Does beef inclusion in a modern diet influence risk factors for obesity-related metabolic disorders using a swine biomedical model

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Using swine as a model for humans, the objectives of this project were to determine if replacing sugar (total Western diet [TWD]) with beef (total Western diet-cooked ground beef [TWD-GB]) in a total Western diet would alter body composition, the onset of puberty and risk factors for obesity-related disorders. The results showed that gilts on the TWD-GB diet had greater growth during the test period due to an increase in fat-free lean growth as evidenced by greater longissimus muscle area. TWD-GB had significantly less total cholesterol due to a decrease in low-density lipoprotein cholesterol (LDLch) and high-density lipoprotein cholesterol (HDLch). The reproductive tracts were prepubertal across both treatments; however, follicular development was observed in the TWD-GB gilts. No differences were observed with blood chemistry at the end of the test period.

Summary

Using swine as a model for humans, this study was conducted to determine if replacing sugar in a total Western diet (TWD) with nutrient-dense beef (TWD-GB) would alter body composition, onset of puberty and risk factors for obesity-related disorders. Twenty-four Berkshire gilts were obtained at weaning, assigned to one of two dietary treatments (TWD vs. TWD-GB) and pair-fed at 3.7 percent body weight (BW) for 91 days. Through time, TWD-GB gilts had superior BW gain ($P < 0.01$). At the end of the test, TWD-GB gilts had larger cross-sectional longissimus muscle area ($P < 0.0001$), less subcutaneous fat depth ($P = 0.0005$) and greater percentage of lean BW ($P < 0.0001$) than swine on the TWD. Reproductive tracts were prepubertal across treatments; however, follicular development was observed in TWD-GB gilts. Sodium, hematocrit, hemoglobin and insulinlike growth factor 1 were higher and ionic calcium lower for TWD-GB gilts, compared with TWD gilts.

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Introduction

Red meat and dietary fat have been targeted as the cause of increased obesity and obesity-related metabolic disorders in children and adults in the U.S. and abroad. Many shifts have occurred in dietary recommendations from the U.S. Department of Agriculture through the years.

Recent dietary advice suggesting limiting the intake of red meat is unnecessarily restrictive and may have led to many unintended health consequences (Binnie et al., 2014). Obesity and Type 2 diabetes mellitus are common and growing, and are related problems (Centers for Disease Control and Prevention, 2011).

Obesity has become so prevalent that it is considered to be a worldwide epidemic and a public health concern; however, the cause of these obesity-related diseases is unclear. The major determinants driving obesity are complex but clearly involve interactions with our environment, particularly related to food supply, eating behaviors and genetics, as well as public policies (Dixon, 2010).

To maintain a healthy lifestyle, an understanding of the importance of a proper diet and nutrition is essential. The nutritional state of an individual might change in accordance with the type (or types) of dietary patterns he/she follows at any given time in his/her life. Ultimately, poor diet can result in decreased metabolic efficiency and may lead to nutrient disorders or an unbalance of nutritional conditions in the body.

Using swine as a model for humans, the objectives of this project were to determine if replacing sugar with nutrient-dense beef in a total Western diet (TWD) will alter body composition, the onset of puberty and risk factors for obesity-related metabolic disorders. The TWD for this project was developed for swine using information obtained from the 2007-2008 National Health and Nutrition Examination Survey, "What We Eat in America."

Nutrient requirements for swine are known definitively down to the specific requirements for individual amino acids. Human diets are much less formulated because of the difficulty in evaluating scientifically the many food combinations available to the average American and feeding habits that typical humans have.

Compared with conventional swine diets, the total Western diet of an average American is more energy dense because of its much higher fat content. It is lower in total carbohydrates yet higher in high glycemic carbohydrate (sucrose) and about equal in crude protein. A TWD is higher in sodium and many of the B vitamins, likely because these vitamins are commonly fortified in human foods.

Compared with conventional swine diets, a TWD is much lower in several minerals, including calcium, phosphorus, magnesium, copper, iron and zinc. The nutritional shortfalls of the diets are obvious to swine nutritionists because of extensive production research, which is absent in human nutrition. However, this study holds relevance to the American dietary pattern because the diets were formulated so that micronutrients in the TWD corresponded to American intakes at the 50th percentile when adjusted for nutrient density (mass of nutrients/calories).

