Utilization of Substrate for the *In vitro* Lipid Synthesis in the Adipose Tissue of Hanwoo Steers

M. K. Song*, H. J. Sohn[1], S. K. Hong[1] and H. C. Kim[1]

Department of Animal Science, Chungbuk National University, Cheong-ju, Chungbuk 361-763, Korea

ABSTRACT : An ability to utilize the substrates (acetate, glucose and lactate) in the lipid synthesis was measured in *vitro* with the adipose tissues of 4 locations (subcutaneous, SUBC; intramuscular, INTR; tail and kidney, KIDN) in 12 Hanwoo (Korean native cattle) steers (26 and 28 months of ages, mean body weight 638.6 kg). The rates of lipid synthesis from acetate were higher than those from glucose in SUBC and ITRA adipose tissues, respectively. In contrast, the rates of lipid synthesis from glucose were higher than those from acetate in the adipose tissues of tail and KIDN, respectively. Lactate utilization was lowest in all the locations while that of acetate or glucose had the different trends of utilization in the lipogenesis. The rate of lipid synthesis from acetate was highest in the SUBC adipose tissue but was lowest in the KIDN while that from glucose was also higher in the SUBC adipose tissue than in the other tissue locations. The rate of lipid synthesis from lactate, however, was highest in the tail adipose tissue among the locations. *(Asian-Aust. J. Anim. Sci. 2001. Vol 14, No. 11 :1560-1563)*

Key Words : Lipogenesis, Adipose Tissue Location, Substrate, Hanwoo Steers

INTRODUCTION

Adipose tissue has been the principle site of fatty acid synthesis in the ruminants (Ingle et al., 1972a, b). Research relating to the utilization of precursor has been one of approaching methods for the examination of the lipid synthesis in beef cattle. The major substrates as precursor in the lipid synthesis of ruminants were acetate and glucose (Bauman et al., 1970; Smith and Crouse, 1984; Miller et al., 1991). An experimental research indicated that lactate was also utilized as a substrate in the lipid synthesis with some differences in the rate of availability, depending upon the adipose tissue location (Prior and Jacobson, 1979a, b).

Smith and Crouse (1984) reported that acetate was the preferable substrate whereas the ability to use the glucose for the lipid synthesis was small in the subcutaneous (SUBC) adipose tissue. Chung et al. (2000) also reported that the amount of lipid synthesized in the adipose tissue of Hanwoo (Korean beef) bulls was higher from acetate than from glucose, and the lipid synthesis in SUBC adipose tissue was higher than in intermuscular adipose tissue. In contrast, more than a half of the glucose was used as a precursor while less than 10% of acetate was utilized for the lipid synthesis in the intramuscular (INTR) adipose tissue (Smith and Crouse, 1984). Thus, the difference may exist between adipose tissues in their ability to utilize the lipogenic substrate. Ingle et al. (1972a) also suggested that fat cannot be considered a homogenous tissue since adipose tissue from the different depots possessed markedly different rates of lipogenesis. In the other hand, L-lactate was used very effectively compared to acetate in the lipid synthesis in sheep (Prior, 1978), and even *in vitro* rate of fatty acid synthesis in bovine adipose tissue from the other studies exceeded that from acetate (Smith and Prior, 1981).

Due to the very limited data, the current in *vitro* study was conducted to examine the ability to utilize the substrates in the lipid synthesis at various locations of adipose tissues in Hanwoo steers.

METERIALS AND METHODS

Animals

Twelve Hanwoo (Korean native cattle) steers (26 and 28 months of ages, mean body weight of 638.6 kg) were used to examine the substrate utilization *in vitro* in the lipid synthesis. Four steers were fed ad *libitum* through the experimental period, and 8 steers were fed the concentrates at the level of 1.2 to 1.5% during growing period (6 to 12 months of ages), 1.7 to 1.8% during fattening I period (13 to 18 months of ages) of body weight. Thereafter, the steers were fed the concentrates *ad libitum* up to 26 or 28 months old (fattening II period). The steers were fed rice straw as roughage ad *libitum*. Chemical compositions of concentrate for each growing stage were in table 1.

