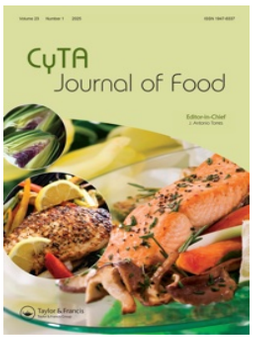
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CyTA - Journal of Food

ISSN: 1947-6337 (Print) 1947-6345 (Online) Journal homepage: www.tandfonline.com/journals/tcyt20

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To cite this article: Meghan L. Spears , Gwendolyn Cooper , Brett Sather , Emily Peterson , Jane A. Boles , Brian Bothner & Mary. P. Miles (2025) Postprandial amino acids and health markers from organic grass-fed and conventional beef in healthy participants – a pilot randomized clinical trial, CyTA - Journal of Food, 23:1, 2554230, DOI: 10.1080/19476337.2025.2554230

To link to this article: https://doi.org/10.1080/19476337.2025.2554230

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CYTA – JOURNAL OF FOOD
2025, VOL. 23, NO. 1, 2554230
https://doi.org/10.1080/19476337.2025.2554230

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Postprandial amino acids and health markers from organic grass-fed and conventional beef in healthy participants – a pilot randomized clinical trial

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ABSTRACT

This study investigates the impact of organic grass-fed (GRA) versus conventional (CON) cattle-feeding systems on human postprandial metabolism. A randomized, double-blind crossover study was used to compare GRA and CON beef consumption in healthy participants ($n = 10$). Blood samples from participants were collected at fasting and postprandially after consuming a 6-ounce steak from each condition. Triglycerides (TG), cholesterol (CHOL), high-density lipoprotein (HDL), glucose (GLU), and insulin (INS), eight inflammation markers, and 16 amino acids were measured. Analysis utilized net area under the curve (AUC), linear models, and repeated measures ANOVA. Postprandial lipid and glucose responses did not differ. INS AUC was significantly greater in CON than GRA ($p = .006$). Inflammation response did not differ. Leucine AUC was greater in CON than GRA ($p < .05$). These results suggest that when consuming CON and GRA beef, lipid and inflammatory responses are similar, but amino acid differences may influence insulin response.

ARTICLE HISTORY
Received 7 March 2025
Accepted 24 August 2025

KEYWORDS
Amino acids; conventional beef; insulin; health implications; organic grass-fed beef; postprandial

Introduction

Diet is one of the key factors influencing markers of health along with postprandial glucose, triglyceride, and inflammation response in humans. Dysregulation of these factors can lead to the development of chronic diseases such as cardiovascular disease (CVD) and metabolic syndrome (Ricordi et al., 2015). Particularly, the habitual consumption of red meat, such as beef in Western diets, has been linked to increased risk for developing type 2 diabetes mellitus (T2DM), CVD, and the promotion of systemic inflammation (Van Hecke et al., 2016; Wolk, 2017). A closer look at feeding methods suggests that cattle-feeding methods could be associated with these health implications (Klopatek et al., 2022). Organic grass-fed feeding systems (GRA) differ from conventional techniques (CON) in that GRA cattle consume more grass and/or forage in their lifetime than CON. It has been shown that grass-fed methods can have a greater content of polyunsaturated fatty acids (PUFA), notably omega-3 PUFA's, compared to CON feeding methods, which can have greater mono-unsaturated fatty acid (MUFA) and saturated fatty acid (SFA) content (Leheska et al., 2008; Nogoy et al., 2022; van Vliet et al., 2021). These nutritional components in GRA cattle have been associated with a favourable lipid response and the attenuation of inflammation (Alfaddagh et al., 2020; Ricordi et al., 2015). However, this has been contradicted when mildly hypercholesterolaemic men consumed pasture fed beef burgers for 3 weeks and observed elevated triglyceride levels compared to grain-fed beef burgers from cattle fed a high oleic diet (Adams et al., 2010). Understanding the human postprandial response to consuming beef from different cattle-feeding systems can help to bridge the gap between beef production and the health implications for consumers.

When considering the impact of dietary habits on an individual's risk for developing chronic diseases, the acute postprandial response to a meal is important to understanding the link between acute responses and long-term impacts (Meessen et al., 2019). A meal with high saturated fat content is known to impact subsequent fat metabolism, contributing to and exacerbating conditions like hypertriglyceridemia (Chan et al., 2013; Yuan et al., 2007). The postprandial glycemic response is also of interest in relation to metabolic diseases, such as T2DM, and how the consumption of a fat and/or protein source can impact this response (Basturk et al., 2021; Pasmans et al., 2022). In general, consumption of a protein source with carbohydrates is considered beneficial for glycemic control, but high red meat consumption has also been linked to increased risk for insulin resistance according to epidemiological data (Van Loon et al., 2000; Zelber-Sagi et al., 2018). There have also been examples of favourable glycemic and insulin responses to red meat consumption, of which they do not contribute to elevated risk factors for T2DM (Sanders et al., 2023). The amino acid composition of protein sources can also impact insulin and glucose postprandial responses, for example, leucine promotes insulin secretion and attenuates glucose responses (Sloun et al., 2020). An increase in inflammatory markers and mediators, such as lipopolysaccharides (LPS), leukocytes, and tumor necrosis factor-α (TNF-α) is expected after a meal due to the metabolism of carbohydrates, fats, and protein (Meessen et al., 2019). The magnitude of this

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Supplemental data for this article can be accessed online at https://doi.org/10.1080/19476337.2025.2554230.

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response may impact chronic inflammation state (Mazidi et al., 2021) and components of the diet can alter this response. For instance, *n*-3 PUFA's have potential anti-inflammatory and antioxidant capabilities, via the production of RvD2 from *n*-3 docosahexanoic acid (DHA), which suppresses the release of pro-inflammatory cytokines, among other pathways (Oppedisano et al., 2020; Shibabaw, 2021). This anti-inflammatory effect of *n*-3 PUFA's is observed when doses of 4 g/day are administered (Hernandez et al., 2021). Grass-fed beef has been reported to have a total *n*-3 PUFA content ranging from 0.0312 to 0.0976 g/100 g beef while grain-fed beef ranges from 0.0235 to 0.0628 g/100 g beef (Nogoy et al., 2022). While this does not meet the full daily dose of *n*-3 PUFA to benefit from anti-inflammatory effects, this showcases that grass and grain fed beef can contribute to the daily intake of *n*-3 PUFAS's. Cattle-feeding systems can impact the nutritional composition of beef. This is because cattle diet contributes to carcass characteristics and nutrient composition (Allen et al., 1996; Leheska et al., 2008). This makes cattle-feeding systems a point of interest to consider for dietary choices of its consumers.

