Glucose and acetate metabolism in bovine intramuscular and subcutaneous adipose tissues from steers infused with glucose, propionate, or acetate

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ABSTRACT: We hypothesized that abomasal infusion of glucose would promote de novo fatty acid biosynthesis from glucose in vitro in bovine intramuscular (i.m.) and subcutaneous (s.c.) adipose tissues to a greater extent than ruminal infusion of acetate, propionate, or glucose. Angus crossbred steers ($n = 24$), 22 mo of age, were fitted with ruminal cannulas, and steers were adapted to another corn/sorghum finishing diet over a 2-wk period while recovering from the placement of the cannulas. After the adaptation period, the steers were fed the second finishing diet at 130% of their voluntary intake and were infused with isocaloric amounts (3.76 Mcal/d) of glucose, propionate, or acetate for 35 d. Glucose was infused either into the rumen or into the abomasum, whereas propionate and acetate were infused into the rumen. Acetate infusion decreased DM and DE intakes ($P < 0.05$). The 5th to 8th longissimus muscle section was removed immediately and transported to the laboratory within 10 min post-exsanguination in 38 °C, oxygenated Krebs Henseleit buffer containing 5 mM glucose and 5 mM acetate. Intramuscular and s.c. adipose tissues were dissected from the muscle and incubated in vitro in 5 mM glucose plus 5

mM acetate (containing [U-¹⁴C]glucose or [1-¹⁴C]acetate). Lipid content was lower ($P = 0.04$) in i.m. adipose tissue of the acetate-infused steers than in the other treatment groups, and i.m. adipocytes from acetate-infused steers were smaller ($P = 0.01$) than those from propionate-infused steers. The rate of incorporation of acetate into glyceride-fatty acids (GFA) in i.m. and s.c. adipose tissues was greater ($P < 0.03$) in steers receiving ruminal or abomasal infusions of glucose than in adipose tissues from steers infused with acetate. The greatest rates of GFA synthesis were observed in s.c. adipose tissue from steers infused ruminally with propionate or abomasally infused with glucose ($P < 0.001$). In i.m. and s.c. adipose tissues, the proportion of acetyl units from acetate incorporated into GFA was greater in steers receiving glucose infusion in the rumen or abomasum than in steers receiving acetate or propionate infusion ($P < 0.05$). Contrary to our hypothesis, abomasal glucose infusion did not promote greater fatty acid biosynthesis from glucose in i.m. adipose tissue than ruminal glucose infusion. However, glucose infusion caused the greatest production of acetyl units from acetate in i.m. and s.c. adipose tissues.

Key words: acetate, adipose tissue, bovine, glucose, propionate

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J. Anim. Sci. 2018.96:921–929

doi: 10.1093/jas/sky017

INTRODUCTION

Propiogenic diets (such as high-grain diets) promote greater marbling development than acetogenic diets (e.g., Schoonmaker et al., 2004; Rhoades et al., 2007, 2009). It is possible that “glucogenic” diets, that is, diets that provide more

Supported by funds from the Beef Checkoff and Texas AgriLife Research.

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Received April 7, 2017.

Accepted January 23, 2018.

glucose for absorption from the small intestine (e.g., dry-rolled corn; [Huntington et al., 2006](#); [Corrigan et al., 2009](#)), would promote marbling development to a greater extent than propiogenic diets. Studies with intramuscular (i.m.) and subcutaneous (s.c.) adipose tissues have indicated that i.m. adipose tissue uses glucose for a greater proportion of the carbon source for de novo fatty acid biosynthesis in vitro than s.c. adipose tissue ([Smith and Crouse, 1984](#); [Lee et al., 2000](#); [Song et al., 2001](#); [Rhoades et al., 2007, 2009](#)). In a recent in vivo study, Nayananjali et al. (2016) infused [$^2\text{H}_3$]acetate or [$^{13}\text{C}_6$]glucose into early-weaned and normal-weaned Angus $\times$ Simmental steers fed a cracked-corn finishing diet from approximately 360 kg to 540 kg BW, and demonstrated that acetate was incorporated into palmitate at rates that are 10-fold greater than glucose in both i.m. and s.c. adipose tissue. It has been difficult to assess the actual effects of increasing glucose availability for fatty acid synthesis on adipose tissue metabolism in growing beef cattle. In this experiment, we tested that the hypothesis by abomasal infusion of isocaloric amounts of glucose (relative to abomasal infusion of acetate, propionate, and glucose) in steers would promote the in vitro incorporation of glucose into glyceride-fatty acids (GFA), particularly in i.m. adipose tissue. Glucose was infused into the rumen and into the abomasum, the latter treatment avoiding ruminal metabolism of the infused glucose. Acetate and propionate were infused into the rumen to mimic the effects acetogenic and propiogenic diets, respectively.

MATERIALS AND METHODS

Animals and Treatments

The experimental protocols for this study were approved by the Institutional Animal Care and Use Committee at Texas A&M University (College Station, TX). Twenty-four ruminally cannulated Angus $\times$ Hereford crossbred steers (initial BW = 549 ± 38.8 kg; approximately 22 mo of age) were used in a one-way, completely randomized, four-treatment experiment designed to evaluate the effects of infusion of acetate or propionate infused ruminally or glucose infused ruminally or abomasally. Steers were fed native pasture until approximately 16 mo of age, after which they gradually adapted to a corn-based finishing diet ([Rhoades et al., 2009](#)) to 22 mo of age. The steers were neither implanted, nor did the diet contain ionophores.

