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ARTICLE

Systemic effects of a high saturated fat diet in grizzly bears (*Ursus arctos horribilis*)

D.R. Rivet, O.L. Nelson, C.A. Vella, H.T. Jansen, and C.T. Robbins

Abstract: Food sources for North America's grizzly bear (*Ursus arctos horribilis* Ord, 1815) population have changed as habitats have fragmented, altering available resources and putting bears in contact with unnatural foods. Bears have evolved mechanisms to tolerate obesity, and do not develop adverse health consequences despite storing massive amounts of body fat. Captive adult grizzly bears were used to determine the effects of dietary fat on health. Group 1 was fed a diet high in polyunsaturated fatty acids (PUFA) wherein 9.5% of available calories came from saturated fatty acids (SFA). Group 2 was fed a diet wherein 28.8% of calories came from SFA. Plasma fatty acids, serum lipid profiles, insulin, inflammatory markers, systolic and diastolic blood pressure, and cardiac function parameters were measured. Serum lipids, SFA, and insulin did not differ between the two groups, although omega-3 fatty acids differed. Bears eating the SFA diet had significantly higher circulating adiponectin, interleukin-7 and interleukin-15, and tumor necrosis factor-alpha. Mild, asymptomatic systolic and diastolic dysfunctions were detected by strain echocardiography in the SFA group. The SFA diet group exhibited higher diastolic arterial pressures. Even though mild metabolic derangements were observed, grizzly bears were remarkably resistant to metabolic effects of diets high in SFA.

Key words: grizzly bears, *Ursus arctos horribilis*, hibernation, cardiac function, diastolic function, polyunsaturated fatty acids (PUFA), saturated fatty acids (SFA), inflammatory marker.

Résumé : Les sources de nourriture de la population nord-américaine de grizzlis (*Ursus arctos horribilis* Ord, 1815) ont changé au fil de la fragmentation des habitats, modifiant du coup les ressources disponibles et exposant les grizzlis à des aliments non naturels. Les grizzlis ont développé des mécanismes de tolérance à l'obésité, et le stockage de grandes quantités de graisses corporelles n'a pas de conséquence néfaste sur leur santé. Des grizzlis adultes en captivité ont été utilisés pour déterminer les effets de la graisse alimentaire sur leur santé. Le groupe 1 a reçu un régime riche en acides gras polyinsaturés (AGPI) dans lequel 9,5 % des calories disponibles provenaient d'acides gras saturés (AGS). Le groupe 2 a reçu un régime dans lequel 28,8 % des calories provenaient d'AGS. Les acides gras plasmatiques, les profils de lipides sériques, l'insuline, des marqueurs d'inflammation, la pression sanguine systolique et diastolique et des paramètres associés à la fonction cardiaque ont été mesurés. Il n'y avait pas de différence entre les deux groupes en ce qui concerne les lipides sériques, les AGS et l'insuline, contrairement aux acides gras oméga-3. Les grizzlis nourris au régime riche en AGS présentaient des concentrations sériques d'adiponectine, d'interleukines 7 et 15 et du facteur de nécrose tumorale alpha plus élevées. De légères dysfonctions systolique et diastolique asymptomatiques ont été décelées par échocardiographie d'effort chez le groupe nourri au régime riche en AGS. Ce dernier était caractérisé par des pressions artérielles diastoliques plus élevées. Même si de légers désordres métaboliques ont été observés, les grizzlis étaient remarquablement résistants aux effets métaboliques de régimes alimentaires riches en AGS. [Traduit par la Rédaction]

Mots-clés : grizzlis, *Ursus arctos horribilis*, hibernation, fonction cardiaque, fonction diastolique, acides gras polyinsaturés (AGPI), acides gras saturés (AGS), marqueur d'inflammation.

Introduction

The grizzly bear (*Ursus arctos horribilis* Ord, 1815) is a North American subspecies of the brown bear (*Ursus arctos* L., 1758) that inhabits Alaska, much of western Canada, and portions of the northwestern United States. Although brown bears are normally thought of as apex predators utilizing meat as a significant part of their diet, many brown bears in North America are more commonly omnivorous in that they feed not only on fish, small mammals, and ungulates, but also consume large quantities of berries, tubers, grasses, forbs, and nuts (Welch et al. 1997; Felicetti et al. 2003; Fortin et al. 2013). In preparation for hibernation, bears feed voraciously in the fall (e.g., hyperphagia) to accumulate fat that will be used as the energy source for hibernation (Stenvinkel et al. 2013). Furthermore, brown bears prefer diets that are fat laden. Grizzly bears given ad libitum access to lipids, carbohydrates, and protein chose a fall diet in which lipids provided 73% ± 3% of metabolizable energy (Erlenbach et al. 2014). Grizzly bears often attain body fat levels of 30%–40% in autumn that are considered "obese to morbidly obese" by human standards (AACE/ACE Obesity Task Force 1998; Grundy 2004).

Food sources for North America's brown bears have changed as habitats have shifted and have fragmented in the last century.

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Diets deemed healthy for humans, which are typically high in monounsaturated fats (MUFA), polyunsaturated fatty acids (PUFA), plant proteins, whole grains, fish, fruits, and have lower quantities of red meats, are thought to be generally more similar to bear's natural diet. However, historically, it is likely that bears living in interior regions of the United States that had access to large ungulates consumed a larger proportion of saturated fatty acids (SFA) compared with those feeding largely on PUFA-rich plant- or marine-based diets (Jacoby et al. 1999). As humans have increasingly occupied bear habitat, bear diets have been altered. For example, bears feeding on human foods left behind by park visitors in dumpsters or in camps is not unusual, as bears in Yellowstone consumed garbage from dump sites for more than 100 years (Craighead et al. 1995; Beckmann and Berger 2003). In addition, brown bear populations have lost access to PUFA-rich anadromous salmon or may have greater access to non-native foods such as livestock carcasses that are generally fatter than wild ungulates. These food resource changes may introduce relatively higher amounts of SFA into the diets of brown bears.

Dietary patterns of fatty acid consumption, particularly the ratio of SFA and PUFA, are thought to be associated with the development of many diseases in humans including but not limited to obesity (Phillips et al. 2012), cardiovascular disease (Siri-Tarino et al. 2010), coronary heart disease (Jakobsen et al. 2009; Siri-Tarino et al. 2010), type-2 diabetes (Tur et al. 2012), and metabolic syndrome (Tur et al. 2012). Although evidence on the effects of consuming a diet high in SFA on cardiovascular health is unclear, the research suggests that replacing SFA with PUFA, particularly omega-3 PUFA, has beneficial effects on cardiovascular morbidity and mortality (Jakobsen et al. 2009; Siri-Tarino et al. 2010; de Lorgeril and Salen 2012). Many of the conditions associated with a less than optimal dietary fat pattern are mediated by altered lipid parameters, insulin resistance, and systemic inflammation and are thus relatively easy to recognize in serum biochemistry profiles (Ärnlöv et al. 2005; Roberts et al. 2009). Obesity alone has also been shown to be an independent risk factor for heart disease in humans, even after accounting for comorbid conditions such as type-2 diabetes and coronary artery disease (Roberts et al. 2009; Orhan et al. 2010). Subclinical cardiac diastolic dysfunction appears to be a common initial consequence of obesity and may be overlooked early in the clinical evaluation (Millen et al. 2014; Sanchez et al. 2015). Such findings highlight the potential for lipid-induced heart dysfunction prior to overt clinical signs.