The hypothesis of this study is that the consumption of red meat as a replacement for sugar in a TWD would decrease the risk factors for obesity and obesity-related metabolic disorders.

Experimental Procedures

Twenty-four Berkshire gilts were obtained at weaning from Newman Farm Heritage Berkshire Pork (Myrtle, Mo.) and transported 918 miles to the NDSU Animal Nutrition and Physiology Center (ANPC; Fargo, N.D.). All gilts represented a common sire and were born within a seven-day window.

Upon reaching approximately 40 pounds, gilts were sorted, blocked by litter and weight, and penned individually. Gilts were assigned to one of two dietary treatments (TWD vs. TWD-GB) and pair-fed at 3.7 percent of body weight (BW) (12 gilts per treatment) for 91 days. Table 2 displays the dietary components expressed as a percentage of total diets for both treatments.

A TWD was developed for swine using the results of the 2007-2008 National Health and Nutrition Examination Survey, "What We Eat in America." In the TWD-GB diet, cooked ground beef (70:30 lean-to-fat blend) replaced sugar in the TWD on a kilocalorie (kcal) for kcal basis. Diets were formulated to be isocaloric.

Blood samples were collected on day 0 prior to treatment administration and then every 28 days. Weekly BW were taken. Subcutaneous fat depth (FD) and longissimus muscle area (LMA) were measured at the 10th thoracic vertebra on day 42, then every 14 days thereafter.

The percentage of lean BW was estimated from an equation using FD, LMA and live BW. A dietary feed analysis was performed on both treatment diets. Data were analyzed using the mixed procedure of SAS (SAS Institute Inc., Cary, N.C.) as repeated measures with fixed effects of treatment, day and treatment × day, with pig serving as the repeated variable. *P*-values for least square means were adjusted using the Tukey-Kramer method.

Diet Formulation and Feeding Protocol

Our TWD was designed for swine by Korry Hintze (Utah State University) by selecting the average (50th percentile) daily intake levels for all reported nutrients for individuals greater than 2 years old reported to the National Health and Nutrition Examination Survey (NHANES), "What We Eat in America," for 2007-2008. Hintze translated the human NHANES diet into a dietary ration suitable for swine. The swine diet provided by Hintze served as the base diet for the TWD treatment (Table 1).

Table 2 displays the dietary components expressed as a percentage of total diets for both treatments. Fifty percent of the total carbohydrates in the TWD were comprised of sugar (sucrose; fine ground table sugar) and the remaining 50 percent was represented by starch. In the TWD-GB diet, cooked ground beef (70:30 lean-to-fat blend) replaced sugar in the TWD on a kcal for kcal basis. Diets were formulated to be isocaloric and administered as described above. The nutrient information necessary to formulate the TWD-GB treatment was obtained from the USDA National Nutrient Database ground beef calculator (http://ndb.nal.usda.gov/ndb/beef/show). Due to the fact that the USDA's ground beef calculator allowed a maximum of 30 percent fat to be entered, 70:30 lean-to-fat percentages were chosen for this project.

Results and Discussion

A feed analysis was performed on both treatment diets. The TWD-GB diet was overall higher in crude protein, crude fat and lower in total carbohydrates when compared with the TWD (Table 3).

The TWD-GB gilts had greater BW gain through time ($P < 0.01$; Figure 1). A linear increase in LMA and calculated lean BW were observed in TWD-GB vs. TWD (Figures 1 and 3). At the end of the test, TWD-GB had a larger cross-sectional LMA (33.1 centimeters $[\text{cm}]^2$ vs. 14.3 cm2; $P < 0.0001$; Figures 2 and 3), less FD depth (2.0 cm vs. 3.1 cm; $P = 0.0005$), and greater percentage lean BW (51.6 percent vs. 34 percent; $P < 0.0001$) than TWD. Stunting of growth, attenuation of muscle depo-

Table 1. Swine TWD[1]. All nutrients supplied at the 50 percent NHANES[2]. Adjusted to calories for swine 50 to 80 kg (1998 NRC).

