Substrate utilization and dose response to insulin by subcutaneous adipose tissue of Angus steers fed corn- or hay-based diets

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ABSTRACT: We hypothesized that, at a common age endpoint, adipose tissue from corn-fed steers would be less sensitive to insulin than adipose tissue from hay-fed steers. Angus steers were assigned to either a corn-based diet (n = 6) or hay-based diet (n = 6) and fed to common days on feed. Steers fed the corn-based diet had 2.44 cm of fat thickness over the 12th thoracic rib, whereas hay-fed steers had 1.04 cm of fat thickness. At slaughter, subcutaneous adipose samples were collected and portions of subcutaneous adipose tissue were incubated with [U-14C]acetate to quantify fatty acid synthesis or with [U- 14C]glucose to assess glucose utilization in the presence of 0, 100, or 500 ng/mL of insulin. Additional subcutaneous samples were used to evaluate glycolytic intermediate concentrations as indicators of glycolytic flux. Data were analyzed as a split-plot with diet in the main plot and insulin concentration and its interaction with diet in the sub-plot. Within diet, linear and quadratic contrasts of insulin concentration were tested. Diet had no effect (P ≥ 0.31) on glucose metabolism or acetate carbon incorporation into total lipids (P = 0.32). Insulin had no effect (P > 0.21) on glucose conversion to CO2, lactate, or total lipids, nor did it affect (P = 0.28) acetate conversion to total lipids. No diet × insulin interaction (P > 0.36) was observed for any measure of subcutaneous metabolism in vitro. Steers fed the corn-based diet exhibited neither a linear (P > 0.22) nor a quadratic (P > 0.24) effect to increasing insulin concentration. However, when steers were fed the hay-based diet, there was a positive linear (P = 0.06) effect for glucose oxidation. These results suggest that subcutaneous adipose tissue may become resistant to stimulation by insulin in steers fed to a fat thickness above the average feedlot steer, but this is independent of diet.

Key words: acetate, adipose tissue, cattle, glucose, insulin

INTRODUCTION

Evidence exists to support the importance of insulin in regulating ruminant adipose tissue metabolism (Yang and Baldwin, 1973; Etherton and Evock, 1986; Rhoades et al., 2007). However, other reports have indicated that fatty acid synthesis from glucose and acetate in bovine subcutaneous (s.c.) adipose tissue is not affected by insulin in vitro (Prior and Smith, 1982; Vernon et al., 1985; Miller et al., 1991). Collectively, the effect of insulin on s.c. adipose tissue metabolism is inconclusive. Diet composition (Scott and Prior, 1980) and total energy intake (Prior, 1979) have been shown to alter activities of several lipogenic enzymes. Propionate is both gluconeogenic and insulinogenic in ruminants (Sano et al., 1995), whereas ketone body accumulation (Tardif et al., 2001) and diet (Waterman et al., 2006) may also affect insulin sensitivity. We previously reported that intramuscular (i.m.) tissue is more sensitive to insulin than s.c. tissue (Gilbert et al., 2003), and recently demonstrated greater insulin sensitivity in i.m. than in s.c. adipose tissue of corn-fed steers, with no response to insulin in adipose tissues of hay-fed steers (Rhoades et al., 2007). This may have been caused by the greater age of the hay-fed steers at sampling (28 mo for hay-fed vs. 24 mo for corn-fed steers; Rhoades et al., 2007). McCann and Reimers (1985) demonstrated that whole-body insulin resistance was overcome when a maximum dose of insulin was administered to obese heifers. We hypothesized that the variation in adipose tissue insulin sensitivity generated by dietary energy source in our previous experiment may have been related to an interaction between diet and age of steers at sampling. Therefore, the objective of this study was to evaluate effects of dietary energy source on the dose response to increasing concentrations of insulin in bovine s.c. adipose tissue metabolism in vitro, from steers sampled at a common age endpoint.
MATERIALS AND METHODS

Animals from which tissues were collected for this experiment were handled according to guidelines established by the Institutional Animal Care and Use Committee at Texas A&M University.

