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The role of estradiol-17β in the regulation of ovine adipose tissue lipid metabolism and feed intake

Green, David Alan, Ph.D.

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U·M·I
300 N. Zeeb Rd.
Ann Arbor, MI 48106

THE ROLE OF ESTRADIOL-17β IN THE REGULATION OF OVINE ADIPOSE TISSUE LIPID METABOLISM AND FEED INTAKE

by

David A. Green

A DISSERTATION

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THE ROLE OF ESTRADIOL-17β IN THE REGULATION OF OVINE ADIPOSE TISSUE LIPID METABOLISM AND FEED INTAKE

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The effect of estradiol 17β (E_2) on adipose tissue metabolism and feed intake was studied in five trials. In trial 1, the relationship of feed intake and E_2 in plasma were characterized in twin-bearing ewes (n=10) during late gestation and during lactation. Feed intake decreased 1.8-fold from 60 d prepartum to parturition as E_2 in plasma increased 3-fold. Feed intake increased 2.8-fold during the first 30 d of lactation when E_2 in plasma was 13-fold lower than the last week of gestation. In trial 2, feed intake was determined (30 d) in ovariectomized ewes implanted with (n=9) E_2 to attain concentrations of E_2 indicative of late gestation or a sham implant (n=9). Intake decreased transiently in ewes implanted with E_2. Trials 1 and 2 indicated E_2 was a potential feed intake regulator during late gestation. In trial 3, subcutaneous adipose tissue was biopsied from thirteen twin-bearing ewes to characterize lipid metabolism during -30, -15, 0, 15 and 30 d relative to parturition. De novo lipogenesis and palmitate esterification decreased as non-esterified fatty acid and glycerol release (lipolysis) increased during late gestation. During lactation lipogenesis and esterification increased while lipolysis decreased. In trial 4, the interaction of body condition and E_2 on lipid metabolism was evaluated in lean (68 kg) and obese (87 kg) ovariectomized ewes receiving implants containing E_2 or sham implants. Biopsies of subcutaneous adipose tissue were taken at 0, 5 and 30 d post-implantation to determine lipogenesis,

esterification and lipolysis. Lipogenesis was inhibited in ewes implanted with E_2 as compared to ewes without E_2. Lean ewes without E_2 had higher rates of lipogenesis over time than obese ewes without E_2. Esterification increased in lean but decreased in obese ewes without E_2. Slices of adipose tissue were cultured (48 h) to evaluate the effect of E_2 on lipid metabolism. Lipogenesis and esterification were decreased by E_2 in the culture. Lipolysis in trials 4 and 5 had little or highly variable responses to E_2. These data indicated increased concentrations of E_2 in plasma during late gestation may directly effect energy metabolism via decreasing feed intake and independently decreasing de novo lipogenesis and palmitate esterification.

DISSERTATION TITLE

The Role of Estradiol-17B in the Regulation of Ovine Adipose Tissue Lipid Metabolism and Feed Intake

BY

David Alan Green

SUPERVISORY COMMITTEE:

	APPROVED	DATE
Signature	Dennis Brink	1/18/91
Typed Name	Dr. Dennis Brink	
Signature	Robert Britton	1/18/91
Typed Name	Dr. Robert Britton	
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INTRODUCTION

The last trimester of gestation in sheep represents a period of rapid fetal growth, increased fetal energy demands and a potential decline of feed intake. The net effect is a negative energy balance. Reid and Hinks (1962) estimated twin-bearing ewes must increase energy intake 75 % during late gestation to meet fetal energy demands. Feed intake may decline during late gestation to further accentuate energy deficiency. Forbes (1986) postulated increasing estrogen levels were a potential negative regulator of feed intake during late gestation. Estrogens have a multi-functional role during late gestation that include increasing myometrial activity, mammary gland development and is inducing prolactin and oxytocin receptors at the myometrial and myoendothelial cells, respectively.

To offset the energy costs of gestation the regulation of maternal tissue mobilization becomes increasingly important. Lodge and Heaney (1973) determined 81 % of the weight loss in gestating ewes was accounted for by losses in adipose tissue and 19 % by losses in protein mass. Non-esterified fatty acids and glycerol are released from adipose tissue as energy sources. Lodge and Heaney (1973) further estimated 20 to 25 % of the total energy mobilized from maternal body tissues was recovered in uterine, fetal tissue and mammary gland development. The deposition of mobilized maternal energy into fetal, uterine and mammary development demonstrates the priority the fetus has for available energy.