Ingredients	Total Amount	Carbohydrates (g/kg)	Protein (g/kg)	Fat (g/kg)	Fiber (g/kg)
Corn (ground, yellow, dent)	420.00	281.23	39.56	19.91	30.66
Sugar	229.33	229.33	0	0	0
Whey protein concentrate 80	165.00	9.08	128.70	13.2	0
Soybean oil	31.40	0	0	31.40	0
Butter	28.47	0	0.24	23.10	0
Olive oil	28.00	0	0	28.00	0
Lard	28.00	0	0	28.00	0
Beef tallow	24.80	0	0	24.80	0
Vitamin mix[3]	35.00	4.01			
Mineral mix[4]	10.00	8.49			
Total g/kg	1,000	532.14	168.50	168.41	
Total kcal/kg	4,318	2,129	674	152	
Total kcal (%)	100	49.3	15.6	35.1	

[1]Total western diet

[2]National Health and Nutrition Examination Survey

[3]Vitamin premix content: niacin (3.6 grams per kilogram [g/kg]); calcium pantothenate (1.7 g/kg); pyridoxine HCL (0.2 g/kg); thiamin HCL (0.38 g/kg); riboflavin (0.35 g/kg); folic acid (0.2 g/kg); biotin (0.03 g/kg); vitamin B12, 0.1 percent in mannitol (1.3 g/kg); vitamin E, DL-alpha tocopheryl acetate (2.0 g/kg); vitamin A palmitate (0.7 g/kg); vitamin D3, cholecalciferol (0.046 g/kg); vitamin K1, phylloquinone (0.013 g/kg); choline bitartrate (140 g/kg); sucrose, fine ground (849.481 g/kg)

[4]Mineral premix content: calcium carbonate (60.57 g/kg); potassium phosphate, monobasic (257.14 g/kg); potassium citrate, monohydrate (71.43 g/kg); sodium chloride (491.43 g/kg); ferric citrate (2.4 g/kg); zinc carbonate (0.686 g/kg); manganous carbonate (1.57 g/kg); cupric carbonate (0.06 g/kg); potassium iodate, (0.0086 g/kg); sodium selenite (0.011 g/kg); sucrose, fine ground (102.4044 g/kg); cholesterol (12.29 g/kg)

sition and increased adiposity were partially alleviated by TWD-GB.

Blood chemistry treatment differences were observed ($P < 0.01$) with blood serum sodium, hematocrit and hemoglobin reading higher for TWD-GB vs. TWD. On day 17 and day 45, gilts on the TWD-GB treatment had less total cholesterol (111.8 vs. 151 and 111.5 vs. 153.1, respectively) than TWD gilts ($P < 0.001$). This was due to a decrease in low-density lipoprotein and high-density lipoprotein cholesterol on those two days ($P < 0.01$). However, at the end of the test period, we found no difference in cholesterol levels or triglycerides ($P > 0.65$) between treatments.

We observed that the animals on the TWD developed porcine acne and thinning of hair, com-

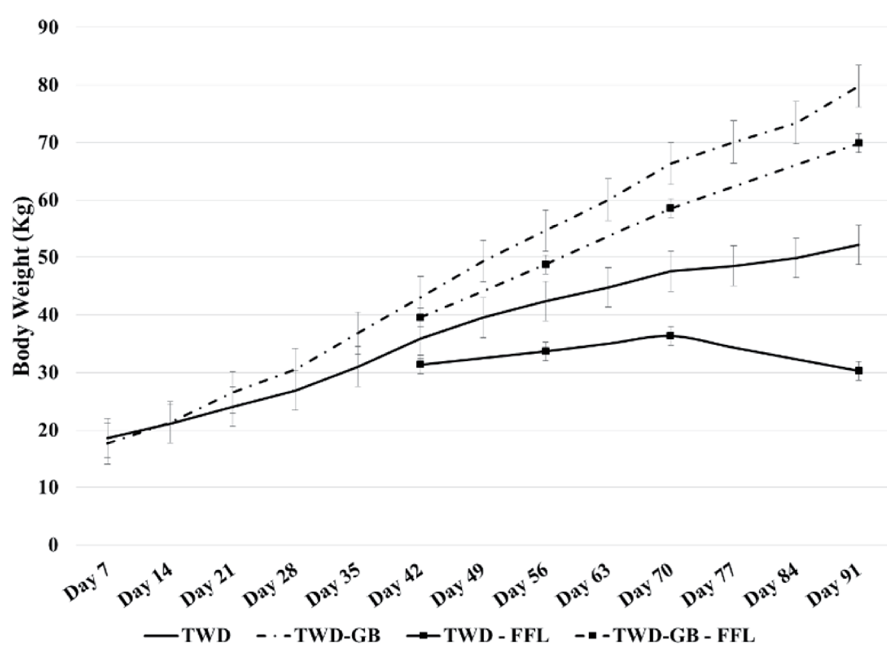

Figure 1. Weekly body weights (TWD and TWD-GB) and lean body weight estimates (TWD – FFL and TWD-GB – FFL) determined by ultrasound measurements.

