Effects of Carbon Precursors and Hormones on the Lipogenesis and Lipolysis of Hanwoo Cattle Adipose Tissues*

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ABSTRACT : This experiment was carried out to determine the contributions of acetate, glucose, amino acids and amino acid metabolites as carbon precursors for the incorporation of radioisotope, in intramuscular and subcutaneous adipose tissue and the effects of insulin on lipogenesis and adrenergic agent, norepinephrine on lipolysis in both tissues. The rate of incorporation of C^{14} labelled acetate, glucose, leucine, isoleucine and α-ketoisocaproic acid into adipose tissue has been measured in subcutaneous and intramuscular adipose tissues. The rate of incorporation was greater (p<0.05) from acetate than glucose in both subcutaneous and intramuscular adipose tissue and the rate of incorporation of carbon precursors into adipose tissues was greater in subcutaneous than in intramuscular adipose tissues. In comparison of amino acids, the rate was highest (p<0.05) with leucine followed by isoleucine and α-ketoisocaproic acid in subcutaneous adipose tissue, in which there were no differences. Also, in intramuscular tissue, leucine was highest (p<0.05), and the rate of incorporation decreased in the same order. The rates of carbon precursor incorporation appeared to be higher in subcutaneous than in intramuscular tissue. For incorporation of radio-labelled acetate and glucose into intramuscular adipose tissue, preincubated for 48 hrs with insulin and IGF-I, insulin was the most effective to stimulate the incorporation of precursors in both substrates but there was no difference between insulin and IGF-1 in glucose incorporation. For glyceride-fatty acid synthesis, acetate was significantly (p<0.05) greater than glucose in both subcutaneous and intramuscular adipose tissue, and glyceride-glycerol synthesis was greater (p<0.05) for glucose than acetate in both adipose tissues. The rates of lipogenesis from both precursors were slightly greater in subcutaneous than intramuscular adipose tissue. There was significant (p<0.05) site effect in insulin treatment for glyceride-fatty acid synthesis. But there were no significance in control and norepinephrine. For glyceride-glycerol synthesis, there was no site effect caused by hormonal treatment. Insulin was the most effective (p<0.05) in glyceride fatty acid synthesis, while norepinephrine was the same as control. Compared with control, glyceride-glycerol synthesis from acetate in insulin treatment was significantly (p<0.05) low in subcutaneous, but high in intramuscular tissue. At the same time, in both tissues, it was lower in norepinephrine treatment than in control. Glyceride-glycerol synthesis from glucose was highest (p<0.05) in norepinephrine treatment followed by insulin although there was no significance between insulin and control. Lipolysis was not affected by insulin but was increased by norepinephrine when added to adipose tissue incubations in vitro. Rates of basal lipolysis were greater in subcutaneous adipose tissue than in intramuscular adipose tissue. (*Asian-Aus. J. Anim. Sci. 2000. Vol. 13, No. 3 : 300-306*)

Key Words : Acylglyceride, Fatty Acid, Glycerol, Subcutaneous, Intramuscular, Acetate, Glucose, Insulin, IGF-1, Norepinephrine

INTRODUCTION

To improve the beef quality in Hanwoo, recent researches have focused on nutritional manipulation to increase fat deposition in intramuscular adipose tissue. The amount of lipid in adipose tissue is determined by the rate of lipid synthesis as well as the rate of lipolysis. Both processes occur simultaneously and continuously, with their relative rates determining if there is net lipid loss or accretion.

Lipogenesis in ruminants occurs primarily in adipose tissue by conversion of acetate to fatty acids (Vernon, 1981). Because the citrate cleavage pathway contributes relatively little to the synthetic pathways in ruminant adipose tissue (Hanson and Ballard, 1967), glucose or glycolytic metabolites are not considered as major precursors for lipogenesis.

Fatty acid synthesis (lipogenesis) requires a source of cytosolic acetyl CoA and NADPH, and the former can be produced from acetate, glucose, lactate and a number of amino acids. Vernon (1986) reported that significance of these precursors varied from species to species; for adult ruminants, acetate was most important whereas glucose and lactate might make more substantial contribution in the fetus, and glucose was the major precursor of fat in birds.

Smith and Crouse (1984) observed that the relative contribution of glucose to lipogenesis was greater than that of acetate in intramuscular adipose tissue. This suggests that different processes exist to regulate the

fatty acid synthesis between subcutaneous and intramuscular adipose tissues.

