THE EFFECTS OF BISON MEAT CONSUMPTION ON BLOOD LIPIDS AND SELECTIVE BIOMARKERS RELATED TO CANCER RISK

by

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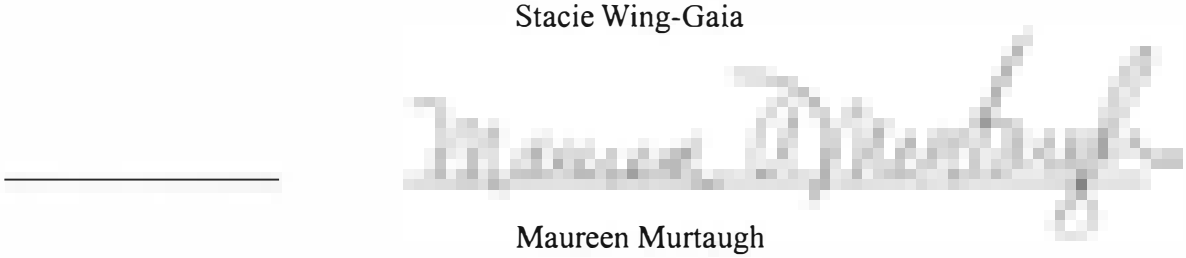

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ABSTRACT

The consumption of animal fats has gained the reputation of being less healthy due to an association with increased inflammation and oxidative stress. However, red meat is also a nutrient-rich food, providing high-quality protein, vitamins B6 and B12, niacin, iron, and zinc, as well as some beneficial lipids such as conjugated linoleic acid, which is believed to have anti-carcinogenic properties. The aim of the study is to investigate the influence of the daily consumption of bison and beef on blood lipids and biomarkers of inflammation and oxidative stress. Twenty-four participants completed a double-blind cross-over randomized trial. They subsisted upon their assigned diet (3-4 oz of beef or bison meat twice a day, 6 days/week) for 6 weeks. Test participants maintained their body weights without a significant gain or loss over the 42-day period. In comparison to beef, bison meat contained higher level of n3 and n6 fatty acids, and PUFA, lower amounts of C14 and C16 fatty acids and SFA, a more favorable P/S and lower n6/n3. Total serum C-14:0 and C-16:0 were significantly increased in the beef fed group ($p<0.01$), but there were no significant differences in TC, LDL, HDL, and TG levels in both groups. Serum high sensitivity CRP levels were unchanged in both groups. PGF2α and urine 8-OHdG were significantly reduced in the beef fed group ($p<0.01$). Serum total alkenals was significantly decreased ($p<0.01$) in the bison group but slightly increased in the beef group. Overall, participants consuming bison meat had a more favorable serum fatty acid composition. The fatty acid profile along with the lower

amount of fat contained in the bison meat is consistent with a decreased risk of cancer. However, there was no significant difference in oxidative stress biomarkers between the two groups. Based upon the limited oxidative stress biomarkers studied, bison meat was not consistently associated with reducing the risk of oxidative stress that has been linked to cancer risk, compared to beef. Therefore, the results of this study suggest that moderate amounts of red meat can be consumed as part of the eucaloric daily diet without negatively influencing the lipid profile and inflammation and cancer risk biomarkers.

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LIST OF ABBREVIATIONS

AHA.....American Heart Association

BrdU.....Bromodeoxyuridine

BMI.....Body Mass Index

CLA.....Conjugated Linoleic Acid

CRP.....C-reactive Protein

CVD.....Cardiovascular Disease

FA.....Fatty Acid

HDL.....High-density Lipoprotein

hsCRP.....High Sensitivity C-reactive Protein

LDL.....Low-density Lipoprotein

MUFA.....Monounsaturated Fatty Acid

n6/n3.....Ratio of n6 Fatty Acid to n3 Fatty Acid

8-OHdG.....8-hydroxydeoxyguanosine

PGF2α.....8-epi-F2-isoprostane

P/S.....Ratio of PUFA to SFA

PUFA.....Polyunsaturated Fatty Acid

SFA.....Saturated Fatty Acid

TC.....Total Cholesterol

TG.....Total Triglyceride

INTRODUCTION

Malignant tumors or neoplasms, collectively known as cancer, are the second leading cause of death linked to diet in the United States. Cancer affects approximately 1.2 million people in the US (1). The age-adjusted incidence rate was 470.1 per 1000,000 men and women per year; the age-adjusted death rate 192.7 per 100,000 (2). It is estimated that 1,437,180 individuals will be diagnosed with and 565,650 will die of cancer of all sites based upon 2008 projections (2).

Epidemiological research suggests that environment and diet may be an important factor in carcinogenesis (13). The environmental factors related to cancer development include tobacco smoking, occupational hazards, a variety of toxic insults and environmental pollutants, alcohol, viruses, chlorinated water, and dietary deficiencies and excesses (14, 15). Diet along with its consequential effect on nutritional status has received increased attention in recent years. The consumption of meat has been shown in epidemiological studies to be associated with increased mortality from cancer due to an association with increased inflammation and oxidative stress (3).

Recent data have expanded the concept that inflammatory cells produce an attractive environment for tumor growth, facilitate genomic instability, and promote angiogenesis in the early neoplastic process (16, 17). Later in the tumorigenic process, neoplastic cells also divert inflammatory mechanisms to favor neoplastic spread and metastasis (16). Superoxide is generated within the mitochondria and is sequentially reduced to hydrogen peroxide and hydroxyl radicals. These reactive

oxygen species damage DNA, producing the mutations such as base modification of DNA, rearrangement of DNA sequence, miscoding of DNA lesion, and gene duplication that lead to cell apoptosis that in turn initiate tumors and sustain progression (18, 50).