Because of the alterations in habitat and food sources, we wanted to know if the type of dietary fat consumed would affect the overall health and hibernation patterns of brown bears. For example, several species of hibernating rodents experience longer hibernation periods, reduced arousal activity, and lower body temperatures after consuming diets high in PUFA compared with diets high in SFA (Geiser and Kenagy 1987; Geiser et al. 1994; Harlow and Frank 2001; Frank et al. 2008). This is thought to be due to the preferential incorporation of PUFAs into the animals' cell membranes and storage lipids where they are presumably more metabolizable at colder body temperatures than SFAs (Geiser and Kenagy 1987; Frank et al. 2008). In addition to hibernation patterns, we wanted to know whether brown bears would show evidence of harmful health conditions that humans tend to develop while eating diets high in SFA and more refined foods.

For this study, we fed two distinct diets to a group of four captive grizzly bears over a 2 year period in a cross-over design. We chose diets to maximize the difference between SFA and PUFA to create the most dramatic challenge to determine whether bears would be affected by these dietary fat parameters. We assessed health effects of the diets by analyzing in vivo cardiovascular parameters, serum chemistries and inflammatory markers, lipid profiles, and hibernation patterns by physical activity monitors. We hypothesized that a diet high in PUFAs, particularly omega-3, and complex carbohydrates would be healthier for the bears than a diet high in SFAs and refined sugars. We further hypothesized that even though the PUFA and complex carbohydrate diet would be healthier for the bears, the bears would be more resistant than humans to major metabolic derangement when consuming diets high in SFAs because of their evolutionary selection for obesity. Likewise, we hypothesized that diets high in SFA would not affect the activity patterns of hibernating brown bears as occurs in rodents because of the higher hibernating body temperature in bears.

Materials and methods

Animals

Four adult female grizzly bears (aged 7–11 years) were studied for two full active and hibernation periods (summer of 2012 through the winter of 2014). All animals were housed at the Washington State University Bear Research, Education, and Conservation Center, and maintained in compliance with guidelines of the American Society of Mammalogists (Sikes et al. 2011) and the *Bear Care and Colony Health Standard Operating Procedures* approved by the Washington State University Institutional Animal Care and Use Committee (IACUC) (ASAF Nos. 3054 and 4476). Animals had routine access to a 0.56 ha enclosure for physical activity. Because of confounding effects of anesthesia on in vivo assessment of certain parameters (Nelson and Robbins 2010), the bears were trained for voluntary echocardiography, blood pressure measurement, and blood collection. Therefore, all four bears were fully conscious for this study and participated in a comfortable handling routine that allowed for data collection.

Feeding for the study began in May of each year and continued through October, when all feeding was ceased in preparation for hibernation. We strived to maintain similar body composition and overall condition between years to avoid the potential confounding effect of one diet group becoming significantly fatter than the other. As hibernation approached, food was gradually reduced until completely withdrawn at the end of October. Water was provided ad libitum year-round. Bears hibernated in unheated, indoor dens (3 m × 3 m × 2.5 m) with continuous access to outdoor runs (3 m × 3 m × 5 m) that enabled the bears to experience regular light and temperature fluctuations throughout hibernation. Dens were monitored with surveillance cameras (OpenEye Digital Video Security Solutions, Spokane, Washington, USA), and bear movement was quantified throughout hibernation with Actical™ physical activity monitors (Minimitter, Bend, Oregon, USA) attached to the skin with epoxy at the neck and shoulder area.

Ethical approval

All applicable international, national, and (or) institutional guidelines for the care and use of animals were followed. All procedures performed in studies involving animals were in accordance with the ethical standards of the institution or practice at which the studies were conducted.

Diet

Two distinct diets were established and fed to the bears for the duration of the active seasons of the study. The diets were a high PUFA, complex carbohydrate diet versus a high SFA, refined carbohydrate diet that were formulated based on recommendations for humans according to the *Dietary Guidelines for Americans* (U.S. Department of Agriculture and U.S. Department of Health and Human Services 2010). To be assured of a challenge on the bears' metabolic systems, the recommended SFA allowance for humans (i.e., <10% of caloric intake coming from saturated fats) was multiplied 2–3 times in formulating the high SFA bear diet. Thus, a diet high in SFAs was designed with the goal of providing 20%–30% of the total calories as SFA, whereas the PUFA diet provided ≤10% of the calories from SFAs (Table 1).

Table 1. Proportions of the ingredients and compositions of the two diets (polyunsaturated fatty acid (PUFA) and saturated fatty acid (SFA)) used to test the physiological responses of grizzly bears (*Ursus arctos horribilis*) to dietary fats with dietary proportions and compositions expressed on a 100% dry matter basis.

	Diet	
Item	PUFA	SFA
Ingredient		
Apples	17.5	22.9
Commercial chow[a]	30.6	40.3
Beef tallow	—	10.1
Cheddar cheese	—	14.1
Ground beef	—	3.9
High-fructose corn syrup	—	8.7
Oats	11.0	—
Salmon	37.0	—
Salmon oil	3.9	—
Dietary composition		
Gross energy (kcal/g dry matter)	5.30	5.18
Crude protein (% dry matter)	38.5	17.5
Total fat (% dry matter)	17.0	25.5
Calories from protein (% of total calories)	39.2	18.2
Calories from fat (% of total calories)	29.8	45.8
Calories from SFA (% of total calories)[b]	9.5	28.8

[a]Science Diet Canine Adult, Hill's Pet Nutrition, Topeka, Kansas, USA.

[b]Available calories in each diet estimated from feeding and digestive studies of Pritchard and Robbins (1990) and Erlenbach et al. (2014).

Chinook salmon (*Oncorhynchus tshawytscha* (Walbaum, 1792)), salmon oil, and cut oats were components of the PUFA diet that provided unsaturated fats and complex carbohydrates. Specifically, Chinook salmon and salmon oil are high in omega-3 and low in omega-6 PUFA. The steel cut oats are high in complex carbohydrates and fiber and low in simple carbohydrates. Together these ingredients allowed for an 18.3% higher level of polyunsaturated fats and a higher ratio of PUFA to SFA in the PUFA diet compared with the SFA diet. Beef fat, cheddar cheese, and high-fructose corn syrup (HFCS) were components of the SFA diet, which provided saturated fats and added sugars (Table 1). HFCS was included in the SFA diet in place of oats to maximize the bears' metabolic challenge because of its association with obesity and metabolic disturbance in humans (Brown et al. 2008). Because human diets often contain HFCS (Brown et al. 2008), we added HFCS to the SFA diet because bears consuming garbage are likely to consume HFCS, which may affect their health. Both diets included a consistent quantity of apples and commercial chow to supply a balanced vitamin and mineral source and an equal amount of dietary fiber from these base foods. We strived to maintain a similar amount of gross energy per diet (Table 1), so this resulted in the proportion of the diet being supplied by commercial chow and apples being slightly different.