Current literature is limited to the assessment of the nutritional composition of GRA and CON beef, lacking investigation into the postprandial amino acid and lipid response of human consumers. Recently, this gap in knowledge was addressed by assessing the impact of CON and GRA beef on human postprandial metabolomics and the metabolomic profiles of the beef itself (Spears et al., 2024). It was shown that these cattle-feeding systems differentiated whole beef and postprandial metabolomics of consumers (Spears et al., 2024). In general, beef from grass-finished animals has been shown to have less total fat content than CON, as well as greater amounts of *n*-3 fatty acids (Leheska et al., 2008; Nogoy et al., 2022). Essential long chain *n*-3 PUFA's have been linked to prevention of CVD, making it a component of interest in the diet (Oppedisano et al., 2020). Red meat consumption has been linked to increased postprandial inflammation, slower triglyceride clearance, and greater insulin secretion in epidemiological data (Wolk, 2017), but intervention studies demonstrate no difference or favourable reductions in insulin after beef consumption (O'Connor et al., 2020; Svelander et al., 2015). Amino acid profiles have not been compared between beef finished either on GRA or CON, and the possible differences in nutritional composition of GRA and CON beef may impact these responses (Alekseeva & Kolchina, 2019; Hollo et al., 2001).

The purpose of this study was to characterize acute postprandial responses to GRA and CON cattle-feeding systems in humans. This included the magnitude and timing of amino acid, triglyceride, glucose, and insulin responses. It was hypothesized that there will be a difference in postprandial measures between beef, either GRA or CON finished. There are some potential differences that may produce a more favorable postprandial response to GRA; however, many factors in beef have the potential to impact postprandial response and the net effect of differences between feeding methods is not known. This study assesses acute postprandial lipid, glycemic, inflammatory, and amino acid responses in humans to GRA and CON beef. The use of a healthy population of men and women was intentional to set a baseline for postprandial responses to beef in this scenario.

Materials and methods

Study population

Participants were recruited from August 2022 to March 2023 in Bozeman, MT on a rolling basis by word of mouth. Inclusion criteria consisted of being between 18 and 45 years old and having a body mass index (BMI) of 18–27 kg/m^2. Anthropometrics, including BMI and visceral adipose tissue (VAT), were measured with segmental multifrequency bioelectrical impedance analysis (Seca mBCA 515, Hamburg, Germany). Waist circumference (WC) was measured in triplicate with an anthropometric measuring tape. Participants were excluded from participation if they had preexisting conditions including allergy to red meat, a gall bladder condition, diabetes, or taking medication to lower cholesterol, blood pressure, and inflammation. Screening of potential participants was managed using REDCap (version 13.10.6) (Harris et al., 2019; Spears et al., 2024).

Experimental design

The study was a randomized, double-blind crossover clinical trial with two conditions, GRA and CON beef. The study protocol was approved by the Institutional Review Board at Montana State University (2022-138-EXPIDITED). This study was prospectively registered with ClinicalTrial.gov (NCT05460754). On three occasions, participants came to the Nutrition Research Laboratory for testing. Informed consent was reviewed verbally in-person and obtained from all participants prior to their participation in the study. The first visit consisted of an informed consent review followed by anthropometric measurements and a general health questionnaire. The second and third visits occurred at least 7 days apart and included the fasting and postprandial blood collection, every half-hour for four hours following a dietary intervention of steak. Primary outcome measures, postprandial lipid, glycemic, and amino acid responses, were analyzed from plasma samples taken at each timepoint. Secondary outcome measures included anthropometric measures taken at baseline. Samples of beef from both CON and GRA were analyzed via proximate analysis to assess fatty acids, fat, protein, moisture, and ash differences (Spears et al., 2024). An overview of the study design is provided in Figure 1.

Dietary intervention and blood sample collection

A randomization protocol determined the order in which participants received steaks at visits 2 and 3. Participants were randomly assigned the order in which steaks from GRA and CON were administered by random number assignment and balanced based on visit number. This process is