There continues to be controversy surrounding the relationship between consumption of red meat and the risk of cancer. Although red meat consumption has gained the reputation of being less healthy due to an association with increased inflammation and oxidative stress, it is also a nutrient-rich food, providing high-quality protein, vitamins B6 and B12, niacin, iron, and zinc, as well as some beneficial lipids such as conjugated linoleic acid (CLA) (4). The major isomer of CLA in natural foods is cis-9, trans-11 (18:2, c9, t11) (5). Over the past two decades, CLA has been shown to possess anti-carcinogenic, -adipogenic, -atherogenic, -diabetogenic, and -inflammatory properties as shown in Table 1 (6, 7). CLA has been shown to inhibit cancer in several animal models. In particular, it inhibits skin tumor initiation and forestomach neoplasia (23, 24, 25). As an anti-initiator, CLA may modulate events such as free radical-induced oxidation, carcinogen metabolism, and carcinogen-DNA adducts formation (26). In recent years, attention has focused on elucidating the mechanisms of CLA that inhibit carcinogenesis during promotion, particularly in the mammary and skin carcinogenesis models (27). The promotion stage involves the clonal expansion of initiated cells to form a benign tumor. In this premalignant state, tumors arise from cells that have increased cell proliferation, reduced programmed cell death (or apoptosis), and dysregulated differentiation. In cultured cells, CLA reduced proliferation of mammary tumor cells in vitro (28) and in vivo (5). In vivo, rats that were carcinogen-initiated with methylnitrosourea and

Table 1

Physiological Properties of Conjugated Linoleic Acid in Carcinogenesis Function[a]

Major of function	Physiological model
Carcinogenesis	↓Chemically induced mamary carcinogenesis in rats
	↓Chemically induced mammary carcinogenesis in rats by either c9t11-CLA or synthetic CLA
	↓Chemically induced mammary carcinogenesis in rats regardless of level of fat or esterification of CLA (in triglyceride) vs. free fatty acid
	↓Growth of transplantable breast cancer tumor cells in nude mice
	↓Growth of transplantable prostate cancer tumor cells in nude mice
	↓Stages of chemically induced colon carcinogenesis in rats
	⊁ Carcinogenesis in Min mice
	↓Chemically induced forestomach

[a]Data from Belury et al (6, 7).

then fed a diet with 1.0% CLA exhibited reduced proliferation of terminal end bud and lobuloalveolar bud structures of mammary epithelium (30). More recently, the reduction of cell proliferation in terminal end bud structures by dietary CLA was accompanied by reduced rates of incorporating bromodeoxyuridine (BrdU) and levels of two cyclins known to regulate the cell cycle, cyclin D1 and cyclin A (31). Therefore, CLA may produce a cytotoxic effect upon cancer cells and even with other beef fatty acids (32).

In addition, CLA also is believed to be anti-inflammatory (33). It is well accepted that inflammation plays a key role in cardiovascular disease (34). It is also biologically plausible that chronic inflammation may also predispose to cancer (35), since cells involved in the immune response generate reactive oxygen and nitrogen species that are directly mutagenic and release autocrine and paracrine factors that stimulate the clonal proliferation of genetically damaged cells.

Food nutrient composition data show that a typical cut of bison meat contains slightly less cholesterol and up to 1/3 less fat per 100g of cooked lean meat, as shown in Table 2 (8). Bison meat also contains more CLA than beef, as shown in Table 3. This has led to the suggestion that the consumption of bison meat may lead to a more favorable human blood lipid profile than similar consumption of feedlot fed beef (10). However, no human clinical trials have substantiated this idea. Although human clinical trials of the relationship between bison meat consumption and cancer-related biomarkers are lacking, our knowledge of the cardioprotective and anti-mutagenic activity of CLA in lower vertebrate models and cell culture studies suggests that this fatty acid, found in relatively high concentrations in range fed bison muscle and adipose tissue, may be a potent naturally occurring anti-carcinogen in the human diet

Table 2

National Comparisons of Several Commercial Cooked Meats[a]

NUTRITIONAL COMPARISONS						
SPECIES	FAT (g)	PROTEIN (g)	CALORIES (Kcal)	CHOLESTEROL (mg)	IRON (mg)	VITMAIN B-12 (mcg)
Bison	2.42	28.44	143	82	3.42	2.86
Beef (Choice)	18.5	27.21	283	87	2.72	2.50
Beef (Select)	8.09	29.89	201	86	2.99	2.64
Pork	9.66	29.27	212	86	1.1	0.75
Chicken (Skinless)	7.41	28.93	190	89	1.21	0.33
Sockeye Salmon	11.0	27.31	216	87	0.55	5.80

[a]Data from USDA Nutrient Data Laboratory (8).

NOTE: Per 100 Gram (3.5 oz.) Serving-Cooked Meat-Updated August 2005

Table 3

Comparison of the Fatty Acid Composition of Uncooked Semitendinosus Muscle of Bison and Beef[a]

Fatty Acid (weight %)	**Range bison**	**Feedlot bison**	**Range beef**	**Feedlot beef**
Saturated Fatty Acid (SFA)	38.1	33.6	39.9	42.0
Polysaturated Fatty Acid (PUFA)	19.9	10.0	14.4	6.1
Omega-3 Fatty Acid (w-3)	6.9	1.7	4.3	1.0
Conjugated Linoleic Acid (CLA)	0.4	0.4	0.3	0.3
Cholesterol (mg/100g)	45.8	51.0	48.7	53.4

[a]Data from Rule et al (9).

(11,12). Therefore, the aim of the study is to investigate the influence of bison meat on the response of oxidative stress and inflammation biomarkers believed to be cancer-related in humans. For this purpose, a study was conducted to compare bison meat with beef cattle meat to see if bison meat is beneficial for lowering blood (8-epi-F2-isoprostane, PGF2α, total alkenals, and C-reactive protein, CRP) and urine markers (8-hydroxydeoxyguanosine, 8-OHdG) associated with the risk of chronic disease such as cancer.