Six steers were assigned to each of the four treatments; however, during treatment administration,

acetate and propionate infusion severely reduced intake in three steers (one from the acetate group and two from the propionate group), and therefore were removed from the study. The following treatments were infused continuously for the last 35 d of the finishing period:

1. Acetic acid, 1.077 kg/d (16.7 mol/d; 3.76 Mcal/d) ruminal infusion ($n = 5$)
2. Propionic acid, 0.758 kg/d (10.15 mol/d; 3.76 Mcal/d) ruminal infusion ($n = 4$)
3. Glucose, 1.0 kg/d (5.55 mol/d; 3.76 Mcal/d) ruminal infusion ($n = 6$)
4. Glucose, 1.0 kg/d (5.55 mol/d; 3.76 Mcal/d) abomasal infusion ($n = 6$)

Before beginning the study, steers were adapted to housing, feeding, and treatment administration protocols for 5 d. Steers were housed in individual pens within a continuously lighted barn, and were provided free access to fresh water and feed. The finishing diet was comprised of dry rolled corn (42.3%), ground milo (18.0%), cottonseed hulls (13.5%), grass hay (10.0%), molasses (6.7%), cottonseed meal (5.4%), vitamin/mineral premix (2.3%), urea (0.9%), and limestone (0.9%) (formulated to meet the requirements of finishing steers; [NRC, 2000](#)) and was provided at 0600 h and offered at 130% voluntary intake of the previous day to ensure ad libitum consumption. For the duration of the study, housing and feeding protocols remained the same.

Steers were continuously infused, using a peristaltic pump set to provide a total infused volume of 5 L/d. Before adapting to treatments, 5 liters of water was infused to familiarize the steers with experimental procedures. For the first 3 d of the study, infusates were administered at increasing levels to prevent sudden changes in intake or animal health. Ruminal infusions were accomplished by inserting lines through the cannula and into the rumen. Abomasal infusions were accomplished by inserting lines through the rumen cannula and passing them through the reticulo-omasal orifice and anchoring them in the abomasum with a 10-cm rubber flange ([Wickersham et al., 2009](#)). The acetate solution was produced by dissolving 6 liter glacial acetic acid in 30 liter water. The propionic acid (Sigma Aldrich, St. Louis, MO) and glucose (Sigma Aldrich) solutions were produced by dissolving the solutes in water. The acetic acid and propionic acid solutions were not buffered to avoid introducing large amounts of Na^+ into the rumen or abomasum. All infusate solutions were made fresh daily.

At the end of the 35-d infusion period (final BW 559 ± 8.1 kg), ruminal contents were manually evacuated to prevent carcass contamination with rumen contents during slaughter. Steers were harvested over a 2-d period immediately following the infusion period. Slaughter day was assigned at random within treatment. Prior to slaughter, steers were transported approximately 9 km to the Texas A&M University Rosenthal Meat Science & Technology Center (College Station, TX), where the cattle were slaughtered by humane, industry standard procedures.

A portion of the 5th to 8th rib section of the longissimus thoracis muscle was removed by cutting through the hide immediately post-exsanguination and transported to the laboratory in oxygenated, 37 °C Krebs Henseleit buffer (pH 7.35–7.40) containing 5 mM glucose and 5 mM acetate. The muscle sample arrived at the laboratory approximately 10 min post-exsanguination. Upon arrival at the laboratory, pieces of i.m. and s.c. adipose tissues were removed from the longissimus thoracis muscle by dissection. Approximately 100 mg of the collected pieces of adipose tissue was incubated fresh per flask, while approximately 100 mg of the adipose tissue was stored at –80 °C for subsequent measurements of composition and enzyme analyses.

Lipogenesis In Vitro

At sample collection, lipogenesis in vitro was measured in i.m. and s.c. adipose tissues as described previously (May et al., 1995). Adipose tissue pieces (~100 mg) were incubated with 5 mM glucose, 5 mM acetate, 10 mM HEPES, 1 μ Ci [U-¹⁴C]glucose or 1 μ Ci [1-¹⁴C]acetate (sodium salt) (American Radiolabeled Chemicals, Inc.) in oxygenated Krebs Henseleit buffer (pH 7.35–7.40). Adipose tissues were incubated in 3 mL of media for 2 h at 38 °C in a shaking water bath. The rate of glucose and acetate incorporation into fatty acids is linear for at least 3 h (Smith, 1983). Reactions were terminated by the addition of 3 mL of 5% trichloroacetic acid. Zero-time incubations contained 3 mL of 5% trichloroacetic acid that was added to the incubation medium before adipose tissues were added to the flasks.

Neutral lipids in adipose tissues were extracted using the procedure of Folch et al. (1957). Total lipids were saponified as described previously (Smith and Prior, 1982) and glyceride–glycerol (GG) and GFA were isolated and resuspended in 10 mL of scintillation cocktail (Bio-safe2, Research Product International Corp., Mount Prospect,

IL). Radioactivity of lipid extracts was counted with a scintillation counter (Packard 1600TR Liquid Scintillation Analyzer, Downers Grove, IL). Results are reported as nmol substrate converted to product/(2 h•100 mg adipose tissue).

Assay of Enzyme Activities

Previously frozen s.c. and i.m. adipose tissue samples (~100 mg) were homogenized in 3 volumes (wt/vol) of 0.1 M K_2HPO_4 (pH 7.4). The homogenate was centrifuged at $14,000 \times g$ for 5 min at 4 °C and the pellet and the fat cake were discarded, after which the infranate was centrifuged at $14,000 \times g$ for 30 min at 4 °C; the pellet and any remaining lipid layer were discarded. The subsequent infranates were used undiluted for all enzyme assays.

