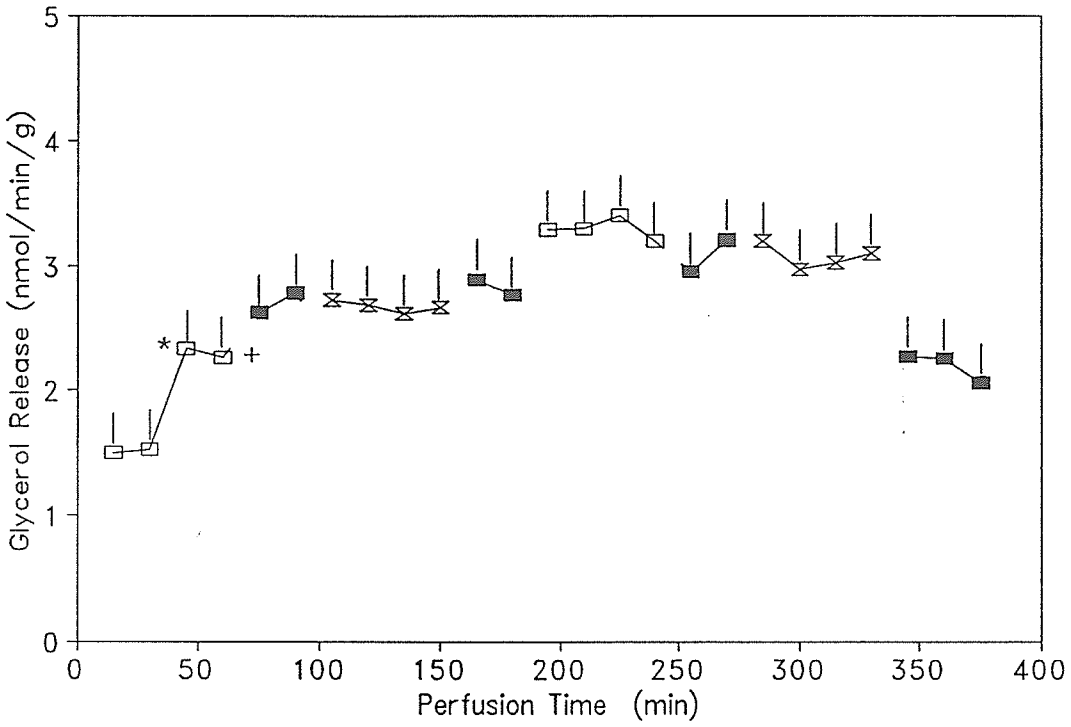


Figure 4. Effects of adrenergic stimulation on glycerol release in the isolated fat-tail depot. Four doses of isoproterenol (□–□) or norepinephrine (X-X) were given consecutively for 15 min (see text for doses) followed by a 30 or 45-min washout period (■–■) between each drug sequence. Mean ± S.E. of 4 sheep. * $p<0.07$, + $p<0.09$ vs zero dose isoproterenol first exposure.

*Glucose uptake*

Overall glucose uptake was highly and significantly ($p<0.05$) variable among tails (4.9, 11.7, 12.3, 14.9 nmol/min/g with the lowest rate of uptake found for the largest (4.1 kg) tail. The mean glucose uptake was greater during the second exposure compared to the first exposure (9.7 vs 12.2 $\pm$ 0.5 nmol/min/ g; $p<0.002$), but the initial rate of uptake was not different ($p=0.64$) from the rate of uptake at 330 min (Figure 5). Neither drug had a significant effect on glucose uptake but it tended to decrease after the initial exposure to isoproterenol ($p>0.1$).

*Acetate uptake*

Acetate uptake was the most variable of metabolite concentrations determined during perfusion. There was a pronounced 90% ($p<0.0001$) decrease in the rate of acetate uptake observed over the perfusion period (Figure 6). No significant drug effects were detected, but there was a tendency for acetate uptake to decline more during the drug infusions than during the saline infusions in the first exposure.

## DISCUSSION

An isolated non-recirculating perfusion of the tail adipose tissue was chosen because it allowed the simultaneous measurement of vascular and metabolic responses to catecholamine stimulation. In addition, accumulation of drugs or metabolites in the perfusate was avoided. In this

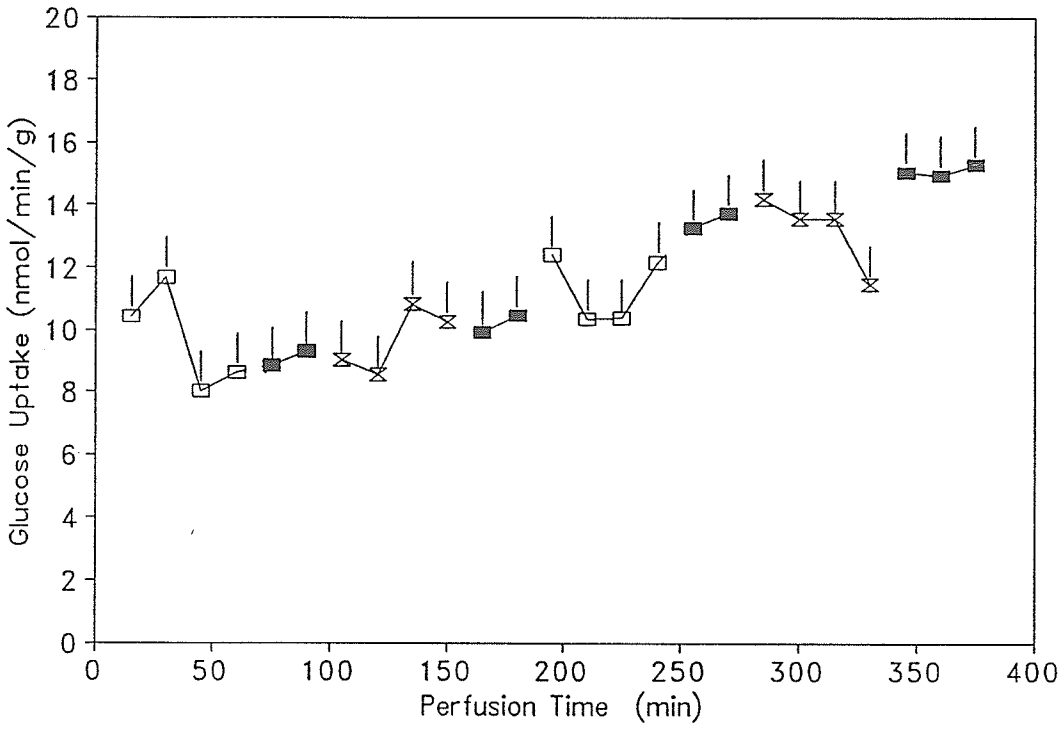


Figure 5. Effects of adrenergic stimulation on glucose uptake in the isolated fat-tail depot. Four doses of isoproterenol (□-□) or norepinephrine (X-X) were given consecutively for 15 min (see text for doses) followed by a 30 or 45-min washout period (■-■) between each drug sequence. Mean ± S.E. of 4 sheep.

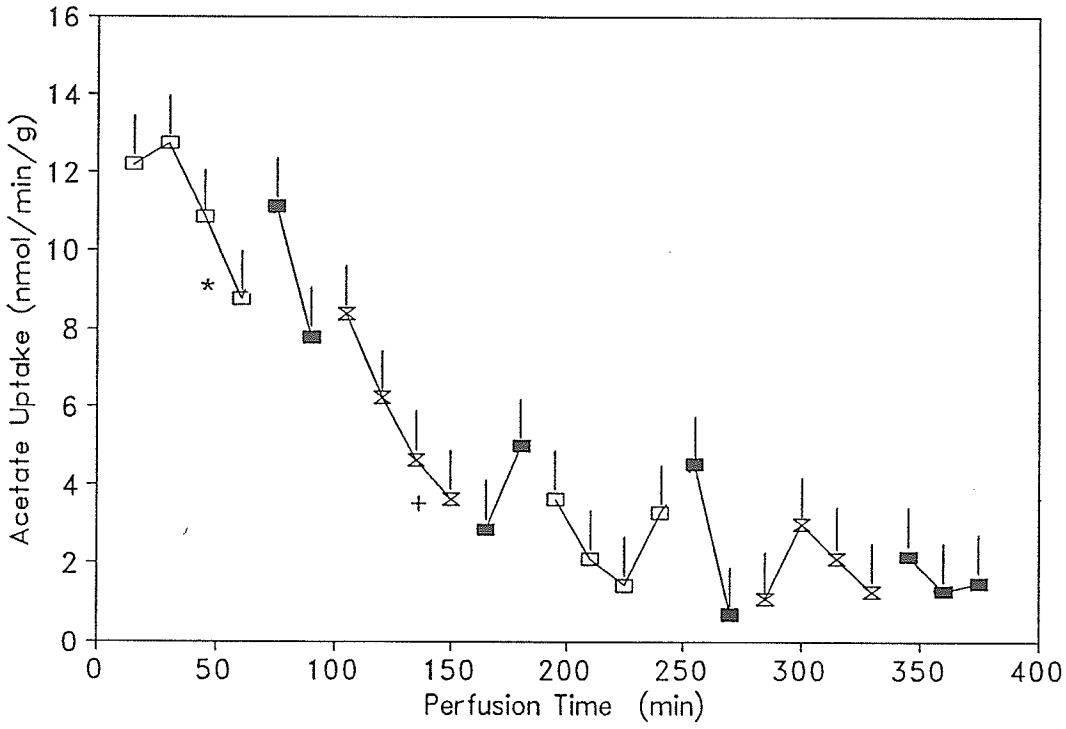


Figure 6. Effects of adrenergic stimulation on acetate uptake in the isolated fat-tail depot. Four doses of isoproterenol (□-□) or norepinephrine (X-X) were given consecutively for 15 min (see text for doses) followed by a 30 or 45-min washout period (■-■) between each drug sequence. Mean ± S.E. of 4 sheep. * $p<0.2$ vs zero dose isoproterenol; + $p<0.05$ vs zero dose norepinephrine.

study, M199 tissue culture medium was diluted with bovine plasma which served as an inexpensive source of plasma protein for the large volumes of perfusate required. Tissue culture medium (M199) supplemented with 4% BSA is sufficient to support adipocyte function *in vitro* (Vernon, 1979; Watt et al., 1991). The perfusate was adequate to support basal metabolism as initial rates of glycerol release and acetate and glucose uptake were similar to values reported in an *in vivo* tail depot study (Gooden et al., 1986) and in isolated ovine adipocytes (Guesnet et al., 1987; Iliou & Demarne, 1987). However, the perfusate may have been suboptimal following isoproterenol-induced lipolysis as indicated by the general rise in glycerol release and the concurrent decline in acetate uptake. As seen in Figure 4, the first exposure to isoproterenol (0.1 μg/min/kg tail) produced a 55% increase in glycerol release, which is modest compared to isoproterenol-induced lipolysis observed in isolated adipocytes from other adipose tissue depots (Guesnet et al., 1987; Iliou & Demarne, 1987; Watt et al., 1991). The magnitude of the response was less than that found in an *in vitro* preparation of adipose tissue pieces biopsied from the tail depot (Manuscript III). Ten-fold higher doses of both isoproterenol and norepinephrine failed to produce an increase in glycerol release (see Appendix III). The initial lipolytic rate was lower than the lipolytic rate found *in vitro* and the highest rate achieved in the present

study was comparable to the basal lipolytic rate found *in vitro* (Manuscript III). Although the modest increase in glycerol release may indicate a low responsiveness of the tail adipose tissue to catecholamine stimulation compared to other depots studied, the failure of glycerol release to return to basal levels following the 45-min saline washout period and the absence of an isoproterenol-induced increase in glycerol release during the second exposure suggests that other factors need to be considered.

Firstly, glycerol release may have failed to return to baseline because the perfusate may not have provided a necessary physiological opposition to lipolysis, for example, insulin. Insulin provides inhibition to both basal and catecholamine-stimulated lipolysis in isolated ovine adipocytes *in vitro* (Cochrane & Rogers, 1990). The perfusate was not supplemented with additional insulin. The perfusate was bovine plasma diluted (1:1) with tissue culture medium (insulin free), therefore, the maximum insulin content could only have been 50% of *in vivo* bovine plasma levels. The insulin level of the perfusate was not determined, but it is possible that the insulin content was not sufficient, therefore, the stimulated lipolysis may have been allowed to continue without opposition.

Secondly, those metabolites which serve as feedback or local regulators of lipid mobilization may have been disrupted because the system was non-recirculating. For

example, adenosine, which is considered a local regulator (Vernon et al., 1991) may have been removed, or accumulation prevented, by the continual flushing with fresh perfusate, thus preventing any reduction in lipolysis expecially during the saline washout periods. Similarly, the accumulation of free fatty acids may have been prevented, which would remove the feedback inhibition of ongoing lipolysis displayed by a build up of free fatty acids (Allen, 1979; Metz et al., 1973; Yang & Baldwin, 1973).

Thirdly, desensitization of the adipose tissue cannot be excluded. Arner et al., 1991 have shown desensitization of ß-receptor responsiveness to be a mechanism of adipocyte regulation of lipolysis. However, sequential infusion of $\beta$-adrenergic agonists with or without a washout period did not reduce their effectiveness in the anesthetized porcine model (Mersmann, 1987; 1989a,b). Also, transfer of epinephrine-stimulated cells to fresh, epinephrine-free buffer, resulted in lipolytic rates returning to prestimulatory levels within 15 min (Birnbaum & Goodman, 1976).

Glucose uptake, although variable, was observed throughout the perfusion duration without the presence of added insulin (see above discussion). $\beta$-adrenergic agonist exposure can lead to increased rates of glucose utilization, usually dependent on the extent of fatty acid re-esterification (see Fredholm, 1985). In the present study, glucose uptake was 50% of that found during norepinephrine

stimulation of a fat-tail *in vivo* preparation (Gooden et al., 1986). The glucose was probably used for glycerol synthesis as glucose incorporation into fatty acids in the presence of acetate is very low (Ballard et al., 1969). Also, fatty acid synthesis from glucose is reduced with exposure to lipolytic agents (Peterla & Scanes, 1990). The reduction in acetate uptake over time was probably a result of increased free fatty acid availability (Baldwin et al., 1973), which would result in an increased rate of re-esterification of the available fatty acids (Edens et al., 1990; Madsen et al., 1986). An increased rate of re-esterification would then account for the uptake of glucose for glycerol synthesis (Vernon, 1980).

The change in flowrate during the duration of the perfusion was minimal, with the exception of the noradrenaline treatment, suggesting that the vascular integrity within the adipose tissue was intact and responsive. Moreover, the dose-dependent vascular response to norepinephrine was demonstrated during both exposures. Maximum vasoconstriction was not achieved with the doses used, as flowrate was found to be reduced by 50% with a norepinephrine dose of 42.5 μg/min/kg tail (see Appendix I). Although flowrate did recover slightly after vasoconstriction, it did not return to the prestimulatory levels. The failure to return to prestimulatory levels may be the result of pressure-induced bursting of vessels during

the vasoconstriction periods because inflow rate was not reduced during the periods of reduced outflow (vasoconstriction). Therefore, if vessels ruptured during constriction they would no longer be available to carry the perfusate and return flowrate to prestimulatory levels.

At no time was vasodilation observed in response to norepinephrine, contrary to that observed in an *in vivo* preparation of the tail depot (Gooden et al., 1986). The observed vasodilation response to noradrenaline was most likely the result of over stimulation resulting in autoregulatory escape, which then produces a $\beta$ adrenergic response (see Fredholm, 1985). Isoproterenol did not induce vasodilation at the doses used nor at a ten-fold higher dose (see Appendix I).

It is possible that in the tail adipose tissue, in the absence of local regulator influence, the vascular mechanisms are more sensitive to adrenergic stimulation than are the lipolytic mechanisms. Furthermore, the lack of a potent lipolytic response to either isoproterenol or norepinephrine suggests that the adipose tissue of the tail is unresponsive to adrenergic stimulation, as seen in an earlier study (Khachadurian et al., 1966). It may be that local regulators such as adenosine, or antilipolytic agents such as insulin play a more important role in regulating lipid mobilization than do catecholamines in this depot. Further studies are needed to evaluate the factors that

contribute to the regulation of lipid metabolism in this unique adipose depot. The non-recirculating perfusion system is suitable for the investigation, by allowing a simultaneous observation of metabolic and vascular events while providing the opportunity to manipulate the perfusate properties.