The diets were freeze-dried, ground, and nutritionally evaluated for total fat, *trans* fat, gross energy, crude protein, and fatty acids (Table 2) by a commercial laboratory (Agricultural Experiment Station Chemical Laboratories, University of Missouri, Columbia, Missouri, USA). Twenty-seven fatty acids were analyzed. Each type of food was weighed and recorded before daily feedings. Two bears received the PUFA diet and two bears received the SFA diet for the active season of 2012. The bears that were fed the SFA diet in 2012 were switched to the PUFA diet in 2013, and vice versa. Thus, all four bears received both diets, and differences in winter weather that might affect hibernation were controlled for between years when examining the effect of diet on hibernation.

Body composition

Bears were weighed 2–4 times/month depending on the need to control mass gain. Body composition was determined monthly by water dilution (Farley and Robbins 1994). A baseline blood sample was taken from a hind limb prior to injection of 8 mL of 99.8% deuterium oxide, which was followed by a flush with sterile saline solution. A blood sample was also taken 1 h after the deuterium oxide injection. Whereas previous studies using anesthetized bears found that 2 h were required for equilibration of the deuterium oxide (Farley and Robbins 1994), our preliminary studies indicated that 1 h was sufficient for complete equilibration in the unanesthetized bear. A commercial laboratory (Metabolic Solutions, Inc., Nashua, New Hampshire, USA) analyzed pre- and post-injection samples by cavity ring-down spectroscopy. This method has been validated against isotope ratio mass spectrometry (Crosson 2008; Thorsen et al. 2011). From these analyses, δ deuterium values for pre- and post-injection samples (δ_{pre} and δ_{post}) were determined, and total body water was calculated from the dilution of the isotope using the equation:

$$\text{TBW (moles)} = \frac{WA}{18.02a} \times \frac{(\delta_{dose} - \delta_{tap})}{(\delta_{post} - \delta_{pre})}$$

where W is the amount of tap water in grams used to dilute the dose, A is the amount of deuterium oxide dose in grams administered to the bear, and a is the amount of dose in grams diluted for analysis. Because deuterium can bind to some acidic amino acids, the total body water measurement was divided by 1.04 to correct for the nonexchange of deuterium (Farley and Robbins 1994; Metabolic Solutions, Inc. 2014). Total body fat was then determined based on the corrected total body water.

Serum chemistries, plasma fatty acids, and inflammatory markers

Blood samples were collected approximately 6 weeks after emergence from hibernation in the spring (April), and approximately 6 weeks prior to hibernation in the fall (September) at a time when the bears had obtained the greatest adiposity. Samples were collected into serum and EDTA tubes. Serum tubes were centrifuged at 4 °C and 1750 rev/min for 20 min, and serum and plasma were transferred by pipette into separate o-ring cryovials for storage. Serum and plasma samples were kept at −80 °C until analyzed. The Clinical Pathology Laboratory at Washington State University ran a routine serum chemistry panel within 90 min of collection.

Frozen serum was submitted in batch to Amgen Clinical Pathology Laboratory (Thousand Oaks, California, USA) for lipid profiles consisting of triglycerides, total cholesterol, high-density lipoprotein cholesterol (HDL), low-density lipoprotein cholesterol (LDL), nonesterified fatty acids (NEFA), and insulin concentration. Serum was analyzed for adiponectin with a commercial ELISA mouse–rat assay kit (B-Bridge International, Inc. San Jose, California, USA) previously validated in bears (Lusby et al. 2008) and inflammatory cytokines with a canine cytokine multiplex assay (EMD Millipore Milliplex® Billerica, Massachusetts, USA) using a Luminex-based assay validated by the manufacturer. With the exception of C-reactive protein (CRP), the majority of these markers have significant homology among species, but validation studies using bears have not been performed with Luminex-based assays. Adiponectin and cytokine serum concentrations were determined via a standard regression curve per manufacturers' instructions. Inflammatory markers examined included granulocyte–macrophage colony stimulating factor (GM–CSF), interleukin (IL) 2, IL-6, IL-7, IL-8, IL-10, IL-15, IL-18, tumor necrosis factor-alpha (TNF-α), interferon-gamma (INF-gamma), CRP, monocyte chemoattractant protein-1 (MCP-1), interferon gamma-induced protein-10 (IP-10), and keratinocyte-derived chemokine (KC) like.

Table 2. Fatty acid composition (% of total fatty acids) of the diet on a 100% dry matter basis and the resulting serum fatty acid and lipid profiles (μmol/L for fatty acids, mg/dL for serum lipids, and ng/mL for insulin).

		PUFA		SFA		
	Fatty acid or lipid profile	Diet	Serum	Diet	Serum	*P*
PUFA omega-3	Eicosapentaenoic	3.66	806.8±229.0	0.01	85.3±40.5	0.001
	Docosapentaenoic	1.64	170.8±29.0	0.01	63.8±39.7	0.005
	Docosahexaenoic	6.09	480.3±93.4	0.02	102.5±21.7	<0.001
	Total omega-3	11.39	1511.6±324.2	0.04	299.5±120.8	0.003
PUFA omega-6	α-Linoleic	9.18	1378.8±361.5	6.75	1884±453.1	0.132
	Arachidonic	4.67	545±102.7	0.15	626.8±107.3	0.313
	Gamma linoleic	—	11.5±3	—	31.8±7.1	0.009
	Dihomogamma linolenic	—	75.3±18.4	—	124.8±11.4	0.017
	Total omega-6	13.85	2058.8±445.6	6.90	2766.8±1539.1	0.847
PUFA omega-9	Docosatetraenoic	—	14.1±2.2	—	32.3±10.2	0.028
	Mead	—	10.1±1.3	—	95.4±36	0.019
MUFA	Myristoleic	0.94	3.1±1.8	0.89	29.1±21.9	0.056
	Palmitoleic	5.67	113.5±72.4	2.52	99.8±49.2	0.764
	Vaccenic	0.49	198.2±57.4	0.11	142±21.6	0.175
	Oleic	27.09	1178.8±350.3	29.92	1614±523.7	0.216
	11-Eicosenoic	3.81	64.5±27.1	0.29	15.9±4.2	0.012
	Nervonic	0.6	11.5±6.0	0.03	1.6±0.3	0.020
	Total MUFA	38.6	1569.5±497.7	33.80	1902.4±576.0	0.614
SFA	Myristic	3.63	71.3±55.3	9.35	142±80.8	0.020
	Palmitic	16.69	1122.3±321.7	24.46	1718±665.8	0.158
	Stearic	5.63	1663.8±319.8	16.87	1688.6±238.3	0.905
	Lignoceric	0.05	3.7±1.3	0.03	2.4±0.5	0.126
	Pentadecanoic	0.16	19.6±10.7	1.17	32.1±18.4	0.282
	Heptadecanoic	0.26	71.7±21.7	1.05	99.4±28.7	0.175
	Total SFA	26.42	3075.4±711.4	52.93	3792.6±1089.4	0.462
Lipid panel	Cholesterol	—	230.3±48.8	—	224.3±37.6	0.850
	Triglyceride	—	282.8±30.8	—	230.3±59.1	0.170
	BUN	—	21.5±6.0	—	16.8±4.3	0.250
	HDL	—	107.5±13.3	—	130.3±28.0	0.190
	LDL	—	11±7.4	—	11.3±5.3	0.960
	NEFA	—	0.2±<0.1	—	0.3±0.1	0.170
	Insulin	—	0.10±0.01	—	0.09±0.03	0.245