Preparation of adipose tissues

Approximate 10 to 20 g of SUBC, tail and kidney (KIDN) fat were taken, and about 50 g of *Longissimus* muscle between 13 and 14 ribs of Hanwoo steers were taken immediately after being slaughtered, and were carried to the laboratory in a 37°C M199 media solution in 5 min.

* Address reprint request to Man K. Song. Tel: +82-43-261-2545, E-mail: mksong@cbucc.chungbuk.ac.kr

[1] National Livestock Research Institute, Rural Development Administration.

Received July 24, 2001; Accepted August 13, 2001

Table 1. Chemical compositions of experimental concentrates for Hanwoo steers by feeding stage (%, DM basis)

Chemical composition	Concentrates by feeding stage [1]		
	Growing	Fattening I	Fattening II
Dry matter	88.2	87.3	87.6
Crude protein	15.1	12.0	11.5
Ether extracts	3.1	2.6	2.9
Ca	1.0	0.7	0.6
P	0.5	0.4	0.4
TDN	68.8	71.4	72.5

[1] Growing period, 6 to 12; Fattening I, 13 to 18 and Fattening II, 19 to 28 months of ages, respectively.

Adipose tissue from ITRA-fat was carefully separated from the *Longissimus* muscle in a beaker containing M199 media solution. All the adipose tissues were sliced to the particles of 20 to 30 mg.

Adipose tissue incubation and measurement of lipogenesis

Twenty ml incubation vials containing 3 ml KRB buffer (0.118 M NaCl + 4.77 M KCl, 1.256 mM $CaCl_2 \cdot 2H_2O$ + 1.232 mM KH_2PO_4 + 1.232 mM $MgSO_4 \cdot 7H_2O$ + 24.79 mM $NaHCO_3$, pH 7.4) were added 25 mM HEPES, 3% bovine serum albumin, insulin (10 ng/lm buffer) and 5mM of each substrate (acetate, glucose or lactate). The KRB buffer was added each 0.5*u*Ci U-^{14}C- substrate, then was flushed with the gas (95% O_2 and 5% CO_2). The sliced adipose tissue was placed in the incubation vial and was incubated in 37°C shaking water bath for 2 h. The incubation was terminated by placing the vials on ice. The incubated adipose tissue was weighed after 30 min. and was placed in a new 20 ml vial. Fat in a vial was extracted from the adipose tissue in 5 ml Dole's solution (isopropanol 40 : n-heptane 10 : 1N H_2SO_4 1, v/v; Dole, 1956) in a ultrasonic water bath for 30 min. After the vial was added 3 ml hexane and 3 ml H_2O the upper (hexane) layer containing fat was transferred to a scintillation vial and diluted with 1.5 ml hexane. The hexane in the vial was evaporated in a dry-bath. The vial was added scintillation cocktail and the specific radioactivity of ^{14}C-fat was measured by liquid scintillation counter (β-Counter). The unit of the lipogenesis was expressed as pM substrate / mg tissue / 2 h.

Statistics

The results obtained were subjected to least squares analysis of variance according to the general linear models procedure of SAS (1985). Since effects of age and body weight were not observed in the rate of lipid synthesis among substrates or adipose tissue locations, S-N-K Multiple Range Test (Steel and Torrie, 1980) was made only for the significant differences among mean values of substrates or tissue locations of all the steers.

RESULTS

Comparison in the rate of lipid synthesis in *vitro* among substrates for various locations of adipose tissues is shown in table 2. Lactate availability was lowest ($p<0.0011$) in all the locations while that of acetate or glucose had the different trend of utilization in the lipogenesis. The rates of lipid synthesis from acetate were slightly and significantly ($p<0.0001$) higher than those from glucose in SUBC and ITRA adipose tissues, respectively. In contrast, the rates of lipid synthesis from glucose were slightly and significantly ($p<0.0001$) higher than those from acetate in the adipose tissues of tail and KIDN, respectively.