Glucose-6-phosphate dehydrogenase (G-6-PDH) and 6-phosphogluconate dehydrogenase (6-PGDH) activities were determined spectrophotometrically by the procedure of Bernt and Bergmeyer (1974). Enzyme activities were measured as the conversion of nicotinamide adenine dinucleotide phosphate (NADP⁺) to its reduced form (NADPH) using a Beckman DU-7400 Spectrophotometer (Palo Alto, CA). All enzymes are expressed as nmol of NADPH produced/(min•100 mg tissue).

Lipid Content of Muscle and Adipose Tissues

Approximately 50 g of muscle from the center of longissimus thoracis muscle from the 5th to 8th rib section was frozen in liquid nitrogen, pulverized with a mortar and pestle, and the lipids were extracted in chloroform:methanol (2:1, vol/vol). Approximately 200 mg of i.m. and s.c. adipose tissues, collected at harvest and frozen at –80 °C, also were homogenized in chloroform:methanol. Lipids were extracted as described by Folch et al. (1957), the solvents were dried exhaustively under N₂, and the lipid weights were measured.

Adipose Tissue Cellularity

Adipose tissue cellularity was measured as described previously (Choi et al., 2014). Previously frozen i.m. and s.c. adipose tissue samples (25 mg) obtained at the day of sampling were sliced thinly as possible on a glass plate on ice. Adipose tissue slices were transferred to scintillation vials and rinsed three times with 37 °C 0.154 M NaCl. A 0.6-mL aliquot of 50 mM collidine•HCl was forcibly added with a pipette to break up the sample; subsequently 0.5 of 3.0% osmium in collidine buffer was added

and the samples were incubated in a 37 °C water bath for 96 h, swirling occasionally. The osmium was removed and 10 mL of 0.154 M NaCl was added. The samples were rinsed continuously with NaCl until the solution was clear, after which 10 mL of 8 M urea was added and samples were incubated at room temperature for 96 h, swirling occasionally. The fixed adipocytes were resuspended with 0.01% Triton in 0.154 M NaCl and used for determination of cell diameter using a bright-field microscope (Olympus Vanox ABHS3, Olympus, Tokyo, Japan), CCD Color Video Camera (DXC-960MD, Sony, Japan). Images were saved as tiff files and diameters were determined using ImageJ (rsbweb.nih.gov/ij/). Diameters were converted to volumes (μm^3) and subsequently converted to pL/cell (at $10^{-3} \mu\text{m}^3/\text{pL}$).

Statistical Analysis

Data for acetyl units from acetate and glucose incorporation into GFA were analyzed separately by one-way analysis of variance. All other data were analyzed using the General linear mixed models (GLMM) of SPSS statistics 20 (IBM, Armonk, NY) as appropriate for completely randomized designs. The model tested main effects of infusate. Means were separated by the Fisher's protected LSD and considered different at $P < 0.05$.

RESULTS

While the focus of this study was not intake and digestion, the results of those measurements are mentioned briefly here to aid in understanding the results. Intake of DM was less ($P < 0.05$) for acetate-infused steers (66.8 g/kg BW^{0.75}) than both ruminal and abomasal glucose-infused steers (92.6 and 92.9 g/kg BW^{0.75}), while propionate infusion resulted in an intermediate level of intake (79.4 g/kg BW^{0.75}). There were no significant differences

in gross energy digestibility ($P \geq 0.21$), which averaged 83.6% across all treatments. Following a similar pattern to DM intake, DE intakes were 25.7, 29.7, 34.4, and 35.5 Mcal/d for steers receiving infusions of acetate, propionate, ruminal glucose, and abomasal glucose, respectively. Intake of DE was less ($P < 0.05$) for acetate-infused steers than both ruminal and abomasal infusion of glucose. The only significant change in BW over the 35-d infusion period was for the acetate-infused steers, which lost approximately 20 kg ($P < 0.01$).

GFA and GG Biosynthesis

Intramuscular adipose tissue. There was no difference in percent intramuscular lipid ($P = 0.13$) among treatment groups (Table 1). Intramuscular adipose tissue from acetate-infused steers contained less lipid than i.m. adipose tissue from the other treatment groups (38.6 vs. 52.0–58.7 mg/100 mg; $P = 0.04$), and i.m. adipocytes from acetate-infused steers were smaller than those from propionate-infused steers (461 vs. 772 pL; $P = 0.01$).

There was less incorporation of acetate into GFA in i.m. adipose tissue from acetate-infused steers than in i.m. adipose tissue from ruminal or abomasal glucose-infused steers (6.95 vs. 16.33 and 16.26 nmol acetate incorporated/[2 h•100 mg], respectively; $P = 0.03$), and acetate incorporation into GG in i.m. adipose tissue was less in acetate-infused steers than in abomasal glucose-infused steers ($P = 0.04$) (Table 2). Glucose incorporation into GFA was greater in i.m. adipose tissue from propionate-infused steers than in i.m. adipose tissue from ruminal or abomasal glucose-infused steers (2.35 vs. 1.41 and 1.16 nmol glucose incorporated into GFA/[2 h•100 mg], respectively; $P = 0.004$), and glucose incorporation into GG was greater in propionate-infused steers than in abomasal glucose-infused steers (2.69 vs. 1.67 nmol glucose incorporated into GG/[2

Table 1. Lipid content of longissimus thoracis muscle, i.m. adipose tissue, and s.c. adipose tissue, and i.m. and s.c. adipocyte volumes of steers ruminally infused with acetate, propionate, or glucose, or abomasally infused with glucose

Item	Treatments ¹					P-value
	A	P	GR	GA	SEM	
Muscle lipid, %	5.66	7.39	4.10	6.03	0.86	0.13
Intramuscular adipose tissue lipid, mg/100 mg	38.6 ^b	52.0 ^a	53.4 ^a	58.7 ^a	5.8	0.04
Subcutaneous adipose tissue lipid, mg/100 mg	80.9	80.5	75.2	80.4	11.3	0.58
Intramuscular adipocyte volume, pL/cell	461 ^b	772 ^a	514 ^b	652 ^{ab}	56	0.01
Subcutaneous adipocyte volume, pL/cell	942	1,048	799	921	82	0.08

¹A, acetate ruminal infusion; P, propionate ruminal infusion; GR, glucose ruminal infusion; GA, glucose abomasum infusion. ^{abc}Means in the same row with common superscripts are not different ($P > 0.05$).