Table2. Dietary components expressed as a percentage of total diet for TWD[1] and TWD-GB[2].

Ingredients	TWD%	TWD-GB%
Corn, ground	42.0	37.0
Whey, dried	16.5	14.5
Cooked Ground Beef	0.0	32.0
Sugar (sucrose)	22.9	0.0
Soy Oil (blend)	3.9	3.4
Butter	2.8	2.5
100% Olive Oil	2.1	1.9
Lard	2.8	2.5
Beef Tallow	2.5	2.2
Vitamin Mix	3.5	3.1
Mineral Mix	1.0	0.9
Total	100	100

[1]total Western diet.
[2]total Western diet with sugar replaced by cooked ground beef.

Table 3. Diet analysis on an as-fed percentage.

Analysis	TWD[1]	TWD-GB[2]
Crude Protein	5.51	11.54
Crude Fat	13.17	20.13
Crude Fiber	0.55	0.62
Moisture	8.10	24.87
Ash	4.22	3.93
Total Carbohydrates[3]	69.55	40.15

[1]TWD refers to total western diet
[2]TWD-GB refers to total western diet with sugar replaced by cooked ground beef
[3]Total Carbohydrates is calculated by Crude Fiber + NFE[4]
[4]Nitrogen free extract is calculated by 100 - (moisture + ash + crude protein + crude fat)

pared with the TWD-GB gilts. A gilt on the TWD-GB diet became nonambulatory and was removed from the test. A modified necropsy revealed a preliminary diagnosis of rickets, resulting in termination of the research project at 91 day. During final processing, we discovered that the animals on both treatments possessed uncharacteristically brittle bones.

Replacing sugar with ground beef in the present study appears to have influenced the release of the anabolic hormone, insulin-like growth factor 1 (IGF-1). Gilts consuming the TWD-GB had higher ($P < 0.0001$) concentrations of IGF-1 by day 17 of the test. These higher IGF-1 concentrations continued throughout the trial. IGF-1 likely is a key player in the superior anabolic accumulation of muscle tissue seen in gilts consuming TWD-GB.

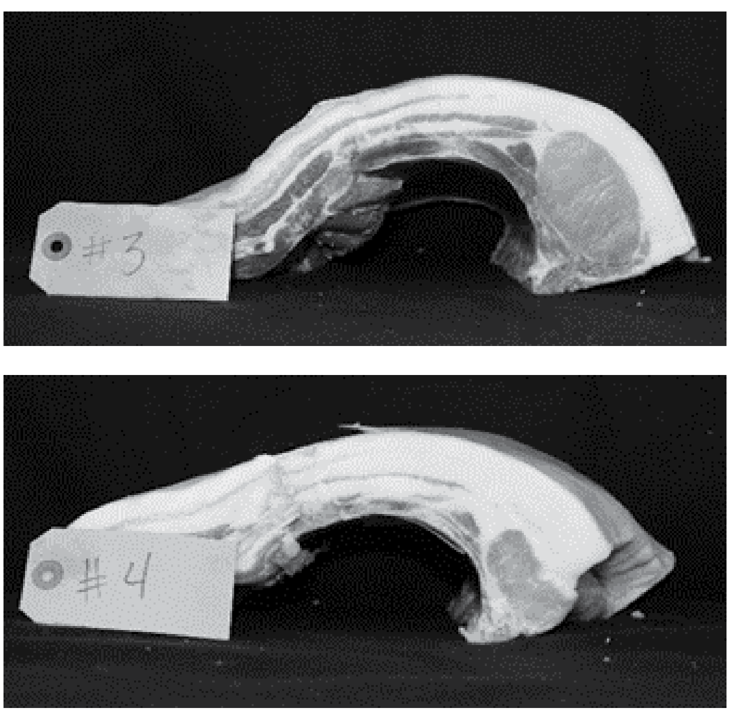

Figure 2. Cross-sectional LMA comparison between littermate gilt pair 3 (TWD-GB) and 4 (TWD) collected at slaughter.