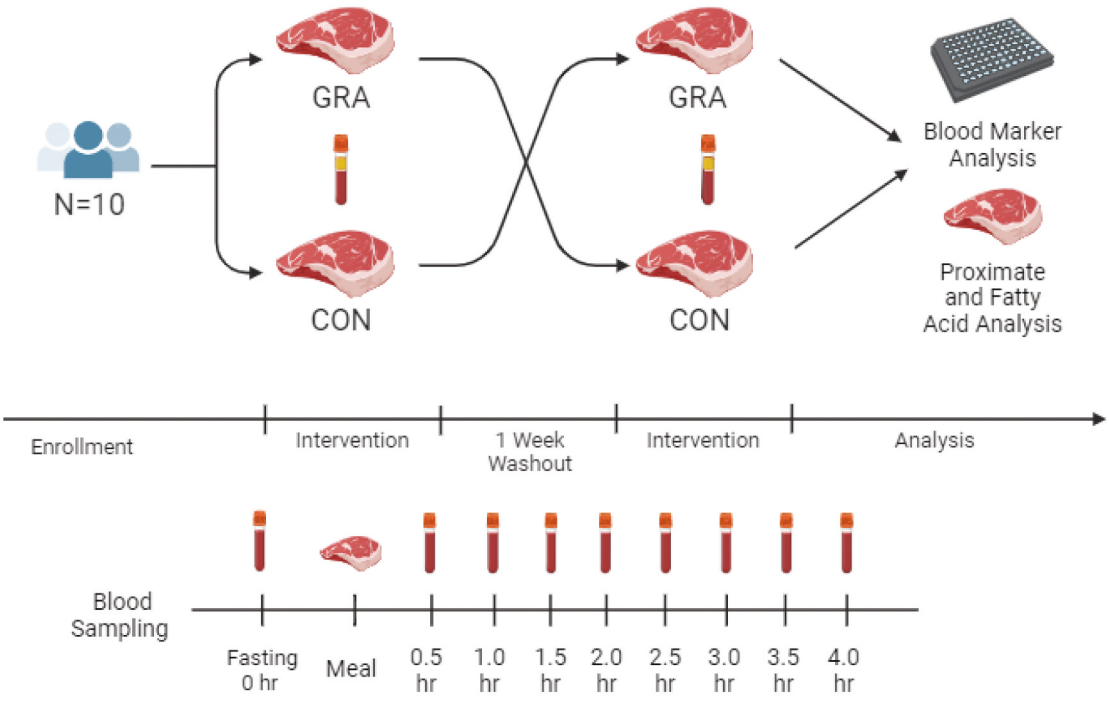


Figure 1. Study design. Created in BioRender. Spears, M. (2024) https://BioRender.com/e88p038.

described in more detail in a previous paper from this study (Spears et al., 2024).

The study followed a cross-over design. All participants received a steak from each condition (GRA and CON). Participants were instructed during participation, beginning 72 h before visit 2 and ending at the completion of visit 3, to not consume any red meat for this period and avoid fatty fish the day before visit 2 and 3. Daily diet before visit 2 and visit 3 was then replicated to improve comparability.

Participants were instructed to arrive at visits 2 and 3 having fasted for 12 h and to not have participated in strenuous activity or the consumption of alcohol for 24 h prior to visits. A phlebotomist or medical doctor inserted an intravenous catheter in the antecubital vein of participants. Blood samples were collected into plasma separating heparin vacutainer tubes (BD Vacutainer, Frankline Lakes, NJ, U.S.A.) after a 3 mL waste withdrawal followed by a sterile saline flush. Fasting samples were taken 15 min after insertion of the catheter. Postprandial blood samples were collected after participants began eating the study steak, every 30 min for 4 h beginning 30 min. Samples were centrifuged at 14,515 × g for 15 min. The plasma was then aliquoted and frozen at −80°C for future analyses (Spears et al., 2024).

Steak preparation

CON beef choice strip loin was sourced from a local purveyor and the GRA beef strip loin was sourced from B Bar Ranch Big Timber, MT. All steaks were cut from the longissimus lumborum muscle and matched for marbling. Two cows per condition were used to source strip loin cuts. Steaks were vacuum sealed and stored at −20°C until needed. All steaks were removed from the freezer and thawed in a refrigerator between 24 and 48 h before participant visits. Weight of the raw steak was recorded before cooking. Steaks were cooked on a clamshell grill with a thermometer (ThermoWorks THS-313-158 probe with Therma Waterproof THS-232-101) inserted into the thickest part of the meat until the thermometer read 70°C. Weight was recorded immediately following removal from the grill. The steak was then wrapped in tin foil and rested for 5 min. After 5 min of rest, the steak was cut to 170.010 g (6 ounces) and served to participants (Spears et al., 2024).

Proximate and fatty acid analysis

Percent moisture and ash, as well as crude protein (%) and crude fat (%) were analyzed using Association of Official Agricultural Chemists (AOAC) methods 950.46, 923.03. 983.23 and 992.15 (Latimer et al., 2006; Cunniff, 1995 Narayanan Nair, 2021). Fatty acid profile analysis followed established protocols utilizing gas chromatography (Phillips et al., 2010). Tests were performed in duplicate with two samples from each GRA and CON.

Blood marker analysis

Total cholesterol (CHOL), high-density lipoprotein cholesterol (HDL), glucose (GLU), and triglycerides (TG) were measured at fasting and hourly timepoints using whole blood collected in a sodium heparin tube using a chemical analyzer (Picollo Xpress Chemistry Analyzer, Abaxis, Union City, CA, U.S.A.).

Insulin analysis

Plasma insulin (INS) was measured with ELISA (MP Biomedicals, Irvine, CA, U.S.A.) at fasting and hourly timepoints. The analysis was performed according to manufacturer's instructions, and samples were run in duplicate. A coefficient of variability between duplicate samples above 15% indicated the need to re-run the sample. The average coefficient of variation for each cytokine ranged

from 3 to 14%. The average of duplicate samples was used for analysis.

Inflammation marker analysis

The concentrations of eight cytokines were measured at hourly timepoints using a high-sensitivity multiplex assay (MILLIPLEX MAP Human Cytokine/Chemokine Magnetic Bead Panel Immunology; Millipore, Billerica, MA, U.S.A.) on a Bio-Rad Luminex. Included cytokines were granulocyte macrophage colony stimulating factor (GMCSF), interferon-gamma (IFN-γ), tumor necrosis factor alpha (TNF-α), and interleukins (IL) IL-1β, IL-23, IL-17, IL-10, and IL-6. Plasma samples from fasting and hourly timepoints were run in duplicate. The analysis was repeated if coefficient of variability was greater than 15%. Average coefficient of variation for each cytokine ranged from 2 to 12%. Hourly timepoints were used for inflammation analysis to coincide with the literature related to postprandial inflammation (Emerson et al., 2017).

Amino acid analysis

For sample extraction, 200 µL of plasma from fasting and half-hourly timepoints were used from frozen 1 mL centrifuge tubes. After thawing and a brief vortex, 100 µL of each sample were pipetted into a fresh 1 mL centrifuge tube. The remainder was stored at −80°C. A 4:1 v/v amount of acetone (400 µL) that had been stored at 3°C was added to the 100 µL thawed sample, briefly vortexed and placed into the 3°C fridge for 30 min followed by a −20°C freezer for 30 min. The samples were then placed into a −80°C freezer for 12 h. Following this, samples were centrifuged at 16,100 × g for 20 min. 100 µL of the supernatant was extracted and then dried down in an Eppendorf Vacufuge plus set to an aqueous vacuum mode at 30°C for 3 h. The remainder was stored at −80°C. Samples were resuspended in 1:1 Acetonitrile/N-tert-butyldimethylsilyl-N-methyltriflueoroacetamide (MTBSTFA) solution (50 µL/50 µL) and heated for 4 h at 80°C in a PCR thermal cycler with a heated lid option that was active. 100 µL of sample was then transferred to mass spectrometry vials with a 300 µL conical, glass insert and run on the Agilent 7890A GC/MS. An amino acid standard was used to validate identification of peaks and spectra (Millipore, Billerica, MA, U.S.A.).