Homeorrhesis is the coordination of a set of adaptations in support of a dominant physiological process such as gestation or lactation (Bauman and Currie, 1980). Adaptation in lipid metabolism is the primary route in which the body accommodates the energy deficiency during gestation and early lactation. McNamara and Hillers (1986a,b,c) in dairy cattle and Vernon et al. (1981) in sheep determined lipogenesis and esterification decreased as parturition approached and increased as lactation progressed. Lipolysis increased as parturition approached and was maintained at elevated levels throughout lactation. The factors regulating lipid metabolism during gestation and lactation are not fully known. Estradiol-17β(E_2) is a potential hormonal regulator of lipid metabolism. Wade and Gray (1979) postulated E_2 has direct effects on adipose tissue as related to energy metabolism. Estradiol-17β concentrations in plasma peak around parturition at the approximate time lipogenesis and esterification are the lowest and lipolysis is the highest. However, the effect of E_2 on lipid metabolism during late gestation has not been established as a direct effect because feed intake decreases as parturition approaches and would be expected to decrease lipogenesis and esterification and increase lipolysis.

The effect of body condition on feed intake and lipid metabolism during late gestation is unclear. Garnsworthy and Topps (1980) found that cows in thin body condition increased feed intake more rapidly after parturition than fat cows. Lipid metabolism is inherently different between animals in lean and obese body condition thus hormonal effectors may have a varied response.

The objectives of this research were to:

1.) Relate the effect of concentrations of E_2 in plasma observed in late gestation on ad libitum feed intake.

2.) Characterize lipid metabolism in adipose tissue and E_2 concentrations during late gestation and lactation in twin-bearing ewes.

3.) Determine if body condition and E_2 interact over time on lipid metabolism in adipose tissue.

4.) Evaluate the direct effect of E_2 on lipid metabolism of adipose tissue cultured in vitro (48 h).

LITERATURE REVIEW

Lipid metabolism in adipose tissue and the regulation of feed intake are two important factors that effect the overall energy balance of the ewe in late gestation and lactation. The literature review will examine each of these factors during late gestation and lactation. Potential factors which may be effecting feed intake and lipid metabolism will be discussed. An overview of feed intake regulation models and principles involved will be discussed first. The research conducted in this dissertation involved long term intake regulation which modulated intake by alterations in metabolism and sustained hormonal secretions. Long term intake regulation will be discussed as it relates to body lipid condition and concentrations of substrates and metabolites.

Gestation and lactation are physiological states in which wide fluctuations in feed intake occur. The relationship of feed intake and gestation are discussed as changes in fetal growth and hormonal secretions both of which may decrease feed intake. Lactation represents increased feed intake which may be related to expulsion of the fetus, increased energy demand for milk production and the removal of hormones which may inhibit intake.

Gestation and lactation also represent physiological stages wherein lipid metabolism of adipose tissue is highly active. The overall factors regulating lipid anabolism and catabolism will be discussed as they relate to substrate availability, enzymatic systems and hormonal profiles. The integration of lipid synthesis, degradation and fatty acid reincorporation will be discussed as it relates to net lipid accumulation

or depletion. Potential hormonal and enzymatic factors which may regulate lipid metabolism as it adapts to energy deficits or surpluses will be summarized in relation to gestation and lactation.

Feed Intake Regulation.

Central Models. The regulation of feed intake is the multiple input of sensory factors located peripherally and in the central nervous system. The biological and economical importance of feed intake regulation during the past 40 years has been recognized as indicated by numerous reviews (Blaxter, 1950; Kleiber, 1961; Balch and Campling, 1962; Jones, 1972; Baile and Forbes, 1974; Forbes, 1986; Martin et al., 1989). A general theme of these reviews reveals that not one but many hormonal and metabolic signals influence feeding behavior. A hierarchy of signal importance influencing feed intake may exist however, absolute control of feeding is not delegated to a single factor or signal. The hierarchy may also shift depending on nutritional or physiological state and(or) species differences. Bray (1978) suggested that feed intake is controlled by multiple factors to assure backup control mechanisms should one or more of the feed intake regulators become inoperative. The diversity of multiple control mechanisms is manifested by the fact that both humoral and metabolic signals are integrated with neural systems modulating feed intake.