Animals and Management

Twelve Angus steers were purchased as calves at weaning (approximately 8 mo of age). Coastal Bermudagrass hay containing 9.5% CP was fed for ad libitum intake for 8 d after the steers were delivered to the Texas AgriLife Research-McGregor Center. Six steers were assigned randomly to receive a high-energy, corn-based diet containing (as-fed basis) 48% ground corn, 20% ground milo, 15% cottonseed hulls, 6.5% molasses, 6% cottonseed meal, 3% limestone, trace mineral salt (NaCl, 98%; Zn, 0.35%; Mn, 0.28%; Fe, 0.175%; Cu, 0.35%; and I, 0.007%), vitamin premix (vitamin A, 2.2 × 10^6 IU/kg; vitamin D, 1.1 × 10^6 IU/kg; and vitamin E, 2.2 × 10^6 IU/kg), and a monensin premix (Elanco Animal Health, Greenfield, IN) to provide 25 mg of monensin/kg of feed (corn-fed). Feeding this diet resulted in an ADG of 1.34 kg/d. The remaining 6 steers were offered coastal Bermudagrass hay for ad libitum intake and supplemented daily with NPN in a cooked molasses carrier to sustain ADG of 0.80 kg/d (hay-fed). All steers were fed for a common time on feed (228 d).

Sample Collection

At the end of the feeding period, steers from each group were slaughtered at the Rosenthal Meat Science and Technology Center, Texas A&M University. At slaughter, blood samples were taken from a jugular vein and collected into a 10-mL heparinized Vacutainer tube (Becton, Dickinson and Company, Franklin Lakes, NJ). Blood tubes were immediately chilled in a cooled container at 7°C. Plasma was harvested from chilled blood tubes by centrifugation at 2,000 × g at 4°C for 20 min. Plasma was stored at −10°C until analysis. A section of s.c. adipose tissue between the fifth and eighth thoracic ribs was removed immediately after hide removal (approximately 20 min postmortem). The s.c. adipose tissue was immediately placed into oxygenated Krebs-Henseleit bicarbonate (KHB) buffer (pH = 7.4; 37°C) with 5 mM glucose and transported to the laboratory. Another 5-g sample of s.c. adipose tissue was excised immediately and immersed in liquid nitrogen for analysis of glycolytic intermediate concentrations. Fresh samples (50 to 100 mg) of adipose tissue were excised and used immediately for measurement of glucose metabolism in vitro, and an additional 50- to 100-mg sample of fresh s.c. adipose tissue was used for measurement of acetate carbon incorporation into total lipids in vitro.

Source of Chemicals

All chemicals and biochemicals were purchased from Fisher Scientific (Pittsburgh, PA) or Sigma-Aldrich Chemical Co. (St. Louis, MO). The [U-14C]glucose and [1-14C]acetate were purchased from Amersham (Arlington Heights, IL).

Substrate Concentrations

Plasma glucose concentrations were determined from blood samples collected at slaughter. Commercial kits (Glucose LIQUI-UV with Hexokinase, Stanbio Laboratory, Boerne, TX) were used for analysis of plasma glucose using 200 µL of provided reagent and 8 µL of sample for reaction, and change in absorbance was measured using a KC4 v3.3 Synergy HT Multi-Detection Microplate Reader (Bio-Tek Instruments Inc., Winooski, VT) at 340 nm as described by Vasconcelos et al. (2009). Glucose, glucose-6-phosphate ([G-6-P]), and fructose-6-phosphate ([F-6-P]) were analyzed from s.c. sample extracts from each animal using assay systems described by Bergmeyer (1974), with modifications as described by Rhoades et al. (2005). Briefly, a buffer system containing 0.9 mM NADP⁺ and 1 mM ATP was added to each cuvette (total volume = 1.0 mL) along with 0.5 mL of s.c. extract. Glucose-6-phosphate dehydrogenase was added to each cuvette catalyzing G-6-P to 6-phosphogluconate, and the change in absorbance was measured using a Beckman DU-7400 Spectrophotometer (Beckman Coulter, Palo Alto, CA) set at 339 nm. In the same cuvette, glucose was converted to G-6-P by the addition of hexokinase, and the change in absorbance was measured at 339 nm. Subsequently, phosphoglucone isomerase was added to each cuvette to convert F-6-P to G-6-P, and the change in absorbance was measured at 339 nm.