Note: PUFA, polyunsaturated fatty acid; MUFA, monounsaturated fatty acid; SFA, saturated fatty acid; BUN, blood–urea–nitrogen; HDL, high-density lipoprotein; LDL, low-density lipoprotein; NEFA, nonesterified fatty acid.

Plasma was analyzed for fatty acid content via capillary gas chromatography – mass spectrometry by a commercial laboratory (Metametrix Clinical Laboratory, Duluth, Georgia, USA) and a complete profile of fatty acids was derived for each bear. Concentrations of omega-3, omega-6, and omega-9 PUFA, MUFA, SFA, odd-chain fatty acids, and *trans* isomers from hydrogenated oils were measured. The specific fatty acids measured were α-linolenic, eicosapentaenoic, docosapentaenoic, docosahexaenoic, linoleic, gamma linoleic, eicosadienoic, dihomogamma linolenic, arachidonic, docosadienoic, docosatetraenoic, mead, myristoleic, palmitoleic, vaccenic, oleic, 11-eicosenoic, nervonic, capric, lauric, myristic, palmitic, stearic, arachidic, behenic, lignoceric, hexacosanoic, pentadecanoic, heptadecanoic, nonadecanoic, heneicosanoic, tricosanoic, palmitelaidic, and total C:18 *trans* fatty acids.

Echocardiography

Trained bears underwent a complete transthoracic echocardiographic examination that included two-dimensional, M mode, spectral and color-flow Doppler evaluations. Echocardiography was performed by the same person (O.L.N.) using commercially available equipment (My Lab 30; Biosound Esaote, Indianapolis, Indiana, USA). The bears were imaged in sternal recumbency with forelegs positioned cranially to optimize the parasternal thoracic windows. Standard imaging planes and global function calculations have been previously described for cats and dogs (Thomas et al. 1993). All measures were performed in accordance with the American Society of Echocardiography for cardiac volume and functional calculations (Schiller et al. 1989; Kuecherer et al. 1991; Ommen et al. 2000; Rajagopalan et al. 2001). Strain echocardiography was also performed to assess regional (vs. global) myocardial diastolic motion, as previously described in dogs (Chetboul et al. 2006; Tidholm et al. 2009).

These examinations were performed in the spring (late April) after hibernation and in the fall (mid-September) prior to any changes in heart function associated with hibernation (Nelson and Robbins 2010). Video images were recorded using a commercially digital echocardiography software program and data were analyzed off-line using a workstation (Biosound Esaote Indianapolis, Indiana, USA). Volume parameters were normalized to body mass to account for disparities in body size between bears, as is customary for large mammals (Schiller et al. 1989). A total of 19 standard echocardiographic parameters assessing global cardiac chamber function (see Supplementary Table S1)[1] and 17 strain echocardiographic parameters assessing regional myocardial motion (Table 3) were collected during the evaluations, with emphasis on determining differences in diastolic function of the heart.

[1]Supplementary Table S1 is available with the article through the journal Web site at http://nrcresearchpress.com/doi/suppl/10.1139/cjz-2016-0271.

Table 3. Echocardiographic regional strain imaging and blood pressure variables (mean ± SD) recorded in unanesthetized grizzly bears (*Ursus arctos horribilis*) eating polyunsaturated fatty acid (PUFA) and saturated fatty acid (SFA) diets in the fall after one season of diet consumption and at the fattest body composition.

Variable	PUFA	SFA	*P*
Strain			
Longitudinal velocity of left ventricle (LV) wall in systole (cm/s)	9.0±2.5	3.8±0.6	0.023
Longitudinal velocity of LV wall in early diastole (cm/s)	−5.4±2.1	−3.0±1.6	0.270
Longitudinal velocity of LV wall during atrial contraction (cm/s)	−6.7±2.9	−3.6±0.6	0.075
Transverse velocity of LV wall in systole (cm/s)	6.6±0.8	5.7±1.1	0.386
Transverse velocity of LV wall in early diastole (cm/s)	−7.6±0.7	−4.3±2.0	0.018
Transverse velocity of LV wall during atrial contraction (cm/s)	−2.9±1.8	−7.0±0.8	0.016
Percent change of LV wall during systole (%)	−21±4.2	−23.5±4.2	0.426
Percent change of LV wall during diastole (%)	0.5±0.4	2.5±1.0	0.426
Strain rate during systole (L/s)	−1.8±0.5	−1.8±0.3	0.055
Strain rate during early diastole (L/s)	1.1±0.7	1.2±0.5	0.934
Strain rate during atrial contraction (L/s)	0.8±0.2	1.3±1.3	0.838
Blood pressure			
Maximum filling volume of heart (mL)	282.5±49.6	340±28.1	0.090
Percent left ventricular ejection fraction (%)	62.5±3.4	53.3±3.9	0.051
Stroke volume (mL)	156.8±9.3	180.5±21.8	0.192
Systolic blood pressure (mm Hg)	228.4±10.3	236.3±8.7	0.172
Diastolic blood pressure (mm Hg)	122.5±2.2	152.6±9.9	0.006
Heart rate (no. of beats/min)	81±9.3	82.8±9.7	0.803

Blood pressure

Systolic and diastolic arterial blood pressure was measured from the bears' left dorsal metatarsal artery using an inflatable cuff and the Doppler technique (Parks Medical Electronics, Inc., Aloha, Oregon, USA) in the pre- and post-hibernating period. The cuff size was determined by taking 40% of the circumference of the limb just proximal to the hock (McLeish 1977; Haskins 1992). The same person (D.R.) performed the test for all bears. Three to four measurements were recorded and averaged to provide a single value for that time period. The indirect Doppler technique was validated in four bears prior to use in this study. Four bears, two adult females (age 12 and 15 years) and two adult males (age 12 years), were anesthetized to compare direct and indirect blood pressure measures. These bears were given an intramuscular injection using a combination of a reversible α2 agonist (6.04 μg/kg dexmedetomidine) and a nonreversible *N*-methyl-D-aspartate (NMDA) agonist with a tranquilizer (1.23 mg/kg tiletamine and 1.23 mg/kg zolazepam; Fort Dodge Animal Health, Fort Dodge, Iowa, USA) (Teisberg et al. 2014). The femoral artery was immediately catheterized for a direct arterial blood pressure measure (BeneView T5; Mindray Medical USA Corp., Redmond Washington, USA). Simultaneously, blood pressure was measured using the indirect Doppler technique on the opposite rear limb. Blood pressures determined by direct artery catheterization did not differ from the indirect Doppler technique, indicating that indirect measures are accurate (systolic: direct 253 ± 24, indirect 260 ± 25 (P = 0.73); diastolic: direct 158 ± 8, indirect 160 ± 10 (P = 0.68)).