Statistical analysis

Statistical power of this study was calculated using G*Power 3.1.9.7. A large effect size (Cohen's d = 0.80) was determined using the TG net area under the curve (AUC) results. A post hoc, paired-samples t-test with alpha level of 0.05 and the sample size of 10 participants achieved power at 77%.

Statistical analysis and visualizations were performed in RStudio (2024.04.0 + 735). Response to beef was analyzed by combining and averaging results from both GRA and CON. Response to cattle-feeding system analyzed results from CON and GRA separately.

AUC was calculated for all markers by subtracting the area below baseline from the area above baseline (Le Floch et al., 1990). Normal distribution of data for analysis was checked with histograms and Shapiro-Wilk test. Log10 was used when necessary to transform data. A one-sample Wilcox test was used to determine if the average AUC for each marker was significantly greater than zero in response to beef consumption. Simple linear modeling and ANOVA were used to compare AUC between beef types. Two-way repeated measures ANOVA (fasting (0), 1, 2, 3, and 4 h) were used to determine the effect of time, beef type, and the interaction of these. The repeated measures accounted for were time and participant. Visceral adiposity was added as a covariate in linear models and repeated measures ANOVA for postprandial inflammation and lipid response as it was significantly higher in male participants and is known to impact these measures (Nishida et al., 2007; Taira et al., 1999). The maximal change (maxchange) in cytokine values after fasting (maximum cytokine value reached between hours one and four (CMAX) – fasting value) was calculated for each cytokine and then compared to zero using a one-sample t-test with and without consideration for condition. The time postprandially at which this maximal value between hours one and four (CMAXtime) occurred was also recorded.

Results

Characteristics of participants

A total of 18 men and women were assessed for eligibility by completing a REDCap survey to assess eligibility criteria between August 2022 and March 2023. Of the 18, 17 met eligibility criteria and were invited to continue participation in the study. Two participants were denied entrance into the study, even if eligibility criteria were met, when the number of participants per sex was previously met (five male, five female). Of the 15 participants who were entered into the study, four dropped out during visits two or three due to inability to maintain intravenous catheter integrity and one voluntarily dropped out due to conflicting commitments (Figure S1). The average age of participants was 26.6 ± 5.8 years with an average

Table 1. Participant baseline characteristics by sequence and total. Fasting values of GLU and TG represent the average fasting value from both study visits. Values are presented as mean ± standard deviation (SD). *indicates a p value < 0.05 between male and female participants. BMI; body mass index, WC; waist circumference, GLU; glucose. TG; triglyceride.

	Condition sequence		
Characteristic	CON then GRA (n = 5)	GRA then CON (n = 5)	Total (n = 10)
Age (years)	29.0 ± 7.3	24.2 ± 2.6	26.6 ± 5.8
Sex (M/F)	3/2	2/3	5/5
BMI (kg/m^2)	25.26 ± 1.14	23.62 ± 2.80	24.44 ± 2.20
WC (cm)	85.98 ± 7.20	78.67 ± 8.40	82.33 ± 8.32*
Fasting GLU (mmol/L)	5.83 ± 1.11	5.12 ± 0.21	5.47 ± 0.81
Fasting TG (mmol/L)	1.15 ± 0.58	0.80 ± 0.16	0.98 ± 0.45

BMI of 24.44 ± 2.20 kg/m2. VAT and WC was significantly greater in male participants than female participants ($p = .003$, $p = .005$). Final participant ($n = 10$) characteristics for those participants successfully completing both conditions are described in Table 1, separated by the condition sequence order. There were no significant differences in these baseline measures when participants are evaluated by condition sequence order ($p > .1$). These baseline characteristics are also reported in a previous paper with these same participants (Spears et al., 2024).

Proximate and fatty acid analysis

Percent moisture, ash, protein, and fat results from the proximate analysis are described in Table 2. Fatty acid analysis results are also described in Table 2.

Postprandial lipid response

The TGAUC and CHOLAUC responses to beef were significantly greater than 0 (TGAUC $p = .005$, CHOLAUC $p = .042$), but not HDLAUC ($p = .348$). Between CON and GRA, TG ($F = 2.46$, $p = .059$), CHOL ($F = 1.71$, $p = .277$), and HDL ($F = 1.58$, $p = .635$) AUC were not significantly different (Figure 2a). Two-way repeated measures ANOVA were performed to evaluate the effect of cattle-feeding system and time on TG, HDL, and CHOL responses. There was a significant main effect of time on TG response ($F(4,36) = 18.7$, $p < .001$). Pairwise T-test with Bonferroni corrections showed that TG response to beef was significantly greater than fasting (0 h) at hours 3 (p.adj = 0.018) and 4 (p.adj = 0.010) as well as between hours 1 and 3 (p.adj = 0.009) and hours 1 and 4 (p.adj = 0.005) postprandially (Figure 2b). There was no interaction between cattle-feeding system and time for TG ($F(1.51,12.2) = 3.18$, $p = .087$), HDL ($F(4, 32) = 0.314$, $p = .866$), or CHOL ($F(1.68,13.5) = 1.15$, $p = .335$) postprandial response.

Table 2. Proximate analysis and fatty acid analysis. Values are presented as the average ± sample SD of both samples run in duplicate for GRA and CON. Total is the sum of all individual fatty acids by saturation and feeding style ± sample SD. ND; not detected.