SUBJECTS AND METHODS

Participants

Participants in the study were males and females between 25-60 years old. Two questionnaires were used to select participants: 1. A telephone initial screening (Appendix A): Telephone screening questions include asking potential participants their age, if they would be willing to eat meat 6 days a week, if they are able to prepare meals at home and eat home-prepared meals 6 days of the week, if they are willing to maintain their weight for the duration of the study, if they are smokers, if they are pregnant or planning to become pregnant, and if they are being treated for cancer, high cholesterol, or heart disease. Those participants who met initial selection criteria were asked to bring a completed medical questionnaire and 3-day food and activity record (Appendix C) to their informational and screening appointment. 2. A medical screening questionnaire (Appendix B): The medical questionnaire included questions about personal past and current medical history concerning heart disease, stroke, cancer, and diabetes, participant's age and gender, and medication and supplement usage and dosages. Once again, participants were asked if they smoke or are planning to become pregnant. Potential participants who still met selection criteria attended an informational meeting and cholesterol screen at a predetermined time. Smokers, pregnant women, cancer patients, vegetarians, and individuals being treated by medication for cholesterol or high blood pressure were excluded from the study. In addition, potential participants were prescreened for high cholesterol levels and hypertension. Those with total cholesterol levels greater

than 230 mg/dl or 10-year risk >2% were excluded from the study based on the Adult Treatment Panel III guidelines (JAMA 2001). Those with blood pressure greater than 140/90 were excluded from the study. Participants with a personal history of heart disease, stroke, or diabetes were excluded from the study.

Experimental design

A double-blind cross-over randomized trial was selected for this study. Twenty-four people were randomly be assigned to one of two meat-component diet groups to consume their assigned diet for 42 days. On day 43, they went through a washout period for 30 days in which each study participant resumed his or her regular diet. After the washout period, participants received their second assigned diet for the following 42 days. The assigned diets were isocaloric with the only variant being the meat: 1) bison meat or 2) beef cattle meat. Study participants were required to eat 6 oz and 8 oz (consisting of one serving of roast or steak and with one serving of ground meat), for females and males, respectively, of the randomly assigned meat treatment, for 6 out of 7 days of the week. Blood and urine measurements were collected four times. Measurements were taken at the beginning of the study period, at day 42 of the first diet treatment, at day 30 of the washout period (before the second treatment begins), and day 42 of the second diet treatment. Height, weight, Body Mass Index (BMI), waist and hip circumference, and body fat composition were measured.

Dietary intervention

Study participants met with the researchers weekly to receive each week's meat issue. Participants submitted a meat consumption checklist at each visit to monitor

compliance. At each visit, participants were weighed and met with the dietitian to ensure that weight was remaining relatively stable. If too much weight was gained or lost (more than 2 lb/week), subjects were given additional food records and counseled by the dietitian. Participants were asked to refrain from vigorous physical activity for 36 hours or to keep physical activity consistent prior to sample collection. Participants submitted 3-day food and activity records for the 3 days proceeding the beginning and end of each treatment. Three-day food and activity records were analyzed by Food Processor software (Version 8.3, 2004, ESHA Research). The activity record included activity type and duration.

Laboratory methods

Three meat packages (roast, steak, and ground) were randomly selected from each type of meat. Each meat package was subsampled by taking cores from three locations in each meat package, and the three subsample cores were combined and extracted for each meat samples. Lipid profile of meat samples were analyzed by Dan Rule and Chuck Murrieta of the Department of Animal Science, University of Wyoming, according to previously published methods (9).

Twenty mL venous antecubital blood were drawn at each sampling period by a trained phlebotomist after the subjects had fasted overnight. The sample refrigerated was centrifuged at 2000 RPM for 15 minutes, divided into two aliquots of serum (4.0, 1.0, and 1.0 mL), processed, and stored at –80 °F until analysis at the end of the study. The first plasma sample (4.0 mL) was analyzed for total cholesterol (TC), low-density lipoprotein (LDL), high-density lipoprotein (HDL), and triglyceride (TG) by Atherotech Laboratories (Birmingham, AL, USA). The second serum sample was determined by Immunoturbidimetric test (Atherotech Laboratories, Birmingham, AL,

USA) for high sensitivity C-reactive protein (hsCRP) which is an acute phase protein produced by liver and it is a general indicator of inflammation. The third serum sample was determined by specrophotometric assay performed on a robotic chemical analyzer and manual ELISA assay (Genox corporation, Baltimore, MD, USA) for total alkenals which are products of lipid peroxides from free radical attacks on cellular lipid membranes and lipoproteins and for peroxidized lipid aldehydes, PGF2α, formed by the free radical catalyzed nonenzymatic peroxidation of arachodonic acid in cellular membranes and lipoproteins. The fourth serum sample was analyzed for its long chain fatty acid profile by Dan Rule and Chuck Murrieta of the Department of Animal Science, University of Wyoming according to previously published methods (20).

Urine samples were stored at –80 °F until analysis at the end of the study. Urine was analyzed by manual ELISA assay (Genox corporation, Baltimare, MD, USA) for 8-OHdG which is hydroxyl radical-damaged quinine nucleotide that has been excised from DNA by endonuclease repair enzymes and it a biomarker of DNA damage. Urinary 8-OHdG levels were subsequently normalized to urinary creatinine levels and expressed as ng/mg creatinine. Data on serum lipid analysis are from the thesis of Rebecca Hurst (39) and included for information purposes.

Statistical procedure

All statistical analyses for data were performed using SPSS (Version 15.0) statistical software. Baseline and the end of intervention were presented as mean values and standard deviation (mean ± SD). Differences were considered significant at $P < 0.05$. At baseline, characteristics of participants in the two groups were compared using the independent t test. The Kolmogorov-Smirnov test was used to

test the distribution of the data. The paired t test was used to assess within-group changes (significance of change from initial and posttreatment from both groups) and between-group differences in change (comparison of bison and beef group).