Baumgardt (1970) described a feed intake regulation system wherein, tissue receptors are sensitive to numerous feedback signals of physical or metabolic origin. The signals are relayed and integrated in the hypothalamus to initiate or cessate feeding. Mayer (1967) earlier had

proposed mechanisms regulating feed intake have five characteristic criteria: 1) Mechanisms must be integrated with metabolism which regulates energy balance. 2) Mechanisms must be logically compatible with known characteristics of the central nervous system and based on cellular changes. 3) Mechanisms should explain the effects of metabolic hormones on intake. 4) Mechanisms may describe why variations in environmental conditions associated with increased energy output influence energy intake. 5) Mechanism may account for the metabolic existence of hunger and satiety.

Baile and Forbes (1974) compiled a comprehensive review of feed intake and energy balance regulation in ruminants. The authors described the inter-relationship of energy balance and feed intake control by integrating factors which regulate feed intake. Energy balance is the difference between energy intake and metabolizable energy output and may be regulated to be positive, negative or zero. Conversely, the authors described feed intake as a controlled function having an on or off response. The inter-relationship of feed intake and energy balance may be illustrated by recognizing both may be affected by external environment, hormones and metabolic substrates and(or) products. Baile and Della-Fera (1981) suggested ruminants tend to maintain a constant energy balance by changing feed intake in proportion to physiological and environmental status. Support for constant energy balance may be found in studies determining feed intake as effected by diet energy dilution. Conrad et al. (1964) determined feed intake was dependent on metabolic size, genetically determined production potential and diet digestibility.

In a summary of 114 digestion trials, Conrad et al. (1964) found lactating dairy cows to decrease energy intake as diet digestibility increased above 67 % or contained 2.95 kcal digestible energy/gram of diet resulting in a relatively constant digestible energy intake. When diet digestibility was below 67 % feed intake was controlled not by digestible energy intake but by physical fill. Mature sheep had a maximal energy intake when the diet has a digestibility of approximately 55 % or 2.45 kcal digestible energy/gram of diet (Jones, 1972). Above this point energy intake declined as dietary energy increased to maintain a constant energy intake. Regulation of feed intake in ruminants reflects factors influencing diet digestibility and rate of passage until diet digestibility reaches 55 to 66 %. Above this level of diet digestibility physiological factors are invoked to maintain a constant digestible energy intake dependent upon age, production level and physiological state (Jones, 1972; Baile and Forbes, 1974; Baile and Della-Fera, 1981).

Long Term Intake Regulation

Lipostatic Control of Feed Intake. Ruminants regulate energy balance dependent upon production conditions although it is not clear what component(s) of the body energy is regulated (Baile and Forbes, 1974). Adipose tissue stores contain 85 to 90 % of the whole body energy in all but the very young ruminant thus it is logical that adipose tissue may be a potential regulator of energy balance and feed intake. Kennedy (1953) initially proposed total body adipose tissue mass acts as a long-term regulator of energy balance and feed intake. The author

hypothesized as adipose stores increased over the long-term a negative feedback signal was generated to cessate feeding. Hervey (1969) described a similar theory in clinical human obesity wherein, as obesity increased food intake declined indicating a potential metabolic feedback from the adipose depot. Garnsworthy and Topps (1982) examined the effect of body condition on feed intake and milk production in dairy cows. Lean compared to obese cows ate more feed (kg/d), produced more milk and increased body condition as lactation progressed whereas obese cows decreased condition. The convergence of condition score as lactation progressed suggested the cows have a target condition to attain. The authors concluded increased body fat levels at calving inhibited feed intake during lactation. Hyer et al. (1986) examined the relationship of body composition and feed intake in beef steers. Four groups of cattle with varying initial weights (280, 322, 364, 419 kg) were fed high grain diets and feed intake was determined. A computer model was used to predict body composition. Feed intake was found to decrease when steers reached a body composition of 32 % fat. The authors concluded intake was regulated by a negative feedback from adipose tissue.

Other potential mechanisms proposed to relate feed intake and adipose tissue depots have dealt with the physical effect of fat depots decreasing ruminal volume and a humoral feedback from the adipose depot (Baile and Della-Fera. 1981). Bines et al. (1969) fed cows of varying body condition a high grain diet and found thin cows to consume 20 % more feed in a 5 h time period after feeding than fat cows. The authors concluded ruminal fill did not play a role in feed intake cessation.