Feeding System	Proximate Analysis and Fatty Acid Analysis								
	Proximate Analysis								
	% Fat		% Protein		% Ash		% Moisture		
GRA	7.3 ± 0.1		22.7 ± 0.6		0.9 ± 0.0		70.3 ± 1.3		
CON	7.6 ± 0.1		20.8 ± 0.2		0.9 ± 0.0		70.9 ± 0.2		
	Saturated fatty acids (SFA)								
	Total	C12:0	C14:0	C16:0	C17:0	C18:0	C20:0	C24:0	
GRA	41.8 ± 7.9	4.7 ± 0.2	2.4 ± 0.1	21.1 ± 0.1	1.1 ± 0.1	12.3 ± 0.4	ND	0.1 ± 0.0	
CON	39.8 ± 7.4	4.8 ± 0.2	2.1 ± 0.3	19.4 ± 1.3	1.1 ± 0.1	12.3 ± 0.9	ND	0.1 ± 0.0	
	Monounsaturated fatty acids (MUFA)								
	Total	C14:1	C16:1	C17:1	C18:1c9	C18:1c11	C20:1	t-vaccenic	
GRA	40.7 ± 13.5	0.6 ± 0.1	3.4 ± 0.1	0.9 ± 0.0	34.2 ± 0.4	1.6 ± 0.0	0.1 ± 0.1	2.9 ± 0.2	
CON	38.8 ± 13.0	0.4 ± 0.1	2.9 ± 0.3	0.9 ± 0.1	33.0 ± 2.0	1.6 ± 0.1	ND	2.5 ± 0.6	
	Polyunsaturated Fatty Acids (PUFA)								
	Total	C18:2	C18:3	C18:2c9t11	C18:2t10c12	C20:2	C20:4	C20:5	C22:6
GRA	4.5 ± 1.2	3.5 ± 0.2	0.03 ± 0.1	0.3 ± 0.0	ND	ND	0.6 ± 0.0	ND	ND
CON	5.2 ± 1.4	4.1 ± 0.5	0.04 ± 0.1	0.3 ± 0.0	ND	ND	0.8 ± 0.2	ND	ND

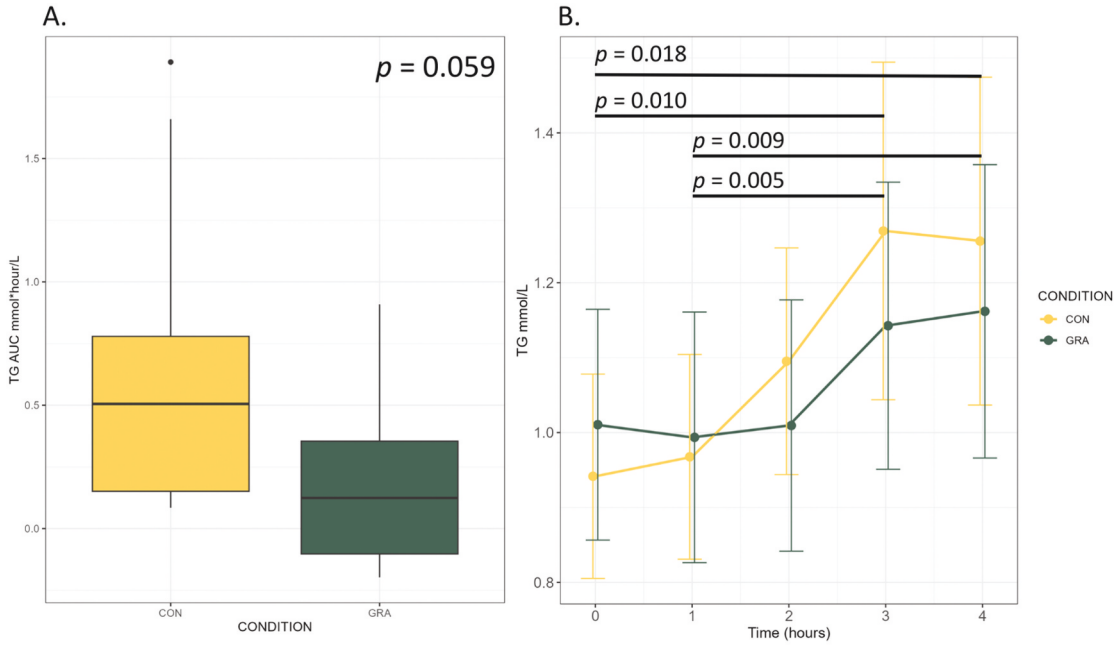


Figure 2. Results from the two-way repeated measures ANOVA of TG response between GRA and CON. (A) TG AUC boxplot. TG AUC was not significantly different between conditions ($p = .059$). (B) TG response from fasting (0) to four hours postprandially. The main effect of time for the two-way repeated measures ANOVA was significant between hours 0–4 ($p = .018$), 0–3 ($p = .010$), 1–3 ($p = .009$), and 1–4 ($p = .005$) were significantly different. Boxplots in a represent the median with whiskers extending to the minimum and maximum values. Error bars of B represent the standard error (SE).