Table 2. GFA and GG synthesis from acetate and glucose in i.m. adipose tissue of steers ruminally infused with acetate, propionate, or glucose, or abomasally infused with glucose

	Treatment ¹					
Item	A	P	GR	GA	SEM	<i>P</i> -value
Acetate incorporation, nmol/(2 h•100 mg)						
GFA	6.95 ^b	7.81 ^b	16.33 ^a	16.26 ^a	1.26	0.03
GG	0.46 ^b	0.50 ^b	0.71 ^{ab}	0.98 ^a	0.06	0.04
Glucose incorporation, nmol/(2 h•100 mg)						
GFA	2.35 ^{ab}	3.17 ^a	1.41 ^b	1.16 ^b	0.19	0.004
GG	2.23 ^{ab}	2.69 ^a	1.91 ^{ab}	1.67 ^b	0.15	0.05
G6PDH, nmol/(min•100 mg)	720 ^b	1,251 ^b	1,226 ^b	2,575 ^a	310	0.002
6PGDH, nmol/(min•100 mg)	265 ^b	273 ^{ab}	359 ^{ab}	532 ^a	64	0.02

¹A, acetate ruminal infusion; P, propionate ruminal infusion; GR, glucose ruminal infusion; GA, glucose abomasum infusion. 6PGDH, 6-phosphogluconate dehydrogenase; G6PDH, glucose-6-phosphate dehydrogenase. ^{abc}Means in the same row with common superscripts are not different ($P > 0.05$).

h•100 mg]; $P = 0.05$). Intramuscular adipose tissue from acetate-infused steers exhibited lower G6PDH and 6-PGDH activities than i.m. adipose tissue from abomasal glucose-infused steers ($P \leq 0.02$).

Subcutaneous adipose tissue. Infusate treatment had no effect on s.c. adipose tissue lipid content (75.2 to 84.0 mg lipid/100 mg; $P = 0.58$), and s.c. adipocyte volumes did not differ across treatment groups (799 to 1,048 pL; $P = 0.08$) (Table 1). There was less in vitro incorporation of acetate into GFA and GG in s.c. adipose tissue of acetate-infused steers than in s.c. adipose tissue from the other treatment groups ($P \leq 0.003$) (Table 3). Acetate incorporation into GFA of ruminal glucose-infused steers was less than in adipose tissues from the propionate- and abomasal glucose-infused steers (104.91 vs. 159.68 and 162.01 nmol acetate incorporated/[2 h•100 mg], respectively; $P = 0.001$), and acetate incorporation into GG was greatest in propionate-infused steers ($P = 0.003$).

There was less glucose incorporation into GFA and GG in s.c. adipose tissue of acetate-infused

steers (1.51 and 5.47 nmol glucose incorporated/[2 h•100 mg], respectively) than glucose incorporation into GFA and GG in s.c. adipose tissue of propionate-infused steers (2.41 and 7.48 nmol glucose incorporated/[2 h•100 mg], respectively; $P \leq 0.03$). Additionally, glucose incorporation into GG was greater in propionate-infused steers than in steers infused with glucose directly into the rumen or abomasum ($P = 0.001$). There were no effects of infusate treatment on G-6-PDH and 6-PGDH activities in s.c. adipose tissue ($P \geq 0.14$).

Acetyl units incorporated into GFA from acetate and glucose were calculated as described previously (Smith, 1983; Smith and Crouse, 1984). Percentages of acetyl units from acetate and glucose in i.m. adipose tissue from acetate-infused steers were 58.8% and 41.2%, respectively (Figure 1). Percentages of acetyl units from acetate and glucose in s.c. adipose tissue from acetate-infused steers were 85.1% and 14.9%, respectively. Similar percentages were observed in adipose tissues from propionate-infused steers. The percentage of acetyl

Table 3. GFA and GG synthesis from acetate and glucose in subcutaneous adipose tissue of steers ruminally infused with acetate, propionate, or glucose, or abomasally infused with glucose

Item	Treatment ¹				SEM	P-value
	A	P	GR	GA		
Acetate incorporation, nmol/(2 h•100 mg)						
GFA	23.25 ^c	159.68 ^a	104.91 ^b	162.01 ^a	11.18	0.001
GG	0.41 ^c	1.07 ^a	0.74 ^b	0.77 ^b	0.05	0.003
Glucose incorporation, nmol/(2 h•100 mg)						
GFA	1.51 ^b	2.41 ^a	1.84 ^{ab}	2.06 ^{ab}	0.10	0.03
GG	5.47 ^b	7.48 ^a	3.87 ^c	4.69 ^{bc}	0.25	0.001
G6PDH, nmol/(min•100 mg)	1,299	1,831	1,728	2,622	403	0.14
6PGDH, nmol/(min•100 mg)	322	477	402	457	75	0.51

¹A, acetate ruminal infusion; P, propionate ruminal infusion; GR, glucose ruminal infusion; GA, glucose abomasum infusion. 6PGDH, 6-phosphogluconate dehydrogenase; G6PDH, glucose-6-phosphate dehydrogenase. ^{abc}Means in the same row with common superscripts are not different ($P > 0.05$).