Activity monitors

Bear activity during the active season and hibernation was monitored with Actical™ physical activity monitors (Phillips Respironics, Bend, Oregon, USA) encased in aluminum protective cases and glued to the skin with two-part epoxy at the shoulder (Ware et al. 2012). Application and removal of the Acticals was performed on bears trained to remain still for a food reward. Incidence of movement and movement velocity data were collected in 1 min epochs via an omnidirectional accelerometer sampling at 32 Hz and converted to counts (resolution 100 counts or 0.02 G at 1 G peak). Activity monitors were removed after the bears emerged from hibernation in the spring, and the data were downloaded into the Actical version 2.12 software (Phillips Respironics, Bend, Oregon, USA). The data were then exported and analyzed for mean daily total activity (counts/day) during the active season (March–October), during the deepest part of hibernation (15 December – 15 January), and throughout the total hibernation duration (November–February).

Statistical analysis

All analyses in this study were performed using IBM SPSS version 22.0. Assumptions for analyses were all conducted independently and met. Each variable was screened for missing data. Each quantitative variable had no missing values. This was largely due to the low sample size. Paired-samples t tests have been shown to be appropriate with extremely small sample size ($N \leq 5$), specifically when the within-pair Pearson coefficient is high (de Winter 2013). The Shapiro–Wilk statistic showed a nonsignificant departure from normality for all variables. Examination of Q–Q plots for all variables did not indicate serious departures from normality. A paired-samples t test was used to determine whether there was a statistically significant mean difference between the test groups. No outliers were detected, defined as more than 1.5 box lengths from the edge of the box in a box plot. Further investigation of the scores did not reveal the need to remove values; thus, they were kept in the analysis. A P value of ≤0.05 was considered significant. All results are presented as means ± SD.

Results

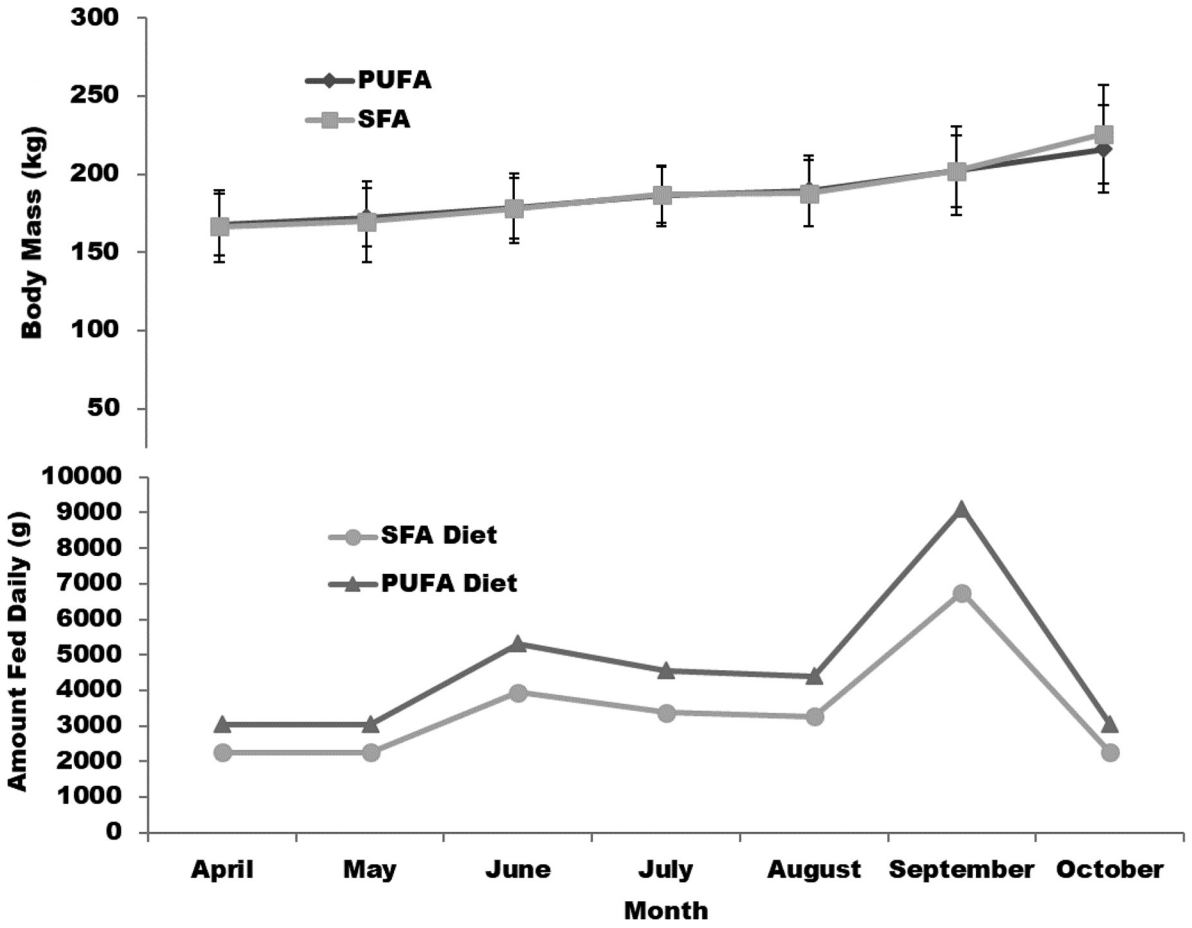
Diet

Approximately 28.8% and 9.5% of the total calories came from saturated fatty acids in the SFA and PUFA diets, respectively (Table 1). The relative concentration of SFAs fed was approximately twice as high in the SFA diet than in the PUFA diet. Similarly, the relative concentration of PUFAs fed in the PUFA diet was approximately three times higher compared with the SFA diet (Table 2).