Although acetate is the major source of carbon for fatty acid synthesis in ruminants, a variety of other substances can also provide carbon for this purpose, including amino acids. Whitehurst et al. (1981) observed in vitro lactate conversion to fatty acids to occur at substantial rates in slices of bovine adipose tissue. Hanwoo, and other ruminants differ from monogastrics in that VFA is the major source of carbon for fatty acid synthesis with minor contributions from other precursors including glucose, lactate, propionate and β-hydroxybutyrate (Vernon, 1980). There have been no reports about utilization of carbon precursors for lipogenesis in Hanwoo.

Insulin is known to have a major role in promoting lipid accumulation, acting acutely to activate key enzymes and inhibit lipolysis, and acting chronically to increase the transcription and hence the amount of various lipogenic enzymes (Vernon, 1992). However, numerous reports have indicated that the role of insulin in regulating lipogenesis in bovine adipose tissue in vitro and in vivo is negligible (Vernon, 1977, 1980; Smith et al., 1983; Vasilatos et al., 1983; Smith and Crouse, 1984; Vernon et al., 1985). In many ways, IGF-1 is known to have similar effects on cells with insulin through their non-specific receptor bindings. However, there have been few reports which compare their effects on lipogenesis in bovine.

Catecholamine, on the other hand, is known to act acutely to enhance lipoylsis and inhibit lipogenesis. Many in vitro studies suggested that the catecholamines are important in regulation of body fat by virtue of their effect on the rate of lipolysis. Consequently, more detailed information is available about site-and sex-related variation in response to adrenergic agonist and antagonist.

Therefore, the purpose of this study was to determine the contributions of acetate and glucose, amino acids and metabolites as carbon precursors for fatty acid and glyceride synthesis in intramuscular and subcutaneous adipose tissue and the effect of insulin on lipogenic capacity and of the adrenergic agent, norepinephrine on lipolytic activity on both tissues.

MATERIALS AND METHODS

Chemicals

Medium 199 (M199), penicillin, streptomycin were from Gibco-BRL, USA. Bovine serum albumin (BSA), insulin, IGF-1, norepinephrine were purchased from Sigma, Chemical Co. USA. [U-^{14}C] acetate, [U-^{14}C] glucose, [U-^{14}C] leucine, [U-^{14}C] isoleucine [U-^{14}C] 2-ketoisocaproic acid were from Du Pont Co. and scintillation cocktail from Amersham.

Animals

Nine Hanwoo steers were fed and managed at a feeding barn in the Livestock Research Institute under a high quality beef production program. Steers were castrated at 3 months old and slaughtered at 24 months of age. Portions of the longissimus dorsi muscle, including the overlying adipose tissue (6th-7th rib), were removed as rapidly as possible after slaughter (approx. 25 minutes, postmortem) and subcutaneous, and intramuscular adipose tissues were taken from this section and kept in 0.9% NaCl sterile saline solution at 37°C until tissue preparation.

Tissue preparation

Adipose tissues were prepared, chopped and rinsed using the procedures described by Lee et al. (2000) for preadipocyte cell preparation. In the first incubation, adipose tissues from the first three cattle were used to compare the incorporation of acetate and glucose; in the second incubation, those from the second three were to compare the amino acids incorporation, and adipose tissues from both were also used to examine the effect of hormones. Adipose tissues from the third trio were used for lipogenesis and lipolysis experiments.

Pieces of intramuscular adipose tissue were dissected from longissimus dorsi muscle. Care was taken not to contaminate with muscular tissue and to not include intermuscular or subcutaneous adipose tissue depots connected to them. Pieces of intramuscular adipose tissue or the overlying subcutaneous adipose tissue weighing 100 mg were cut with scissors, placed in a petri-dish, rinsed and preincubated for about 20 minutes in M199 containing insulin (1.6 μg/ml) and antibiotics, and buffered with 25 mM Hepes, pH 7.3. Flasks contained 2.5 ml of culture medium, M199 containing 5 mM D-glucose, 2.0 mM acetate and 1.6 μg/ml insulin.

Carbon precursor incorporation into adipose tissue

M199 conditioned as above was added to 1 μCi of [U-^{14}C] acetate or of [U-^{14}C] glucose in the first incubation with adipose tissue from three cattle, and either [U-^{14}C] leucine, [U-^{14}C] isoleucine or [U-^{14}C] 2-ketoisocaproic acid in the second incubation with adipose tissue from the other three cattle. The pieces of adipose tissue were incubated in M199 for 3 hours at 37°C in a shaking water bath. After 3-hour incubation, reactions were terminated by transferring the tissue to a scintillation cocktail and radioactivity was measured by scintillation counter.