# MANUSCRIPT II

## Lipid Metabolism in the Perfused Isolated Fat-Tail Depot of Clonidine-Treated Lambs

## ABSTRACT

Flowrate, glycerol release and glucose and acetate uptake in response to isoproterenol and clonidine were examined over 7 h in the isolated perfused fat-tail depot of six Karakul ram lambs treated for 84 days with clonidine or saline. The mean perfusate flowrates were 32.9 ± 1.3 ml/min and 33.7 ± 3.2 ml/min in the control and clonidine-treated groups, respectively. Initial rates were monitored during saline infusion, which was subsequently replaced with isoproterenol infusion for 4 consecutive hours. Clonidine was infused with isoproterenol during the second hour. The flowrate declined over time in both groups and was reduced 27% by the clonidine infusion compared to the previous isoproterenol infusion in the control group only. The lipolytic rate, as determined by glycerol release, increased 2-3 fold prior to the drug treatments in both groups and was further increased (25%) with isoproterenol in the control group only. Following the initial increase, glycerol release declined gradually during the perfusion period in both groups. Glucose uptake was initially higher (65%) in the control group but was reduced to the level of the clonidine group after the initial exposure to isoproterenol. Acetate uptake declined rapidly in the control group prior to any drug treatment and continued to decline in both groups over time. The isolated perfusion model allowed simultaneous observation over an extended period of time, of

the metabolic and vascular response to adrenergic stimulation. Chronic exposure to clonidine desensitized the vascular response to clonidine during perfusion and possibly altered the metabolism of glucose and acetate in the tail adipose tissue.

# INTRODUCTION

Clonidine, an α-adrenergic agonist, has been shown to affect growth and fattening patterns in lambs (Kennedy et al., 1987, 1991) and endocrine profiles in lambs (Kennedy et al., 1988) and cattle (Schaefer et al., 1990; Veenhuizen et al., 1982). It has been suggested that clonidine effects are mediated through alterations in the endocrine profile, notably growth hormone secretion, in ruminants (Kennedy et al., 1987, Schaefer et al., 1990).

Increased circulating growth hormone results in a significant increase in protein accretion at the expense of fat deposition in meat producing animals (see Etherton & Smith, 1991). In addition, β-adrenergic agonists cause increases in the lean to fat ratio (see Etherton & Smith, 1991). As catecholamines are the major endogenous regulators of adipose tissue function (Fain & Garcia-Sainz, 1980), chronic clonidine treatment of meat producing animals may alter the adrenergic responsiveness of adipose tissue, thus altering energy metabolism and the fattening process.

The present study examined the effects of ß-adrenergic (isoproterenol) and α-adrenergic (clonidine) stimulation on glucose and acetate uptake and glycerol release in an isolated perfusion preparation of the unique fat-tail depot of saline and 84-day clonidine-treated growing Karakul lambs. The vascular responsiveness to isoproterenol and clonidine was also monitored. The objective was to

determine if prior *in vivo* exposure to an α-adrenergic agonist alters the lipolytic responsiveness of the tail adipose tissue to α- or ß-adrenergic agonist stimulation.

## MATERIALS AND METHODS

### *Animals*

Nine Karakul ram lambs weaned at five weeks of age were fed *ad libitum* a pelleted 17% protein diet containing 49% rolled barley, 40% dehydrated alfalfa meal, 10% canola meal and 1% premix containing vitamins A and D each at 500 IU/kg. The diet exceeded NRC requirements for growing lambs (NRC, 1985). Lambs were housed individually at six weeks of age in the university animal housing facility. Water and cobalt-iodinized salt were available at all times.

### *Clonidine treatment*

At nine weeks of age the lambs were implanted, in the lower shoulder region, with Alzet® osmotic pumps (Alza Corp., Palo Alto, CA), which delivered either sterile 0.9% saline (n=4) or clonidine dissolved in sterile 0.9% saline (n=5). Clonidine was administered at a dose rate of 20 μg/kg/day. The pumps, which have a delivery life of 28 days, were removed and replaced twice at 28-day intervals adjusting the clonidine dosage for body weight at each interval.

### *Surgical preparation*

Feed was withheld for 16 h prior to surgery. A 20 ml blood sample was collected via jugular venous puncture and

through the same puncture, 26 mg/kg sodium pentobarbitone (Somnotol, MTC Pharmaceuticals, Cambridge, Canada) was administered as initial anaesthetic. The sheep was intubated and anesthesia was maintained with halothane while isolation of the tail circulation was perfomed as previously described (Manuscript I). Some minor modifications were necessary. The catheters were smaller because of the smaller vessels of the younger rams (2 mm I. D. and 3 mm O. D.). The inflow catheter was positioned in the most caudal portion of the aorta via the deep circumflex iliac artery instead of the external iliac artery due to the small size and the weakness of the external iliac artery (see Figure 2). In addition, the testicular arteries were ligatured to prevent circulation. Perfusion was initiated when the aorta and vena cava were ligatured and the sheep was euthanized.

*Experimental design and analyses*

The perfusate consisted of a Hepes-buffered 1:1 mixture of M199 tissue culture media and bovine plasma, which was prepared and maintained as described previously (Manuscript I and Appendix I). The final concentrations of acetate, glucose and glycerol in the perfusate are shown in Table 3. A stable flowrate was established after blood was replaced by the perfusate infused with heparin (approximately 45 min). Saline was infused in the perfusate for 1 h then replaced with isoproterenol delivered into the arterial catheter at 1 ml/min administering 1 $\mu$g/min/kg tail for 4 h.

Table 3. Components and final concentrations of the perfusate mixture

| Component | Concentration |
|---|---|
| Bovine plasma | 50% v/v |
| M199 solution[1] | 50% v/v |
| EDTA[2] | 0.5 mg/ml |
| HEPES[3] | 12.5 mM |
| Sodium Bicarbonate | 13.2 mM |
| Glucose | 5.61 ± 0.07 mM |
| Acetate | 2.47 ± 0.08 mM |
| Glycerol | 0.02 ± 0.01 mM |

[1]M199 Tissue culture media with Earle's salts obtained from Sigma (St. Louis, MO; catalog number M2520).
[2]EDTA (ethylenediaminetetraacetate) used as an anticoagulant in blood collection from rural abattoir.
[3]HEPES N-2-Hydroxyethylpiperazine-N'-2-ethanesulfonic acid.

The second h of isoproterenol infusion was accompanied with 10 μg/min/kg tail of clonidine. The final h of perfusion was again saline for 1 h. Flowrate was measured by continuously weighing (corrected for sample removal) the effluent from the vena cava-placed catheter (outflow). Effluent aliquots of 15 ml were collected at 20-min intervals and selected samples were later analyzed for glycerol, acetate and glucose concentration. Determination of metabolite concentrations in the collected perfusate and plasma have been described earlier (Manuscript I).

*Calculations* and statistics

Rates of uptake or release were calculated as follows: [(input-output concentration nmol/ml) x effluent flowrate ml/min]/[tail + rump fat g dry weight]=nmol/min/g. Data were analyzed using the General Linear Model (SAS, 1985) in a 2 x 7 Treatment (control, clonidine implant) x Drug (perfusate (P), saline 1 ($S_1$), isoproterenol 1 ($I_1$), isoproterenol plus clonidine (IC), isoproterenol 2 ($I_2$), isoproterenol 3 ($I_3$), and saline 2 ($S_2$)) factorial with repeated measures on the last variable. The error terms were the interactions including animal. Pairwise comparisons were made using the Least Squared Means (SAS, 1985) and significance level was $p<0.05$. Data are expressed as mean $\pm$ S. E. except in the control group following the IC infusion period where n=1.

*Materials*

Clonidine hydrochloride (2-(2,6-dichloroanilino-2-imidazoline) was a gift from Bohringer Ingelheim (Canada). Alzet® osmotic pumps were purchased from Alza Corp., Palo Alto, CA. Glycerol enzymatic kits were purchased from Boehringer Mannheim, (Canada). All other chemicals and reagents were purchased from Sigma (St. Louis, MO) or Canlab (Winnipeg, Canada).

## RESULTS

The perfusate was a mixture of tissue culture medium and bovine plasma (Table 3). Tissue culture medium has been shown to adequately support adipocyte function up to several days (Vernon, 1982) and the bovine plasma was an inexpensive source of protein for the large quantities of perfusate required.

The data were obtained from six (3 control and 3 clonidine) ram lambs although nine preparations were attempted. Three preparations failed during the first 80 min of perfusion and following the clonidine infusion only one control preparation was functioning (see Appendix IV).

A moderate flowrate was maintained in both treatment groups for a period of 7 h, but did decline 49% ($p<0.006$) and 51% ($p<0.001$) over the reported perfusion period in the control and clonidine-treated groups, respectively (Figure 7). The mean overall flowrate was 32.9 $\pm$ 1.3 ml/min (control group) and 33.7 $\pm$ 3.2 ml/min (clonidine-treated group). The overall mean specific flowrate was 0.8 $\pm$ 0.1

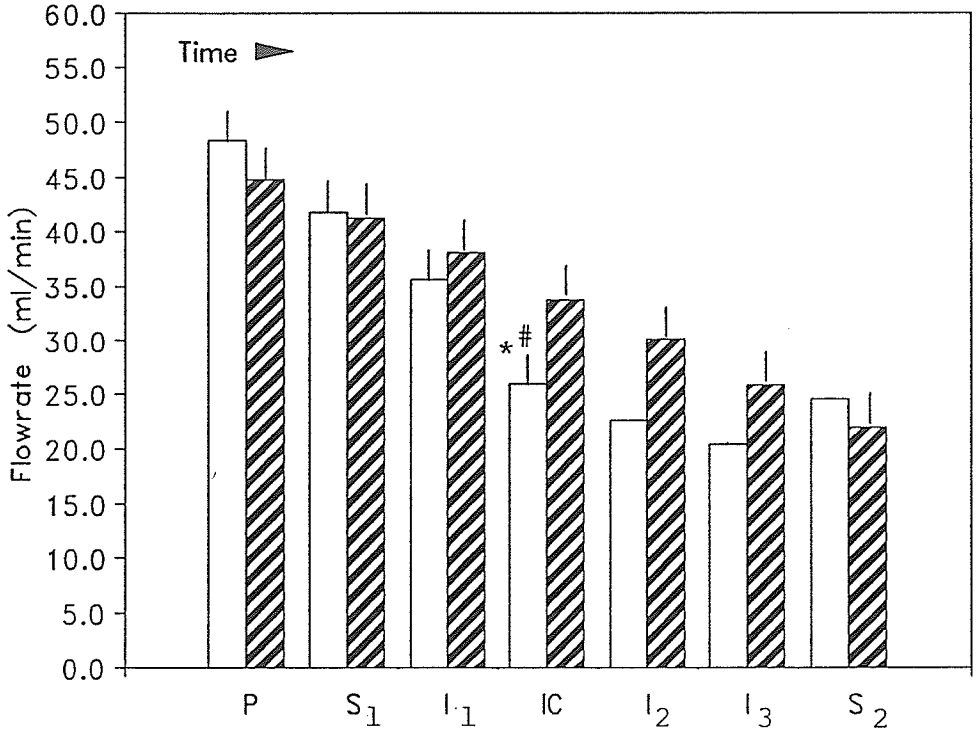


Figure 7. Effects of adrenergic agonists on flowrate over time (420 min) in the perfused fat-tail depot in control (open bars) and 84-day clonidine-treated (hatched bars) Karakul ram lambs. Drugs were prepared in saline and infused into the perfusate inflow. P=perfusate alone, S=saline infusion, I=isoproterenol, IC=isoproterenol and clonidine consecutively. Mean $\pm$ S.E. of 3 sheep per group taken after 1 h of infusion treatment. * Comparisons within control group $S_1$ vs IC, $p<0.0001$; $I_1$ vs IC, $p<0.009$; # Control vs Clonidine at IC, $p<0.03$.

ml/min/100 g in the control group and 0.9 ± 0.1 ml/min/100 g in the clonidine-treated group. The initial specific flowrate after blood had been replaced by perfusate, approximately 30-45 min, was 1.2 ± 0.1 ml/min/100 g and 1.3 ± 0.1 ml/min/100 g in the control and clonidine-treated groups, respectively.

The clonidine infusion reduced the flowrate in the control group 27% ($p<0.009$) compared to the previous isoproterenol flowrate and 38% ($p<0.0001$) compared to the initial saline flowrate (Figure 7). During the IC infusion the control flowrate was reduced compared to the clonidine-treated group ($p<0.03$), and the clonidine group showed no response to the clonidine infusion. Isoproterenol did not induce vasodilation in either group during any of the exposures.

*Glycerol release*

The glycerol concentration in the effluent was determined at the conclusion of each drug or drug combination session. The lipolytic rate increased significantly from the initial infusion period (perfusate) to the conclusion of the first saline infusion period in both groups (Figure 8). The lipolytic rate then decreased continually in the clonidine group, but tended to increase an additional 25% with isoproterenol infusion in the control group (Figure 8). Following the isoproterenol infusion the control group also displayed a continual decrease in

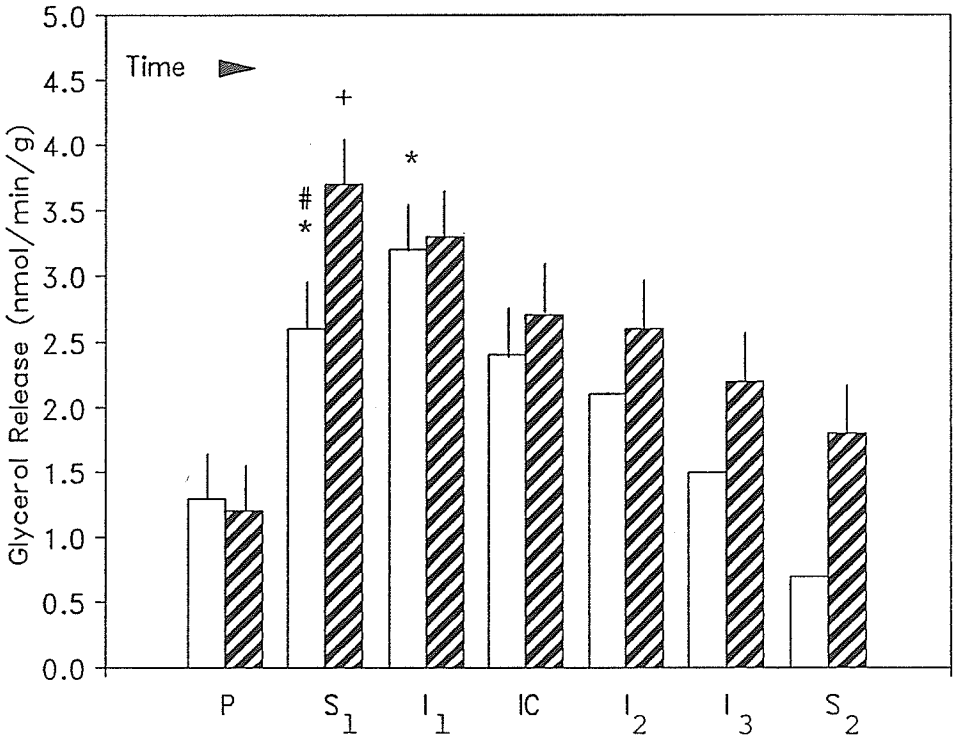


Figure 8. Effects of adrenergic agonists on glycerol release over time (420 min) in the perfused fat-tail depot in control (open bars) and 84-day clonidine-treated (hatched bars) Karakul ram lambs. Drugs were prepared in saline and infused into the perfusate inflow. P=perfusate alone, S=saline infusion, I=isoproterenol, IC=isoproterenol and clonidine consecutively. Mean ± S.E. of 3 sheep per group taken after 1 h of infusion treatment. * Comparisons within Control group, P vs $S_1$, $p<0.05$; P vs $I_1$, $p<0.005$, + within Clonidine group P vs $S_1$, $p<0.0005$; # Control vs Clonidine at $S_1$, $p<0.08$.

lipolytic rate over time regardless of drug treatment.

*Glucose uptake*

Glucose uptake continued over the entire perfusion period but was more stable in the clonidine-treated group (Figure 9). The rate of glucose uptake was greater in the control group compared to the clonidine-treated group during the initial perfusate (P) and saline (S) infusion periods. However, a 35% isoproterenol-induced reduction in glucose uptake in the control group resulted in similar uptake rates in the two groups for the remaining duration of the perfusion.

*Acetate uptake*

Acetate uptake declined 87% ($p<0.02$) in the clonidine-treated group and was completely abolished in the control group by the completion of the perfusion period (Figure 10). The most dramatic decline in acetate uptake in the control group occurred during the first saline infusion. No significant drug effects were detected compared to the saline infusion in the control group, but acetate uptake was lower in the control group than the clonidine group for the first 3 h.