Body composition

Food intake and body mass increased as the bears prepared for hibernation (Fig. 1). No significant difference in percent body fat was found between the two diet groups (P = 0.86). Percent body fat in May and September for the SFA group was 23% ± 5% and 34% ± 2%, respectively, whereas the percent body fat in May and September for the PUFA group was 22% ± 5% and 35% ± 7%, respectively.

Fig. 1. Mean amount of food (fresh mass basis) consumed per day during the active season (May–October) by grizzly bears (*Ursus arctos horribilis*) involved in the dietary study and their corresponding body masses (mean ± SD).



Blood chemistries and inflammatory markers

Serum biochemistries and lipids, including cholesterol, HDL, LDL, SFA, and NEFA, did not differ between the two diet groups in the spring or fall. However, total cholesterol, HDL, LDL, and NEFA in bears were elevated compared with human standards (Table 2). Bears eating the PUFA diet had significantly higher levels of omega-3 fatty acids in the fall than did those on the SFA diet. Bears eating the SFA diet had higher levels of some omega-6 fatty acids, including gamma linoleic and dihomogamma linolenic, as well as the omega-9 fatty acids docosatetraenoic and mead acid, than did bears consuming the PUFA diet (Table 2).

Serum adiponectin levels were significantly higher ($P < 0.001$) in bears consuming the SFA diet (17.2 ± 2.9 μg/mL) than in bears consuming the PUFA diet (9.5 ± 2.5 μg/mL). Of the 13 inflammatory cytokines analyzed, only 3 were significantly different between diet groups. TNF-α was higher in bears eating the SFA diet (11.7 ± 1.8 pg/mL) than those consuming the PUFA diet (9.7 ± 1.8 pg/mL) ($P < 0.01$). IL-7 was higher in bears eating the SFA diet (56.1 ± 40 pg/mL) than those eating the PUFA diet (39.8 ± 33.1 pg/mL) ($P = 0.03$). IL-15 was higher in bears eating the SFA diet (60.7 ± 29 pg/mL) than those eating the PUFA diet (49 ± 29.1 pg/mL) ($P = 0.02$) (Fig. 2).

Heart and circulatory function

There were no differences detected in standard echocardiography parameters between the two diet groups in the spring or fall (see Supplementary Table S1).[1] However, myocardial strain imaging detected significant differences in regional left ventricular wall motion between the two diet groups in the fall (Table 3). Bears in the SFA group compared with those in the PUFA group had slower ventricular wall motion in systole (longitudinal direction of wall contraction) and in early diastole (transverse velocity of wall relaxation; Figs. 3*a*, 3*b*). Bears in the SFA group compared with those in the PUFA group had higher atrial contraction velocities in late diastole (both longitudinal and transverse directions), indicating decreased ventricular compliance during atrial contraction. These parameters indicate left ventricular diastolic impairment in the SFA group. Blood pressure did not differ between to two groups of bears in the spring. Bears eating the SFA diet exhibited higher diastolic arterial pressures than the PUFA group in the fall, but systolic arterial blood pressures did not differ (Table 3).

Activity

Although mean total daily activity was significantly different across seasons ($P < 0.01$) in that bears displayed more daily activity during the active season than the hibernation season, there were no differences in activity between the diet groups during the deeper part of hibernation ($P = 0.48$) or for the total hibernation period ($P = 0.33$) (Fig. 4).

Discussion

Although bears were obese in the fall by human standards (Gallagher et al. 2000; Pescatello and the American College of Sports Medicine 2014), there were fewer metabolic derangements compared with that expected for obese humans consuming similar diets (Fung et al. 2001; Brown et al. 2008; Heidemann et al. 2008; Fung et al. 2009; Estruch et al. 2013). One consideration for the observed difference between species is that metabolic dysfunction is something that develops over time in humans who

Fig. 2. Mean (±SD) serum concentrations of inflammatory markers analyzed from grizzly bears (*Ursus arctos horribilis*) eating both the polyunsaturated fatty acid (PUFA) diet and the saturated fatty acid (SFA) diet. A *P* value of <0.05 was considered significant. TNF-α, tumor necrosis factor-alpha; MCP-1, monocyte chemoattractant protein-1; IL-18, interleukin-18; IL-10, interleukin-10; KC-like, keratinocyte-derived chemokine-like; IP-10, interferon gamma-induced protein-10; IL-15, interleukin-15; IL-8, interleukin-8; IL-7, interleukin-7; IL-6, interleukin-6; IL-2, interleukin-2; GM–CSF, granulocyte–macrophage colony stimulating factor.