Glucose Metabolism In Vitro

Glucose metabolism in vitro was measured using fresh s.c. adipose tissue from each animal as described by Espinal et al. (1983). Briefly, 50 to 100 mg of adipose tissue was incubated in 3 mL of 10 mM glucose, 10 mM sodium acetate, KHB and 20 mM HEPES buffer, and 1 µCi [U-14C]glucose (5 µCi/mmol). Bovine insulin (27 USP units/mg) from bovine pancreas was added to these flasks at 0, 100, or 500 ng/mL (i.e., pharmacological dose added) and replicated twice within each animal at each concentration of insulin addition. Flasks were gassed for 1 min with 95% O₂:5% CO₂, capped with hanging center wells containing fluted filter paper, and incubated in a shaking water bath for 2 h at 37°C. Addition of 0.5 mL of 1 M H₂SO₄ to incubation media stopped all reactions. Determination of [U-14C]glucose carbon conversion to CO₂ trapped (in fluted filter paper saturated with 2 M NaOH in the hanging center wells) was performed according to Smith (1983), and...
the glucose carbon from incubation media was recovered as lactate and determined according to Smith and Freeland (1981). This method of lactate recovery also traps pyruvate and other carboxylic acids. The total lipids synthesized from glucose were extracted from the adipose tissue samples and separated into the glyceride-fatty acids and glyceride-glycerol fractions by saponification methods described by Hood et al. (1972). All measures of radioactivity were counted using a Beckman liquid scintillation spectrometer (Beckman Coulter).

Lipogenesis In Vitro

Total lipid synthesis from acetate was measured in fresh s.c. adipose tissue from each animal according to Page et al. (1997). Briefly, 50 to 100 mg of adipose tissue was incubated in 3 mL of 10 mM sodium acetate, 10 mM glucose, KHB, 20 mM HEPES buffer (pH 7.40; 37°C), and 1 µCi [1-14C]acetate (57 µCi/mmol) for 2 h. Bovine insulin (0, 100, or 500 ng/mL) was added to these flasks and replicated twice within animal at each concentration of insulin addition. Addition of 3 mL of 5% trichloroacetic acid to incubation media stopped all reactions. Measurement of [1-14C]acetate incorporation into fatty acids was conducted as described by Page et al. (1997). Radioactivity was counted using a Beckman liquid scintillation spectrometer (Beckman Coulter).

Cellularity

Adipocyte number per gram of tissue was determined by the method of Etherton et al. (1977) as modified by Smith et al. (1996). Adipose tissue samples were sliced into 1-mm-thick sections, placed into 20-mL scintillation vials, and then processed as described by Smith et al. (1996). The fixed cells (50 cells per sample) were sized using a microscope fitted with an ocular micrometer and recorded for further calculations. Mean cell diameter was calculated as the average diameter of counted and recorded cells from each sample. The mean cell volume was calculated based on mean cell size. The number of adipose cells per gram of tissue was then calculated based on mean cell volume. Calculations involving glucose and acetate utilization measures were expressed on a per-cell basis.