Lipogenesis

M199 conditioned as above was added to 1 μCi of [U-^{14}C] acetate or of [U-^{14}C] glucose. Pieces of

adipose tissue prepared as above were incubated in M199 for 3 hours at 37°C in a shaking water bath. After 3-hour incubation, reactions were terminated by transferring the tissue to lipid extraction solution (Chloroform:Methanol=1:1), and triglycerides in adipose tissues were extracted as described in Folch et al. (1957). Glyceride fatty acids were separated from glyceride-glycerol by the saponification procedure of Hood et al. (1972).

After fractionation, scintillation cocktail was added to each fraction and radioactivity was measured by scintillation counter.

Lipolysis

Pieces of adipose tissues prepared as above were rinsed and transferred to flasks containing 2.5 ml of Krebs-Ringer solution containing 3% fatty acid free BSA, 5 mM D-glucose, 2.5 mM acetate and 1.6 μg/ml insulin or 100 nM norepinephrine. After 3 h incubation, reactions were terminated by transferring 1 ml of lipolysis medium to tubes containing 65% $HClO_4$ and then the contents in tubes were centrifuged at 2,500 rpm for 15 min, and the supernatants were neutralized with 5M KOH and saturated $KHCO_3$. After centrifuging at 2,500 rpm for 15 min, supernatant was stored in -20°C freezer until glycerol assay.

Statistical analysis

All data were analysed by analysis of variance. The differences of means between treatments were compared by Duncan's multiple range test, using General Linear Model (GLM) procedures of SAS package (1989).

RESULTS AND DISCUSSION

The rate of incorporation was greater ($p<0.05$) from acetate than glucose in both subcutaneous and intramuscular adipose tissue, and the rate of incorporation of carbon precursors was greater in subcutaneous than in intramuscular adipose tissues, as shown in table 1. There was a significant ($p<0.05$) site effect in acetate incorporation, which means that subcutaneous adipose tissue used acetate more efficiently than glucose.

As shown in table 2, the incorporation rate of labelled amino acid varied with precursors. Site effect was significant ($p<0.05$) only in isoleucine. In subcutaneous adipose tissue, the rate was highest ($p<0.05$) with leucine, followed by isoleucine and α-ketoisocaproic acid, between which there was no difference. Also in intramuscular tissue, leucine was significantly highest ($p<0.05$), and the rate of incorporation decreased in the same order. The rates of carbon precursor incorporation appeared to be

higher in subcutaneous than in intramuscular tissue.

Table 1. Incorporation of radioisotope labelled acetate and glucose into adipose tissues of Hanwoo ($nmol/3h/10^6$ cells)

Sites	[U-^{14}C] isotopes	
	Acetate	Glucose
Subcutaneous	2423.3[a]	1502.0[b]
Intramuscular	1703.7	1381.0
Site effect	*	NS

[a,b] Means in the same row with different superscripts significantly differ ($p<0.05$).
* Significant ($p<0.05$), NS=not significant.

Table 2. Incorporation of radioisotope labelled amino acids and KIC into adipose tissues of Hanwoo ($nmol/3h/10^6$ cells)

Sites	[U-^{14}C] isotopes		
	Leucine	Isoleucine	α-Ketoisocaproic acid
Subcutaneous	190.0[a]	66.5[b]	42.0[b]
Intramuscular	136.5[a]	41.6[b]	46.1[b]
Site effect	NS	*	NS

[a,b] Means in the same row with different superscripts significantly differ ($p<0.05$).
* Significant ($p<0.05$), NS=not significant.

The relative rates of utilization of amino acid carbon for fatty acid synthesis by adipose tissue from Hanwoo were in accordance with previous studies (Rosenthal et al., 1974). Feller (1965) also found that the rate of fatty acid synthesis from amino acids in rat adipose tissue decreased in the order leucine, alanine, isoleucine, valine.