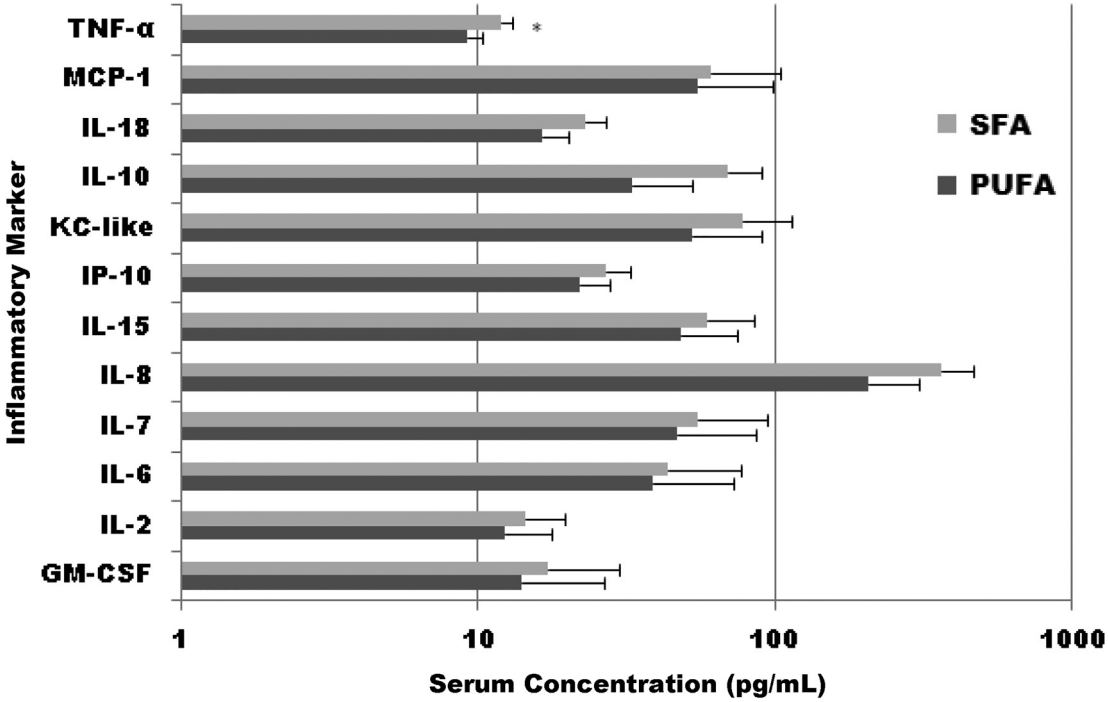
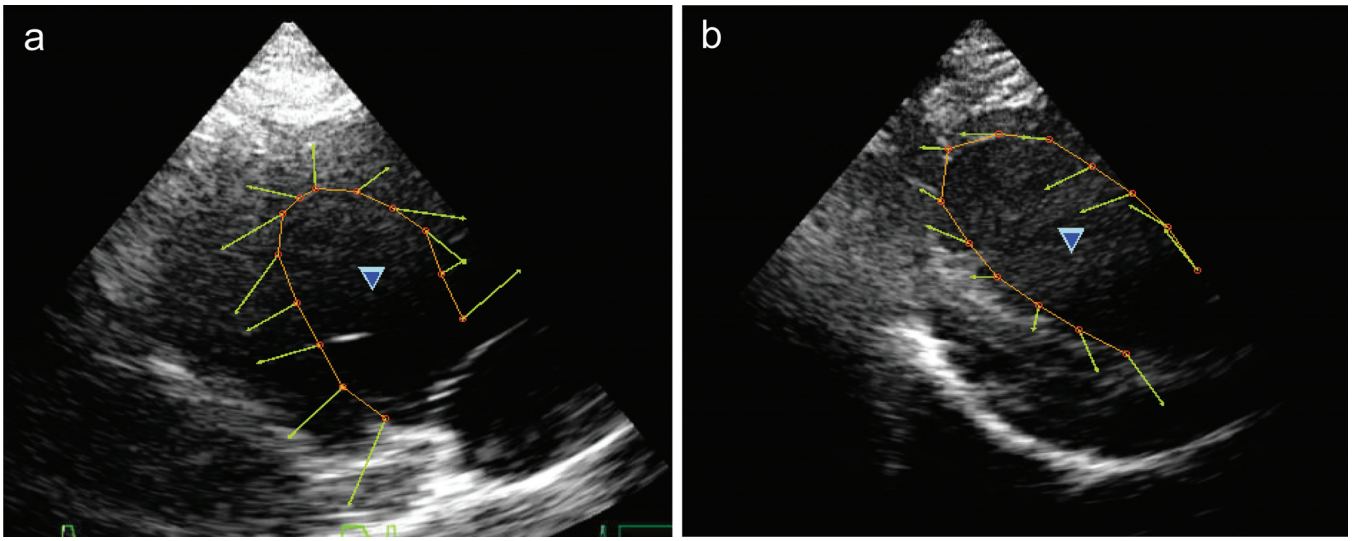


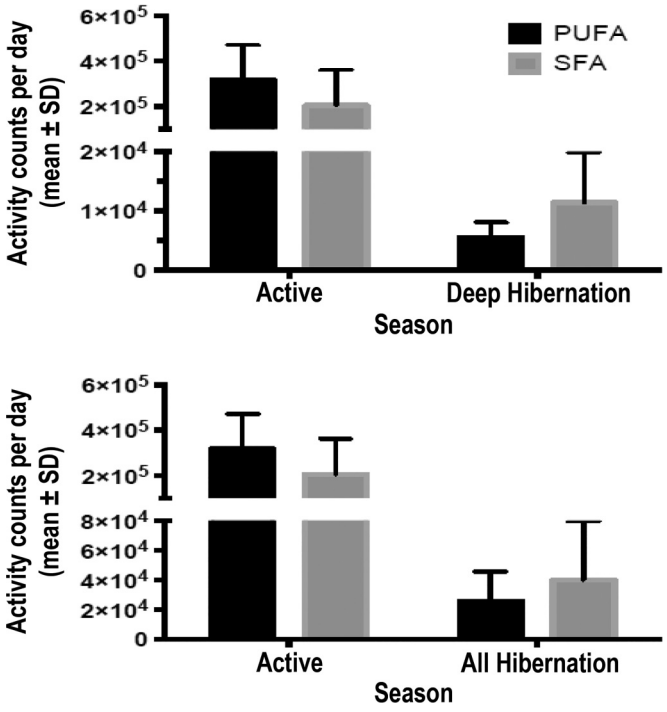
Fig. 3. Representative transverse velocities of left ventricular wall motion indicating the direction of myocardial movement and the velocity or vigor of the myocardial movement taken at the same point in early diastole (cm/s) in grizzly bears (*Ursus arctos horribilis*) on the two different diets: (*a*) polyunsaturated fatty acid (PUFA) and (*b*) saturated fatty acid (SFA). Colour online.



remain obese. Bears do not remain obese throughout the year, but gain and then lose mass seasonally. Moreover, insulin resistance and hyperinsulinemia, two hallmarks of metabolic syndrome, develop only during hibernation in bears (Rigano et al. 2017) and only at specific times of the year. Together these results suggest that bears have experienced extensive evolutionary selection to accumulate large amounts of fat to survive hibernation while remaining healthy and reproductively active (LeBlanc et al. 2001; Robbins et al. 2012; Viscarra and Ortiz 2013; Lopez-Alfaro et al. 2013). For example, females with greater adiposity produce larger cubs with a better chance of survival compared with leaner bears (McLellan 2011; Robbins et al. 2012), and larger bodied males do most of the breeding (Kovach and Powell 2003; Dahle et al. 2006). This has resulted in bears with dramatically increased appetites in the fall and a preference for high-fat diets (Robbins et al. 2012; Erlenbach et al. 2014). As such, obesity as defined by human standards may be healthy or even necessary for this species to thrive and reproduce.

The significant differences in serum fatty acid profiles in response to the two diets suggested that the diets were sufficient

Fig. 4. Activity counts recorded per day during the active season (March–October), the deepest part of hibernation (15 December – 15 January), and total hibernation duration (November–February) compared by diet type (polyunsaturated fatty acid (PUFA) vs. saturated fatty acid (SFA)). Active season mean (±SD) counts are 317 271 ± 77 413 for PUFA and 204 774 ± 78 529 for SFA. Deep hibernation mean (±SD) counts are 5 570 ± 1 253 for PUFA and 11 381 ± 4 240 for SFA, whereas all hibernation mean (±SD) counts are 25 587 ± 9 860 for PUFA and 40 011 ± 19 693 for SFA.



to induce biochemical changes in the circulation by the fall. The degree of alteration observed in plasma fatty acids due to the diets would have health consequences in humans. For example, omega-3 fatty acids (e.g., docosapentaenoic, docosahexaenoic, and eicosapentaenoic acids) are known to have many health benefits, including protecting against inflammation, coronary artery disease, and ischemic stroke (Esposito et al. 2004; Ruxton 2004; Chen et al. 2011; Tur et al. 2012). The omega-6 fatty acids (e.g., gamma linoleic and dihomogamma linolenic acids) that were elevated in bears consuming the SFA diet are associated with increased inflammation, coronary artery disease, and ischemic stroke and often inhibit the production of omega-3 fatty acids in humans (Guebre-Egziabher et al. 2008). Furthermore, high levels of docosatetraenoic acid, which was twice as high in the serum of the SFA bears, are correlated with obesity and implicated in insulin resistance in humans (Kusunoki et al. 2007). Interestingly, levels of serum MUFA and SFA did not differ significantly between the two diet groups. In hibernators, fatty acids provide the major source of energy and despite the high rate of hepatic β-oxidation, long-chain PUFA are selectively conserved (Xia et al. 1993). Evidence from other studies on black bears (*Ursus americanus* Pallas, 1780) and northern elephant seals (*Mirounga angustirostris* (Gill, 1866)) suggest that SFA are preferentially mobilized and metabolized for mitochondrial oxidation, whereas PUFA are spared and likely conserved for other purposes (LeBlanc et al. 2001; Viscarra and Ortiz 2013). Therefore, bears may be more resistant to the effects of high SFA diets that would otherwise be detrimental to humans.