The rate of lipid synthesis from acetate was highest in the SUBC adipose tissue but was lowest in the KIDN among the four locations ($p<0.0001$) while the difference was small between the adipose tissues of ITRA and tail (table 2). The rate of lipid synthesis from glucose was also higher in the SUBC adipose tissue than in the other

Table 2. The in *vitro* rate of lipid synthesis in the adipose tissues of Hanwoo steers by locations and by substrate (pm/mg/2 h)

Location of adipose tissue	Substrates [1]			SEM [2]	Pr>F [3]
	Acetate	Gucose	Lactate		
Subcutaneous	323.1^{aA}	257.7^{aA}	50.2^{bB}	26.31	0.0001
Intramuscular	154.6^{aB}	121.5^{bB}	45.3^{cB}	12.40	0.0001
Tail	168.1^{aB}	173.7^{aB}	65.9^{bA}	13.07	0.0001
Kidney	85.3^{bC}	135.6^{aB}	42.9^{cB}	9.606	0.0001
SEM [2]	21.37	18.85	4.395	-	-
Pr>F [3]	0.0001	0.0001	0.0011	-	-

[1] Means in the same column with different superscripts (capital letters) or same row with different superscripts (small letters) differ as shown in Pr>F values.

[2] Standard error of the means.

[3] Probability levels.

Table 3. Comparison among substrates in the *in vitro* lipid synthesis of Hanwoo steers (pm/mg/2 h)

Substrates			SEM[2]	Pr>F[3]
Acetate	Glucose	Lactate		
182.8[a]	172.1[a]	51.1[b]	10.05	0.0001

[1] Means in same row with different superscripts differ as shown in Pr>F values.
[2] Standard error of the means.
[3] Probability levels.

Table 4. Comparison among locations of adipose tissue in the *in vitro* lipid synthesis of Hanwoo steers (pm/mg/2 h)

Locations of adipose tissue				SEM[2]	Pr>F[3]
Subcutaneous	Intramuscular	Tail	Kidney		
210.4[a]	107.2[bc]	135.9[b]	87.9[c]	12.13	0.0001

[1] Means in same row with different superscripts differ as shown in Pr>F values.
[2] Standard error of the means
[3] Probability levels.

locations which had similar rates ($p<0.0001$). The rate of lipid synthesis from lactate, however, was highest in the tail adipose tissue among the locations ($p<0.0011$).

The comparison in the pooled rate of lipid synthesis from the four locations of adipose tissues indicated no difference between acetate and glucose with the lowest rate ($p<0.0001$) from lactate (table 3). The pooled rate of lipid synthesis from 3 substrates showed the highest value in the SUBC but lowest in KIDN ($p<0.0001$) whereas no difference between adipose tissues in ITRA and tail was observed (table 4).

DISCUSSION

An acetate has been the predominant precursor for the lipid synthesis in ruminant adipose tissue from both and SUBC fat depots (Ingle et al., 1972b; Smith and Crouse, 1984; Miller et al., 1991). Smith and Crouse (1984) reported that more than 80% of the acetate added to the medium was used as a substrate whereas 10 to 25% of glucose was utilized for the lipid synthesis in the subcutaneous (SUBC) adipose tissue. But the ability to use the carbon substrate may differ depending on the location of adipose tissue since 50 to 75% of the glucose was used while less than 10% of acetate was utilized for the lipid synthesis in the ITRA adipose tissue (Smith and Crouse, 1984).

In the current *in vitro* experiment, lipid synthesis from acetate was higher than that from glucose in both SUBC and ITRA adipose tissues (table 2), and the pooled data from the 4 adipose tissue locations also indicate the slightly increased utilization of acetate compared to that of glucose (table 3). Glucose, however, was slightly more utilized than acetate in the lipid synthesis of tail and KIDN adipose tissues (table 2). The rate of lipid synthesis from lactate was lowest among 3 substrates in all locations of adipose tissues (tables 2 and 3). The present result in the utilization of lactate was far below from those of the previous reports (Prior, 1978; Smith and Prior, 1981) where lactate was used up to 70% as effectively as acetate in the lipid synthesis in sheep, and the *in vitro* rate of fatty acid synthesis from lactate in bovine adipose tissue was even higher that from acetate. Thus, the difference should exist between adipose tissues in their ability to utilize the lipogenic substrate in the current *in vitro* study with Hanwoo. Meanwhile, information relating to the comparison in the rate of lipid synthesis among the adipose tissue locations is limited. Chung et al. (2000) reported that the amount of lipid synthesized in the adipose tissue of Hanwoo bulls was higher in SUBC adipose tissue than in intermuscular adipose tissue. Pooled data from the 3 substrates also showed the highest lipid synthesis in the SUBC adipose tissue and followed by the order of tail, ITRA and KIDN adipose tissues (table 4).