RESULTS

Lipid profiles of bison and beef cattle meats

Bison and beef cattle meat contain beneficial lipids such as anti-cancer lipid CLA, anti-atherogenic lipid monounsaturated fatty acid (MUFA) and polyunsaturated fatty acid (PUFA), and anti-inflammatory lipid n3 fatty acids, as shown in Table 4. Major sources of CLA in both bison and beef are cis-9, trans-11 (18:2, c9, t11) as shown in Table 4. In comparison to the beef cattle meat, bison meat contains higher levels of n3 fatty acids, n6 fatty acids, and PUFA, lower amounts of the more atherogenic C14 and C16 fatty acids and saturated fatty acids (SFA), a more favorable ratio of PUFA to SFA (P/S) and a lower ratio of n6 fatty acid to n3 fatty acid (n6/n3) (Table 4). Bison is considerably lower in total fat than beef, containing up to 1/3 less fat (Table 4). Previous food nutrient composition data show that bison meat contains more CLA than beef (Table 3). However, there was no significant difference in amounts of CLA between bison and beef cattle meat in the meat fed in this study (Table 4).

Baseline characteristics

Twenty-four participants (16 male and 8 females) aged 44.3 ± 8.6y completed this cross-over study. There were 24 participants in the bison meat treatment with BMI of 25.3±4.2 kg/m^2 and mean body fat composition of 23.5±7.3%. The 24 participants in the beef cattle meat treatment had mean BMI of 25.1±4.2 kg/m^2 and body fat composition of 23.3±8.2%. BMI and body fat composition did not differ between groups at baseline and did not change during the study as shown in Table 5.

Table 4

Weight Percentage of Fatty Acids in Bison and Beef Cattle Meats

	BEEF			BISON		
	Roast	Steak	Burger	Roast	Steak	Burger
Fatty Acid	Weight %	Weight %	Weight %	Weight %	Weight %	Weight %
14:0[a]	2.84	2.43	3.03	1.28	1.05	1.63
14:1[b]	0.58	0.41	0.81	0.27	0.09	0.33
15:0[a]	0.41	0.37	0.5	0.32	0.4	0.6
15:1[b]	0.1	0.21	0.13	0.26	0.28	0.44
16:0[a]	26.01	26.67	24.18	15.97	16.87	17.99
16:1t9[b]	0.37	0.46	0.42	0.52	0.47	0.66
16:1c9[b]	3.66	2.27	3.23	1.88	1.53	1.47
16:1c/t11[b]	0.15	0.07	0.2	0	0	0
17:0[a]	1.19	1.13	1.27	1.24	1.16	1.37
17:1[b]	0.93	0.59	0.91	0.7	0.6	0.5
18:0[a]	12.63	16.04	13.71	22.3	21.71	28.25
18:1t9[b]	0.11	0.17	0.11	0.46	0.38	0.64
18:1t10[b]	0.19	0.28	0.2	0.56	0.52	0.59

Table 4 Continued

	BEEF			BISON		
	Roast	Steak	Burger	Roast	Steak	Burger
Fatty Acid	Weight %	Weight %	Weight %	Weight %	Weight %	Weight %
18:1t11[b]	2.25	3.28	4.47	1.81	1.73	2.5
18:1c9[b]	40.86	38.33	38.54	41.96	37.96	32.29
18:1c11[b]	1.67	1.25	1.53	1.61	1.78	1.18
18:2c9, 12[c, g]	2.74	3.03	1.69	5.47	8.6	2.17
18:3c9,12,15[c, f]	0.16	0.15	0.23	0.65	0.8	0.32
18:2c9t11[c, e]	0.31	0.32	0.44	0.38	0.3	0.3
18:2t10c12[c, e]	0	0	0	0	0	0
20:4[c, g]	0.47	0.29	0.08	0.61	1.31	0.1
20:5[c, f]	0	0	0	0	0	0
22:4[c, g]	0	0	0	0	0	0
22:5[c, f, g]	0	0	0	0	0	0
22:6[c, f]	0	0	0	0	0	0
Unknown	2.35	2.27	4.34	1.74	2.45	3.68
Total fatty acid (mg)	4471.48	4611.30	15021.30	813.48	1175.234	7278.595
Total lipid %	5.354	4.916	17.047	1.569	1.801	8.576

Table 4 Continued

	BEEF			BISON		
	Roast	Steak	Burger	Roast	Steak	Burger
Fatty Acid	Weight %	Weight %	Weight %	Weight %	Weight %	Weight %
Total CLA	0.31	0.32	0.44	0.38	0.30	0.30
Total n3	0.16	0.15	0.23	0.65	0.80	0.32
Total n6	3.21	3.32	1.77	6.08	9.91	2.27
Total n6/n3	20.06	22.13	7.70	9.35	12.39	7.09
Total SFA	43.08	46.64	42.69	41.11	41.19	49.84
Total MUFA	50.87	47.32	50.55	50.03	45.34	40.60
Total PUFA	3.68	3.79	2.44	7.11	11.11	2.89
P/S[d]	0.07	0.08	0.05	0.14	0.24	0.07
C-14 + C-16	28.85	29.1	27.21	17.25	17.92	19.62

[a]SFA = Saturated fatty acid

[b]MUFA = Monounsaturated fatty acid

[c]PUFA = Polyunsaturated fatty acid

[d]P/S = PUFA/MUFA

[e]CLA = Conjugated linoleic acid

[f]n3 = ω-3 or omega-3 fatty acid

[g]n6 = ω-6 or omega-6 fatty acid

Table 5

Body Weight, BMI, Dietary Intake, and Physical Activity Level at Baseline and at 6 Weeks (Post)[a]