Figure 1. Percent acetyl units from acetate or glucose incorporated into GFA in vitro in intramuscular (IM) or subcutaneous (SC) adipose tissues. A, acetate ruminal infusion; P, propionate ruminal infusion; GR, glucose ruminal infusion; GA, glucose abomasum infusion. ^{abcde}Means in the acetate portions of the columns with common superscripts are not different ($P > 0.05$). ^{abcd}Means in the glucose portions of the columns with common superscripts are not different ($P > 0.05$).

units from glucose in i.m. adipose tissue from ruminal and abomasal glucose-infused steers was approximately 18%, and the percentages of acetyl units from glucose in s.c. adipose tissue from ruminal and abomasal-infused glucose steers were 6% and 3%, respectively.

DISCUSSION

In this study, there were differences between ruminal and abomasal glucose-infused steers in our in vitro measures of fatty acid synthesis, the proportion of acetyl units from acetate (ruminal glucose < abomasal glucose in s.c. adipose tissue), the proportion of acetyl units from glucose in s.c. adipose tissue (ruminal glucose > abomasal glucose in s.c. adipose tissue), and G6PDH activity (ruminal glucose < abomasal glucose in i.m. adipose tissue). We conclude that abomasal glucose infusion may have increased the daily glucose supply sufficiently to elicit these differential in vitro responses between abomasal glucose and ruminal glucose infusion. Also, propionate infusion increased [^{14}C] acetate and [^{14}C]glucose incorporation into GFA in s.c. adipose tissue (relative to ruminal glucose infusion) and glucose incorporation into GFA and GG in i.m. adipose tissue in vitro, whereas glucose infusion had no effect on [^{14}C]glucose incorporation into the GFA or GG fractions in i.m. adipose tissue.

The design of this study did not allow us to distinguish if acetate infusion depressed GFA

synthesis from acetate or if propionate and glucose infusion stimulated GFA synthesis from acetate in s.c. adipose tissue. It is expected that the depression in DM and DE intakes caused by acetate infusion would have depressed lipogenesis in vitro, but in most cases rates of acetate and glucose incorporation into GFA and GG were not lower in adipose tissues from acetate-infused steers than in adipose tissues from propionate- or glucose-infused steers. Urrutia et al. (2017) infused sodium acetate (7 mol/d) in dairy cows and demonstrated that acetate infusion did not significantly affect lipid synthesis from acetate in vitro. However, acetate infusion depressed *fatty acid synthase*, *acetyl-CoA carboxylase*, and *stearoyl-CoA desaturase* gene expression in s.c. adipose tissue, relative to adipose tissue from control cows. Urrutia et al. (2017) did not report DM intake, but in this study acetate infusion significantly depressed DM intake. We cannot rule out the possibility that, in this study, the depression in DM intake caused by acetate infusion was responsible for the markedly lower in vitro GFA synthesis from acetate, relative to propionate and/or glucose infusion, in i.m. and s.c. adipose tissues.

Paradoxically, although in vitro GFA synthesis from acetate in s.c. adipose tissue was markedly lower than observed in s.c. adipose tissue from propionate- and glucose-infused steers, s.c. adipose tissue lipid content and adipocyte size were not different among treatment groups. The basis for this discrepancy is unknown although acetate may have depressed lipolysis via the G-coupled protein receptor-43 (GPR43). The GPR43 receptor binds volatile fatty acids and inhibits lipolysis by depressing adenylate cyclase activity (Hong et al., 2005), and we have documented *GPR43* expression in bovine s.c. adipose tissue (Smith et al., 2012). However, *GPR43* also is highly expressed in i.m. adipose tissue (Chung et al., 2016), yet i.m. adipocyte size and lipid content were lowest in the acetate-infused steers. Therefore, we have no explanation for the discrepancy in responses to acetate infusion between s.c. and i.m. adipose tissues.

Feeding high-grain diets increases the activity of lipogenic enzymes such as acetyl-CoA carboxylase (ACC), ATP-citrate lyase (ATP-CL), NADP-malate dehydrogenase (NADP-MDH), G6PDH, and 6-PGDH in s.c. adipose tissue, which is associated with greater carcass adiposity (Smith et al., 1984; Smith and Crouse, 1984; Schoonmaker et al., 2004). It is possible that feeding beef cattle “gluconogenic” diets, which putatively would provide more glucose for absorption from the small intestine, would promote adiposity to a greater extent than

propiogenic diets. [Prior and Scott \(1980\)](#) intravenously infused glucose (2.75 mol/d), propionate (5.50 mol/d), acetate (8.25 mol/d), and lactate (5.50 mol/d) in beef cattle and demonstrated that acetate infusion did not increase lipogenesis from acetate in vitro, relative to saline-infused steers. In [Prior and Scott \(1980\)](#) propionate, glucose, and lactate increased lipid synthesis from acetate by 107%, 165%, and 77%, respectively, indicating that glucose infusion was more effective in stimulating lipogenesis in vitro than propionate or lactate. Additionally, intravenous glucose infusion increased plasma glucose approximately 50% ([Prior and Scott, 1980](#)).