Feed Intake, Substrates and Body Condition. Forbes (1980) suggested at least two ways in which increased fat depots may contribute to the homeostatic balance of body energy in ruminants. The author assumed a limit to triglyceride synthesis in adipose tissue. As the synthesis limit was approached more of the lipogenic substrates appear in the plasma thus receptors sensitive to energy availability may signal a cessation of feed intake. Bines and Morant (1983) found a higher rate of fatty acid synthesis in adipose tissue from thin cows and a resultant lower plasma acetate level. The authors suggested feed intake of the thin cows was increased due to lower plasma acetate levels stimulating intake. Bines (1971) had earlier proposed alterations in volatile fatty acid (VFA) utilization between thin and fat cows regulated feed intake. The rate of VFA absorption is dependent upon the concentration gradient between the rumen and peripheral blood system. Ruminal VFA concentrations were determined to be similar between thin and fat cows suggesting at a higher intake level thin cows absorbed and oxidized more VFA or synthesized more VFA into lipid than fat cows.

A second mechanism Forbes (1980) suggested dealt with long-term feed intake control as regulated not by adipose tissue substrate uptake but by degradation products from adipose. Non-esterified fatty acids (NEFA) are released from triglycerides and may serve as an indicator of body energy stores (Russell, 1978) Plasma NEFA levels were higher in fat as compared to thin dairy cows (Reid et al., 1986). Plasma NEFA levels have been reported to be inversely related to ruminal VFA and plasma

glucose levels. McNiven (1984a) examined plasma glucose, NEFA and insulin in thin (55 kg) and fat (90 kg) wethers fed four levels of energy (5 d fast, 6, 10 and 17 MJ/d). In thin wethers, plasma glucose was negatively correlated (-.542) to NEFA, NEFA was negatively correlated (-.520) to insulin and insulin was positively correlated (.307) to glucose. Fat sheep had similar plasma relationships however the correlations were lower. Metabolizable energy intake was negatively correlated to plasma NEFA in thin wethers (-.771) and fat wethers (-.534). McNiven (1984a) concluded increased body fat effects the relationship of plasma insulin, glucose and NEFA in ruminants. Although a negative correlation exists between energy intake and plasma NEFA the cause and effect relationship is not definitive.

Trenkle and Kuhlemeier (1966) were interested in plasma NEFA and glucose as affected by ruminal VFA in fasted sheep. Ruminal infusion of acetate did not effect plasma NEFA while propionate increased blood glucose and decreased plasma NEFA. Ruminal butyrate infusion also decreased plasma NEFA as did jugular glucose injections. The authors suggested decreased plasma NEFA was altered by the appearance of glucose and(or) gluconeogenic precursors at the liver suggesting a role of glucose or its precursors in regulating energy balance. If plasma NEFA concentrations are regulators of feed intake an apparent paradox occurs in fasted or obese animals. In both the obese and fasted states plasma NEFA are higher than in animals in moderate condition and thus, are probably of little use as a signal to the hypothalamus on the state of energy depots (Kennedy, 1966).

Brobeck (1975) speculated the ability or lack of ability of adipose tissue to extract and incorporate plasma glucose into lipids determines body condition in non-ruminants. Forbes (1983) also theorized adipose tissue becomes less sensitive to the lipogenic effects of insulin as the cells fill with lipid in ruminants. The rate of precursor uptake by adipose tissue is reduced leaving higher precursor levels in the plasma to induce satiety. Experimental support of these theories is available. Insulin resistance has been defined in non-ruminants as hyperinsulinemia is coupled to increased plasma glucose (Wangness et al., 1981). Insulin resistance occurs in large adipocytes accompanied by increased rates of glucose recycling in obese sheep (McNiven, 1984b) and obese pigs (Cote et al., 1982). McCann et al. (1989) concluded basal hyperinsulinemia in obese sheep was maintained compared to lean sheep due to excessive insulin secretion not decreased insulin catabolism. The obese sheep had greater basal hepatic glucose output and similar glucose removal from the hindquarter as lean sheep even though insulin arterial concentrations were four-fold higher in obese sheep. The role of glucose in metabolism of adipose tissue in ruminants is primarily to provide carbon for the glycerophosphate moiety of triglycerides and secondarily for lipogenesis. In the obese animal lipid synthesis may be lowered thus less glucose is needed for glycerophosphate synthesis. Although the metabolic feedback signals from adipose tissue to regulate intake are unclear the combination of glucose, glycerol and NEFA status may act on the energy sensitive monitors in the liver and hypothalamus.