	BISON			***BEEF***		
Measurement	*Baseline*	*Post*	*P*	*Baseline*	*Post*	*P*
Weight (lb)	167.4±40.52	166.8±39.7	**NS**	166.3±39.9	165.9±39.6	**NS**
BMI (kg/m^2)	25.3±4.2	25.2±4.1	**NS**	25.1±4.2	25.1±4.2	**NS**
Body fat composition (%)	23.5±7.3	23.6±7.8	**NS**	23.3±8.2	23.5±8.4	**NS**
Waist-Hip ratio	0.83±0.08	0.84±0.07	**NS**	0.84±0.07	0.84±0.07	**NS**
Energy Intake (kcal)	2169.8±692.8	2152.8±667.2	**NS**	2205.9±938.3	2476.3±706.1	**< 0.05**
Protein (%kcal)	16.0±4.0	23.2±5.1	**< 0.01**	16.8±4.6	19.6±3.5	**< 0.05**
Fat (%kcal)	33.1±6.2	32.8±5.5	**NS**	34.8±7.8	39.5±5.9	**< 0.05**
Saturated Fat (%kcal)	10.3±3.0	11.0±2.7	**NS**	11.5±3.7	13.7±2.5	**< 0.05**
Physical activity level (minutes/day)	71.0±88.9	65.4±95.9	**NS**	64.8±78.3	71.5±84.9	**NS**

[a]Baseline and postvalues are mean ±SD, n=24

Energy and nutrient intakes

Energy and nutrient intakes did not differ between groups at baseline as shown in Table 5. Participants complied with the set dietary changes. Protein intake significantly increased in the bison meat group throughout the intervention ($p< 0.01$). Intakes of energy ($p< 0.05$), protein ($p< 0.05$), fat ($p< 0.05$), and SFA ($p< 0.05$) significantly increased in the beef cattle meat group from baseline to end of intervention. Percent of SFA intake in both groups exceeded AHA guidelines of <7% (Bison meat group: 11.0±2.7%; Beef cattle meat group: 13.7±2.5%). However, body weight did not change during this study in both groups as shown in Table 5. Physical activity level did not differ between groups at baseline and did not significantly change during the study (Table 5).

Fatty acid distribution of serum

After bison meat treatment period, SFA's 15:0 ($p < 0.01$), 17:0 ($p < 0.01$), and 18:0 ($p < 0.05$), MUFA's 18:1*t*11 ($p < 0.01$) and 18:1*c*9 ($p < 0.01$), and PUFA's 18:1*c*11 ($p < 0.05$), 18:2n-6 ($p < 0.05$), 20:4n-6 ($p < 0.01$), and 22:5n-3 ($p < 0.05$) were significantly increased (Table 6). In contrast, SFA's 14:0 ($p < 0.01$), 15:0 ($p < 0.05$), 16:0 ($p < 0.01$), 17:0 ($p < 0.05$), and 18:0 ($p < 0.01$), MUFA's 14:1*c*9 ($p < 0.05$), 16:1*t*9 ($p < 0.01$), 16:1*c*9 ($p < 0.01$), 18:1*t*10 ($p < 0.01$), 18:1*t*11 ($p < 0.01$), 18:1*c*9 ($p < 0.01$), and 18:1*c*11 ($p < 0.01$), and PUFA's 18:2n-6 ($p < 0.01$), 20:4n-6 ($p < 0.01$), 20:5n-3 ($p < 0.05$), 22:5n-3 ($p < 0.01$), and 22:6n-3 ($p < 0.01$) were significantly increased after the beef cattle meat treatment period (Table 6). Total serum fatty acid was significantly increased in the beef group ($p < 0.01$). In comparison to the bison meat group, beef cattle meat contained a significantly increased amount of serum PUFA 18:2n-6 (18:2 c9, 12) ($p < 0.01$) but a significantly

Table 6

The Effects of Intervention on the Human Serum Fatty Acid Profile

	BEEF			BISON		
Serum Fatty acid	**Pre (mg/100mL)**	**Post (mg/100mL)**	**P**	**Pre (mg/100mL)**	**Post (mg/100mL)**	**p**
14:0[a]	0.0082±0.005	0.0135±0.008	**< 0.01**	0.0092±0.006	0.0124±0.007	**NS**
14:1 c9[b]	0.003±.001	0.0039±.002	**< 0.05**	0.0035±0.002	0.0038±0.001	**NS**
15:0[a]	0.0094±0.003	0.0116±0.004	**< 0.05**	0.0094±0.003	0.0110±0.002	**< 0.01**
16:0[a]	0.2553±0.093	0.3717±0.107	**< 0.01**	0.2956±0.129	0.3560±0.116	**NS**
16:1t9[b]	0.0028±0.001	0.0048±0.002	**< 0.01**	0.004±0.002	0.0046±0.002	**NS**
16:1c9[b, g]*	0.0100±0.006	0.0210±0.012	**< 0.01**	0.0136±0.007	0.0191±0.013	**NS**
17:0[a]	0.0073±0.003	0.0094±0.003	**< 0.05**	0.0067±0.003	0.0117±0.004	**< 0.01**
17:1[b]	0.0037±0.001	0.0045±0.002	**NS**	0.0039±0.002	0.0040±0.002	**NS**
18:0[a]	0.1159±0.035	0.1540±0.030	**< 0.01**	0.1287±0.049	0.1584±0.042	**< 0.05**
18:1t9[b]	0.0035±0.001	0.004±0.002	**NS**	0.0050±0.003	0.0038±0.002	**NS**
18:1t10[b]	0.003±0.002	0.004±0.002	**< 0.01**	0.0038±0.002	0.0039±0.002	**NS**
18:1t11[b]	0.0034±0.001	0.0065±0.003	**< 0.01**	0.004±0.002	0.007±0.004	**< 0.01**
18:1c9[b]	0.1600±0.081	0.2595±0.087	**< 0.01**	0.1878±0.107	0.2648±0.122	**< 0.01**
18:1c11[b]	0.0148±0.007	0.0228±0.007	**< 0.01**	0.0178±0.010	0.0226±0.010	**< 0.05**
18:2c9,12[c, f, g]**	0.3188±0.187	0.5000±0.163	**< 0.01**	0.3781±0.243	0.4839±0.184	**< 0.05**
18:3c9,12,15[c, e]	0.0096±0.007	0.0102±0.004	**NS**	0.0100±0.007	0.0100±0.004	**NS**