The plasma glucose, acetate and glycerol levels prior to surgery are shown in Table 4. Body weights and perfused adipose mass were similar in the saline and clonidine-treated rams (Table 4). Serum insulin levels (ng/ml) were measured in blood samples collected 1 week prior to the

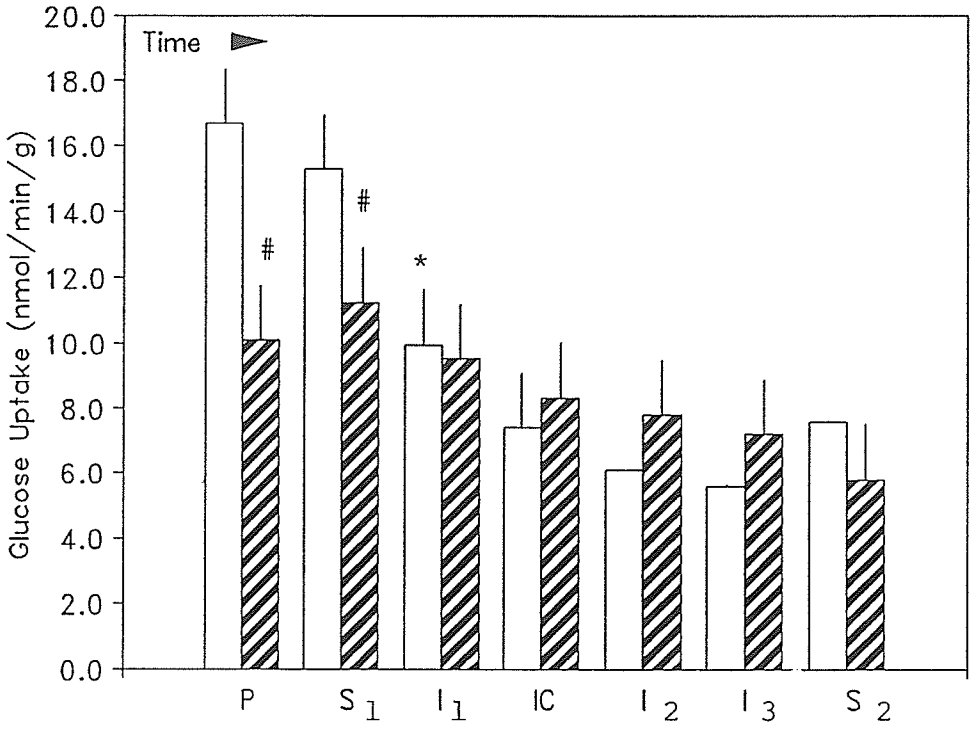


Figure 9. Effects of adrenergic agonists on glucose uptake over time (420 min) in the perfused fat-tail depot in control (open bars) and 84-day clonidine-treated (hatched bars) Karakul ram lambs. Drugs were prepared in saline and infused into the perfusate inflow. P=perfusate alone, S=saline infusion, I=isoproterenol, IC=isoproterenol and clonidine consecutively. Mean $\pm$ S.E. of 3 sheep per group taken after 1 h of infusion treatment. * Comparisons within Control group, $I_1$ vs P, $p<0.0001$; $I_1$ vs S $p<0.0004$; # Control vs Clonidine group at P, $p<0.0001$,; at $S_1$, $p<0.005$.

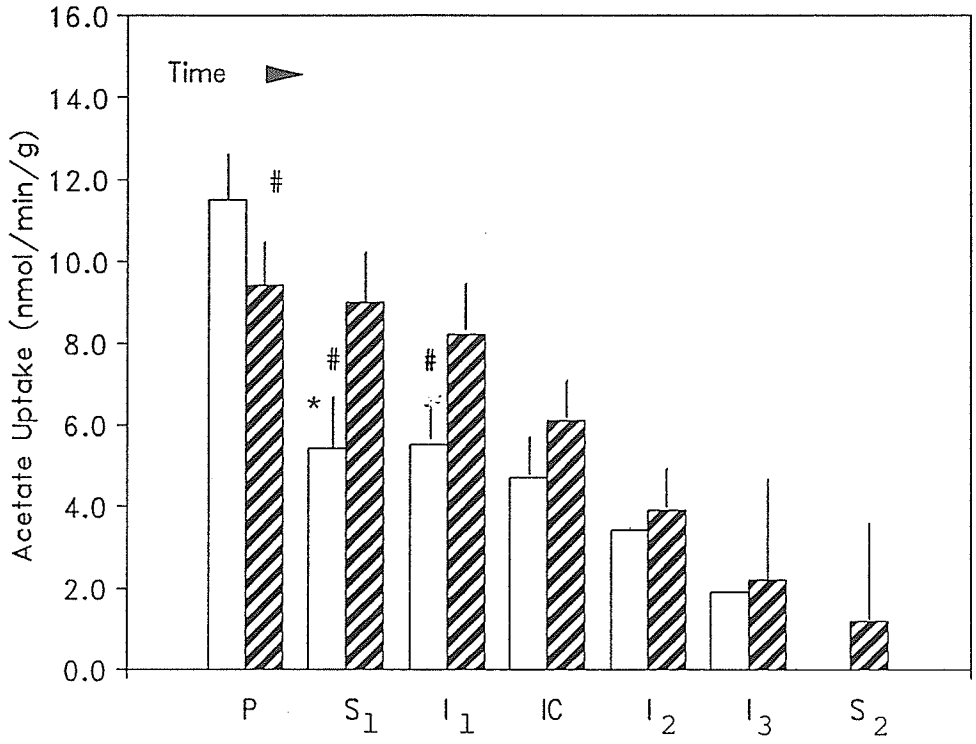


Figure 10. Effects of adrenergic agonists on acetate uptake over time (420 min) in the perfused fat-tail depot in control (open bars) and 84-day clonidine-treated (hatched bars) Karakul ram lambs. Drugs were prepared in saline and infused into the perfusate inflow. P=perfusate alone, S=saline infusion, I=isoproterenol, IC=isoproterenol and clonidine consecutively. Mean $\pm$ S.E. of 3 sheep per group taken after 1 h of infusion treatment. * Comparison within Control group P vs $S_1$, $p<0.0001$; # Control vs Clonidine group at P, $p<0.01$; at $S_1$, $p<0.01$; at $I_1$, $p<0.04$.

Table 4. Plasma concentrations, body weights and mass of perfused adipose tissue of the Karakul ram

| | Variable | | | | |
|---|---|---|---|---|---|
| | Plasma (mM) | | | Body Wt. | Tail Wt. |
| Animal[1] | Glucose | Acetate | Glycerol | (kg) | (kg)[2] |
| S-74 | 7.1 | 0.23 | 0.19 | 48.6 | 5.5 |
| S-67 | 5.6 | 0.44 | 0.05 | 42.0 | 3.2 |
| S-03 | 5.3 | 0.26 | 0.19 | 45.8 | 3.3 |
| C-75 | 4.6 | 0.22 | 0.19 | 40.6 | 3.6 |
| C-62 | 5.0 | 0.27 | 0.19 | 40.1 | 3.0 |
| C-02 | 7.4 | 0.29 | 0.22 | 42.6 | 4.3 |

[1]Animal includes individual animal number and treatment condition: S=saline implant, C=clonidine implant.
[2]Tail represents the dried weight of perfused adipose mass including the associated rump fat.

perfusion study (Kennedy et al., in preparation). Blood samples were collected from an indwelling catheter at 20-min intervals over an 8-h period. The mean and S. E. (ng/ml) of these 25 insulin values for each sheep were 5.14 (0.3), 7.61 (0.5) and 6.64 (0.4) in control lambs and 3.73 (0.3), 4.04 (0.2) and 6.56 (0.4) for the clonidine-treated lambs.

## DISCUSSION

The isolated perfusion model was functional for approximately seven hours; however, the preparation proved to be more difficult in the younger rams than a similar model prepared in adult ewes (Manuscript I). Blood vessels were smaller and less resilient to surgical preparation in the younger rams, which necessitated use of an alternate vessel for the inflow catheter (see Figure 2). The decreased vascular resilience required smaller catheters to be used; however, the smaller catheters were prone to blocking with blood clots during the initial phases, hence compromising the circulation. Also, the blood vessels were less hardy compared to similar blood vessels of older sheep, which resulted in vessel rupture and leaking during the perfusion period of the younger lambs. Nine perfusion preparations were attempted; three were unsuccessful during the first 20-80 min of perfusion. The data reported here were collected from six preparations that continued for a minimum of 220 min and a maximum of 460 min. The procedure has been previously maintained for up to 10 h (Manuscript

The mean flowrates and specific flowrates for each group were lower than that found in an *in vivo* preparation of a fat-tail depot (Gooden et al., 1986) and lower than values found in adipose depots of other species (see Vernon & Clegg, 1985). The significant clonidine-induced reduction in flowrate in the control group supports an earlier finding (Manuscript I) that the α-induced vasoconstrictive properties of the tail were more sensitive than were the ß-induced lipolytic properties. However, flowrate did not return to the preclonidine rate after termination of the clonidine infusion. As in the earlier study, it was suspected that vasoconstrictive-induced increased pressure caused weaker vessels to rupture, whereby they were no longer capable of carrying perfusate and returning flowrate to prestimulatory levels. The clonidine-induced vasoconstriction was so intense in one preparation (Animal # 67) that the preparation ceased to function following clonidine infusion. Clonidine infusion failed to induce a vasoconstrictive response in the clonidine-treated group. Cheung (1986) found that repeated exposure to an α-adrenergic agonist reduced the α-mediated vasoconstriction response in the tail artery and saphenous vein of the rat. Thus, prior 84-day exposure to clonidine may have desensitized the vascular network of the tail to α-induced vasoconstriction.

The basal lipolytic rate in this study was similar to

the basal values (2 μmol/min/kg tail) demonstrated in an *in vivo* preparation of a fat-tail depot (Gooden et al., 1986). Furthermore, the basal rate of lipolysis found in this study was similar to basal rates observed in an earlier perfusion study of the tail depot (Manuscript I). The basal lipolytic rate was, however, less than values found in an *in vitro* preparation of tail adipose tissue (Manuscript III). After the initial rise in lipolytic rate there was a general decline in lipolysis over time and lipolysis was not stimulated with isoproterenol infusion. However, the rate of glycerol release did not decline below basal release rates (except during the last saline in the control group which was represented by only one ram). This contrasts an earlier finding in a similar preparation where glycerol release increased over time regardless of drug treatment (Manuscript I).

In Manuscript I it was suggested that the increase in lipolytic rate over time following tail adipose isolation was related to the removal of endogenous antilipolytic agents (e.g., insulin). Possibly, the antilipolytic effects of insulin were greater in the older sheep, thus sublevels of insulin in the perfusate were not reflected in an increase in glycerol release over time in the younger rams.

An antilipolytic effect of clonidine was not observed during the clonidine infusion period. However, the antilipolytic effect of clonidine is not always displayed in

adipose tissue of sheep (Manuscript III) and other species (Carpene et al., 1983; Mersmann, 1984c). In adult ewes, a 77% ($p<0.1$) $\alpha_2$-induced reduction in lipolytic rate was observed in the fat-tail depot with epinephrine and propranolol (Manuscript III) and $\alpha$-adrenergic receptors have been found in other adipose depots of mature sheep (Kennedy & Vernon, 1990; Watt et al., 1991). The age of the rams may have been a factor in the functional contribution of the $\alpha_2$ antilipolytic effect. The $\alpha_2$-induced decrease in lipolytic rate has been shown to increase with age in the rabbit (Lafontan, 1979) and the rat (Rebourcet et al., 1988), therefore, the $\alpha_2$ antilipolytic effect may not be demonstrated in these younger animals.

Glucose uptake in this preparation was similar to the value found in an *in vivo* study of a tail depot (Gooden et al., 1986) and similar to that seen *in vitro* (see Vernon, 1980) for ovine adipose tissue. The initial acetate uptake rates were similar to those found with *in vitro* preparations of ovine adipose tissue, however, a wide range of values have been reported (see Vernon, 1980).

The overall reduced rate of acetate uptake and the continual uptake of glucose are similar to the effects found in cultured ovine adipocytes without insulin supplementation (Vernon, 1979) and a previous perfusion preparation (Manuscript I). However, during the initial infusion periods there were significant reductions in glucose and

acetate uptake in the control group only. The reason for the clonidine-treated group not displaying the sudden decline in acetate and glucose uptake during the first 2 h is unclear. It is possible that the same dramatic reductions occurred in the clonidine group prior to the first measurement, which took place 45-60 min after the onset of perfusion, but the initial changes in glucose and acetate uptake required at least 1-2 h to become evident in the control group and it is unlikely they would occur within 45 min in the clonidine group.

The major differences in metabolic events between the control and clonidine groups occurred prior to drug infusions. The initial perfusion effects on glucose uptake in the control group of this study were similar to those in the perfused depot of the older ewes (Manuscript I). Both groups displayed a similar reduction in glucose uptake following the initial 2 h of perfusion. Conversely, the perfused tissue of the clonidine-treated rams did not exhibit the same reduction in glucose uptake in the same perfusion period. It may be that the stable rate of glucose uptake displayed in the perfused tissue of the clonidine-treated group was a consequence of adaptation to low circulating insulin levels, thereby, when exposed to the perfusate the challenge was not as great to this group. The mean insulin level was 26% lower in the clonidine-treated group compared to the saline-treated group (6.5 $\pm$ 0.8 versus

4.8 ± 1.1; mean ± S.E.M for the saline and clonidine groups, respectively). Kennedy et al (1988) have shown that clonidine reduces circulating insulin levels in certain breeds of sheep, and as well, clonidine treatment reduced circulating insulin levels in humans (Okada et al., 1986) and rats (Gotoh et al., 1988). Possibly, the alteration in circulating insulin levels affected the role of acetate and glucose in the lipid metabolism of the tail adipose tissue and removal of the contributing factors with the onset of perfusion and substitution of perfusate for blood was expressed in the shifts in acetate and glucose uptake.

In conclusion, the isolated perfusion model of the fat-tail depot allowed simultaneous monitoring, over an extended period of time, of vascular and metabolic responses to adrenergic agonists. Increased vascular resistance was detected with clonidine infusion, but the effect was abolished with prior chronic clonidine exposure, suggesting a desensitization of vascular α adrenergic response in the tail depot. Chronic exposure to clonidine also appeared to alter the rates of glucose and acetate uptake compared to the control group, but the mechanisms responsible for this alteration are unknown. Lipolytic rates were unaffected by either ß or α adrenergic stimulation suggesting that the adipose tissue of this depot has a reduced responsiveness and possibly a low sensitivity to adrenergic agonists.

# MANUSCRIPT III

# EFFECT OF ANATOMICAL LOCATION AND BREED ON THE RESPONSIVENESS AND SENSITIVITY OF OVINE ADIPOSE TISSUE TO ADRENERGIC STIMULATION

## ABSTRACT

The responsiveness of adipose tissue to epinephrine ($10^{-7}$-$10^{-4}$ M) and isoproterenol ($10^{-10}$-$10^{-6}$ M) stimulation was examined in the omental and subcutaneous (SC) depots of Outaouais (OTA) and Karakul ewes as well as in the unique fat-tail adipose depot of the Karakul ewes. Basal lipolytic rates were not significantly different in any of the depots tested. The addition of adenosine deaminase (ADA) increased the lipolytic rate in the adipose tissue of the Karakul-tail depot during Experiment 1, only. Epinephrine significantly increased the lipolytic rate in all depots examined and the order of maximal responsiveness was: Karakul omental (11.9 $\mu$mol/$10^6$ cells/3 h) > Karakul SC (9.4) = OTA omental (9.8) > OTA SC (7.1) > Karakul tail (5.2) (S.E.=0.64; $p<0.05$). The $\alpha_2$-antilipolytic component of epinephrine action (epinephrine in the presence of propranolol) did not have a significant effect on the lipolytic rate in the OTA omental and SC depots, but inhibited lipolysis in the Karakul omental ($p=0.05$) and tail ($p=0.1$) depots. Maximal responsiveness to isoproterenol stimulation was in the order: Karakul omental (11.1 $\mu$mol/$10^6$ cells/3 h) = Karakul SC (10.2) > OTA omental (8.8) = OTA SC (8.3) > Karakul tail (5.5) (S.E.=0.64; $p<0.05$). The depots of the Karakul ewes tended to be more sensitive to isoproterenol stimulation than the OTA omental depot ($p=0.1$) and were significantly more sensitive than the SC depot (Karakul: 0.22 to $0.79 \cdot 10^{-8}$

M isoproterenol vs OTA: 3.73 and $5.40 \cdot 10^{-8}$ M isoproterenol; S.E.=$1.28 \cdot 10^{-8}$ M). Results suggest that depot-specific variation in responsiveness to epinephrine is modulated by a greater complement of ß receptors in the adipose tissue of the omental depots and not an increased $\alpha_2$-antilipolytic component in the SC tissue. The reduced lipolytic capacity of the tail adipose depot may be due to a combination of a decreased ß-adrenergic receptor number and a greater effectiveness of the $\alpha_2$-adrenergic response. Furthermore, the Karakul-tail depot may rely on the influence of local regulators, such as adenosine, to control lipid metabolism. The data suggest that the omental depots serve as a readily mobilizable source of stored energy regulated by adrenergic input; whereas, the tail depot is likely a long-term energy storage depot that is regulated by additional factors.

# INTRODUCTION

Individual adipose depots display different rates of fat accretion (Allen, 1976). However, the factors that regulate the different rates of fattening displayed by individual depots are unknown. Variations in the sensitivity and responsiveness of individual depots to catecholamine stimulation may be the primary regulator of depot contribution to overall energy balance. Recently, Rule et al (1992) found depot-specific variations in responsiveness to adrenergic stimulation in bovine adipose tissue. Dual α- and ß-mediated regulation of adipose tissue displayed by the catecholamines has been demonstrated in humans (Fain & Garcia-Sainz, 1983) and in the hamster (Dieudonne et al., 1992).