In general, beef cattle in late fattening stage are fed very high level of concentrates compared to those at growing stage, thus similar amount of propionate, lactate and acetate are possible to provide as substrate in the lipid synthesis. The rate of utilization of acetate, however, was higher than those of glucose and lactate in the SUBC and ITRA adipose tissue of Hanwoo while opposite trend was observed in tail and KIDN tissues although the difference between acetate and glucose was small. These results may indicate that there was the substrate preference for each location of adipose tissue in the lipid synthesis in Hanwoo steers.

Based on the in vitro study with adipose tissue of Hanwoo steers, it is concluded that the rate of substrate utilization in the lipid synthesis is dependent upon the location of adipose tissue with the highest preference of acetate or glucose in the adipose tissues being SUBC and ITRA or tail and KIDN, respectively. Lactate was the minor contributor in all adipose tissue locations.

REFERENCES

Bauman, D. E., R. E. Brown and C. L. Davis. 1970. Pathways of fatty acid synthesis and reducing equivalent generation in mammary gland of rat, sow and cow. Arch. Biochem. Biophys. 140:237-244.

Chung, C. S., N. S. Kim, M. K. Song, Y. I. Choi, Y. S. Won and J. G. Kim. 2000. Effects of age and feeding level of concentrates on adipose tissue lipogenesis and adipocyte size in Hanwoo bulls. J. Anim. Sci. & Technol. (Kor.) 42:459-466.

Ingle, D. L., D. E. Bauman and U. S. Garrigus. 1972a. Lipogenesis in the ruminants : in *vitro* site of fatty acid synthesis in sheep. J.

Nutr. 102:617-624.

Ingle, D. L., D. E. Bauman and U. S. Garrigus. 1972 b. Lipogenesis in the ruminants : in *vitro* study of tissue site, carbon source and reducing equivalent generation for fatty acid synthesis. J. Nutr. 102:609-616.

Miller, M. F., H. R. Cross, D. K. Lunt and S. B. Smith. 1991. Lipogenesis in acute and 48-hour cultures of bovine intramuscular and subcutaneous adipose tissue explants. J. Anim. Sci. 69:162-170.

Prior, R. L. 1978. Effect of level of feed intake on lactate and acetate metabolism and lipogenesis *in vivo* in sheep. J. Nutr. 108:926-935.

Prior, R. L. and J. J. Jacobson. 1979 a. Lactate, pyruvate and acetate interactions during *in vitro* lipogenesis in bovine adipose tissue. J. Anim. Sci. 49:1410-1417.

Prior, R. L. and J. J. Jacobson. 1979 b. Effects of fasting and refeeding and intravenous glucose infusion on in *vitro* lipogenesis in bovine adipose tissue. J. Nutr. 109:1279-1284.

SAS. 1985. SAS User's Guide. Statistical Analysis Systems Institute, Inc., Cary, NC.

Smith, S. B. and R. L. Prior. 1981. Evedence for a functional ATP-citrate lyase: NADP-malic dehydrogenase pathway in ruminant adipose tissue, enzyme and metabolite levels. Arch. Biochem. Biophys. 202:192-201.

Smith, S. B. and J. D. Crouse. 1984. Relative contributions of acetate, lactate and glucose to lipogenesis in bovine intramuscular and subcutaneous adipose tissue. J. Nutr. 14:792-800.

Steel, R. G. D. and J. H. Torrie. 1980. Principles and Procedures of Statistics. McGraw Hill Book Co., NY.