Table 6 Continued

	BEEF			BISON		
Serum Fatty acid	**Pre (mg/100mL)**	**Post (mg/100mL)**	**P**	**Pre (mg/100mL)**	**Post (mg/100mL)**	**p**
18:2c9t11[c, d]	0.0025±0.002	0.0030±0.001	**NS**	0.0032±0.002	0.0028±0.001	**NS**
20:4c5,8,11,14[c, f]	0.0810±0.040	0.1388±0.040	**< 0.01**	0.0978±0.053	0.1355±0.051	**< 0.01**
20:5c5,8,11,14,17[c, e]	0.0073±0.006	0.0134±0.012	**< 0.05**	0.0101±0.012	0.0119±0.014	**NS**
22:5c7,10,13,16,19[c, e]	0.0062±0.003	0.0108±0.005	**< 0.01**	0.0076±0.005	0.0098±0.004	**< 0.05**
22:6c4,7,10,13,16,19[c, e]	0.0203±0.011	0.0317±0.015	**< 0.01**	0.0254±0.020	0.0303±0.017	**NS**
Total mg FA	2.184±0.985	3.354±0.870	**< 0.01**	2.605±1.372	3.148±0.988	**NS**

[a]SFA = Saturated fatty acid

[b]MUFA = Monounsaturated fatty acid

[c]PUFA = Polyunsaturated fatty acid

[d]CLA = Conjugated linoleic acid

[e]n3 = ω-3 or omega-3 fatty acid

[f]n6 = ω-6 or omega-6 fatty acid

[g]Significant difference after intervention between groups, *p < 0.05 (paired t test); **p < 0.01 (paired t test).

decreased amount of serum MUFA 16:1 *c*9 ($p < 0.05$) as shown in Table 6.

Lipid profile

Blood lipid data is included for informational purposes from the thesis of Rebecca Hurst (39). The groups did not differ in lipid profile at baseline (Table 7). Also, there were no significant differences in TC, LDL, HDL, and TG from baseline to end of intervention in both groups as shown in Table 8. In comparison to the beef cattle meat group, there were lower energy ($p < 0.01$), and higher protein ($p < 0.01$) intake in bison meat group (Table 5); however, levels of TC, LDL, HDL, and TG did not significantly differ from baseline to end of intervention between groups as shown in Table 8.

Markers of oxidative stress

Serum PGF2α, total alkenals, and urine 8-OHdG did not differ at baseline (Table 7). PGF2α and urine 8-OHdG were significantly reduced in beef cattle meat group ($p<0.01$) but not in the bison meat group (Table 9). However, PGF2α and urine 8-OHdG were no different from baseline to end of intervention between groups. Serum total alkenals were reduced from baseline to end of intervention only in the bison meat group ($p<0.01$) and slightly increased in beef cattle meat group (Table 9).

Markers of inflammation and oxidative stress

Serum hsCRP level was not different at baseline (Table 7). Levels did not increase from baseline to end of intervention in both groups and between groups as shown in Table 9, indicating that the meat consumption did not increase inflammation.

Table 7

Baseline Data of Bison and Beef Groups at the Beginning of the Intervention Period[a]

	BISON		BEEF		
Measurement	Mean	SD	Mean	SD	P
Weight (lb)	167.4	40.5	166.3	39.9	< 0.05
BMI (kg/m^2)	25.3	4.2	25.1	4.2	NS
Body fat composition (%)	23.5	7.3	23.3	8.2	NS
Waist-Hip ratio	0.83	0.08	0.84	0.07	NS
Total cholesterol (mg/dL)[c]	197.2	33.5	200.1	32.7	NS
Triglycerides (mg/dL)[c]	79.8	27.0	76.7	24.4	NS
HDL-cholesterol (mg/dL)[c]	59.0	19.8	56.6	17.8	NS
LDL-cholesterol (mg/dL)[c]	120.5	24.3	124.9	24.5	NS
8-OHdG ng/mg creatinine[b]	0.13	0.06	0.15	0.07	NS
PGF2α (pg/mL)[c]	27.25	19.32	32.88	27.69	NS
Total alkenals (μmol/L)[c]	3.35	0.65	3.14	0.75	NS
hsCRP (mg/L)[c]	1.61	2.06	1.48	1.76	NS

[a]Baseline and post values are mean ±SD, n=24
[b]Urine
[c]Serum

Table 8

Lipid Profiles at Baseline and the End of Intervention (Post)[a]

	BISON		BEEF	
Lipid profile	Baseline	Post	Baseline	Post
Total cholesterol (mg/dL)	197.2±33.5	198.9±299	200.1±32.7	197.8±28.0
Triglycerides (mg/dL)	79.8±27.0	81.1±33.2	76.7±24.4	77.6±24.4
HDL-cholesterol (mg/dL)	59.0±19.8	57.3±18.9	56.6±17.8	58.3±15.8
LDL-cholesterol (mg/dL)	120.5±24.3	122.9±20.6	124.9±24.5	121.6±22.5

[a]Baseline and post values are mean ±SD, n=24
NOTE: Lipid profile data from Hurst (39).