In humans (see Arner, 1992) the regional variation in responsiveness to catecholamines is influenced primarily by α- and ß-adrenergic receptor complement. Ovine adipose tissue has both α- and ß-adrenergic receptors present (Kennedy & Vernon, 1990; Watt et al., 1991); however, depot-specific differences in response to adrenergic stimulation has received limited attention.

A perfusion study involving the unique fat-tail depot found tail lipolysis unresponsive to norepinephrine stimulation (Khachadurian et al., 1966;); however, a later *in vitro* study showed a modest epinephrine-stimulated increase in fatty acid release in the fat-tail depot, a

response which was greater than that found in the abdominal subcutaneous adipose tissue and similar to the mesenteric adipose tissue (Khachadurian et al., 1967). Another approach, involving an isolated non-recirculating perfusion of the tail depot, found a low lipolytic response to isoproterenol (Manuscript I) and no antilipolytic effect of clonidine (Manuscript II).

The purpose of this study was to use an *in vitro* approach to examine the contribution of the α- and ß-adrenergic components in the regulation of lipid metabolism in adipose tissue obtained from various depots in Outaouais (OTA) and Karakul sheep. Depot-specific differences in sensitivity and responsiveness to the ß-adrenergic agonist isoproterenol was compared and the α-adrenergic component was examined using clonidine in the presence of theophylline, a phosophodiesterase inhibitor, and by treating with epinephrine (mixed α and ß agonist) in the presence of propranolol (ß blocker).

## MATERIALS AND METHODS

### *Animals*

Data were collected from nonpregnant, nonlactating ewes during Sept-Nov, 1991 (Experiment 1) and July, 1992 (Experiment 2). Outaouais (OTA) (n=8 Experiment 1 and n=5 Experiment 2) and Karakul (n=7 Experiment 1 and n=5 Experiment 2) ewes were fed alfalfa hay at maintenance (NRC, 1985) and were provided with free access to water and

cobalt-iodinized salt.

*Materials*

Pyruvate kinase (E.C.2.7.1.40), L-lactic dehydrogenase (E.C.1.1.1.27), collagenase (Type II; E.C.3.4.24.3; Lot # 11H6820), protease (Lot # 89F1065), (-)-epinephrine, (-)-isoproterenol and bovine serum albumin (Fraction V, # A4503) were purchased from Sigma (St. Louis, MO). The BSA was defatted and dialysed by the procedure of Chen (1967). Clonidine hydroxychloride (2-(2,6-dichloroanilino-2-imidazoline) was a gift from Boehringer Ingleheim (Canada). Glycerokinase (E.C.2.7.1.30), adenosine deaminase (ADA; E.C.3.5.4.4) were purchased from Sigma (St. Louis, MO) and Boehringer Mannheim (Canada). All other chemicals and materials were purchased from Sigma or Canlab (Canada).

*Tissue incubation*

Adipose tissue samples (c.a. 10-15 g) were collected either by biopsy under local Lidocaine anesthetic (MTC Pharmaceuticals, Cambridge, Canada) (Experiment 1) or immediately following stunning and exsanguination (Experiment 2). Adipose tissue from the (SC) and omental depots was collected from both breeds through a 6-8 cm incision on the left side behind the last rib. Tail adipose tissue was collected from a 6 cm incision on the left dorsal portion of the fat-tail depot of the Karakul ewes. Adipose tissue samples were collected in Krebs buffer (1.22 mM $CaCl_2$, 2 mM acetate, 5 mM glucose, 25 mM HEPES, pH 7.4) at

38°C for the SC and tail samples and 40°C for the omental samples. The onset of incubation was within 1 h of tissue removal and tissue was maintained in buffer at either 38 or 40°C (tissue dependent) throughout the entire experimental procedure. To maintain a warm controlled environment (28-30°C) a plastic hood (0.5 $m^3$) was constructed, which housed a 30 cm X 30 cm Thermolyne hot plate (Sybron, USA). The collected adipose tissue was transferred from the collection container to a petri dish containing Krebs buffer maintained at 38°C or 40°C with the aid of the hot plate.

Approximately 5 g of collected tissue was cleared of obvious connective tissue and blood vessels and minced into small pieces (each approximately 5-10 mg) with warmed scissors. The pieces were floated in Krebs buffer and approximately 5-10 pieces were transferred to each polypropylene screw-top vial containing 2 or 3 ml of aerated (95% $O_2$/5% $CO_2$) Krebs buffer with 3% BSA, 20 $\mu$g/ml ascorbic acid and appropriate drug concentrations. The vials were capped and incubated for 3 h at physiological temperatures (38°C tail and SC; 40°C omental) with shaking (120 cycles/min). The drug treatments for Experiment 1 were as follows: **1)** basal--no additions, **2)** ADA (0.2 U/ml), **3)** theophylline (2 mM), **4-7)** clonidine $10^{-7}$ - $10^{-4}$ M plus theophylline (2 mM), and **8-11)** isoproterenol $10^{-7}$ - $10^{-4}$ M. The drug treatments for Experiment 2 were as follows: **1)** basal--no additions, **2)** ADA (0.2 U/ml), **3)** clonidine $10^{-4}$ M,

4) propranolol $2 \cdot 10^{-5}$ M, 5-8) epinephrine $10^{-7}$ - $10^{-4}$ M, 9-12) epinephrine $10^{-7}$ - $10^{-4}$ M plus propranolol ($2 \cdot 10^{-5}$ M), 13-20) isoproterenol $10^{-10}$ - $10^{-6}$ M. All drug treatments (basal not included) were performed in the presence of 0.2 U/ml ADA to prevent adenosine accumulation. In Experiment 1 four determinations were conducted on treatment conditions 1-3 and all others (4-11) were done in triplicate. The Karakul SC tissue was not included in the lipolytic portion of Experiment 1. Epinephrine and epinephrine plus propranolol were included in Experiment 2 to provide a second measure of the $\alpha$-mediated response. A more extensive dose range of isoproterenol was included to provide measures of sensitivity and threshold doses. In Experiment 2 treatment conditions 1-4 were performed in triplicate and all others (5-20) were done in duplicate.

*Glycerol determination*

The concentration of glycerol in the incubation medium was used as an indicator of lipolytic activity. All the tissue peices were removed from each vial, dried and weighed at the end of the incubation period. One ml of incubation medium was removed from below the surface and transferred to polypropylene tubes to be deproteinized by a modified method from Honnor & Saggerson (1980). Briefly, the medium was added to polypropylene tubes containing 120 $\mu$l of ice-cold $HClO_4$ (45% w/v). The tubes were mixed and centrifuged for 15 min at $1050 \cdot g$. An 800 $\mu$l aliquot of the supernatant was

taken and neutralized with a mixture of 80$\mu$l KOH (5 M) and 160 $\mu$l $KHCO_3$ (saturated). The solution was mixed and centrifuged as before. Glycerol determinations were performed on an aliquot of the final supernatant as per the method of Garland and Randle (1962) and corrected with the necessary dilution factor (see Appendices III & IV).

*Adipocyte isolation and lipid extraction*

The adipose tissue was maintained in a controlled temperature environment using a fiberglass hood (0.5 $m^3$) heated with a 1500 watt electric furnance (Holmes Products Corp., Mississauga, ON). The tissue preparation took place on a hot plate and instruments were warmed in a Tempblock module heater (Lab-line Instruments, Melrose Park, IL). Adipocytes were isolated in 15 ml aerated Krebs at 38°C or 40°C containing 3% BSA, 1.7 mg/ml collagenase and 0.05 mg/ml protease. Pieces of adipose tissue weighing about 5 g were minced in the Krebs collagenase solution. The mixture was transferred to a polypropylene, screw-top flask and incubated at the appropriate temperature for the depot site for 30-60 min without shaking. The digested slurry was poured through a warmed nylon sieve and washed three times with Krebs wash buffer (1% BSA collagenase/protease free) by adding the buffer to the cells and siphoning the infranatant off with a 14 ga needle and syringe. A small aliquot (ca. 50 $\mu$l) was placed in a warmed counting chamber (Weber Scientific International Ltd., England) and cell diameters

were determined by measuring displayed adipocytes on a precalibrated screen attached to a microscope. The free adipocyte cell diameters of 100 to 250 cells from each depot preparation were determined within 10 min of isolation.

Total lipid was extracted from a known weight of adipose tissue (ca. 500-800 mg) to provide the measure of g total lipid/g wet weight tissue · 100 (i.e., percent lipid). Briefly, the adipose tissue was extracted with 20 volumes of chloroform/methanol (2/1, v/v) (Floch et al., 1957). The tissue residue (nonlipid matter) was removed with tweezers, dried and weighed. The weight of the residue was then expressed as a percentage of the wet tissue weight (percent residue). The remaining chloroform/methanol extract containing the lipid was separated into two phases with the addition of 2 volumes of 0.73% NaCl. The upper water phase was discarded and the lower phase was evaporated to complete dryness and the weight of the extracted lipid was determined. Density was determined at 50°C by weighing a known volume of the previously extracted lipid. The conversion of incubated tissue weight to total number of cells/incubated tissue weight was determined by the method of DiGirolamo et al (1972) (see Appendix V).

*Statistical analyses*

Lipolytic data, adipose tissue and adipocyte characteristics were analyzed by General Linear Model procedures (SAS, 1985) using depot site (OTA-omental, OTA-

SC, Karakul-omental, Karakul-SC and Karakul-tail) and drug treatment (described earlier) as main effects. To compare analogous treatments in Experiment 1 and Experiment 2, year was included as a variable. Pairwise comparisons were made using Least Squared Means (SAS, 1985) following a significant depot site x drug treatment interaction. Body weights were analyzed in a 2 x 2; breed (OTA, Karakul) x year (1991, 1992) factorial design. The $ED_{50}$ dose of isoproterenol for each depot was estimated by performing linear regression analysis of the $\log_{10}$ values for the dose-response curves for each animal in Experiment 2. The $ED_{50}$ dose was 50% of the difference between the dose required to produce a maximal response minus the ADA baseline (zero dose) response (Kather et al., 1987). Significance was $p<0.05$ unless otherwise indicated.

## RESULTS

### *Experiment 1*

Basal lipolytic rates were higher in the omental depots than in either the OTA-SC or the Karakul-tail depots although no significant differences were found (Table 5). Addition of ADA to the incubation medium increased the lipolytic rate from a minimium of 81% in the OTA-omental depot to a maximum of 349% in the Karakul-tail depot. The ADA-induced increase in lipolytic rate was significant in the Karakul-tail depot only ($p<0.05$). The lipolytic rate was increased ($p<0.05$) compared to ADA alone in all depots

Table 5. Lipolytic response (glycerol release $\mu$mol/$10^6$ cells/3 h) to isoproterenol and clonidine in adipose tissue from Outaouais (OTA) and Karakul sheep

| Condition | OTA | | Karakul | |
|---|---|---|---|---|
| | Omental | SC | Omental | Tail |
| Basal | $0.70^{a,x}$ | $0.56^{a,x}$ | $0.81^{a,x}$ | $0.61^{a,x}$ |
| ADA | $1.27^{a,x}$ | $1.66^{a,x}$ | $1.88^{a,x}$ | $2.74^{b,x}$ |
| Theophylline | $4.63^{b,x}$ | $4.34^{b,x}$ | $7.74^{bc,y}$ | $4.89^{c,x}$ |
| Clonidine[1] M | | | | |
| $10^{-7}$ | $4.58^{b,x}$ | $4.22^{b,x}$ | $6.17^{bc,y}$ | $4.88^{c,xy}$ |
| $10^{-6}$ | $4.31^{b,x}$ | $4.24^{b,x}$ | $7.06^{bc,y}$ | $4.65^{c,x}$ |
| $10^{-5}$ | $4.55^{b,x}$ | $4.02^{b,x}$ | $7.02^{bc,y}$ | $4.78^{c,x}$ |
| $10^{-4}$ | $4.54^{b,x}$ | $4.23^{b,x}$ | $7.39^{bc,y}$ | $5.13^{c,x}$ |
| ISO (M) | | | | |
| $10^{-7}$ | $6.09^{bc,x}$ | $5.30^{b,x}$ | $8.18^{c,y}$ | $4.49^{bc,x}$ |
| $10^{-6}$ | $6.79^{c,xy}$ | $5.24^{b,xz}$ | $8.39^{c,y}$ | $4.68^{c,z}$ |
| $10^{-5}$ | $6.95^{c,xy}$ | $5.58^{b,xz}$ | $8.72^{c,y}$ | $4.49^{bc,z}$ |
| $10^{-4}$ | $7.06^{c,xy}$ | $5.65^{b,xz}$ | $8.81^{c,y}$ | $4.54^{bc,z}$ |
| S.E. | 0.64 | 0.64 | 0.69 | 0.69 |

[1] Clonidine in the presence of theophylline (2mM)
Values sharing a common letter $p>0.05$;
$^{a,b,c}$ comparisons in a column; $^{x,y,z}$ comparisons in a row

by the addition of theophylline from 81% in the tail depot to 312% in the Karakul-omental depot (Table 5). Clonidine ($10^{-7}$-$10^{-4}$ M) in the presence of theophylline-induced lipolysis did not reduce the rate of ongoing lipolysis in any depot.

Isoproterenol at the highest dose significantly increased the lipolytic rate compared to ADA alone 5.6 and 3.4 fold in the OTA omental and SC adipose tissues, respectively and 4.7 fold in the Karakul-omental tissue (Table 5). A significant response to all lower doses was also observed in these tissues. The lipolytic rate was increased 64-71% in the tail adipose tissue but only the response at $10^{-6}$ M was significant. The Karakul-tail depot displayed a reduced responsiveness to isoproterenol compared to the OTA and Karakul omental depots, but the lipolytic capacity of the tail depot was not significantly different that did from the OTA-SC depot (Table 5).

Isoproterenol produced a greater lipolytic response than did theophylline in all depots except the tail, but this difference was only significant for the OTA-omental tissue at the higher isoproterenol doses ($10^{-6}$-$10^{-4}$ M).

*Experiment 2*

Based on the results of Experiment 1, lower doses of isoproterenol were studied to determine the sensitivity and responsiveness of adipose tissue from individual depots. A maximum dose of $10^{-6}$ M was chosen as $10^{-7}$ M yielded a maximum

response in Experiment 1. The Karakul-SC depot was included in all portions of this study and because clonidine failed to reduce theophylline-induced lipolysis in Experiment 1, a second approach was conducted to further investigate the $\alpha_2$-adrenergic antilipolytic component. In this approach, the $\alpha$ adrenergic effect of epinephrine was examined by blocking the ß adrenergic component with propranolol.

The basal lipolytic rates were not different among the depots examined in Experiment 2 (Table 6). Addition of ADA produced a slight, but nonsignificant increase in the lipolytic rate in all depots from 29% in the OTA-SC adipose tissue to, 208% in the Karakul-tail depot (Table 6).