Central adiposity is yet another risk factor associated with metabolic syndrome. Central adiposity in humans is the result of accumulation of adipose tissue between the internal and external abdominal muscles, as well as internally, where adipose accumulates around the kidneys (referred to as perirenal fat). In the present study, TWD-fed gilts had nearly two times more perirenal fat accumulation than TWD-GB (Table 4).

Beef consumption has been targeted by some researchers as the cause of early (precocious) puberty in young American girls. We previously addressed the role of beef consumption and reproductive (puberty) development (see: Magolski et al., 2014) and found no link. In the present study, the reproductive tracts were pre-pubertal across both treatments; however, follicular development was observed in the

TWD-GB gilts. Furthermore, uterine weights expressed as a percentage of eviscerated body weight from gilts receiving the TWD tended (P = 0.0695) to be larger relative to eviscerated body weight than those from TWD-GB (Table 4).

Gilts fed ground beef had more muscle mass and less body fat. Furthermore, subjective evaluation showed that swine on the TWD-GB treatment had fewer skin acne lesions and less hair thinning.

The nutritional shortfalls of the diets are obvious to swine nutritionists because of extensive production research, which can be absent in human nutrition. However, this study still holds relevance to the American dietary pattern because the diets were formulated so that micronutrients in the TWD corresponded to American intakes at the 50th percentile when adjusted for nutrient density (mass of nutrient/calorie).

Both treatment groups exhibited brittle bones, and TWD-GB gilts were less ambulatory by day 91. Further analysis is necessary to determine the physiological reason and relationship to human nutrition.

Acknowledgments

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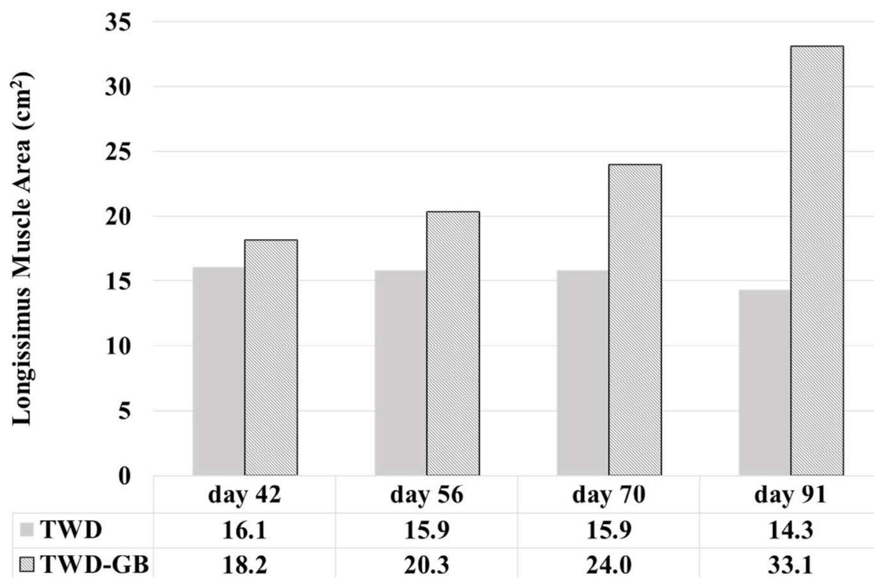

Figure 3. Longissimus muscle area (cm2) at the 10th thoracic vertebra through time for gilts consuming a total Western diet (TWD) versus a TWD-GB where cooked ground beef replaced dietary sugar on a kcal for kcal basis.

Table 2. Least squares means (standard error) for organ weights expressed as a percentage of eviscerated body weight obtained from gilts consuming a total western diet (TWD) versus a TWD where cooked ground beef (GB) replaced dietary sugar on a kcal for kcal basis.

	Perirenal fat	Kidneys	Pancreas	Uterus
TWD	2.55 (0.1836)	0.33 (0.012)	0.101 (0.0059)	0.45 (0.0124)
TWD-GB	1.32 (0.2053)	0.41 (0.014)	0.098 (0.0066)	0.17 (0.0139)
P-value	0.0004	0.0011	0.7643	0.0695