Data Analysis

Data were analyzed as a split-plot. Diet served as the whole-plot effect and was tested using animal nested within diet as the error term. For response variables related to substrate concentrations (plasma glucose, tissue glucose, G-6-P, F-6-P), diet served as the whole-plot effect and was tested using animal nested within diet as the error term because these samples represent the whole-plot experimental unit. For response variables related to glucose utilization (glucose conversion into CO2, lactate, and fatty acids) and acetate utilization (acetate incorporation into fatty acids), insulin concentration (0, 100, or 500 ng/mL) and diet x insulin were the subplot effects and were tested with residual mean square as the error term. When overall F-tests were significant, means were separated using Fisher’s protected LSD. To characterize the nature of the insulin dose response among diets, preplanned linear and quadratic contrasts of insulin concentration were tested and coefficients were adjusted for unequal spacing using IML procedures (SAS Institute, Cary, NC) to generate appropriate contrast coefficients. All analyses were performed using the mixed linear models procedures of SAS.

RESULTS

Carcass Traits and s.c. Adipose Tissue Cellularity

Steers used in this study were fed a different amount and source of dietary energy throughout the entire finishing period and were slaughtered at common time on feed. Therefore, by design, large differences in carcass characteristics were generated due to treatment (Table 1). Corn-fed steers had greater HCW (P < 0.01), and carcass-adjusted fat thickness and marbling scores were 123 and 48% greater, respectively, for corn-fed steers than for hay-fed steers at slaughter (P < 0.01). Differences in HCW were not sufficient to overcome the greater adiposity of corn-fed steers; therefore, numerical yield grade was greater (P < 0.01) in corn-fed than in hay-fed steers.

Adipocytes per gram of s.c. adipose tissue were similar (Table 1; P = 0.52) between dietary treatment groups. Additionally, diet did not affect (P = 0.53) mean s.c. adipocyte diameter or volume.

Glucose and Metabolites

A numerical difference in plasma glucose concentration was observed between dietary treatment groups, but differences were not significant (Table 1; P = 0.17). Similarly, there appeared to be a numerical difference in tissue glucose concentrations, but concentrations were not significantly different (P = 0.27) between corn- and hay-fed steers and were less than 20% of plasma concentrations. Likewise, concentrations of G-6-P and F-6-P were not significantly different (P > 0.31) in s.c. adipose tissue from steers fed corn- or hay-based diets. The lack of statistical significance among all glucose and metabolite measurements is likely due to the large variation among samples.

Substrate Utilization in Response to Insulin

Glucose conversion to lactate (per 100 mg of tissue) was not influenced by dietary energy source (Figure 1; P = 0.24), insulin (P = 0.47), or the interaction between diet and insulin (P = 0.35). Additionally, there were no linear (P = 0.82) or quadratic (P = 0.50) ef-
effects of additional insulin on lactate production when steers were fed hay. There was no effect of insulin ($P = 0.12$) on lactate production in steers fed the corn-based diet.

Adipose tissue from steers fed the corn- or hay-based diets did not differ in the incorporation of acetate into fatty acids (Figure 1; $P > 0.11$). The rate of acetate incorporation into fatty acids was not influenced by insulin ($P = 0.20$) addition to culture media, nor was there a significant interaction between diet and insulin concentration ($P = 0.23$). However, there was a tendency ($P = 0.09$) for a quadratic effect of supplemental insulin on acetate incorporation into fatty acids in steers fed the hay diet. For steers fed corn, there were no linear ($P = 0.27$) or quadratic ($P = 0.76$) effects of supplemental insulin on acetate incorporation into fatty acids.

Subcutaneous adipose tissue from steers fed either diet converted similar amounts of glucose to CO$_2$ per 100 mg of adipose tissue (Figure 2; $P = 0.11$). The rate of glucose conversion to CO$_2$ was not influenced by insulin ($P = 0.19$), nor was there an interaction between diet and insulin ($P = 0.48$). However, there was a positive, linear (Figure 2; $P = 0.05$) effect of increasing insulin concentrations on CO$_2$ production when steers were fed hay but neither a linear ($P = 0.56$) nor a quadratic ($P = 0.65$) effect of supplemental insulin on CO$_2$ production when steers were fed corn.