[Lemosquet et al. \(2009\)](#) infused glucose into the abomasum and propionate into the rumen of dairy cows (5.15 Mcal/d for each) and documented that the whole-body rate of glucose appearance was 24% greater in glucose-infused dairy cows than in propionate-infused cows and 33% greater than in cows fed a grass-silage-based diet; plasma glucose concentrations were not affected by glucose infusion. Similarly, [Bailey et al. \(2012\)](#) reported that ruminal infusion of glucose at 1.2 kg/d (4.5 Mcal/d) had no effect on plasma glucose concentrations. Therefore, it is not certain if ruminal infusion of glucose at 3.76 Mcal/d would have affected plasma glucose concentrations in this study. However, the results of this study produced results similar to [Prior and Scott \(1980\)](#) in that, relative to acetate infusion, propionate and glucose infusion may have stimulated [^{14}C]acetate incorporation into fatty acids in s.c. adipose tissue incubations, and only glucose infusion increased [^{14}C]acetate incorporation into fatty acids in i.m. adipose tissue, relative to acetate infusion. The similarities in results between this study and [Prior and Scott \(1980\)](#) suggest that both ruminal and abomasal glucose infusion increased plasma glucose concentrations.

Previous studies demonstrated that continuous intraruminal infusion of propionate increased plasma glucose concentrations in beef and dairy cattle ([McAtee and Trenkle, 1971](#); [Oba and Allen, 2003](#)) although a later investigation ([Oh et al., 2015](#)) demonstrated that propionate infusion had no effect on plasma glucose concentrations. However, it is noteworthy in this study that the greatest amount of intramuscular lipid (numerically), the largest adipocytes, and the greatest rates of [^{14}C]glucose incorporation into GFA and GG were observed in i.m. adipose tissue from the propionate-infused steers. This is, in spite of the reduction in DM and DE intakes, caused by propionate infusion. These results suggest that the feeding of propiogenic diets

promotes marbling development by increasing plasma glucose. This in turn may have increased plasma insulin, which would have promoted glucose uptake (for review, see [Smith, 2017](#)).

The results of this study also indicate that glucose is replaced by acetate as a significant source of carbon for fatty acid biosynthesis in both i.m. and s.c. adipose tissues as the rate of acetate incorporation into fatty acids increases, similar to results of a recent study from our laboratory ([Choi et al., 2014](#)). This may explain why different laboratories have reported vast differences in the contribution of glucose to fatty acid biosynthesis in i.m. adipose tissue. Our first study of the use of glucose carbon for fatty acid biosynthesis demonstrated that glucose provided 1% to 10% of the acetyl units in s.c. adipose tissue, but 50% to 75% of the acetyl units in i.m. adipose tissue in vitro ([Smith and Crouse, 1984](#)), and subsequent studies reported similar results ([Song et al., 2001](#); [Rhoades et al., 2007](#)). In contrast, other studies of in vitro fatty acid biosynthesis demonstrated that glucose provided only approximately 4% of the acetyl units for fatty acid biosynthesis in i.m. adipose tissue, with acetate providing the remainder ([Miller et al., 1991](#); [Lee et al., 2000](#)).

Rather than studying fatty acid synthesis in vitro, [Nayananjali et al. \(2016\)](#) continuously infused [$^2\text{H}_3$]acetate and [U^{13}C_6]glucose into steers; measured plasma enrichment of acetate, glucose, and palmitate; and calculated the fractional synthesis rate (FSR) of palmitate from each isotope. [Nayananjali et al. \(2016\)](#) demonstrated that FSRs from acetate and glucose were not different among s.c., i.m., and visceral fat depots. Also, the FSR for acetate was approximately 10-fold higher than for glucose in all three depots, indicating that acetate was the primary precursor for fatty acid biosynthesis in vivo regardless of adipose tissue depot. In this study, the most consistent effect of glucose infusion (relative to acetate and propionate infusion) was a greater absolute rate of acetate incorporation into GFA in vitro and a greater proportion of acetyl units from acetate into GFA in i.m. adipose tissue. These relative contributions of acetate and glucose to GFA synthesis are consistent with [Nayananjali et al. \(2016\)](#). Taken together with our previous report ([Choi et al., 2014](#)), these data indicate that, under conditions in which overall capacity for fatty acid biosynthesis increases (i.e., in growing, grain-fed beef cattle), glucose would provide a lesser proportion of the acetyl units to lipid synthesis in vivo than acetate in both i.m. and s.c. adipose tissues.

It should be noted that in vitro and in vivo studies provide different information about the capacity of adipose tissues to synthesize fatty acids *de novo*. This in vitro study provided information about the maximum capacity of i.m. and s.c. adipose tissues to produce GFA and GG from acetate and glucose. In vitro conditions can never completely mimic in vivo conditions, but neither can in vivo conditions yield information about the maximal capacity of adipose tissue to synthesize fatty acids. For example, Nayananjalie et al. (2016) provided FSRs for i.m., s.c., and visceral adipose tissues, but could not provide information about the relative rates of fatty acid biosynthesis from acetate or glucose between i.m. and s.c. adipose tissues. The results of this study are consistent our previous reports (Smith and Crouse, 1984; Miller et al., 1991; May et al., 1994, 1995; Archibeque et al., 2005) that acetate is incorporated into GFA at rates up to 10-fold higher in s.c. adipose tissue than in i.m. adipose tissue, whereas GFA synthesis from glucose was similar between i.m. and s.c. adipose tissues. Additionally, this study provided novel information about the relative contributions of acetate and glucose to GFA synthesis under widely varying total rates of GFA synthesis.