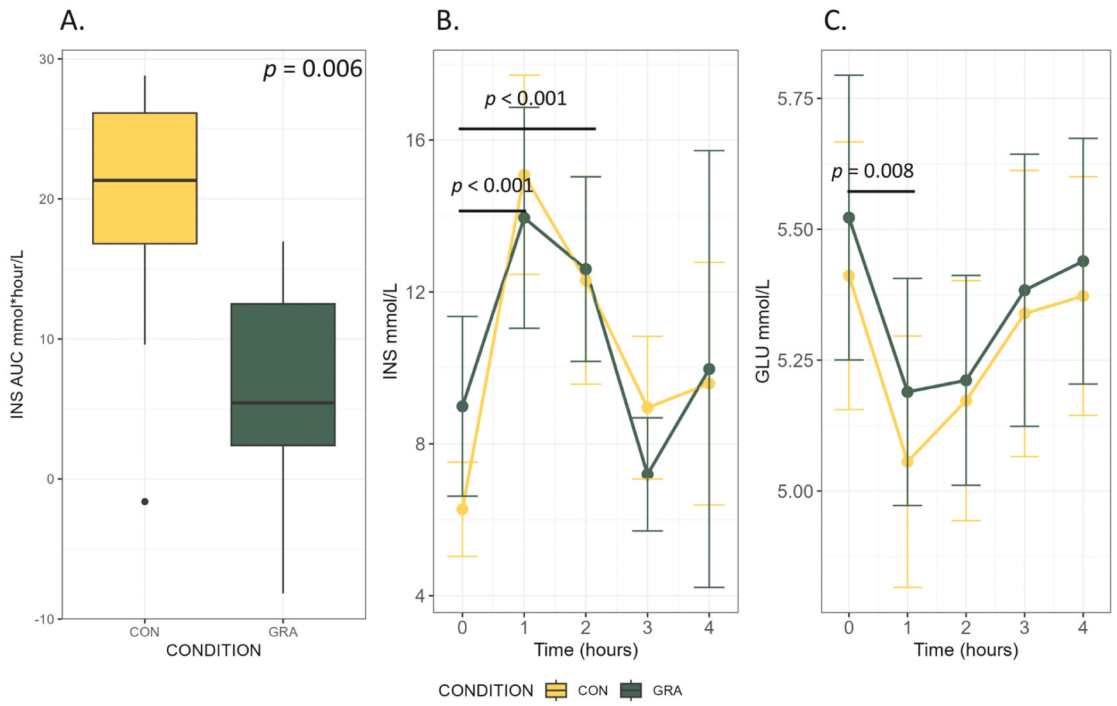


Figure 3. Results from the two-way repeated measures ANOVA of INS and GLU response between GRA and CON (A) insulin AUC boxplot. INS AUC was significantly greater in CON ($p = .006$). (B) Insulin and (C) glucose response from fasting (0) to 4 hours postprandially. The main effect of time for the two-way repeated measures ANOVA was significant between fasting and hours 1 ($p < .001$) and 2 ($p < .001$) in insulin and fasting and hour 1 ($p = .008$) in glucose. Boxplots in a represent the median with whiskers extending to the minimum and maximum values. Error bars of B and C represent the standard error (SE). One outlier was removed from both INS AUC and INS postprandial responses for statistical analysis and visualization.

Postprandial glycemic response

The INS AUC was significantly increased from 0 ($F = 5.13$, $p = .007$), but not GLU AUC ($F = 0.851$, $p = .744$). The INS AUC was significantly greater in CON ($F = 9.71$, $p = .006$) than GRA (Figure 3a). Two-way repeated measures ANOVA showed that there was a main effect of time for the INS ($F(4,32) = 8.87$, $p < .001$) and GLU ($F(4,32) = 5.34$, $p = .002$) responses to beef (Figure 3b and 3). Pairwise t-test showed that INS responses significantly differed from fasting (0) at hours 1 (p.adj < 0.001) and 2 (p.adj < 0.001). GLU responses significantly differed from fasting (0) at hour 1 (p.adj = 0.008). The interaction of cattle-feeding system and time did not impact INS and GLU postprandial responses ($p > .05$).

Postprandial inflammation response

Only IFNγ AUC ($p = .042$) was significantly greater than zero after consuming beef. There were no differences between the AUC in each of the eight cytokines between GRA and CON ($p > .05$). The maxchange in response to beef was significantly greater than zero for all eight cytokines ($p < .05$). CMAXtime favored hour three for five out of eight cytokines. Two-way repeated measures ANOVA showed that postprandial inflammation was not impacted by main effects or interaction of cattle-feeding system and time ($p > .05$). The maxchange within GRA and CON was significantly greater than 0 for all by one cytokine in GRA (Table S1). Between GRA and CON, the maxchange was not significantly different. CMAXtime was evenly distributed between hours two and three within GRA and CON.

Postprandial amino acid response

Amino acids detected via gas chromatography included alanine, glycine, valine, leucine, isoleucine, proline, methionine, serine, threonine, phenylalanine, aspartic acid, glutamic acid, lysine, histidine, tyrosine, and cysteine. All AUC were significantly greater than 0 ($p < .007$), except lysine ($p = .05$), aspartic acid ($p = .111$), and tyrosine ($p = .803$) in response to beef. In response to cattle-feeding system, leucine AUC ($F = 21.4$, $p = .0002$) was significantly greater in CON than GRA (Figure 4). Two-way repeated measures ANOVA were performed to evaluate the effect of the cattle-feeding system over time on amino acids responses. There was a main effect of time for leucine ($F(F(3.31,29.8) = 4.825$, $p = .006$), valine ($F(2.76,24.9) = 3.461$, $p = .034$), threonine ($F(3.14,28.3) = 3.261$, $p = .034$), and isoleucine ($F(3.11,27.9) = 5.681$, $p = .003$). Significant differences between hours are shown in Figure S2. There was not an interaction between cattle-feeding system and time for any amino acids ($p > .05$).

Discussion

This study adds insights into the topic of human lipid, glycemic, inflammatory, and amino acid postprandial responses to beef, with and without consideration for the cattle-feeding system that produced the beef. Key findings include a significant increase in postprandial TG, CHOL, inflammation, and multiple amino acids. A significantly greater INS response to CON but no difference in inflammation or lipid response between grass-fed beef and conventional beef was also observed. Leucine response was greater when CON beef was consumed than GRA and significantly increased over time along with valine, threonine, and isoleucine. The beef used in this study was matched for marbling before analysis,