Incorporation of glucose into glyceride-fatty acids (per 100 mg of tissue) was not influenced by diet ($P = 0.76$), insulin ($P = 0.99$), or the interaction between diet and insulin ($P = 0.77$). Similarly, there were no linear ($P = 0.74$) or quadratic ($P = 0.63$) effects of additional insulin on glucose conversion to glyceride-fatty acids in steers fed either diet.

Adipose tissue from steers fed the corn- or hay-based diets had similar ($P > 0.13$) rates of incorporation of

Table 1. Least squares means for carcass characteristics, subcutaneous adipose tissue cellularity, and metabolites of steers fed hay- or corn-based diets

<table>
<thead>
<tr>
<th>Item</th>
<th>Hay-fed</th>
<th>Corn-fed</th>
<th>SEM$^1$</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carcass characteristics</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HCW, kg</td>
<td>284</td>
<td>364</td>
<td>14.6</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Adjusted fat thickness, cm</td>
<td>1.04</td>
<td>2.43</td>
<td>0.23</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Marbling score$^2$</td>
<td>440</td>
<td>600</td>
<td>18.2</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Yield grade</td>
<td>3.03</td>
<td>4.82</td>
<td>0.36</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Subcutaneous adipose tissue cellularity</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cells per gram, $10^{-5}$</td>
<td>9.7</td>
<td>8.4</td>
<td>1.45</td>
<td>0.52</td>
</tr>
<tr>
<td>Mean diameter, µm</td>
<td>59.9</td>
<td>62.8</td>
<td>3.25</td>
<td>0.53</td>
</tr>
<tr>
<td>Mean volume, pL</td>
<td>1,174</td>
<td>1,353</td>
<td>194</td>
<td>0.53</td>
</tr>
<tr>
<td>Metabolites</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plasma glucose, mg/100 mL</td>
<td>112</td>
<td>146</td>
<td>16.4</td>
<td>0.17</td>
</tr>
<tr>
<td>Tissue glucose, µmol/g</td>
<td>1.33</td>
<td>0.60</td>
<td>0.44</td>
<td>0.27</td>
</tr>
<tr>
<td>G-6-P$^3$, µmol/g</td>
<td>0.064</td>
<td>0.098</td>
<td>0.035</td>
<td>0.51</td>
</tr>
<tr>
<td>F-6-P$^3$, µmol/g</td>
<td>0.28</td>
<td>0.13</td>
<td>0.096</td>
<td>0.31</td>
</tr>
</tbody>
</table>

$^1$n = 12.

$^2$Marbling score, small = 500.

$^3$G-6-P = glucose-6-phosphate; F-6-P = fructose-6-phosphate.
glucose into the glyceride-glycerol fraction when expressed per 100 mg of tissue (Figure 2). Also, incorporation of glucose into glyceride-glycerol was not influenced by insulin ($P = 0.40$) or the interaction between diet and insulin ($P = 0.38$). No linear ($P = 0.32$) or quadratic ($P = 0.33$) effects of additional insulin on glucose conversion to the glycerol-glyceride fraction of total lipid were observed.

When responses were expressed per $10^5$ cells, there was no effect of diet, insulin, or their interaction on the rate of acetate incorporation into fatty acids ($P > 0.28$; Table 2). Similarly, when the rate of glucose incorporation into CO$_2$ was expressed per $10^5$ cells, dietary treatment had no effect on CO$_2$ production ($P = 0.63$; Table 2). A linear effect of insulin addition on CO$_2$ production was still observed in s.c. tissue from steers fed the hay-based diet. Subcutaneous adipose tissue from hay-fed steers produced 11 and 15% more CO$_2$ with increasing insulin concentrations ($P = 0.06$) when expressed on a per-cell basis. No linear or quadratic trends in glucose conversion to CO$_2$ were observed in s.c. adipose tissue from corn-fed steers.

Glucose conversion to lactate (per $10^5$ cells) was not influenced by diet, insulin, or their interaction ($P \geq 0.31$; Table 2). There was no effect of insulin concentration on lactate production when data were expressed on the basis of cell number ($P = 0.22$).