Adipose tissue is thought to be an important site of amino acid metabolism in the rat, particularly with respect to the degradation of branch-chain amino acids (Rosenthal et al., 1974; Snell and Duff, 1977; Tischler and Goldberg, 1980). The metabolite of leucine has been studied in greatest detail and one of the major products is fatty acid (Meikle and Klain, 1972; Rosenthal et al., 1974; Goodman, 1977). The role of adipose tissue in amino acid metabolism in ruminants is not clear. Roberson et al. (1982) and Ballard et al. (1969) reported that sheep adipose tissue differs from rat adipose tissue in that it has relatively low activities of pyruvate dehydrogenase and ATP-citrate lyase; low ATP-citrate lyase activity restricts the use of leucine and isoleucine as well as glucose and alanine. Thus the low ATP-citrate lyase activity of adipose tissue from ruminants is probably a major reason for the low rate of fatty acid synthesis from amino acids in the

tissue.

The total contribution of amino acid carbon to fatty acid synthesis in bovine adipose tissue has not been assessed whereas adipose tissue is thought to be an important site of amino acid metabolism in the rat, particularly with respect to the degradation of branch-chain amino acids (Rosenthal et al., 1974; Snell and Duff, 1977; Tischler and Goldberg, 1980), but the present study suggests that the three amino acids chosen make a significant contribution of that of glucose carbon.

The metabolism of leucine has been studied in greatest detail and one of the major products is fatty acid (Meikle and Klain, 1972; Rosenthal et al., 1974; Goodman, 1977). Wijayasinghe et al. (1984) have also shown that fatty acid synthesis from leucine is very low in sheep adipose tissue. Thus even if adipose tissue is found to be an important site of amino acid metabolism in sheep, it appears unlikely that their metabolism will make a significant contribution to fatty acid synthesis.

It also appears that fatty acids are a minor product of amino acid metabolism. In addition, the study provides further evidence for a key role of ATP-citrate lyase in restricting the use of acetyl CoA generated in the mitochondria for fatty acid

Incorporation of radioisotope labelled acetate and glucose in intramuscular adipose tissue preincubated for 48 h with insulin and IGF-1 is shown in table 3. There were highly significant (p<0.01) substrate effects which means that the incorporation rate was remarkably higher for acetate than glucose, regardless of hormone treatments. Insulin was most effective to stimulate the incorporation of precursors but there was no difference between insulin and IGF-1 in glucose incorporation.

Table 3. Incorporation of radio-labelled acetate and glucose in intramuscular adipose tissue of Hanwoo preincubated for 48 h with hormones (nmol/3h/10^6 cells)

Substrates	Treatments		
	Control	Insulin	IGF-1
Acetate	1788.3[c]	3956.7[a]	3190.9[b]
Glucose	1095.3[a]	1533.3[b]	1403.7[b]
Substrate effects	**	**	**

[a,b,c] Means in the same row with different superscripts significantly differ (p<0.05).
** Significant (p<0.01), NS=not significant.

The potency of IGF-1 seemed to be slightly lower than for insulin. The finding that IGF-1 was less potent than insulin in stimulating lipogenesis agrees with previous studies conducted with rat adipocytes (Zapf et al., 1979, 1981).

This suggests that *in vivo* it would be expected that the somatomedin, IGF-1, would exhibit insulin-like effects on adipose tissue metabolism. But Zapf et al. (1979, 1984) reported that the ability of free IGF-1 to stimulate lipogenesis *in vitro* is abolished when the carrier protein for IGF-1 is added to the incubation based on studies with rats. It has been shown that while the free IGF-1 has noted insulin-like effects in vitro on adipose tissue metabolism the fact that essentially all the IGF-1 in the circulation is bound to the carrier protein in rats and humans (Zapf et al., 1984) suggests that in normal situations the circulating IGF-1 carrier protein complex would not have any appreciable insulin-like effects on bovine adipose tissue metabolism.

The rate of lipogenesis from acetate and glucose in subcutaneous and intramuscular adipose tissue of Hanwoo steers is given in table 4. Incorporation into glyceride-fatty acids was significantly (p<0.05) greater from acetate than glucose in both subcutaneous and intramuscular adipose tissue, and that into glyceride-glycerol was greater from glucose than acetate in both adipose tissues. The rates of lipogenesis from both precursors were slightly greater in subcutaneous than intramuscular adipose tissue.

Table 4. Lipogenesis from acetate and glucose in Hanwoo adipose tissues (nmol/3h/10^6 cells)

Glyceride fractions	Sites	Substrates	
		Acetate	Glucose
Fatty acid[1]	Subcutaneous	2344.5[a]	112.0[b]
	Intramuscular	1703.7[a]	33.0[b]
Glycerol	Subcutaneous	18.6[b]	192.6[a]
	Intramuscular	17.8[b]	145.8[a]

[a,b] Means in the same row with different superscripts significantly differ (p<0.05).