Addition of acetate to in vitro incubations of s.c. adipose tissue strongly depresses the incorporation of glucose carbon into GFA (Smith, 1983; Smith and Crouse, 1984). Conversely, supplementing incubation media with glucose increases acetate incorporation into GFA approximately twofold in vitro (Smith and Prior, 1982). Glucose-6-phosphate dehydrogenase and 6PGDH, enzymes in the pentose cycle, generate NADPH that can be used to support *de novo* fatty acid biosynthesis (Flatt and Ball, 1964; Smith, 1983). In vitro, in the absence of acetate, 12% of glucose is metabolized via the pentose cycle (Smith, 1983). Addition of acetate to incubation media increases glucose utilization by the pentose cycle to over 20%, and the pentose cycle provides 33% to 43% of the NADPH required for lipogenesis from acetate in the presence of glucose. The greater rate of acetate incorporation into fatty acids observed in s.c. and i.m. adipose tissues of glucose-infused steers, relative to acetate-infused steers, indicates a greater demand for NADPH, which would cause the consumption of more glucose carbon via the pentose cycle. This would provide the mechanism by which the proportion of glucose-derived acetyl units incorporated into fatty acids decreased as the

magnitude of fatty acid biosynthesis from acetate increased.

CONCLUSION

Abomasal and ruminal glucose infusion resulted in greater rates of in vitro lipogenesis from acetate in i.m. and s.c. adipose tissues than acetate infusion, which was consistent with our hypothesis. However, this likely was the result of depressed DM and DE intake in the acetate-infused steers. Regardless, this in vitro investigation confirmed that glucose provides a greater proportion of acetyl units to *de novo* fatty acid biosynthesis in i.m. adipose tissue than in s.c. adipose tissue. The results of this study also suggest that when overall rates of fatty acid biosynthesis are relatively high (such as with feeding grain-based diets), glucose contributes only a small proportion of acetyl units to fatty acid biosynthesis relative to the contribution by acetate, both in i.m. and s.c. adipose tissue.

LITERATURE CITED

- Archibeque, S.L., D.K. Lunt, R.K. Tume, and S.B. Smith. 2005. Fatty acid indices of stearoyl Co-A desaturase activity do not reflect actual stearoyl Co-A desaturase enzyme activity in adipose tissues of beef steers finished with corn-, flaxseed-, or sorghum-based diets. *J. Anim. Sci.* 83:1153–1166.
- Bailey, E.A., E.C. Titgemeyer, K.C. Olson, D.W. Brake, M.L. Jones, and D.E. Anderson. 2012. Effects of ruminal casein and glucose on forage digestion and urea kinetics in beef cattle. *J. Anim. Sci.* 90:3505–3514. doi:10.2527/jas.2011.4459
- Bernt, E., and H.U. Bergmeyer. 1974. Hexokinase. In: Bergmeyer, H.U., and K. Gawehn, editors. *Methods of enzymatic analysis*. New York: Academic Press; p. 473–474.
- Choi, S.H., D.T. Silvey, B.J. Johnson, M.E. Doumit, K.Y. Chung, J.E. Sawyer, G.W. Go, and S.B. Smith. 2014. Conjugated linoleic acid (t-10, c-12) reduces fatty acid synthesis *de novo*, but not expression of genes for lipid metabolism in bovine adipose tissue *ex vivo*. *Lipids* 49:15–24. doi:10.1007/s11745-103-3869-0
- Chung, K.Y., S.B. Smith, S.H. Choi, and B.J. Johnson. 2016. Oleic acid enhances G protein coupled receptor 43 expression in bovine intramuscular adipocytes but not in subcutaneous adipocytes. *J. Anim. Sci.* 94:1875–1883. doi:10.2527/jas.2015-0010
- Corrigan, M.E., G.E. Erickson, T.J. Klopfenstein, M.K. Luebke, K.J. Vander Pol, N.F. Meyer, C.D. Buckner, S.J. Vannes, and K.J. Hanford. 2009. Effect of corn processing method and corn wet distillers grains plus solubles inclusion level in finishing steers. *J. Anim. Sci.* 87:3351–3362. doi:10.2527/jas.2009-1836
- Flatt, J.P., and Ball, E.G. 1964. Studies on the metabolism of adipose tissue. XV An evaluation of the major pathways of glucose catabolism influenced by insulin and epinephrine. *J. Biol. Chem.* 239:675.