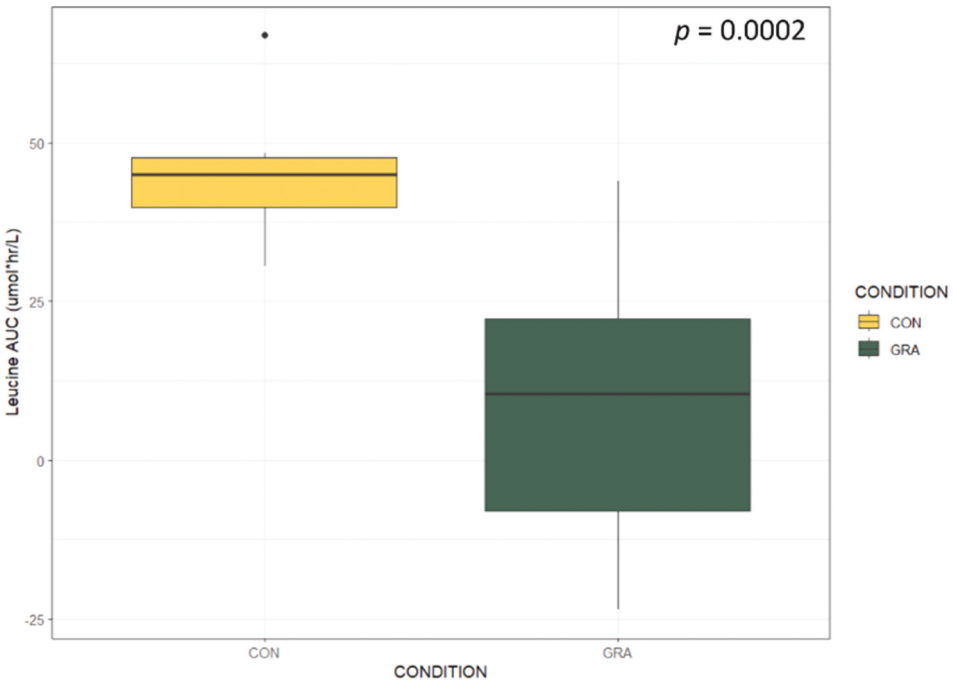


Figure 4. Leucine AUC boxplot. Leucine AUC was significantly greater in CON ($p = .0002$). Boxplot represents the median with whiskers extending to the minimum and maximum values.

resulting in beef that did not differ in fatty acid composition (Table 2). Matching for marbling allowed our comparison to identify fundamental differences between GRA and CON beyond known impacts of higher fat content. When using matched beef from grass-fed and conventional cattle-feeding systems postprandial lipid, glucose, and inflammation responses were similar.

The results of the proximate and fatty acid analysis suggest a minimal difference between the composition of GRA and CON beef meat (Table 2). To achieve a comparable comparison of meat samples from GRA and CON, samples used for proximate and fatty acid analysis were matched for marbling. A similar strategy was utilized in another study to relieve total fat intake as a confounding variable to postprandial response (Gilmore et al., 2011). These results differ from research done by Leheska et al., analyzing nutrient composition of grass-fed and conventional feeding systems on beef strip steaks using proximate analysis and fatty acid gas chromatography analysis. In this study the moisture percent was significantly greater in GRA beef and fat percent was significantly greater in CON beef. Both protein and ash percent were higher in conventional, but not significantly. The fatty acid composition between GRA and CON beef showed significant difference between total SFA and MUFA composition, but not total PUFA composition (Leheska et al., 2008). In our findings, all four proximate analysis measures differed by 1–1.9% between GRA and CON. Our findings suggest no more than a 2% difference between total SFA and MUFA composition and less than a 1% difference in PUFA composition between GRA and CON. Omega-3 fatty acid composition was greater in CON, while omega-6 fatty acids were not detected in this analysis.

While there were no significant differences in the postprandial lipid response to GRA and CON, beef consumption did increase CHOL and TG. The postprandial lipid response suggest that TG and CHOL significantly increased whereas HDL did not change. The beef used in this study was between 7.3 and 7.6% fat (about 12.6 g of fat per 6oz steak). This is less fat than other studies assessing postprandial responses to beef that use beef containing 19% fat but concurs with the findings that postprandial TG and CHOL responses increase after consuming beef or beef fat (Muesing et al., 1995; Svelander et al., 2015). The beef used in our study was lean, and the opposite HDL response was observed. This corresponds with other research showing that leaner beef (5% fat) decreased HDL concentrations after 5 weeks (Lytle et al., 2023). In our study, CON and GRA beef had similar postprandial lipid responses. AUC values of TG, CHOL, and HDL were not different between cattle-feeding systems and no difference at certain timepoints from fasting to 4 h differed between groups. Since the fat content and fatty acid composition of CON and GRA beef used in this study were comparable, similar postprandial lipid responses can be expected. We did observe a significant increase in TG and CHOL in response to beef. Similar findings have been reported that consumption of beef can increase CHOL and TG when compared to lean beef and coconut oil (O'Dea et al., 1990; Reiser et al., 1985).

Glycemic responses are a critical dietary measure; therefore, we measured postprandial INS and GLU. In response to consuming beef, INS increased significantly while GLU did not. To understand these results, we must note that the meal given to participants in this study contained only cooked, unseasoned steak. Other studies tracking postprandial response to a beef only meal have not measured glycemic response (Arya et al., 2010; Turner et al., 2016). A study providing a mixed meal containing red meat and a carbohydrate source reports that GLU and INS increase 30 min after consumption (Turner et al., 2016). The lack of GLU response above fasting levels is consistent with findings showing no change in glucose concentration after consuming only protein (Westphal et al., 1990).

A significant INS response to beef was present one hour after consumption as well as a significantly greater INS AUC

in CON compared to GRA conditions. This INS response to consuming beef may be related to the insulinotropic effect of leucine in which leucine-rich foods increase insulin concentration (Sloun et al., 2020). Leucine simulates mammalian target of rapamycin complex 1 (mTORC1) and S6L1 kinase signalling, increasing insulin secretion (Melnik, 2012). Our data suggest that the leucine AUC was greater in CON than GRA condition which is consistent with the larger INS response to CON beef.

Postprandial inflammation is another important health marker that was measured. Inflammation did increase in response to beef; however, there was no difference between finishing approach. The maxchange response of all cytokines (GMCSF, IFN-γ, TNF-α, IL-1β, IL-23, IL-17, IL-10, and IL-6) were significantly increased, demonstrating that the response to beef rose above fasting values within the four-hour postprandial window. These findings are congruent with current knowledge on the impact of short-term beef consumption on inflammation in rodents. Van Hecke et al. used multiple measures of inflammation (Trimethylamine N-oxide (TMAO), C-Reactive protein (CRP), and oxidative stress parameters) and found that after 14 days of ad libitum consumption of beef, inflammation increased in rats (Van Hecke et al., 2016). The findings of this study were that inflammation response did not differ between CON and GRA. It has been shown that leaner kangaroo meat compared to fattier beef has a more favorable inflammation response (Arya et al., 2010). Nutritional components, like SFA and *N*-6 PUFA's, are known to be related to increase inflammation response in humans. SFA has potential proinflammatory effects by promoting TLR4 signaling which stimulates the transcription of cytokines like TNF-α (Ruiz-Núñez et al., 2016). *N*-6 PUFA's have potential proinflammatory effects by promoting eicosanoid production (Poli et al., 2023). Since the GRA and CON finished beef used in our study were similar in SFA composition and *N*-6 PUFA's (arachidonic and linoleic acid), our inflammation results are expected.