The dose-response curves for isoproterenol are shown in Figures 11 and 12. The response to isoproterenol was similar in the two depots of the OTA breed (Figure 11). Similar maximal responses were displayed and the threshold doses, or the lowest dose required to produce an increase in lipolytic rate above ADA levels, were not different between depots. The maximal response to isoproterenol was also similar in the SC and omental tissues of the Karakul ewes but, the threshold dose was lower in the omental tissue than in the SC or tail (Figure 12). The tail adipose tissue displayed a limited lipolytic capacity compared to all other depots examined in both breeds and the threshold dose was similar to the SC tissue. The maximal lipolytic response ($\mu$mol/$10^6$ cells/3 h) of the adipose tissue to isoproterenol

Table 6. Lipolytic response (glycerol release $\mu$mol/$10^6$ cells/3 h) to adenosine deaminase (ADA), propranolol and clonidine compared to basal lipolysis in adipose tissue from Outaouais (OTA) and Karakul sheep

| Depot | Glycerol release ($\mu$mol/$10^6$ cells/3 h) | | | |
|---|---|---|---|---|
| | Basal | ADA | Propranolol | Clonidine |
| OTA-Omental | $0.89^{a,x}$ | $1.53^{ab,x}$ | $1.68^{a,x}$ | $1.76^{a,x}$ |
| OTA-SC | $0.76^{a,x}$ | $0.90^{a,x}$ | $0.92^{a,x}$ | $1.20^{a,x}$ |
| Karakul-Omental | $1.44^{a,x}$ | $2.80^{b,xy}$ | $3.47^{b,y}$ | $2.03^{a,xy}$ |
| Karakul-SC | $0.64^{a,x}$ | $1.27^{ab,x}$ | $1.47^{a,x}$ | $1.43^{a,x}$ |
| Karakul-Tail | $0.53^{a,x}$ | $1.63^{ab,x}$ | $1.84^{ab,x}$ | $1.13^{a,x}$ |
| S.E. | 0.64 | 0.64 | 0.64 | 0.64 |

Values sharing a commom letter $p>0.05$
$^{a,b}$Comparisons within a column
$^{x,y}$Comparisons within a row

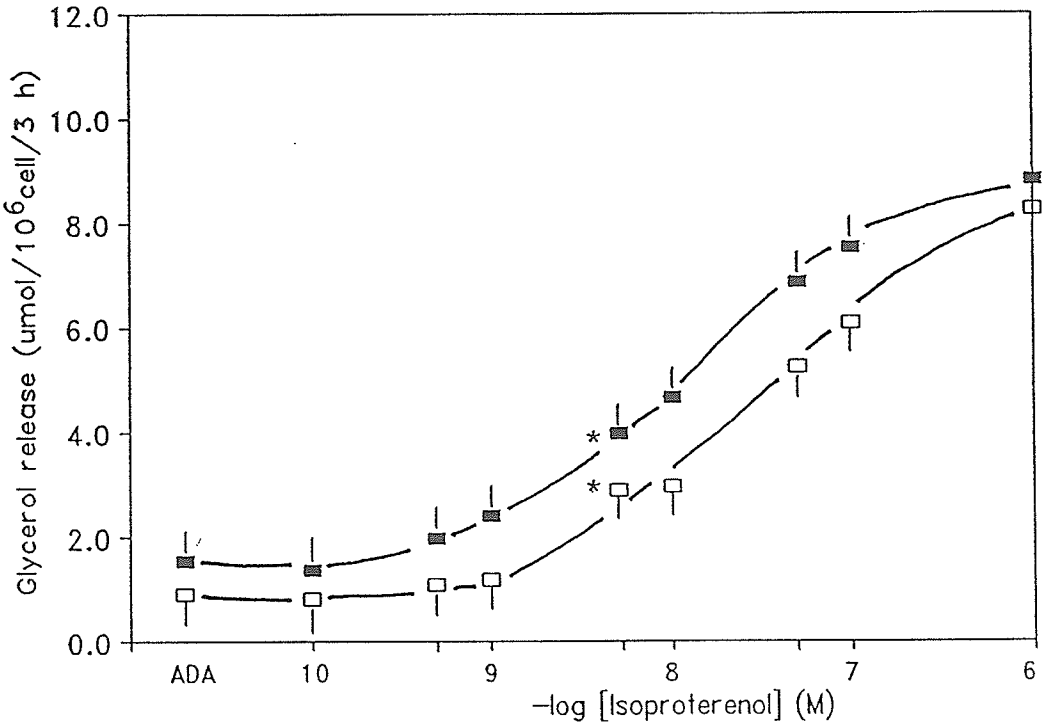


Figure 11. Dose-response relationship for isoproterenol in the omental (■-■ and SC (□-□) adipose tissue of OTA ewes. Isoproterenol incubations include ADA (.2 U/ml). Values are the mean ± S.E. of duplicate determinations of 5 sheep. * $p<0.05$ vs ADA (threshold dose).

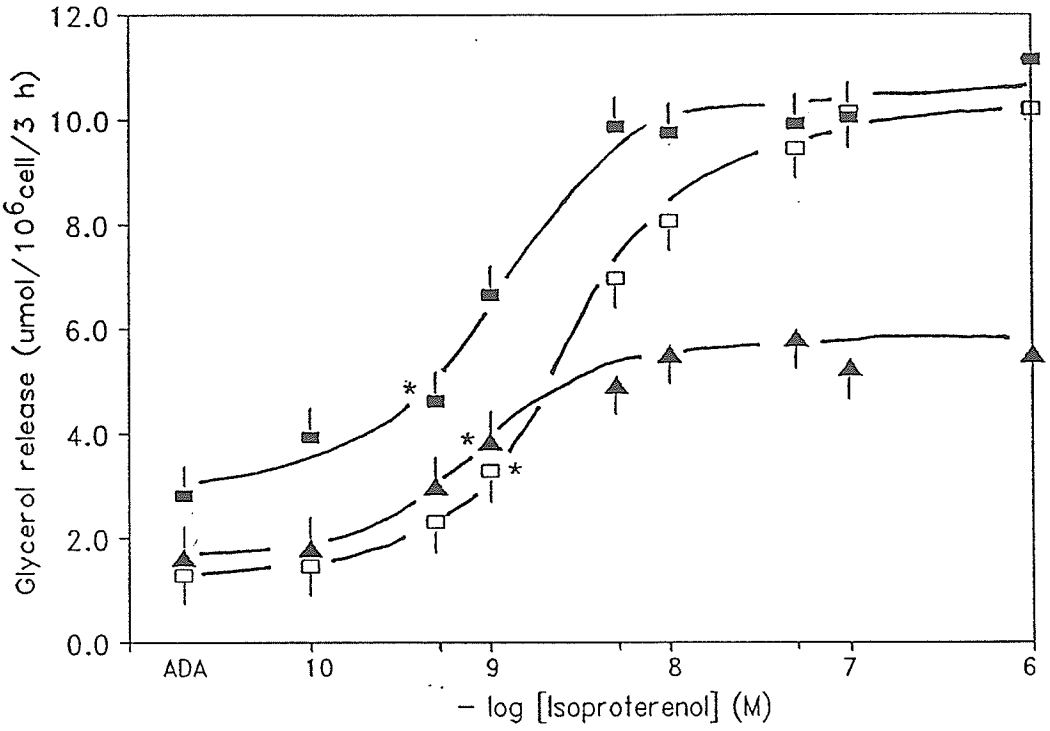


Figure 12. Dose-response relationship for isoproterenol in the omental (■-■), SC (□-□) and tail (▲-▲) adipose tissue of Karakul ewes. Isoproterenol incubations include ADA (.2 U/ml). Values are the mean ± S.E. of duplicate determinations of 5 sheep. * $p<0.05$ vs ADA within depot comparisons (threshold dose), + $p<0.001$ depot comparisons at $10^{-6}$ M isoproterenol.

was Karakul omental (11.1) = Karakul SC (10.2) > OTA omental (8.8) = OTA SC (8.3) > Karakul tail (5.5) ($p<0.05$).

The $ED_{50}$ values for isoproterenol for each depot are shown in Table 7. Adipose tissue of the Karakul sheep was approximately 10-fold more sensitive to isoproterenol stimulation than was the OTA adipose tissue. The OTA-SC depot was the least sensitive and was significantly less sensitive than the three Karakul depots ($p<0.05$). The was a tendency for the Karakul omental ($p<0.07$), SC ($p=0.12$) and tail ($p=0.12$) adipose tissue to be more sensitive than the OTA omental tissue. No significant depot-specific differences in sensitivity were found within a breed, but isoproterenol was more potent and displayed a lower threshold dose in the Karakul omental tissue compared to the SC and tail depots within this breed (Figure 12).

To examine the $\alpha_2$-induced antilipolytic effects of epinephrine in each depot, tissue pieces were subjected to epinephrine ($10^{-7}$-$10^{-4}$ M; Figures 13 and 14) or epinephrine plus propranolol ($2 \cdot 10^{-5}$ M; Figures 15 and 16). Epinephrine proved to be lipolytic in all depots at all doses compared to ADA-induced lipolysis. Sensitivity to epinephrine was not calculated as an insuficient dose range was used. The maximal respones to epinephrine ($10^{-4}$ M) differed both within breed and among depot site. The order of depot maximal responsiveness ($\mu$mol/$10^6$ cells/3 h) to epinephrine was Karakul omental (11.9) > Karakul SC (9.4) = OTA omental

Table 7. ß-adrenergic sensitivity to isoproterenol (ISO) and ratio of epinephrine/isoproterenol response in adipose tissue from Outaouais (OTA) and Karakul sheep

| Depot Site | Variable | |
|---|---|---|
| | $ED_{50}$[1] ($\cdot 10^{-8}$ M) | EP/ISO[2] |
| OTA-Omental | 3.73[a,b] | 1.08[a] |
| OTA-SC | 5.40[a] | 0.88[a,c] |
| Karakul-Omental | 0.22[b] | 1.04[a] |
| Karakul-SC | 0.79[b] | 0.88[a] |
| Karakul-Tail | 0.68[b] | 0.94[a] |
| S.E. | $1.29 \cdot 10^{-8}$ | 0.08 |

[1]Dose of isoproterenol producing 50% maximal response
[2]Response to Epinephrine ($10^{-4}$ M)/Response to Isoproterenol ($10^{-6}$ M)
[a,b] Values sharing a common letter $p>0.05$
[c] OTA-SC vs OTA-Omental $p=0.09$

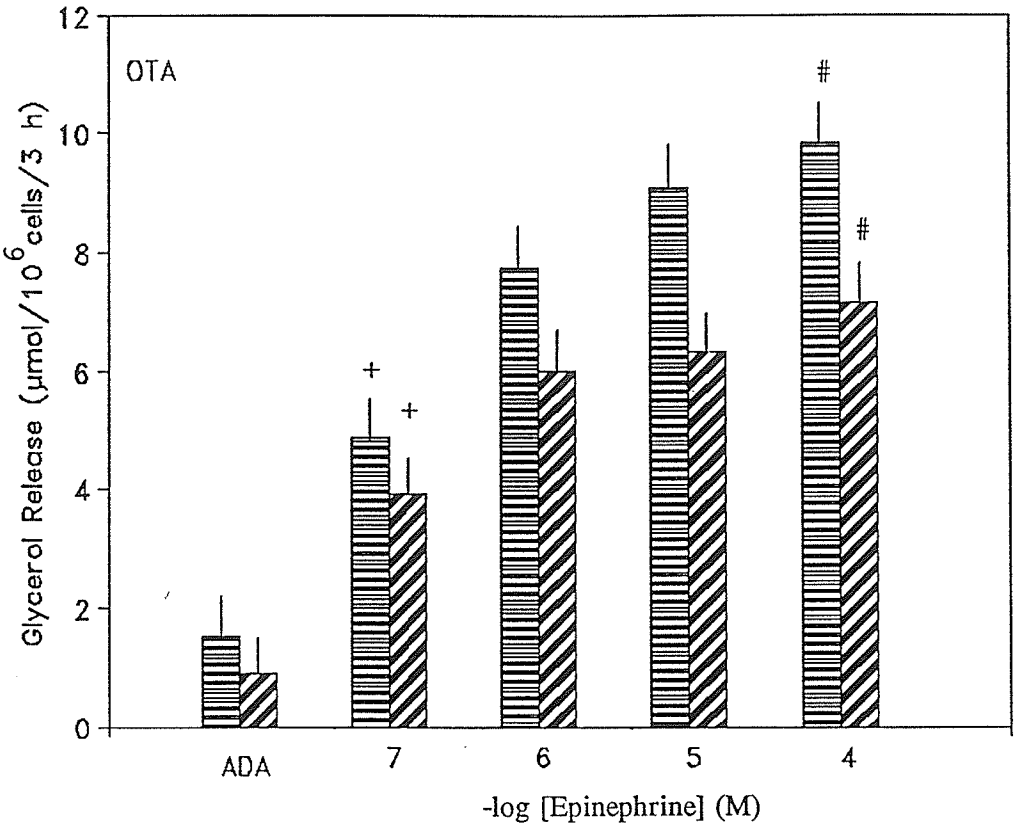


Figure 13. Epinephrine-induced increase in lipolytic rate compared to baseline levels in the presence of adenosine deaminase (ADA) in OTA omental (▤) and S.C. (▨) tissue. Epinephrine treatments contain ADA (0.2 U/ml). Values are the mean ± S.E. of duplicate determination from 5 sheep. + $p<0.001$ vs ADA; # $p<0.001$ vs epinephrine $10^{-7}$ M.

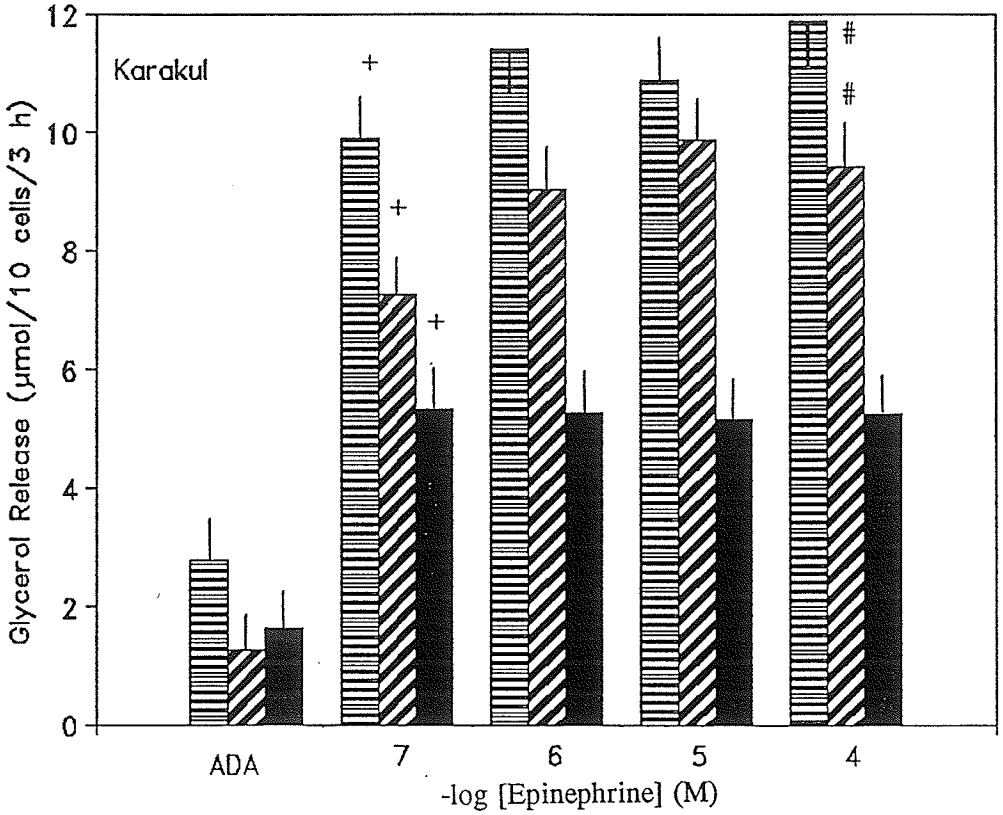


Figure 14. Epinephrine-induced increase in lipolytic rate compared to baseline levels in the presence of adenosine deaminase (ADA) in Karakul omental (▤), S.C. (▨) and tail (■) tissue. Epinephrine treatments contain ADA (0.2 U/ml). Values are the mean ± S.E. of duplicate determination from 5 sheep. + $p=0.0001$ vs ADA; # $p<0.03$ vs epinephrine $10^{-7}$ M.

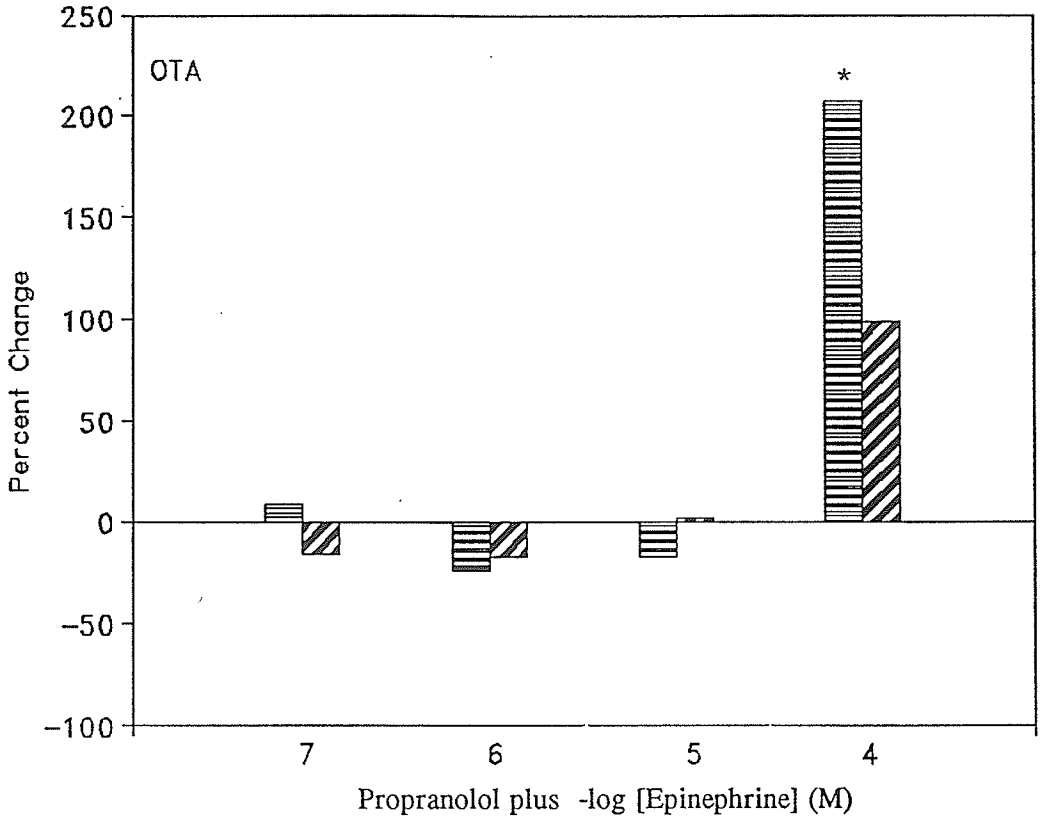


Figure 15. Percent change in lipolytic rate from propranolol baseline levels in OTA omental (▤) and S.C. (▨) tissue exposed to epinephrine and propranolol ($2 \cdot 10^{-5}$ M). Treatments contain adenosine deaminase (0.2 U/ml). Values are the mean percent change of duplicate determinations from 5 sheep. * p=0.0002 vs propranolol alone.