The rates of utilization of glucose and acetate carbon for fatty acid synthesis by adipose tissue from Hanwoo are in accord with previous studies with sheep and rat (Ballard et al., 1969; Vernon, 1980).

As shown in other reports, acetate provided 70-80% of the acetyl units to fatty acid synthesis in subcutaneous adipose tissue in Hanwoo (Smith and Prior, 1982); acetate's contribution to lipogenesis in intramuscular adipose tissue was substantially less (table 3) than that of glucose.

The amount of acetate and glucose incorporated into the glyceride-glycerol moiety during the incubation period is shown in table 4. Some label from [U-^{14}C] acetate was recovered in glyceride-glycerol; however, this does not represent a net synthesis of glycerol-3-

phosphate from acetate, rather a net flow of tricarboxylic acid cycle intermediates to glyceride- glycerol. The incorporation of two precursors into glyceride-glycerol seemed to be greater in subcutaneous than in intramuscular adipose tissue.

Several earlier studies have demonstrated that intramuscular adipose tissue depots have smaller adipocytes (Moody and Cassen, 1968; Hood and Allen, 1973) and lower lipogenic rates (Whitehurst et al., 1981) than does subcutaneous adipose tissue from the same animals. Smith and Crouse (1984) determined the relative contributions of the three major lipogenic precursors, acetate, lactate and glucose, to fatty acid synthesis at levels approaching physiological concentrations. Robertson et al. (1981) utilizing ovine perirenal adipose tissue and more physiological concentrations of acetate, lactate and glucose (0.6, 1 and 3 mM, respectively) in combination, demonstrated that under these conditions, lactate and glucose provided only 3 and 2%, respectively, of the acetyl units to lipogenesis, the remainder coming from acetate.

Smith and Prior (1982) utilizing 10 mM acetate and lactate, and 2 mM glucose, demonstrated that in bovine subcutaneous adipose tissue slices incubated in the presence of all three substrates, 70, 30 and less than 1% of the acetyl units for lipogenesis were provided by acetate, lactate and glucose, respectively. Later, when there were somewhat more physiological concentration of substrates (5 mM), similar results were reported in the subcutaneous adipose tissue samples. However, in the intramuscular adipose tissue slices, absolute incorporation of glucose into fatty acids, and especially the relative contribution of glucose to fatty acids were shown to be greater than in subcutaneous adipose tissue, even though the incorporation of glucose into glyceride-glycerol was markedly less in intramuscular adipose tissue, relative to the subcutaneous adipose tissue. They concluded the importance of acetate as a lipogenic precursor was reduced in the intramuscular adipose tissue and the basis for this major differences between depots was unknown. Such a preference to glucose as a carbon precursor in intramuscular adipose tissue was not shown in this study.

Acetate conversion to glyceride-fatty acids in intramuscular adipose tissue was 5 to 16% of the rate observed in subcutaneous adipose tissue. Conversely, glucose carbon recovery in glyceride-glycerol was approximately equal in both tissues.

Effects of insulin and norepinephrine on glyceride-fatty acid and glyceride-glycerol synthesis in adipose tissue of Hanwoo are shown in table 5 and 6. For glyceride-fatty acid synthesis (table 5), there was significant (p<0.05) site effect in insulin treatment but

not in control and norepinephrine. Insulin was the most effective in glyceride fatty acid synthesis, while norepinephrine was the same as control.

Table 5. Effects of insulin and norepinephrine on glyceride-fatty acid synthesis in adipose tissues from Hanwoo (nmol /3h/10^6 cells)

Substrates	Sites	Treatments		
		Control	Insulin	Norepinephrine
Acetate	Subcutaneous	578.2[b]	2609.2[a]	754.0[b]
	Intramuscular	532.3[b]	1464.0[a]	581.2[b]
Glucose	Subcutaneous	13.8[b]	35.5[a]	18.7[b]
	Intramuscular	13.8[b]	31.3[a]	17.0[ab]
Site effect		NS	**	NS

[a,b] Means in the same row with different superscripts significantly differ (p<0.05).
* Significant (p<0.05), ** Significant (p<0.01).