Our analysis tracked 16 amino acids before and after beef consumption. Changes in serum abundance could be attributed to both beef and the cattle-feeding system. Beef is an important source of essential and non-essential amino acids, and postprandial amino acid responses have been documented in a variety of male participants (Uhe et al., 1992; Pennings et al., 2013; Prodhan et al., 2020). We found a higher abundance of L-threonine, aspartyl-serine, and derivatives of phenylalanine in GRA beef along with higher abundance of L-tryptophan in CON for the beef samples used in this study (Spears et al., 2024). The content of amino acids in beef is an important consideration for consumer health as amino acids have been associated with T2D and other aspects of metabolism (Rose, 2019). Elevated fasting levels of BCAA's have been associated with an increased risk for T2D, while elevated glutamine levels have been associated with the opposite (Chen et al., 2019; Ottosson et al., 2016). In this study, 13 of the 16 amino acids had a significant increase after consumption of beef. The three amino acids that did not show a response included lysine, tyrosine, and aspartic acid. These three amino acids have been shown to exist in smaller quantities compared to other amino acids in beef samples, so lacking a postprandial response of these is not abnormal (Oh et al., 2016). When comparing the response between CON and GRA, leucine displayed a differential response. The leucine AUC was significantly greater in CON beef than GRA. The larger postprandial leucine response in CON may be indicative of a larger amount of leucine in CON than GRA beef or quicker metabolism of leucine; however, our metabolomic analysis of the beef samples used in this study did not detect greater abundance of leucine in CON (Spears et al., 2024). Other studies of the composition of beef have shown that CON beef contains greater amounts of leucine compared to GRA beef (Gómez et al., 2022). Leucine is an essential branched chain amino acid that has been shown to reduce sarcopenia in older adults, play an important role in protein synthesis in a wide range of human tissues, and regulate insulin and glucose responses in skeletal muscle (Duan et al., 2015; Layman & Walker, 2006; Martínez-Arnau et al., 2019). This could be linked to the beneficial health implication of consuming beef for populations with health concerns pertaining to muscle mass maintenance and/or glycemic regulation. Postprandial timing also impacted leucine, isoleucine, threonine, and valine regardless of cattle-feeding system, suggesting that these four essential amino acids displayed significant increases over time from fasting compared to other detected amino acids.

This study improves our understanding of the postprandial responses to consuming beef and how cattle-feeding systems may impact this. There are limitations to the findings of this study that we recognize. First being the healthy population of individuals recruited. The use of a healthy population of men and women in this study allowed us to baseline postprandial responses to consuming beef from different cattle-feeding systems. Men and women were not expected to have different responses to beef consumption and the difference in VAT between our male and female participants did not impact results as explored in the statistical analysis (Arya et al., 2010). It is known that obesity and other metabolic health components can impact inflammation, glucose, insulin, and lipid values at fasting and after a meal (Kempf et al., 2006; Onat et al., 2006). The interpretation of these results applies to this specific study population, but further research is needed to understand the impact of beef and cattle-feeding systems in populations other than healthy adults. The sample size of 10 participants may also be considered a limitation as daily variation in metabolic measures may contribute to unexpected variations in response to the beef steaks. The baseline anthropometric, TG, and GLU measures of participants did not differ between subjects based on condition sequence order, reducing the external variations that could have influenced results. Power of this study was also slightly below (77%) generally accepted values (80%), a factor of the small sample size. Finally, the disparity in information between the cattle-feeding practices used to source GRA and CON beef used in the study limits inferences that can be made to specific cattle-feeding practices. The GRA beef was directly sourced from a local ranch in Big Timber, MT practicing grass-fed and finishing. The CON condition beef was sourced from a purveyor in Bozeman, MT, for which there is a lack of knowledge of the specific feeding practices, and rather information was derived from the label. The reason for sourcing the CON meat from a purveyor was to mimic the options a consumer would have when purchasing beef. While this is beneficial to mimicking realistic consumer scenarios, the lack

of information about the CON meat was sourced from limits direct comparison to the GRA condition.

In conclusion, the results of this study suggest that beef produced by CON cattle-feeding system produces a greater insulin and leucine response in healthy adult participants compared to beef produced by GRA cattle-feeding. When matched to the confounding influence of fat content differences between these two types of beef, this study highlights that consumption of CON and GRA beef elicits similar postprandial lipid, glucose, and inflammation responses. Knowing these differences in insulin and leucine response can be beneficial for populations with glycemic control concerns, pertaining to their dietary choices. Further investigation is needed to understand the impact of long-term consumption of beef from various cattle-feeding methods.

Acknowledgements

We thank Dr. Terry Engle of Colorado State University for the analysis of beef samples. We also thank the Montana State University Mass Spectrometry Facility members, Jesse Thomas and Don Smith, for their assistance with the instrumentation. Funding for the Montana State Mass Spectrometry Facility used in this publication was made possible in part by the MJ Murdock Charitable Trust, the National Institute of General Medical Sciences of the National Institutes of Health under Award Numbers P20GM103474 and S10OD28650, and the MSU Office of Research and Economic Development.

Author contributions

CRediT: **Meghan L. Spears:** Data curation, Formal analysis, Investigation, Software, Visualization, Writing – original draft, Writing – review & editing; **Gwendolyn Cooper:** Data curation, Writing – review & editing; **Brett Sather:** Data curation, Writing – original draft; **Emily Peterson:** Data curation, Methodology, Writing – review & editing; **Jane A. Boles:** Conceptualization, Data curation, Supervision, Writing – review & editing; **Brian Bothner:** Conceptualization, Funding acquisition, Resources, Supervision, Writing – review & editing; **Mary. P. Miles:** Conceptualization, Funding acquisition, Methodology, Project administration, Resources, Supervision, Validation, Writing – review & editing.

Funding

This work was supported by the Organic Advisory and Education Council.

Disclosure statement

No potential conflict of interest was reported by the author(s).

Data availability statement

Data presented in this study are available on request from the corresponding author. The data are not publicly available due to participant confidentiality.

Informed Consent Statement: Informed consent was obtained from all participants involved in this study.

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