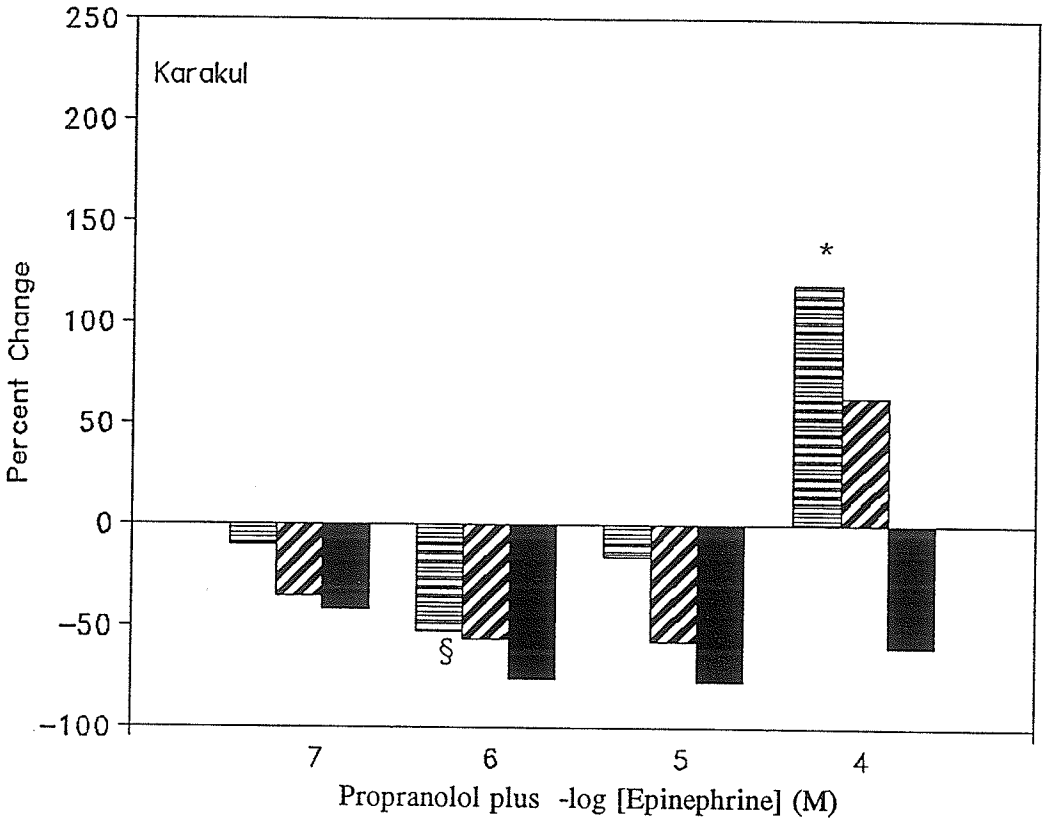


Figure 16. Percent change in lipolytic rate from propranolol baseline levels in Karakul omental (▤), S.C. (▨) and tail (■) tissue exposed to epinephrine plus propranolol ($2 \cdot 10^{-5}$ M). Treatments containe adenosine deaminase (0.2 U/ml). Values are the mean percent change of duplicate determinations from 5 sheep. § p=0.05 and * p=.0001 vs propranolol alone.

(9.8) > OTA SC (7.1) > Karakul tail (5.2) (S.E.=0.64; $p<0.05$). Maximal response was greater in the OTA omental tissue compared to the SC tissue ($p<0.05$), but the maximal response in the SC tissue was achieved with a 10-fold lower dose (Figure 13). In the Karakul tail depot maximal response was achieved with the lowest dose of epinephrine examined, whereas, the omental and SC tissue required a 10-fold higher dose to induce maximal response (Figure 14). In a comparison of the omental and SC depots, 10 to 100-fold greater doses were required to achieve maximal response in the OTA depots compared to the Karkaul depots. These results suggest that the Karakul adipose tissue has a higher sensitivity to epinephrine than does the OTA adipose tissue.

Clonidine did not inhibit lipolysis in the presence of ADA in any of the depots examined (Table 6). To further investigate the $\alpha_2$-antilipolytic component, response to epinephrine plus propranolol was compared to the response of propranolol alone. The addition of propranolol had no effect on the lipolytic rate compared to ADA alone (Table 6). The percent change in lipolysis from the propranolol baseline are shown in Figures 15 and 16. The magnitude of antilipolytic effect of epinephrine was dependent on the dose of epinephrine. In the OTA depots there was no significant reduction in lipolysis with epinephrine and propranolol and the two depots responded similarly at the 3 lowest doses (Figure 15). The highest dose of epinephrine

($10^{-4}$ M) was lipolytic even in the presence of propranolol and the increase was significant compared to propranolol alone in the OTA omental ($p<0.0002$), but not in the OTA SC adipose tissue ($p=.35$; Figure 15).

The antilipolytic effect of epinephrine was more dramatic in Karakul adipose tissue (Figure 16). A 52% ($p=0.05$) reduction in lipolytic rate was displayed in the Karakul-omental depot at $10^{-6}$ M epinephrine plus propranolol (Figure 16). There was a nonsignificant 57% ($p=0.36$) reduction in the lipolytic rate at the same dose of epinephrine in the Karakul-SC adipose tissue (Figure 16). Tail adipose tissue displayed a strong tendency for inhibition at all doses of epinephrine and attained a maximum 77% inhibition at $10^{-5}$ M epinephrine in the presence of propranolol (Figure 16; $p=0.12$).

Also, as seen in the adipose tissue of the OTA ewes, the highest dose of epinephrine plus propranolol was lipolytic compared to propranolol alone in the omental depot of the Karakul ewes ($p<0.0001$). Similar to the OTA SC tissue, the lipolytic rate of the Karakul SC was not different from propranolol alone ($p=0.33$). The highest dose of epinephrine plus propranolol was not lipolytic in the tail adipose tissue and there was a tendency for lipolysis to remain inhibited ($p=0.23$; Figure 16).

The antilipolytic component of epinephrine can also be represented by calculating the ratio of the maximal response

to epinephrine over the maximal response to isoprterenol, as was done by Watt et al (1991) in examining the antilipolytic component of norepinephrine. An examination of the epinephrine to isoproterenol ratios for each depot is given in Table 7. The omental depots from both breeds displayed a ratio near 1, indicating that at maximal ß stimulation there was no $\alpha$ antilipolytic component of epinephrine in these depots (Table 7). The ratio in the other depots are less than 1 indicating a slight $\alpha$-mediated component of epinephrine in these depots. However, only difference between the OTA-omental and OTA-SC ratios approached significance ($p<0.09$). As shown in Table 8 comparisons were made between Experiment 1 and Experiment 2. Basal lipolytic rates were similar between the two years. There were no significant differences in the response to ADA, but the response tended to be lower in the second year in the tail adipose tissue compared to the response in the first year ($p<0.07$). The lipolytic response to isoproterenol at $10^{-7}$ and $10^{-6}$ M was compared across experiments. The maximal lipolytic response tended to be greater in the second year in the omental tissue of both breeds and was significantly greater in the second year in the OTA SC tissue (see Table 8). The maximal response of the tail adipose tissue was unaffected by experimental year.

As shown in Tables 9 (OTA) and 10 (Karakul) there were breed and depot differences in the properties of adipose

Table 8. Comparison of lipolytic response (glycerol release $\mu$mol/$10^6$ cells/3 h) to adenosine deaminase (ADA) and isoproterenol (ISO) for Experiment 1 and Experiment 2 in adipose tissue from Outaouais (OTA) and Karakul sheep

| Breed and Treatment | Experiment | | | |
|---|---|---|---|---|
| | 1 | 2 | 1 | 2 |
| OTA | Omental | | SC | |
| Basal | 0.70 | 0.89 | 0.81 | 0.76 |
| ADA | 1.27 | 1.53 | 1.88 | 0.90 |
| ISO $10^{-7}$M | 6.09 | 7.52 | 5.30 | 6.07 |
| ISO $10^{-6}$M | 6.79 | 8.81* | 5.24 | 8.25+ |
| S.E. | 0.64 | 0.64 | 0.64 | 0.64 |
| Karakul | Omental | | Tail | |
| Basal | 0.81 | 1.44 | 0.61 | 0.53 |
| ADA | 1.88 | 2.80 | 2.74 | 1.63§ |
| ISO $10^{-7}$M | 8.18 | 10.03 | 4.49 | 5.23 |
| ISO $10^{-6}$M | 8.39 | 11.13# | 4.54 | 5.50 |
| S.E. | 0.69 | 0.64 | 0.69 | 0.64 |

Comparisons made between years within depot, breed and treatment
*$p<0.09$, +$p<0.05$, #$p<0.06$, §$p<0.07$

tissue and the individual adipocytes. All adipocytes were larger in the second year compared to the first year. The omental adipocytes were consistently larger than the SC adipoctyes in both breeds. The Karakul omental adipocytes tended to be larger than those of the OTA omental depot in the first year ($p<0.06$), but were not different in the second year ($p=0.26$). The Karakul SC adipocytes were not different from the OTA SC adipocytes in the first year ($p=0.35$) or the second year ($p=0.26$). The tail adipocytes tended to be smaller than the Karakul SC adipocytes in the first ($p<0.08$) and second year ($p<0.06$), but were similar to the OTA SC adipocytes in the first year ($p=0.35$). However, they were significantly smaller than the OTA SC adipocytes in the second year ($p<0.01$).

In Experiment 2 the adipocyte volumes were 80 and 89% greater in the OTA omental and SC depots, repectively, than they were in Experiment 1 ($p<0.05$). The adipocyte volumes of the Karakul ewes increased 31, 43 and 57% in the omental, SC and tail depots respectively from Experiment 1 to Experiment 2 ($p<0.05$). Ewe body weights are shown in Table 11. The OTA sheep were consistently heavier than the Karakul sheep and both groups were heavier in Experiment 2 compared to Experiment 1 ($p<0.05$).

The percent frequency distributions of cell diameters are shown in Figures 16 (OTA) and 17 (Karakul). All distributions were monophasic and reflect the larger cell

Table 9. Properties of adipose tissue and adipocytes from onemtal and SC tissue from the Outaouais (OTA) ewe

| Variable | Depot | |
|---|---|---|
| Experiment 1 (1991) n=7 | Omental | SC |
| Lipid[1] | 85.40 (.91) | 82.79 (.93) |
| Cell Volume (pl) | 1046 (114)[a] | 653 (48)[b] |
| Cells/g tissue ($10^6$) | 1.00 (.10)[x] | 1.5 (.20)[y] |
| Density (g/ml) | 0.910 (.001) | 0.915 (.001) |
| Residue[2] | 1.23 (.05) | 1.91 (.08) |
| Experiment 2 (1992 ) n=5 | | |
| Lipid | 92.40 (.78) | 83.2 (1.54) |
| Cell Volume (pl) | 1889 (166)[c] | 1237 (105)[d] |
| Cells/g tissue ($10^6$) | 0.56 (.08)[z] | 0.74 (.08)[x,z] |
| Density (g/ml) | 0.899 (.002) | 0.895 (.003) |
| Residue | 1.41 (.13) | 1.92 (.13) |

[1] Lipid (g)/Tissue Wet Weight (g) · 100
[2] Residue (g)/Tissue Wet Weight (g) · 100
[a,b,c,d] Adipocyte volumes differ, within breed $p<0.05$
[x,y,z] Cells/g tissue differ, within breed $p<0.05$
(S.E.)

Table 10. Properties of adipose tissue and adipocytes from omental, SC and tail tissue from the Karakul ewe

| Variable | Depot | | |
|---|---|---|---|
| Experiment 1 n=7 | Omental | SC | Tail |
| Lipid[1] | 85.06 (.75) | 84.09 (.89) | 78.90 (.67) |
| Cell Volume (pl) | 1302 (137)[a] | 779 (122)[b] | 532 (73)[b] |
| Cells/g tissue·$10^6$ | 0.80 (.09)[x] | 1.30 (.20)[y] | 1.8 (.30)[z] |
| Density (g/ml) | 0.908 (.002) | 0.914 (.001) | 0.919 (.001) |
| Residue[2] | 1.08 (.04) | 1.48 (.07) | 1.98 (.07) |
| Experiment 2 n=5 | | | |
| Lipid | 93.29 (.39) | 87.65 (.89) | 90.20 (.21) |
| Cell Volume (pl) | 1712 (41)[c] | 1144 (34)[d] | 839 (73)[d] |
| Cells/g tissue·$10^6$ | 0.60 (.01)[x] | 0.84 (.03)[x] | 1.24 (.12)[y] |
| Density (g/ml) | 0.893 (.002) | 0.892 (.003) | 0.889 (.001) |
| Residue | 0.88 (.06) | 1.37 (.07) | 1.61 (.06) |

[1] Lipid (g)/Tissue Wet Weight (g) · 100
[2] Residue (g)/Tissue Wet Weight (g) · 100
[a,b,c,d] Adipocyte volumes differ, within breed $p<0.05$
[x,y,z] Cells/g tissue differ, within breed $p<0.05$
(S.E.)

Table 11. Mean ± S.E. body weights of the Outaouais (OTA) and Karakul sheep used in Experiment 1 and Experiment 2

| Body Weights (kg) | | | |
|---|---|---|---|
| Experiment 1 (1991) | | Experiment 2 (1992) | |
| OTA | Karakul | OTA | Karakul |
| 78 ± 3[a] | 54 ± 4[b] | 92 ± 4[c] | 67 ± 4[d] |

$p<0.05$ when letters differ

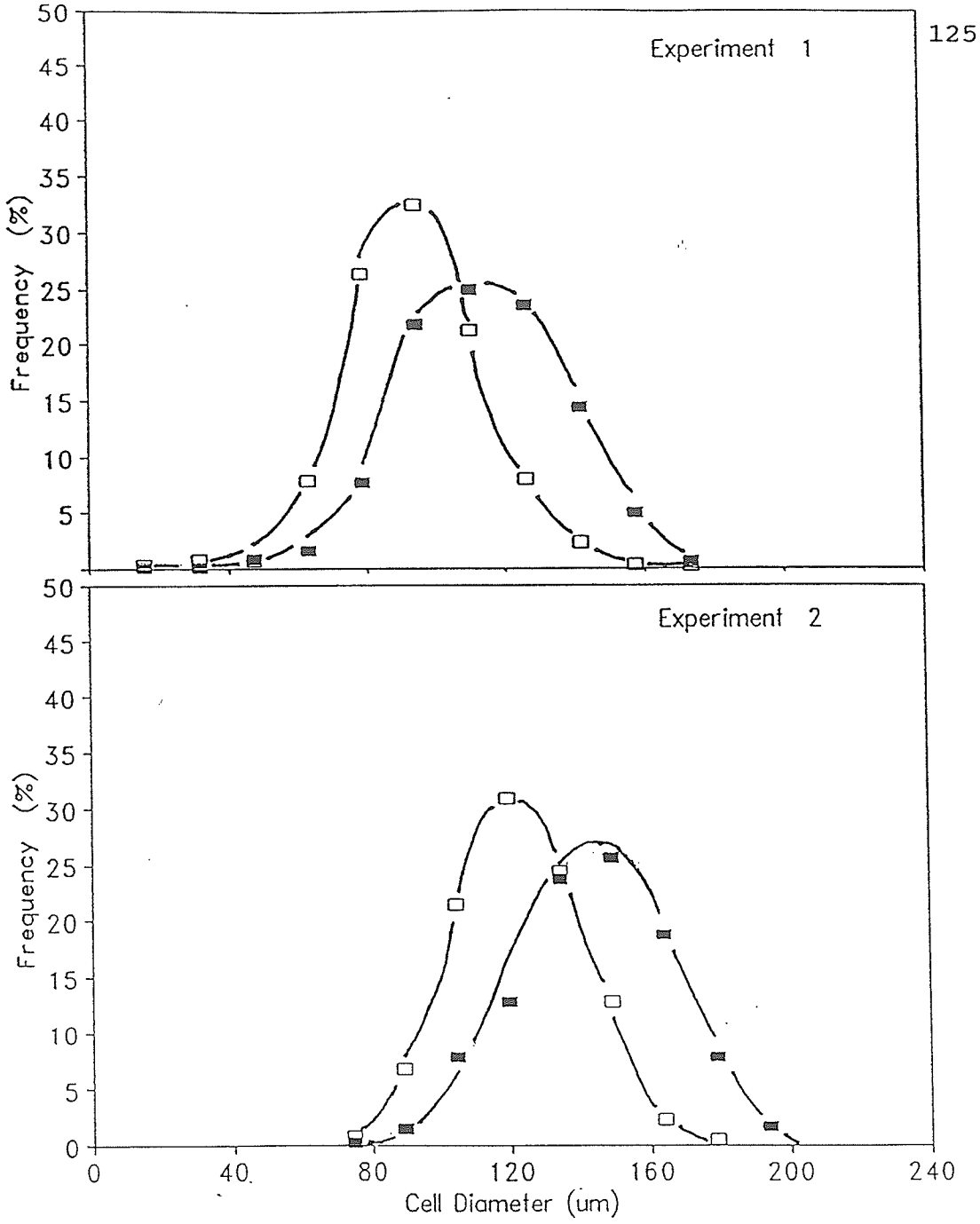


Figure 17. Percent frequency distributions of diameters from collagenase isolated adipocytes from omental (■) and SC (□) depots of OTA ewes. Approximately 100-250 isolated adipocytes were measured for each depot from 8 (Experiment 1) or 5 (Experiment 2) sheep.