Table 9

Markers of Oxidative Stress and Inflammation at Baseline and End of Intervention (Post)[a]

Measurement	BISON			BEEF		
	Baseline	Post	P	Baseline	Post	P
8-OHdG ng/mg creatinine[c]	0.13±0.06	0.11±0.05	NS	0.15 ±0.07	0.11±0.06	< 0.01
PGF2α (pg/mL)[d]	27.25±19.32	21.98±12.46	NS	32.88±27.69	23.73±21.59	< 0.01
Total alkenals (μmol/L)[b, d **]	3.35±0.65	3.14±0.79	NS	3.14±0.75	3.40±0.88	NS
hsCRP[d]	1.61±2.06	1.56±2.12	NS	1.48±1.76	1.34±2.01	NS

[a]Baseline and post values are mean ±SD, n=24
[b]Significant difference after intervention between groups,**p < 0.01 (paired t test)
[c]Urine
[d]Serum

DISCUSSION

The consumption of animal fats has gained the reputation of being less healthy due to an association with increased inflammation and oxidative stress, leading to chromosomal instability, mutations, loss of organelle functions, membrane damage and eventually cancer (50). A common dietary recommendation to reduce fat intake is to decrease consumption of red meat due to the positive association between dietary fat and the etiology of some cancers (22, 29, 36-37). Epidemiological studies have revealed that increasing the fat content of the diet from 2 or 5% to 20 or 27% increased tumor incidence and resulted in earlier tumor appearance in animals (22). Furthermore, epidemiological studies showed that Westernization of the diet, that is, a high-calorie and high-fat diet, increased incidence of cancers of digestive organs (22, 38), breast (36, 38), and prostate (38). In this study, we found that beef cattle meat had higher amounts of total fat compared to bison meat, suggesting that bison meat is potentially more potent than beef cattle meat for lowering risk of cancer. However, the results demonstrated that PGF2α and urine 8-OHdG were no different from baseline to end of intervention between bison meat and beef cattle groups.

Evidence from both epidemiologic and experimental studies suggest that the types of fats consumed such as saturated fats, as well as the amounts, may influence the development and subsequent progression of some types of cancer (36). A meta-analysis published in 2003 showed that SFA was significantly associated with breast cancer risk (40). In this current study, the results indicated that serum SFA 14:0 and 16:0 were only significantly increased in beef cattle group, so bison meat

might be expected to be associated with decreased risk of cancer. However, there were no differences between the two groups in the levels of oxidative stress biomarkers.

Epidemiological studies have indicated a positive association between the dietary intake of n-6 fatty acids and enhancement of the promotional phase of experimental mammary carcinogenesis (29, 38, 41-42), whereas n-3 fatty acids exert inhibitory effects (38, 40, 43-44). The bison meat in our study had higher amounts of n-3 fatty acids so it was potentially more potent than beef cattle meat for lowering risk of cancer. Additionally, n-3 and n-6 fatty acids are converted into eicosanoids by the same enzymatic system (45). Therefore, lower n-6/n-3 ratio may contribute to inhibition of the early stages of carcinogenesis. In comparison to beef cattle meat, the results showed that bison meat had higher n-3 fatty acids, higher n-6 fatty acids, and lower n-6/n-3, so it was potentially more potent than beef cattle meat for lowering risk of cancer. However, the results of oxidative biomarkers did not reveal a significant difference between bison meat and beef cattle groups, as demonstrated in other studies as well (46, 47). Furthermore, the results showed that PGF2α and urine 8-OHdG were significantly reduced only in the beef cattle group.

Baer et al demonstrated that consumption of dietary intakes of total fat and SFA are associated with inflammation (48). In comparison to bison meat, the results showed that beef cattle meat had higher amount of total fat and SFA, so bison meat was potentially more potent than beef cattle meat for lowering risk of cancer. However, CRP, one of the acute-phase proteins involved in inflammation, was not significantly different from baseline to end of intervention in both groups and between bison meat group and beef cattle group. This was also demonstrated by Nanri et al

(49).

In conclusion, the consumption of lean beef for 42 days as part of a mixed diet for free living adults did not increase inflammation or greatly perturb the oxidative stress burden, as was also demonstrated by Hodgson et al (21). The results indicated that moderate amounts of red meat can be consumed as part of the daily diet without negatively influencing inflammation and cancer risk biomarkers. Interestingly, there was a significant difference between beef cattle meat group and bison meat group on serum total alkenals biomarkers. Both 8-epi-PG2α and total alkenals are products of lipid peroxidation. However, serum total alkenals were reduced only in bison meat group. On the contrary, serum total alkenals were slightly increased in beef cattle meat group. This interesting result of this pilot clinical trial provided useful data for planning future studies which investigate why bison have reduced serum total alkenals but beef do not are needed to confirm and extend this finding. In addition, future studies could investigate influence of bison meat on various mechanisms promoting and inhibiting cancer. Consequently, bison meat might be expected to be potentially more beneficial than beef cattle meat for reducing risk of cancer considering the changes in cancer biomarkers in this study.

CLA has been suggested to have antioxidant properties (19). Although this claim is disputed, it is recognized that CLA does inhibit the production of lipid peroxidation products (19). According to previously published investigations, bison meat contains more CLA than beef as shown in Table 3. This has led to the suggestion that the consumption of bison meat may lead to a more favorable human blood lipid profile than similar consumption of feedlot fed beef (10). However, according to the limited meat analysis from this study, there was no difference in the

amount of CLA between the bison meat and beef cattle meat used in this study. The outcome seems not to be consistent to the research from Rule et al (2002). A possible reason could be that the feeding regimen of the bison used in this study may have been different from Rule's research. The bison of this study were pastured and then put on feed for the last 120-150 days. The feeding regimen consisted of 50% silage (either barley or hay) and 50% mill run wheat which may have contained some wheat screening. By contrast, the range-raised bison of Rule's study were obtained from a local bison producer that raised bison exclusively on forage. Also, the beef cattle of this study were from standard commercial wholesale channels so they most likely were fed high grain-diets for 6 months before harvest. Therefore, the differences in CLA between two studies and the lack of differences in CLA between bison and beef may relate to the finishing processed used in preparing the meat for market. Based upon the limited oxidative stress biomarkers we studied, bison meat is not associated with on higher reduced risk of cancer comparing to beef cattle meat.