When glucose incorporation into fatty acids was expressed on a cellular basis, the results were similar to those reported on unit weight of tissue basis because neither diet, insulin, nor their interaction influenced incorporation rate ($P \geq 0.47$; Table 2). Hay-fed steers tended to convert more glucose into glyceride-glycerol than corn-fed steers when expressed per gram of tissue, but when rates were calculated on a per-cell basis, diet did not influence the incorporation rate ($P = 0.73$).

**DISCUSSION**

Circulating glucose in corn-fed steers was numerically greater than in hay-fed steers, which could have been due to the greater production of propionate from starch fermentation associated with the corn-based diet (Orskov et al., 1991) and the preferential use of propionate as a gluconeogenic substrate by ruminants (Danfaer et al., 1991). Conversely, adipose tissue and muscle insulin sensitivity may have been depressed in the corn-fed steers, consistent with their greater carcass adiposity (McCann and Reimers, 1985).

Tissue glucose concentrations reflect the balance between glucose uptake and subsequent tissue utilization of glucose. The concentrations of G-6-P and F-6-P observed in this study were greater than those we previously reported for bovine s.c. adipose tissue (Rhoades et al., 2007). The greater accumulation of G-6-P and F-6-P in this study, relative to concentrations we reported in heavy-BW Angus and Wagyu steers, would suggest decreased flux through 6-phosphofructokinase (6-PFK; Rhoades et al., 2005) and therefore reduced glucose utilization. Activities of hexokinase and 6-PFK are low in bovine adipose tissue (Smith and Prior, 1981; Miller et al., 1991), and 6-PFK limits glycolytic flux in bovine s.c. adipose tissue (Smith, 1984).

Alternatively, decreased G-6-P and F-6-P concentrations reported in Rhoades et al. (2007) may have resulted from reduced glucose uptake from the periphery, perhaps due to depressed insulin receptor or glucose transporter number or activity because insulin had no effect on glucose metabolism in s.c. adipose tissue in that study. The long-fed cattle in Rhoades et al. (2007) that served as the source of s.c. and i.m. adipose tissue had 2.02 cm of adjusted fat thickness in corn-fed steers and 1.60 cm of adjusted fat thickness in the hay-fed steers (Lunt et al., 2005). By design, the steers of Rhoades et al. (2007) were fed for either 480 d (corn-fed) or 600 d (hay-fed) to achieve similar BW at slaughter (approximately 600 kg). The cattle used in the current study were considerably younger, and the adipose tissue of the hay-fed steers was less developed. In the
previous study, the hay-fed steers were 120 d older than the corn-fed steers at slaughter, and there was no effect of supplemental insulin on glucose carbon incorporation into CO₂, lactate, or lipids. Thus, in older and fatter Angus and Wagyu steers, hay feeding eliminated sensitivity to insulin, whereas, in the younger Angus steers of this study, insulin sensitivity of glucose carbon incorporation into CO₂ was apparent only in the lighter-weight, hay-fed steers.

Vasilatos et al. (1983) reported that insulin binding to isolated bovine s.c. adipocytes was low to nonexistent, depending on the group of cattle that was sampled. Subsequently, Abe et al. (1997) reported that GLUT4 gene expression was detectable only at very low concentrations in bovine perirenal adipose tissue. Both of these reports are consistent with the limited effects of insulin in vitro on bovine adipose tissue glucose and acetate metabolism. Aso et al. (1995) similarly reported that an i.m. preadipocyte clonal cell line derived from the longissimus thoracis muscle of long-fed Japanese Black cattle did not express GLUT4 protein, consistent with the lack of effect of insulin on in vitro glucose metabolism in i.m. adipose tissue of long-fed, hay-fed steers that we demonstrated previously (Rhoades et al., 2007).