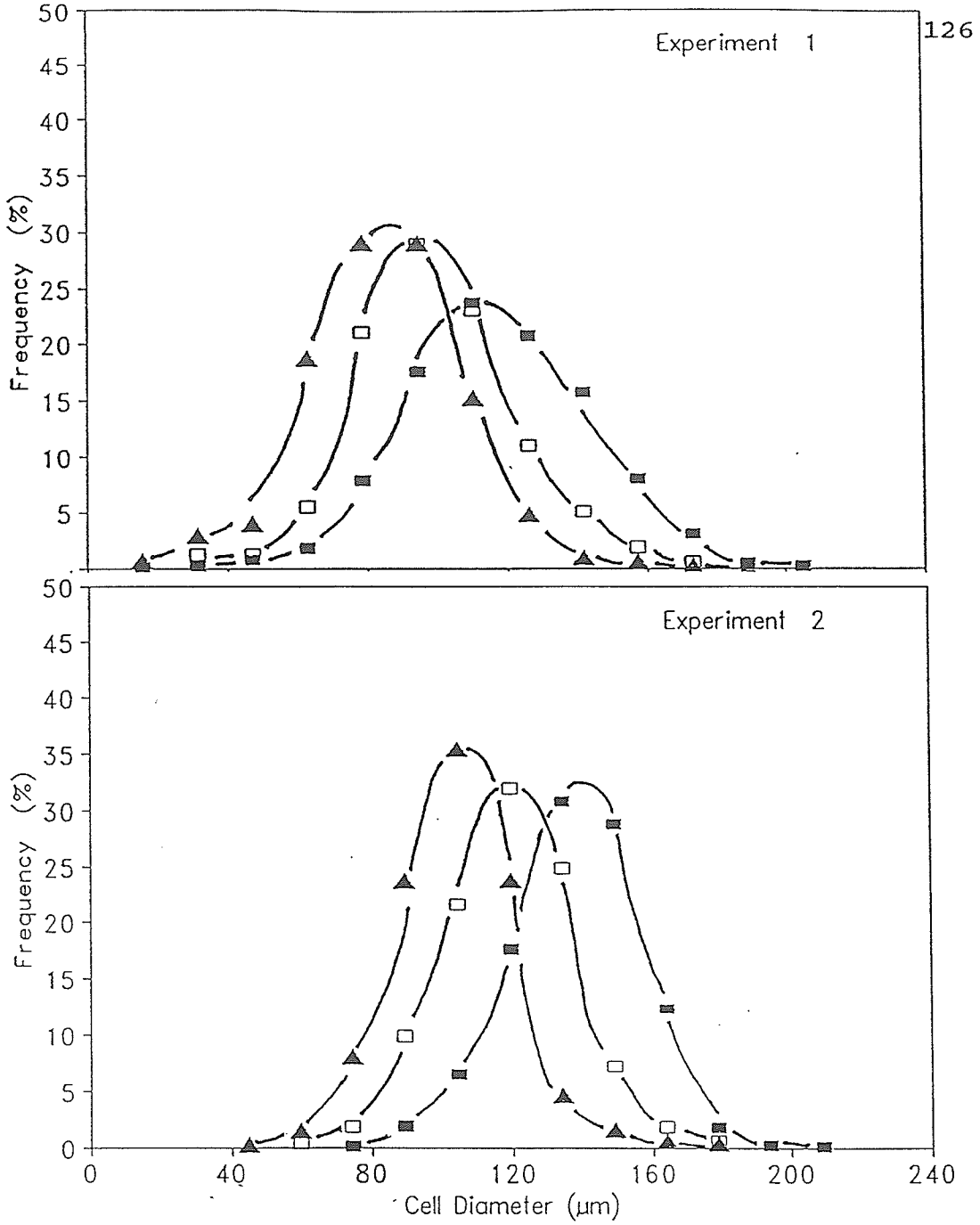


Figure 18. Percent frequency distributions of diameters from collagenase isolated adipocytes from omental (■), SC (□) and tail (▲) depots of Karakul ewes. Approximately 100-250 isolated adipocytes were measured for each depot from 7 (Experiment 1) or 5 (Experiment 2) sheep.

size in Experiment 2 compared to Experiment 1.

## DISCUSSION

The genetic variation in fat accretion in sheep has been known for many years (Wood et al., 1980). However, the effects of breed and anatomical location of the adipose depot have received limited attention regarding lipid mobilization. The present study provides evidence for both breed and depot-specific differences in the responsiveness and sensitivity of ovine adipose tissue to adrenergic stimulation.

Basal and stimulated lipolytic rates were similar in magnitude to values found for isolated ovine adipocytes (Guesnett et al., 1987; Watt et al., 1991). The doses of isoproterenol used in Experiment 1 were 10 to 100-fold higher than doses used in other studies with ovine adipose tissue (Guesnett et al., 1987; Iliou & Demarne, 1987), but were employed because perfusion studies with Karakul tail adipose tissue suggested that this tissue had a reduced sensitivity to adrenergic stimulation. The lower isoproterenol dose range used in Experiment 2 proved to be ideal, as sensitivity and maximal responsiveness to isoproterenol were determined in all depots. The doses of epinephrine used were higher than doses of norepinephrine used by Watt et al (1991), but this was done to ensure that a maximal antilipolytic effect would be induced in all depots.

The demonstration of within breed depot-specific differences in lipolytic responsiveness to adrenergic agonists was more evident with epinephrine than isoproterenol. In both breeds, responsiveness to epinephrine was reduced in SC compared to omental tissue and further reduced in the tail depot of the Karakul ewes. Only a depot effect due to the tail was seen in response to isoproterenol.

Epinephrine is a mixed α and ß agonist, while isoproterenol is a pure nonspecific ß agonist, these results suggest depot differences might be due to an increase in α-mediated effect in the SC adipose tissue and possible alterations in both the α- and ß-adrenergic component in the tail depot. Regarding the tail adipose tissue, an increment in α-mediated antilipolytic effect of epinephrine was supported by the large (77%) inhibition seen with epinephrine in the presence of propranolol. Antilipolytic effects were minimal and not different in the omental and SC depots of the OTA ewes, therefore, α-adrenergic receptor involvement was apparently not responsible for the depot difference in responsiveness to epinephrine in this breed. A significant α-mediated effect was seen in the Karakul omental depot but the magnitude of the effect was similar for the omental and SC depots and again cannot explain differences in maximal responsiveness to epinephrine.

Human adipose tissue displays a similar pattern of

maximal responsiveness, where adipose tissue from a deep abdominal depot is more lipolytically responsive than is adipose tissue from the gluteal SC depot (Leibel & Hirsch, 1987). This pattern of decreased lipolytic capacity in the more superficial depots has also been demonstrated in dogs (Berlan et al., 1982; Taouis et al., 1987) and hamsters (Dieudonne et al., 1992). The reverse was found for omental and SC backfat in cattle, but the data were expressed per mg protein rather than per cell number, which may have influenced the data interpretation (Rule et al., 1992).

Numerous studies have attempted to define the differences in lipolytic capacity in terms of an alteration in either the $\alpha$- or ß-adrenergic receptor density within a depot. There is evidence which suggests the lower lipolytic capacity of the SC depot is due to an increase in the $\alpha_2$-mediated response (Berlan et al., 1982; Lafontan et al., 1979). A second body of evidence has suggested that a preponderance of a ß-mediated effect is responsible for the enhanced lipolytic capacity of the internal depots (Arner et al., 1990a; Taouis et al., 1987).

The adrenergic receptor status of the depots was not examined in this study, but further consideration of the lipolytic response to epinephrine plus propranolol suggests depot differences in ß-adrenergic receptor population (Figures 15 and 16). In all depots propranolol prevented a ß-mediated response in the presence of $10^{-5}$ M epinephrine.

However, the higher $10^{-4}$ M dose elicited a lipolytic response from nonblocked receptors in the omental depots from both breeds. A high density of ß receptors or spare ß receptors may have been responsible for the depot-specific differences in maximal response to epinephrine. Arner et al (1988) demonstrated that occupancy of only a fraction of the ß receptors is necessary to induce a full lipolytic response. The lower lipolytic capacity of the tail adipose tissue and the inability to overcome the propranolol blockage suggests that this depot is lacking in a ß-mediated response compared to the other depots.

Comparisons of ß-adrenergic receptor density in ovine adipocytes from breeds other than those used in this study do not support the above hypothesis, as depot-specific differences in ß receptor density were not found in nonlactating ewes (Bowen et al., 1992; Kennedy & Vernon, 1990). Bowen et al (1992) however, demonstrated depot-differences in sensitivity regarding a specific ß receptor subtype in sheep adipose tissue. The $ß_2$-adrenergic receptors were the predominant population in all depots. The $ß_2$ receptors in SC tissue were more sensitive to adrenergic stimulation than were those in omental tissue, but the less abundant $ß_1$ receptor was more sensitive in the omental depot (Bowen et al., 1992). We found that the Karakul omental tissue had a lower threshold to pure, but nonselective ß stimulation than either the SC or the tail

tissue. The lipolytic rate was increased significantly above ADA levels in the Karakul omental tissue at a lower isoproterenol dose than was required for a similar increase in the SC or tail depots (Figure 12). Also, the adipose tissue from the Karakul ewes was more sensitive than reported values for other breeds (Guesnet et al., 1987; Watt et al., 1991).

It is also possible that there was a population of lipolysis-inducing atypical ß receptors that were not blocked by the propranolol and were only engaged at high doses of adrenergic stimulation. However, the competition curves of propranolol and selective ß stimulation were not explored in this study.

Dieudonne et al (1992) recently demonstrated that depot-specific differences in lipolytic response were not related to adrenoreceptor status of the depot but rather depot differences in a post-receptor ability to stimulate the adenylate cyclase cascade, which was reduced in the SC depot. Similar results were found in human adipocyte ghosts (Kather et al., 1977). Thus, breed and depot-specific differences in responsiveness found in the present study may be related to post-receptor alterations.

The mechanisms involved in the depot differences in response to adrenergic stimulation were not investigated, but the Karakul sheep, with the unique tail adipose depot, would be an excellent model for further examination. The

nonhomongeneous nature of its adipose tissue is displayed in the dramatic difference in lipolytic capacity between the omental adipose tissue and that of the tail. Depot-differences too subtle to detect in other breeds of sheep may be detectable using comparisons of the omental and tail depots in this breed. These depot differences may also be enhanced by limiting the amount of fat deposited as depot-differences are reduced in cattle at heavier weights (Rule et al., 1992)

The Karakul breed displays a genetic predisposition to deposit greater quantities of fat compared to other breeds of comparable weights (Edwards et al., 1982; Shelton et al., 1991). This ability to deposit large quantities of fat when feed is available is believed to be an adaptation that has allowed them to cope with the fluctuations in feed availability of their native environments. Similar increases in fat accretion rate were found when rats were forced to consume their daily caloric requirement in shortened feeding period (Levielle & Hanson, 1966). In a study comparing total fat content of Rambouillet and Karakul breeds, the fat content was 9% of dressing percentage in Rambouillet compared to 20% in the Karakul (Shelton et al., 1991). The difference was almost entirely accounted for by the tail-adipose depot. Farid (1991) has suggested that the hardiest of the native breeds has the greatest propensity to fatten and possessed the largest tail depot. The ability of

the fat-tailed breed to survive physiological stresses, such as restricted intake, better than other breeds may be due to the greater body fat content. However, the Karakul may also have a more sensitive control of mobilizing the lipid from each of the storage depots. This ability may also include limiting the lipolytic capacity of the tail depot until situations such as feed shortages, pregnancy or lactation which increase demands on energy reserves.

The present results suggest that the Karakul ewe has a greater sensitivity to ß-adrenergic stimulation in all depots compared to OTA sheep. They also appear to regulate the lipolytic capacity of the individual depots through different mechanisms. Removal of adenosine with adenosine deaminase significantly increased the lipolytic rate only in the tail adipose tissue in Experiment 1 and tended to have a greater effect in the tail in Experiment 2 compared to all other depots. The results suggest that adenosine release may be partially responsible for the reduced lipolytic capacity of tail adipose tissue.

The percent frequency distributions shown in Tables 16 and 17 indicated that all depots displayed a monophasic distribution of cell diameters. If further increases in cell diameter or volume become limiting to adipose deposition a second population of smaller cells would begin to fill, thereby displaying two populations of cells reflected by a biphasic distribution (Allen, C. E., 1976).

Also, the lack of skewness in the curves suggested that the collagenase preparations did not preferentially damage either small or large cells.

In conclusion, isoproterenol and epinephrine induced a 3 to 9-fold increase in lipolytic rate in various adipose depots from two breeds of sheep. The Karakul omental depot had the greatest lipolytic capacity, whereas, the Karakul tail displayed the lowest capacity. The Karakul depots were the most sensitive to isoproterenol-stimulated lipolysis. A significant $\alpha$-mediated antilipolytic component was displayed in the Karakul omental depot and strong evidence for an $\alpha$-mediated inhibition was displayed in the other depots. However, the functional role of an $\alpha$-mediated effect in adrenergic reponsiveness in the omental and SC depots of nonpregnant, nonlactating sheep is inconclusive. The data suggest that increased ß-adrenergic receptor responsiveness are responsible for the increased lipolytic capacity of the omental adipose tissue compared to the SC tissue, but the contribution of alterations in post-receptor mechanisms were not studied. In the tail depot, a reduced ß-mediated response coupled with a potential $\alpha_2$-mediated response appear to be primarily responsible for the decreased lipolytic capacity, but the role of adenosine in this depot cannot be excluded.

If the sensitivity and the capacity of adipose tissue to respond to adrenergic stimulation is a reflection of that

depot's role in energy balance, then presumably the role of the omental depot is to rapidly mobilize large quantities of stored lipid, whereas, that of the Karakul tail depot is to provide slow release requiring increased adrenergic stimulation.

To provide a greater insight into adrenergic regulation and the role of the fat-tail depot in energy metabolism, future studies should examine the effects of pregnancy and lactation or negative energy balance on the sensitivity and responsiveness of the depots to adrenergic stimulation. Also, the dramatic heterogeneity of the adipose tissue of the Karakul breed makes it an appropriate model for examining depot-specific differences in lipid mobilization. Finally, Karakul tail adipose tissue could be studied by perfusion, tissue biopsy and *in vivo* by examining arterial-venous differences in metabolites (Gooden et al., 1986). Thus, the Karakul breed is ideal for comparisons of *in vivo* and *in vitro* effects of modulators of lipid metabolism.

# GENERAL DISCUSSION

The distribution of adipose tissue throughout the sheep is influenced by breed (Wood et al., 1980) and the predisposition of a breed to partition adipose tissue to specific depot is related to the environmental origin of the breed (Palsson, 1940). Because a greater proportion of adipose tissue is directed to a particular depot, for example, in dam breeds, a greater proportion of adipose tissue is found in the internal depots compared to sire breeds, it would suggest that the omental depot plays an important role in energy balance of the animal.

The adipose tissue in two breeds of sheep studied displayed similar patterns in depot-specific differences in lipolytic capacity as those of humans (Leibel & Hirsch, 1987), dogs (Berlan et al., 1982) and hamsters (Deiudonne et al., 1992). The data suggest that there was an enhanced ß-mediated response in the omental tissue compared to the SC tissue. The α-mediated antilipolytic effect of epinephrine was significant in the Karakul omental depot, but a similar effect was found in the SC depot. Thus, the α-mediated effect cannot explain the difference in epinephrine-induced lipolysis in these two depots of the Karakul sheep. Also, the α-mediated response of adipose tissue was examined with

clonidine in both the stimulated (theophylline-induced) and basal (ADA) states. Clonidine did not induce an inhibition to lipolysis in any of the depots studied, including the Karakul omental tissue, which displayed a significant α-mediated inhibition to epinephrine in the presence of propranolol.