Table 6. Effects of insulin and norepinephrine on glyceride-glycerol synthesis in adipose tissues from Hanwoo (nmol/3h/10^6 cells)

Substrates	Sites	Treatments		
		Control	Insulin	Norepinephrine
Acetate	Subcutaneous	37.7[a]	26.2[b]	23.3[b]
	Intramuscular	34.7[b]	45.2[a]	25.0[b]
Glucose	Subcutaneous	75.7[b]	143.8[ab]	257.5[a]
	Intramuscular	83.0[b]	145.0[ab]	235.8[a]

[a,b] Means in the same row with different superscripts significantly differ (p<0.05).

For glyceride-glycerol synthesis (table 6), there was no site effect caused by hormonal treatment. Insulin was the most effective in glyceride-glycerol synthesis, while norepinephrine was the same as control. Glyceride-glycerol synthesis from acetate in insulin treatment was significantly (p<0.05) lower in subcutaneous, but higher in intramuscular. At the same time, in both tissues, it was lower in norepinephrine treatment than in control. Glyceride-glycerol synthesis from glucose was highest (p<0.05) in norepinephrine treatment followed by insulin although there was no significance between insulin and control.

Insulin is generally known to be important with regard to anabolic functions; in addition to its effect on lipoprotein lipase, insulin increases glucose transport and the uptake of other metabolites, stimulates triglyceride synthesis and inhibits lipolysis. It was observed that insulin stimulated lipogenesis although an apparent maximal dose of insulin for stimulation of lipogenesis was not established from this experiment. Lipogenesis was stimulated by 1.6 μg/ml insulin and

the presence of insulin in the culture media stimulated the lipogenic activity during culture for both subcutaneous and intramuscular adipose tissues. The results for insulin are in direct contrast to those shown by norepinephrine. While norepinephrine inhibited lipogenesis and increased the lipolytic activity, insulin seemed to activate the enzymes and increase lipogenesis.

The mechanism, however, is still obscure. Various possibilities have been suggested. Insulin causes dephosphorylation of acetyl CoA carboxylase at the AMP-stimulated kinase sites, perhaps by activating phophatase-1; insulin has been reported to cause the covalent binding of a low molecular weight activator to the enzyme; insulin also causes phosphorylation of the enzyme on at least two serine residues (Hardie, 1989). Thus although it is now clear that changes in phosphorylation status play a major role in the acute control of acetyl CoA carboxylase, the detailed mechanisms of how insulin and catecholamines elicit such changes remain to be elucidated. O'Hea and Leveille (1970) also found that insulin increased lipid synthesis in adipose tissue of young pigs, although all other reports have indicated that lipogenesis was only negligibly stimulated by insulin *in vitro* (Romsos et al., 1971a, b; Steele et al., 1974; Etherton and Chung, 1981; Chung et al., 1983).

Norepinephrine, nut not insulin, affected lipolysis when added to adipose tissue incubations *in vitro* (figure 1). Rates of basal lipolysis were greater in subcutaneous adipose tissue. Basal lipolysis in intramuscular adipose tissue was approximately at the rate observed in subcutaneous adipose tissue.
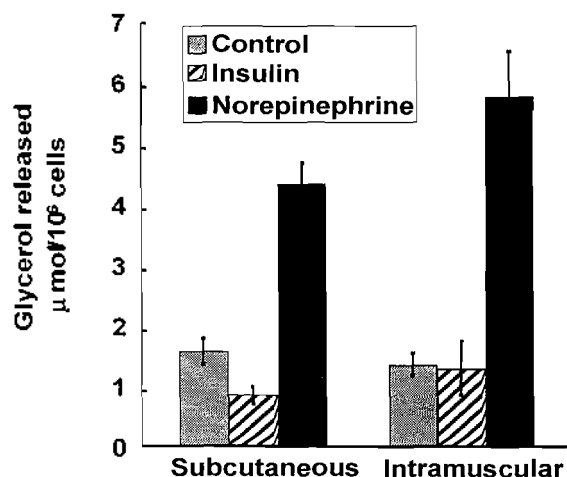
The metabolic function of adipose cells strongly depends on their anatomical position. For instance, omental adipocytes are more sensitive to lipolytic agents than are adipocytes from the gluteal-femoral depot; conversely, however, lipoprotein lipase activity is reportedly higher in the gluteal-femoral region than in abdominal adipose tissue (Arner and Ostman, 1976).

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Figure 1. Effects of insulin and norepinephrine on lipolysis in adipose tissues from Hanwoo steers

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