Though differences in consumption of PUFA and SFA in hibernating rodents have been shown to affect torpor bout duration and arousal activity (Florant et al. 1993; Geiser et al. 1994; Harlow and Frank 2001), no significant differences were noted in hibernation activity levels between the diet groups in bears. Metabolism of fatty acids may be less problematic for bears because they hibernate at a warmer body temperature than do rodents. Although the difference in hibernation activity by bears on the PUFA diet appeared to show a similar trend to that observed in rodents (i.e., decreased activity in hibernation), the required sample size of bears to detect a statistical difference based on the observed variation suggests that our current sample size would represent a limitation to detect a significant difference.

Serum adiponectin was highest in bears consuming the SFA diet. Adiponectin is a metabolically active cytokine exclusively secreted from adipose tissue that modulates several metabolic processes, including insulin resistance and fatty acid oxidation, preventing fatty liver disease (You et al. 2005), and preventing arterial inflammation and atherosclerosis (Libby et al. 2010; Arinell et al. 2012). In humans, adiponectin is inversely associated with obesity, type-2 diabetes, atherosclerosis, and other derangements linked with metabolic syndrome (Fernández-Real et al. 2005; You et al. 2005; Murakami et al. 2013; Santos et al. 2013). Therefore, lower adiponectin levels in the blood of humans are often coupled with higher percent body fat and increased intake of SFA (Fernández-Real et al. 2005; Reis et al. 2010). Unlike humans, bears displayed a positive correlation between percent body fat, high SFA diet, and circulating adiponectin levels. Some of these differences between bears and humans in adiponectin secretion may occur because bears accumulate virtually all of their fat in subcutaneous deposits, whereas humans tend to accumulate visceral fat, which is associated with decreases in adiponectin levels and negative health consequences (Asayama et al. 2003; Lenchik et al. 2003; Fontana et al. 2007). In addition, seasonal changes in adiponectin are expected for hibernating mammals. For example, adiponectin is lower in winter because these animals switch from lipogenesis during the active season to lipolysis and insulin resistance during hibernation (Florant et al. 2004; McCain et al. 2013). As such, adiponectin may serve a different physiologic process in hibernators than it does in humans, meaning high or low serum levels in bears do not imply the same pathologic processes.

Of the 13 inflammatory cytokines tested, only TNF-α, IL-7, and IL-15 showed significant differences between diet groups, suggesting little inflammation in bears regardless of adiposity or diet, though many inflammatory markers were elevated compared with human standards (Fig. 2). All three of these cytokines are known to have prominent biological effects on metabolic and cardiac health in humans and other mammals (Pagani et al. 1992; Lang et al. 2002; Alam et al. 2004; Saidijam et al. 2014).

For example, TNF-α is a proinflammatory cytokine thought to play a role in the pathogenesis of heart failure because intravenous infusion of TNF-α in animal models produces immediate negative effects on myocardial contraction and promotes chamber dilation (Oral et al. 1995; Lang et al. 2002). The suspected cell signaling pathways responsible for these effects are believed to be production of nitric oxide (NO), secondary to TNF-α induced expression of the inducible form of nitric oxide synthase (iNOS) (Mann and Young 1994; Oral et al. 1995). Continuous TNF-α infusion in dogs alters the diastolic elastic properties of the ventricle by altering expression of ECM proteins and their regulatory proteins (Pagani et al. 1992), whereas other studies have documented impairment of cardiac muscle mRNA protein translation by TNF-α infusion (Mauviel et al. 1991; Lang et al. 2002). Thus, it is possible that increased TNF-α in the SFA group is related to the cardiovascular differences noted in these bears. However, even though the difference in TNF-α is significant, the overall levels are relatively low compared with humans.

Interleukins also play a key role in inflammation and modulate metabolic functions. IL-7, known as B-cell precursor growth factor,

has a key role in lymphocyte homeostasis, especially in basal metabolism of glucose. It maintains high glucose uptake and expression of GLUT1, which results in adequate glycolytic flux (Saidijam et al. 2014). IL-15 is a more recently studied cytokine that appears to have functions in regulating adipose tissue deposition (Quinn and Anderson 2011). Both cytokines have been implicated in the mechanism underlying metabolic syndrome and cardiovascular dysfunction. In addition, cell adhesion molecules, growth factors, and proinflammatory cytokines have been identified in excessive concentrations in the setting of acute coronary syndrome associated with obesity. These factors lead to vascular endothelial dysfunction, plaque vulnerability, and thrombogenicity (Alam et al. 2004).

Clinical studies support that indices of adiposity and proinflammatory cytokine levels in obesity are (*i*) correlated with dyssynchrony in ventricular contraction and relaxation and (*ii*) are an independent predictor of cardiac mortality (Marfella et al. 2004). Although bears did not exhibit clinical symptoms of cardiovascular disease during this study, we did note significant decreases in ventricular contraction and primarily relaxation rates in bears eating the SFA diet, as well as higher diastolic blood pressures in this group. Subclinical cardiac diastolic dysfunction is a common initial consequence of human obesity and may be overlooked early in the clinical evaluation if exercise testing is not performed. Over time, functional changes may deteriorate into clinical disease, although the time frame for progression is variable. It is unknown whether the cardiovascular differences seen in these bears could be the result of inflammatory cytokines or tissue/endothelial oxidative changes as described for other species eating high SFA diets (Sun et al. 2012). The mechanisms behind these observations warrant further investigation.

Our findings suggest a less dramatic degree of metabolic disturbance and cardiac dysfunction associated with consumption of foods containing high levels of saturated fats and HFCS compared with humans. Although the SFA diet in our study appears less healthy for bears, the animals remained relatively resistant to developing metabolic derangements or severe clinical disease in a time frame when such anomalies would be expected to occur in humans. Though bears in the SFA group were asymptomatic, it is important to note that our study is small and occurred over a single feeding season for each group. Wild bears relying heavily on human refuse or bears residing in captive facilities could potentially be more negatively affected over a longer term by similar diets.

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