The reduced lipolytic capacity of the tail adipose tissue adds an additional component to the heterogeneity of adipose tissue. The regulation of adrenergic-induced lipolysis in this depot was different from both the omental or SC depots. The limited lipolytic responsiveness to isoproterenol would suggest that the tail depot has a reduced ß-mediated response. Whether this is a receptor or post-receptor deficit was not determined. In addition, there was also a strong tendency for this depot to display an α-mediated inhibition of lipolysis.

Isolated perfusion studies of the tail depot also suggested a reduced lipolytic capacity of this depot. Concentrations of isoproterenol which induced a maximal response *in vitro* did not induce a similar response in the perfusion preparation (Manuscript I). Adenosine may have limited the response in the perfusion study as its levels were not controlled with ADA addition. Adensosine has been shown to reduce the sensitivity of ovine adipose tissue to adrenergic stimulation (Vernon & Finley, 1985) and to increase the action of insulin in rat adipose tissue (Joost

& Steinfelder, 1982).

A vasoconstrictive response was displayed in two separate isolated perfusion preparations where norepinephrine (Manuscript I) and clonidine (Manuscript II) were used. Because the vasoconstrictive properties of the tail were evident at doses that were expected to induce lipolysis as well, it suggests that the vasoconstrictive response may be more sensitive in the tail than was the lipolytic response.

The Karakul breed has adapted to harsh environments. It is able to deposit greater quantities of fat when food is available and to regulate the mobilization of this fat to increase its survival rate under harsh conditions. This is the first study that has examined the lipid mobilizing capabilites of three depots within an individual. It would appear that adaptations have occurred in mobilization of lipid as well. The Karakul breed has adipose tissue with an increased sensitivity to adrenergic stimulation compared to non-fat-tailed breeds. It is likely that, because this breed has three large depots to rely on during energy demands, it has developed a greater sensitivity of control in each depot. Also, the additional storage depot in the tail provides a reserve that is slow releasing and of limited lipolytic capacity. The results of this study suggest that the tail adipose tissue is mobilized only in times of increased adrenergic stimulation. That is, the

greater adenosine response and the vasoconstrictive properties within the tail depot would limit the contribution this depot can have under nonstressed conditions.

The tail depot of the Karakul sheep adds an additional dimension to the heterogeneity of adipose tissue within an animal. This breed would be an appropriate model to examine the effects of feed restriction or pregnancy and lactation on those factors that regulate lipid mobilization.

# CONCLUSIONS

There are breed and depot-specific differences in the responsiveness and sensitivity of ovine adipose tissue to adrenergic stimulation. The differences in lipolytic capacity of ovine adipose tissue are modulated through alterations in ß-mediated responsiveness, sensitivity of the response and an α-mediated contribution.

An enhanced response to adenosine and a vasoconstrictive response to adrenergic stimulation may contribute to the limited lipolytic capacity of the Karakul tail depot.

In future studies, individual depots differences in responsiveness and sensitivity should be examined in the three depots of the Karakul sheep under various physiological conditions, such as pregnancy, lactation or negative energy balance. Capitalizing on the dramatic heterogeneity of adipose tissue in the Karakul sheep could only expand our knowledge of adipose tissue metabolism.

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# APPENDIX I

Concentration of oxygen dissolved in the perfusate.

$$C = k \cdot PO_2$$

C=concentration (ml oxygen/l $H_2O$)
$k$=oxygen solubility coefficient 0.029
$PO_2$=partial pressure of oxygen in mmHg

Theoretical concentration with 95% $O_2$/5% $CO_2$ aeration:

C=0.029 · 722 mmHg
=20.9 ml oxygen/l perfusate

Measured[1] concentration for $PO_2$ in the inflow and outflow perfusate:

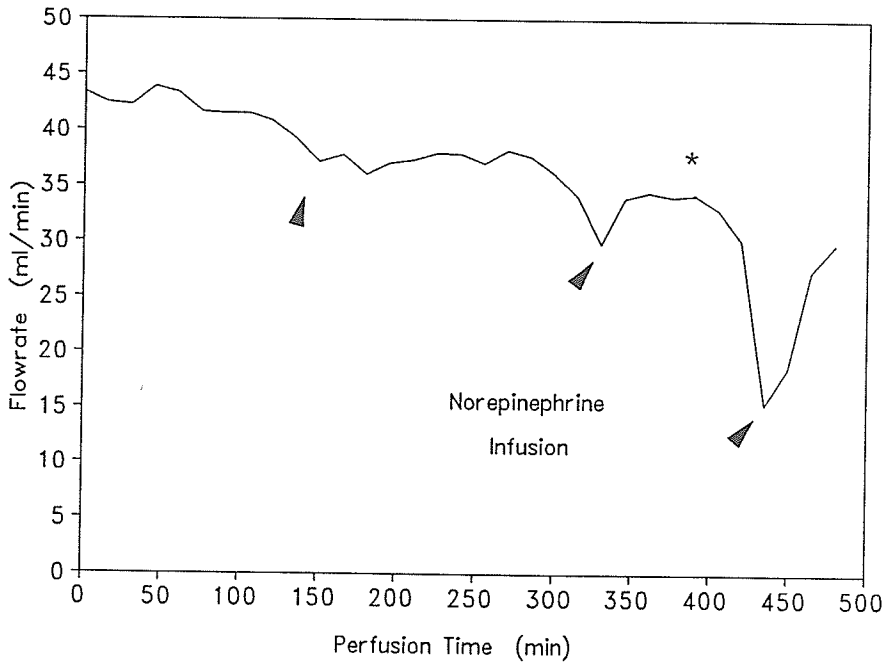
**Inflow**
C=0.029 · 346.4 ± 44.1 mmHg (mean ± S.D.; n=3)
=10.0 ml oxygen/l perfusate

**Outflow**
C=0.029 · 85.7 ± 15.8 mmHg (mean ± S.D.; n=3)
=2.49 ml oxygen/l perfusate

[1]The partial pressure of oxygen was determined from samples collected during the course of perfusion (2 values from one preparation and 1 value from a second preparation). Samples were collected in a syringe using a 3-way stopcock located in the inflow and outflow catheters. Collected samples were analyzed immediately using a Corning 165/2 pH/Blood Gas Analyzer.

# APPENDIX II

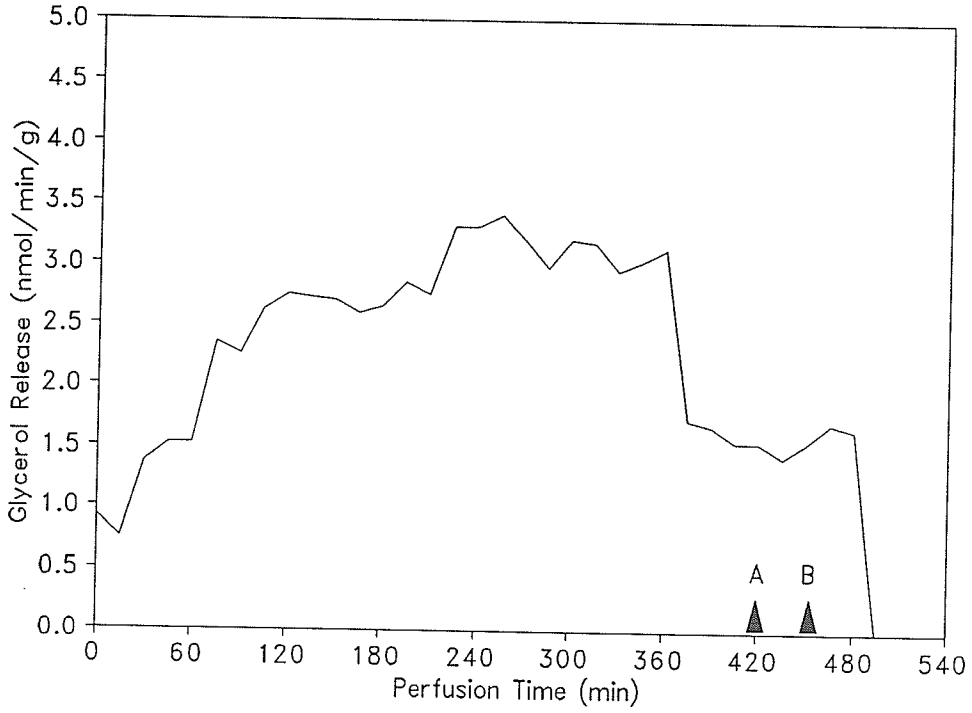
Flowrate data over time including vascular response to norepinephrine infusion in the Karakul ewe (Manuscript I).




Flowrate of perfusate (bovine plasma:M199 tissue culture medium, 1:1) through the isolated fat-tail depot of the adult Karakul ewe. Arrows indicate the flowrate after 15 min of norepinephrine infusion at 4.25 μg/min/kg tail (160 and 320 min) or 42.5 μg/min/kg tail (445 min). * indicates flowrate after 15 min of 5 μg/min/kg tail isoproterenol. Each is followed by saline infusion for 30 min. Flowrate values were taken every 5 min and represent the mean of four sheep.

# APPENDIX III

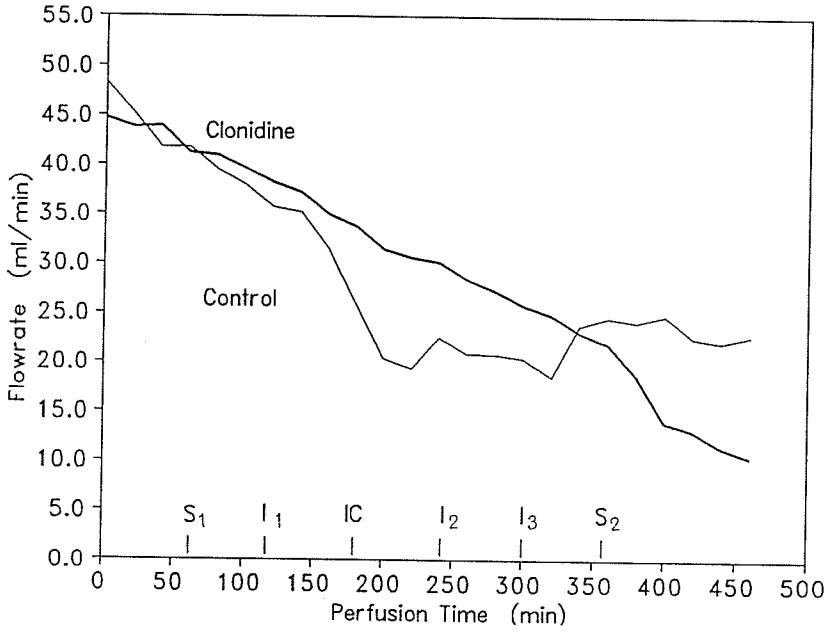
Glycerol release (nmol/min/g perfused adipose tissue) following infusion of norepinephrine or isoproterenol (Manuscript I).




Rate of glycerol release (nmol/min/g perfused adipose tissue) following a 15 min infusion of 425 μg/min/kg tail isoproterenol (A;420 min) or a 15 min infusion of 42.5 μg/min/kg tail norepinephrine (B;465 min).

# APPENDIX IV

Flowrate data over time in the saline and clonidine-treated Karakul ram lambs (Manuscript II).




Flowrate of perfusate (bovine plasma:M199 tissue culture medium, 1:1) through the isolated fat-tail depot of 84-day control (n=3) or clonidine (n=3) treated Karakul ram lambs. Values represent the mean of 3 sheep per group (except the control group which had only 1 sheep contributing after 200 min) taken every 5 min. Infusion additions to the perfusate are S=saline, I=isoproterenol, IC=isoproterenol plus clonidine.

# APPENDIX V

Dilution factor--correction for deproteinization with $HClO_4$.

**Dilution Factor (F) =**

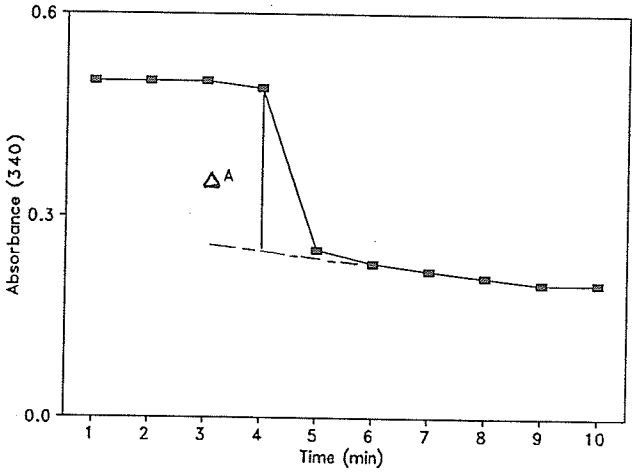
[aliquot incubation medium · sg · $\%H_2O$ + volume $HClO_4$/ aliquot incubation medium] x [volume supernatant + volume KOH + volume $KHCO_3$/volume supernatant].

sg=specific gravity.

see Bergmeyer et al., 1979.

# APPENDIX VI

Determination of metabolite concentration with end-point methods (Bergmeyer et al., 1984).




Enzymatic determination of glycerol concentration by monitoring the decrease in NADH concentration over time.

$$C = \Delta A \cdot V / \epsilon \cdot d \cdot v = [\mu mol/ml]$$

C=concentration of metabolite per ml solution

$\Delta A$=change in absorbance

V=final assay volume

v=sample volume

$\epsilon$=extinction coefficient for NADH [6.3 $cm^2/\mu mol$]

d=distance of light path [cm]

# APPENDIX VII

Calculation of the number of cells per known tissue weight.

**Number of Cells/Adipose Tissue Weight=**

[Tissue weight·Lipid:Tissue ratio]/[Mean Cell Lipid content]

**Mean Cell Lipid Content** ($\mu$g/cell)=

Mean Cell Volume ($\mu$l/cell) · Lipid Density ($\mu$g/$\mu$l)

**Mean Cell Volume** = $\Sigma$ ($V_i$ · $F_i$)/n

Where: $V_i$ is the average of upper and lower volumes for each class (volume = $\pi$·diameter$^3$/6).

$F_i$ is the frequency.

n is the number of cells contributing to the mean.

**Lipid Density** = weight of known volume of extracted lipid.

per DiGirolamo et al., 1971.

# APPENDIX VIII

Least squared means for glycerol release ($\mu$mol/$10^6$ cells/3 h) for each breed-depot combination.

| Treatment | OTA omental | OTA-s.c. | Karakul omental | Karakul s.c. | Karakul tail |
|---|---|---|---|---|---|
| Basal | 0.89 | 0.76 | 1.44 | 0.64 | 0.53 |
| ADA (.2 U/ml) | 1.53 | 0.90 | 2.80 | 1.27 | 1.63 |
| Clonidine (100$\mu$M) | 1.76 | 1.20 | 2.03 | 1.43 | 1.13 |
| Propranolol (20$\mu$M) | 1.68 | 0.92 | 3.47 | 1.47 | 1.84 |
| Ep 0.1 | 4.87 | 3.91 | 9.91 | 7.26 | 5.32 |
| Ep 1.0 | 7.73 | 6.00 | 11.41 | 9.04 | 5.26 |
| Ep 10 | 9.07 | 6.32 | 10.87 | 9.88 | 5.14 |
| Ep 100 | 9.83 | 7.14 | 11.88 | 9.41 | 5.23 |
| Prop + Ep 0.1 | 1.83 | 0.77 | 3.13 | 0.96 | 1.06 |
| Prop + Ep 1.0 | 1.27 | 0.76 | 1.68 | 0.64 | 0.45 |
| Prop + Ep 10 | 1.40 | 0.94 | 2.91 | 0.63 | 0.42 |
| Prop + Ep 100 | 5.16 | 1.83 | 7.58 | 2.35 | 0.72 |
| Iso .0001 | 1.37 | 0.80 | 3.92 | 1.46 | 1.79 |
| Iso .0005 | 1.96 | 1.09 | 4.61 | 2.31 | 2.97 |
| Iso .001 | 2.39 | 1.17 | 6.64 | 3.27 | 3.83 |
| Iso .005 | 3.96 | 2.90 | 9.87 | 6.95 | 4.91 |
| Iso .01 | 4.65 | 2.95 | 9.74 | 8.05 | 5.49 |
| Iso .05 | 6.84 | 5.24 | 9.90 | 9.43 | 5.79 |
| Iso .1 | 7.52 | 6.07 | 10.03 | 10.15 | 5.23 |
| Iso 1 | 8.81 | 8.25 | 11.13 | 10.21 | 5.50 |
| S.E. | 0.64 | 0.64 | 0.64 | 0.64 | 0.64 |

See Manuscript III for a complete discussion of the treatment conditions. Values presented are the least squared means (and pooled S.E.M.) of triplicate determinations from 5 sheep per breed-depot combination. OTA=Outaouais; ADA=adenosine deaminase; Ep=epinephrine ($\mu$M); Prop=propranolol; Iso=isoproterenol ($\mu$M); s.c.=subcutaneous.