- Folch, J., M. Lees, and G.H. S. Stanley. 1957. A simple method for the isolation and purification of total lipids from animal tissues. *J. Biol. Chem.* 226:497–509.
- Hong, Y.-H., Y. Nishimura, D. Hishikawa, H. Tsuzuki, H. Miyahara, C. Gotoh, K. Choi, D.D. Feng, C. Chen, H. Lee, K. Katoh, S. Rho, and S. Sasaki. 2005. Acetate and propionate short chain fatty acids stimulate adipogenesis via GPCR43. *Endocrinology* 146:5092–5099. doi:10.1210/en.2005-0545
- Huntington, G.B., D.L. Harmon, and C.J. Richards. 2006. Sites, rates, and limits of starch digestion and glucose metabolism in growing cattle. *J. Anim. Sci.* 86(E. Suppl.):E14–E24.
- Lee, S.C., H.J. Lee, D.W. Kim, J.G. Park, and I.K. Han. 2000. Effects of carbon precursors and hormones on the lipogenesis and lipolysis of Hanwoo cattle adipose tissues. *Asian Austral. J. Anim. Sci.* 13:300–306. doi:10.573/ajas.14.0481
- Lemosquet, S., E. Delamaire, H. Lapierre, J.W. Blum, and J.L. Peyraud. 2009. Effects of glucose, propionic acid, and nonessential amino acids on glucose metabolism and milk yield in Holstein dairy cows. *J. Dairy Sci.* 92:3244–3257.
- May, S.G., N.S. Burney, J.J. Wilson, J.W. Savell, A.D. Herring, D.K. Lunt, J.F. Baker, J.O. Sanders, and S.B. Smith. 1995. Lipogenic activity of intramuscular and subcutaneous adipose tissues from steers produced by different generations of Angus sires. *J. Anim. Sci.* 73:1310–1317.
- May, S.G., J.W. Savell, D.K. Lunt, J.J. Wilson, J.C. Laurenz, and S.B. Smith. 1994. Evidence for preadipocyte proliferation during culture of subcutaneous and intramuscular adipose tissues from Angus and Wagyu crossbred steers. *J. Anim. Sci.* 72:3110–3117.
- McAtee, J.W., and A. Trenkle. 1971. Metabolic regulation of plasma insulin in cattle. *J. Anim. Sci.* 33:438–442.
- 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 bovine adipose tissue explants. *J. Anim. Sci.* 69:162–170.
- Nayananjalie, W.A.D., T.R. Wiles, D.E. Gerrard, M.A. McCann, and M.D. Hanigan. 2016. Acetate and glucose incorporation into subcutaneous, intramuscular, and visceral fat of finishing steers. *J. Anim. Sci.* 93:2451–2459. doi:10.2527/jas.2014-8374.
- NRC. 2000. Nutrient requirements of beef cattle. 7th rev. ed. Washington, DC: National Academy Press.
- Oba, M., and M.S. Allen. 2003. Extent of hypophagia caused by propionate infusion is related to plasma glucose concentration in lactating dairy cows. *J. Nutr.* 133:1105–1112.
- Oh, Y.K., J.S. Eun, S.C. Lee, G.M. Chu, S.S. Lee, and Y.H. Moon. 2015. Responses of blood glucose, insulin, glucagon, and fatty acids in intraruminal infusion of propionate in Hanwoo. *Asian Austral. J. Anim. Sci.* 28:200–206. doi:10.5713/ajas.14.0481
- Prior, R.L., and R.A. Scott. 1980. Effects of intravenous infusions of glucose, lactate, propionate or acetate on the induction of lipogenesis in bovine adipose tissue. *J. Nutr.* 110:2011–2019.
- Rhoades, R.D., J.E. Sawyer, K.Y. Chung, M.L. Schell, D.K. Lunt, and S.B. Smith. 2007. Effect of dietary energy source on in vitro substrate utilization and insulin sensitivity of muscle and adipose tissue of Angus and Wagyu steers. *J. Anim. Sci.* 85:1719–1726. doi:10.2527/jas.2006.498
- Rhoades, R.D., J.E. Sawyer, C.H. Ponce, D.K. Lunt, C.W. Choi, and S.B. Smith. 2009. Substrate utilization and dose response to insulin by subcutaneous adipose tissue of Angus steers fed corn- or hay-based diets. *J. Anim. Sci.* 87:2338–2345. doi:10.2527/jas.2008.1365
- Schoonmaker, J.P., F.L. Fluharty, and S.C. Loerch. 2004. Effect of source and amount of energy and rate of growth in the growing phase on adipocyte cellularity and lipogenic enzyme activity in the intramuscular and subcutaneous fat depots of Holstein steers. *J. Anim. Sci.* 2004. 82:137–148.
- Smith, S.B. 1983. Contribution of the pentose cycle to lipogenesis in bovine adipose tissue. *Arch. Biochem. Biophys.* 221:46–56.
- Smith, S.B. 2017. Practical application of the basic aspects of GLUT4 membrane trafficking and insulin signaling on issues related to animal agriculture. *J. Anim. Sci.* 95:2185–2197. doi:10.2527/jas.2016.0984
- 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.* 114:792–800.
- Smith, S.B., G.W. Go, B.J. Johnson, K.Y. Chung, S.H. Choi, J.E. Sawyer, D.T. Silvey, L.A. Gilmore, G. Ghahramany, and K.H. Kim. 2012. Adipogenic gene expression and fatty acid composition in subcutaneous adipose tissue depots of Angus steers between 9 and 16 months of age. *J. Anim. Sci.* 90:2505–2514. doi:10.2527/jas.2011-4602
- Smith, S.B., and R.L. Prior. 1982. The effect of 3-mercaptopicolinic acid and substrate interactions on the incorporation of lipogenic precursors into glyceride-glycerol, glyceride-fatty acids and nonesterified fatty acids in bovine adipose tissue. *Biochim. Biophys. Acta* 712:365–373.
- Smith, S.B., R.L. Prior, C.L. Ferrell, and H.J. Mersmann. 1984. Interrelationships among diet, age, fat deposition and lipid metabolism in growing steers. *J. Nutr.* 114:153–162.
- Song, M.K., H.J. Sohn, S.K. Hong, and H.C. Kim. 2001. Utilization of substrate for the in vitro lipid synthesis in the adipose tissue of Hanwoo steers. *Asian Austral. J. Anim. Sci.* 14:1560–1563.
- Urrutia, N., Y. Ying, and K.J. Harvatine. 2017. The effect of conjugated linoleic acid, acetate, and their interaction on adipose tissue lipid metabolism in nonlactating cows. *J. Dairy Sci.* 100:5058–5067. doi:10.3168/jds.2016-12232
- Wickersham, T.A., E.C. Titgemeyer, R.C. Cochran, and E.E. Wickersham. 2009. Effect of undegradable intake protein supplementation on urea kinetics and microbial use of recycled urea in steers consuming low-quality forage. *J. Anim. Sci.* 101:225–232. doi:10.1017/S0007114508995672