Blood lipid profiles and selective biomarkers related to cardiovascular diseases (CVD) were also discussed, and these data are reported in Hurst's research (39). Surprisingly, this pilot study suggested that there were no significant differences in overall blood lipid profiles. These results were unexpected based upon differences in the total fat and SFA amounts between bison meat and beef cattle meat. The results in this study also demonstrated that moderate consumption of lean red meat does not increase risk of CVD (39).

There are some limitations to a human clinical trial such as the one used in this study. First, since this was an observational study, we did not have total control over participant's diets. Second, the quantity of meat consumed might not be typical

compared to that of an average person. Based upon the three day food records, some of the subjects ate larger portions than the allotted 3-4 oz per serving and some consumed other meats during the study. This may confound the results in terms of the biomarkers selected. Third, it is unlikely that a strong treatment effect related to cancer biomarkers will be manifested in a short duration pilot study such as this, but changes in blood fatty acid composition and cancer biomarkers should at least be directional, permitting inferences that will aid in planning a longer study. A more comprehensive long term study related to disease outcome or carcinogenesis is not economically feasible or practical at this time but would be useful to elucidate the relationship between different types of meat consumption and cancer risk. Fourth, body weight, waist circumference, and BMI are positively related to risk of colon cancer in men, whereas weak or no associations exist in women (53). However, we have total controlled participants' weight. If participants gained or lost 2 pounds, we gave them a dietitian counseling. Perhaps if weight had not been controlled, differences in oxidative stress and inflammation biomarkers between bison and beef group may have been more evident.

This study is encompassed in a larger one to evaluate the influence of bison meat on the response of CVD- and cancer-related biomarkers in humans. In future studies, the serving size of meat consumption (6oz for male and 4oz for female/per meal) can be increased in order to meet typical meat consumption of the average population as it may produce a different outcome. Duration of the study can also be increased from six weeks to seven. In addition, in vitro experiments may be considered to enhance the scope of the study. For this purpose, pure CLA and CLA mixtures with other fatty acids from bison meat samples could be tested on cancer cell

lines as describe previously (32). Two kinds of fatty acids (FA) extracted from beef cattle meat and bison meat could be tested on cancer cell lines. Cancer cells would be exposed for 48hr to medium containing 100μm FA and their proliferation will be determined by quantifying cellular DNA content (Hoechst 33342 dye).

Although there were no significant differences in this study, the results of this pilot clinical trial still provide data useful for planning future studies which investigating relationship between red meat consumption and risk of cancer. According to American Cancer Society, 3 or more ounces per day for men and 2 or more ounces per day for women is considered "high" consumption of red meat and will increase risk of cancer (51, 52). However, neither oxidative nor inflammation biomarkers increased from baseline to end of intervention in this study. In conclusion, the results suggest that moderate amounts of red meat can be consumed as part of the eucaloric daily diet without negatively influencing the lipid profile and inflammation and cancer risk biomarkers.

APPENDIX A

TELEPHONE INITIAL SCREENING QUESTIONNAIRE

Bison Study

Initial Screening Questionnaire

Name:

1. Do you like to eat meat? Yes/No

2. How often do you currently eat meat?

3. Can you eat meat 6 days a week? Yes/No

4. Do you (or spouse) prepare meals at home? Yes/No

5. Do you have time to eat home-cooked meals 6 days a week? Yes/No

6. Do you currently smoke? Yes/No

7. Females: Are you pregnant or planning pregnancy in the next 4 months? Yes/ No

8. Are you currently taking medication to treat cholesterol? Yes/No

9. Do you have a personal history of heart disease? Yes/No

10. Are you currently being treated for cancer? Yes/No

If the caller answered Yes to Questions 1-4 and No to Questions 4-8, please ask if they are still interesting in participating in the study. Please get contact information and let participant know that they will be receiving a medical questionnaire and food record packet in the mail in the next week. Otherwise, thank them for responding to the study and let them know that unfortunately they do not meet the selection criteria.

Contact information:

Name:

Address:

Phone:

APPENDIX B

MEDICAL SCREENING QUESTIONNAIRE

Bison Study

Medical Questionnaire

Name:

Address:

Phone number (Please indicate if work/home/cell)

Gender (Please circle): Male / Female

Age:

Medical History:

Please indicate with an 'X" if you have you ever had or been treated for the following:

____heart disease ____stroke ____high blood pressure

____heart attack ____diabetes ____cancer

____other heart problems

Do you currently smoke? Yes / No Please circle.

Are you pregnant or planning to become pregnant in the next four months? Yes/No Please circle.

Please indicate with an 'X' if you are currently being treated for the following:

____heart disease ____stroke ____high blood pressure

____heart attack ____diabetes ____cancer

List medications currently taking. Include dosages

_____

_____

_____

APPENDIX C

THREE-DAY FOOD AND ACTIVITY RECORD FORM

3 DAY FOOD RECORD

*ID#*_____
*Age:*_____
*Height:*_____
*Weight:*_____

Instructions:

- Record everything you eat and drink in a 24 hour period (including water). **Record for the 3 days prior to your appointment date.**
- Accurately list the **amounts** (cups, tablespoons, etc.) of food you eat.
- Describe how the food was **prepared** (fried, boiled, baked, etc.) and any spices or condiments used (gravies, salad dressings, barbeque sauce, etc.)
- When eating out, specify the restaurant. When you eat convenience foods, please specify brands.
- Bring 3 day food record and 3 day activity record with you to your appointment.

Office Use Only

Subject No. _____

Date: ____________________

Time	Description of Food	Amount	For Office Use Only

3 DAY ACTIVITY RECORD

Please complete for the 3 days immediately prior to your appointment as well as the day of appointment.

Dates:__________________

Time of Day	Description of Activity	Duration of Activity	For Office Use Only

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