Robertson et al. (1982) reported that a large proportion of glucose carbon metabolized by adipose tissue is converted to lactate. Smith (1983) demonstrated that 55% of glucose carbon was converted to lactate in s.c. adipose tissue when steers were fed a high-energy diet. Results in this study are similar to those reported by Rhoades et al. (2007), in that at least 85% of glucose carbon was converted to lactate from either diet. Previous studies have demonstrated that rates of acetate incorporation into fatty acids are increased when glucose is added to the culture media, due to the additional NADPH available for fatty acid synthesis (Hanson and Ballard, 1967; Smith, 1983). Early work demonstrated that a change in NADPH generation was in response to an increase in fatty acid synthesis (Ingle et al., 1973; Martin et al., 1973). Smith (1983) demonstrated that 33 to 43% of the NADPH required for lipogenesis from acetate can be derived from the pentose cycle.

Both glucose oxidation to CO₂ and acetate incorporation into fatty acids responded to insulin in s.c. adipose tissue of hay-fed steers. Thus, the greater oxidation of glucose was a reflection of increased flux through the pentose cycle to produce more reducing equivalents (NADPH) in response to the greater rate of acetate incorporation into fatty acids observed in hay-fed s.c. adipose tissue.

Previous studies have indicated that roughage feeding inhibits insulin action. Smith et al. (1983) indicated that when steers were fed an alfalfa diet, insulin failed to stimulate measures of lipogenesis. Few reports exist that demonstrate an effect of insulin on acetate or glucose utilization in s.c. adipose tissue, particularly when cattle were fed a hay-based diet. Smith et al. (1983) reported that injections of insulin tended to increase
acetate incorporation and lipogenic enzyme activity, when simultaneously infused with glucose in steers fed a roughage-based diet. More recently, Schoonmaker et al. (2003) reported greater insulin concentrations in steers fed a high-concentrate diet than in steers fed a high-forage diet during the growing phase.

Several studies have demonstrated that insulin effects on adipose tissue metabolism are increased with amount of concentrate in the diet (Baldwin et al., 1973; Miller et al., 1989, 1991). Most recently, we reported that when steers were fed a high-concentrate diet, glucose metabolism was stimulated by insulin in adipose tissue samples (Rhoades et al., 2007). However, in our previous study, the effect of diet on insulin sensitivity was most noticeable in i.m. adipose tissue. McCann and Reimers (1985) demonstrated that obese heifers were resistant to the glucoregulatory effects of insulin. However, these authors reported that whole-body insulin resistance was overcome when a maximum dose of insulin was administered in vivo to obese heifers. McCann and Reimers (1985) concluded that in obese heifers the decreased insulin response to administration of a low dose was a result of a decrease in the number of receptors, consistent with reduced insulin receptor density in bovine adipose tissue reported by Vasilatos et al. (1983). Schoonmaker et al. (2004) established that greater lipid filling or complete maturity of adipocytes occurs sooner when cattle are fed corn-based diets than when they are fed hay-based diets. Our corn-fed steers had a greater amount of carcass fat thickness even though there were no differences in s.c. adipocyte mean volume. These data suggest that corn-fed cattle had a greater number of s.c. adipocytes that were less sensitive to insulin.

The data of the current study are consistent with numerous earlier reports that suggest insulin has little or no effect on ruminant s.c. adipose tissue metabolism (Prior and Smith, 1982; Vasilatos et al., 1983; Vernon et al., 1985; Miller et al., 1991). We had predicted that adipose tissue from corn-fed steers would be more sensitive to supplemental insulin based on our previous results (Rhoades et al., 2007). However, the substantial differences in animal age, carcass weight, and carcass adiposity make it difficult to reconcile the different responses to insulin observed in the 2 studies. Results from the present study suggest that feeding cattle to a fat thickness above the average feedlot steer may have depressed the sensitivity of bovine s.c. adipose tissue to supplemental insulin in vitro. In general, s.c. adipose tissue, unlike i.m. tissue, is only marginally sensitive to insulin and any previous sensitivity disappears as age or adiposity is increased. In this experiment, increased insulin dose failed to elicit a meaningful suite of responses regardless of the diet fed to steers.

**LITERATURE CITED**