The inter-relationship of intake and energy balance as reflected by adipose tissue mass produces interest in the role of adipocyte lipids in the genesis and maintenance of obesity. Adipose tissue may play a passive role in obesity by filling with lipid excessively as controlled by substrate availability and endocrine factors. Signals derived from adipose tissue may be important in controlling whole body energy balance by influencing intake (Forbes, 1983). Lipostatic models of the regulation of energy balance have hypothesized an adipocyte-produced circulating regulator. Adipsin a serine protease produced by the adipocyte may be a potential regulator of energy stores in adipose tissue (Cook et al., 1987). Adipsin mRNA was greatly reduced (100-fold) in obese rodents compared to lean counterparts (Flier et al., 1987). Speigelman et al. (1989) administered corticosterone to mice and determined the time-course relationship of fat pad size and adipsin mRNA. Adipsin mRNA decreased 43 % of the control value prior to an increase in fat pad weight indicating changes in adipsin levels is not secondary to the change in adiposity. Adipsin has the potential ability to serve as a feedback regulator of adipose tissue depot size which has implications in energy balance and feed intake.

Feed Intake Regulation During Pregnancy

During late pregnancy the feed intake of cattle (Aitken and Preston, 1964), sheep (Forbes, 1970) and swine (Friend, 1971) may decrease 30 to 60 % of the mid-pregnancy level. The decline of intake may be regulated via two mechanisms. As fetal and uterine tissues grow

during late gestation more abdominal space is taken up by these tissues thus decreasing ruminal and gastrointestinal space. A second mechanism relates to the large changes in steroid hormone profiles especially estrogens and progesterone. However, the decline of feed intake probably is not regulated by a single factor but by a summation of factors.

Fetal Growth. The growth of fetal tissue occurs rapidly in the last one-third of pregnancy in sheep. Lodge and Heaney (1973) evaluated the growth (g/d) of the uterine contents in pregnant ewes at days 35, 70, 105 and 140 of pregnancy. The total weight of the fetuses, fluids and membranes increased as pregnancy progressed; day 35, 70, 105 and 140 had weights of 169, 1476, 2926 and 8147 g, respectively. Fetuses were 3, 19, 62 and 70 % of the total weight of the uterine contents at days 35, 70, 105 and 140, respectively. The uterus also increased in weight as pregnancy progressed 133, 1400, 1154 and 1222 g on days 35, 70, 105 and 140, respectively. Non-pregnant controls had uterine weights that averaged 80 g. Uterine and fetal tissues comprised 17.6 % of live body weight at day 140 of pregnancy. Ferrell et al. (1976) in beef heifers also found large increases in fetal, uterine and mammary gland components as pregnancy progressed. The uterine contents during 134, 189, 237 and 264 days of pregnancy were 6.28, 16.88, 30.96 and 48.03 kg, respectively. Uterine weight increased 1.94, 3.56, 5.68 and 6.92 kg at days 134, 189, 237 and 264 of pregnancy, respectively. Mammary gland weight increased from 3.8 kg at day 134 of pregnancy to 5.41 kg at day 264 of pregnancy. Prior et al. (1979) in a similar study found the weight of the fetus,

fluids and membranes to be 23 kg which equated to 4.9 to 6.4 % of the heifers' liveweight.

The growth and physical expansion of the uterus, fetus and associated tissues may compete for physical abdominal space thus reducing ruminal and gut size. The reduction in gut size potentially would decrease feed intake. Lagerlof (1929) in cows and Forbes (1968) in ewes found ruminal volume to decrease as pregnancy progressed due to an increased size of the uterus and its contents. These studies were conducted by killing, freezing and cross-sectioning the animals to determine gut and uterine volumes. Forbes (1968) found ruminal volume to decrease from 9 to 5.5 liters at days 98 and 140 of pregnancy, respectively. Gunter et al. (1990) and Vanzant and Cochran (1990) with beef cows found ruminal volume to decline from 120 to 112 liters at days 88 to 15 prior to parturition. The smaller percentage decline in ruminal volume for cows may be due to the smaller percentage of body weight related to uterine contents and uterus weight, 6.4 to 8.4 % as calculated from Ferrell et al. (1976) and Prior et al. (1979). Lodge and Heaney (1973) found uterine contents and uterine tissue to comprise 17.6 % of the live body weight of ewes at 140 days of pregnancy. Gunter et al. (1990) found ruminal volume to be 9.5 liters/kg body weight in non-pregnant ewes and 7.5 liters/kg in pregnant ewes. The decrease of feed intake during pregnancy may not totally be accounted for by a lowered ruminal volume from increased size of the fetus. Aitken and Preston (1964) fed a complete pelleted diet to heifers during pregnancy and found intake to decrease during the last three months of pregnancy. Forbes