European Journal of Clinical Nutrition (2002) 56, 181–191
 0954–3007/02 $25.00
www.nature.com/ejcn

REVIEW ARTICLE

Fatty acid analysis of wild ruminant tissues: evolutionary implications for reducing diet-related chronic disease

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Hypotheses: Consumption of wild ruminant fat represented the primary lipid source for pre-agricultural humans. Hence, the lipid composition of these animals' tissues may provide insight into dietary requirements that offer protection from chronic disease in modern humans.
Method: We examined the lipid composition of muscle, brain, marrow and subcutaneous adipose tissue (AT) from 17 elk (*Cervus elaphus*), 15 mule deer (*Odocoileus hemionus*), and 17 antelope (*Antilicapra americana*) and contrasted them to wild African ruminants and pasture and grain-fed cattle.
Results: Muscle fatty acid (FA) was similar among North American species with polyunsaturated fatty acids/saturated fatty acids (P/S) values from 0.80 to 1.09 and n-6/n-3 FA from 2.32 to 2.60. Marrow FA was similar among North American species with high levels (59.3 – 67.0%) of monounsaturated FA; a low P/S (0.24 – 0.33), and an n-6/n-3 of 2.24 – 2.88. Brain had the lowest n-6/n-3 (1.20 – 1.29), the highest concentration of 22:6 n-3 (elk, 8.90%; deer, 9.62%; antelope, 9.25%) and a P/S of 0.69. AT had the lowest P/S (0.05 – 0.09) and n-6/n-3 (2.25 – 2.96). Conjugated linoleic acid (CLA) isomers were found in marrow of antelope (1.5%), elk (1.0%) and deer (1.0%), in AT (deer, 0.3%; antelope, 0.3%) in muscle (antelope, 0.4%; elk, trace), but not in brain.
Conclusions: Literature comparisons showed tissue lipids of North American and African ruminants were similar to pasture-fed cattle, but dissimilar to grain-fed cattle. The lipid composition of wild ruminant tissues may serve as a model for dietary lipid recommendations in treating and preventing chronic disease.
European Journal of Clinical Nutrition (2002) **56,** 181 – 191. DOI: 10.1038/sj/ejcn/1601307

Keywords: fatty acids; CLA; wild game tissues; evolution; diet

Introduction

Both quantitative and qualitative aspects of dietary lipid intake exert important influence upon human health and the expression of chronic disease. It is likely that human dietary lipid requirements are genetically determined (Eaton, 1992; Eaton *et al*, 1998), and that the evolutionary, nutritional selective pressures that have acted upon the ancestral human lineage over the past 2.4 million years since the emergence of our genus (*Homo*), may provide important insight into optimal, present day, lipid intakes (Eaton, 1992; Eaton *et al*, 1998; Simopoulos, 1999; Simopoulos *et al*, 1999). There is substantial evidence from both the archaeological and ethnographic literature to show that consumption of wild animal tissues played a predominant role in the diet of early humans (Marean & Assefa, 1999; Milton, 1999; Stanford & Bunn, 1999) as well as in historically studied hunter-gatherers (Cordain *et al*, 2000). Recent mean estimates of the plant-to-animal subsistence ratios in 229 hunter-gatherer societies, the best surrogates of Stone Age humans, demonstrated that meat and other animal-derived foods would have provided on average 68% of the total energy, and the remaining 32% of the average daily

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Received September 2000; revised June 2001; accepted July 2001

energy would have come from plant sources (Cordain *et al*, 2000). Consequently, fats derived from wild game animals almost always represented the primary lipid source in pre-agricultural human diets.

Neither fowl nor fish became common dietary constituents until about 20 000 y ago (Eaton, 1992), so the main nutritional adaptation over most of humanity's 2.4 million y existence was to the tissues of mammals (Eaton, 1992), particularly ruminants (Defleur *et al*, 1999; Marean & Assefa, 1999; Stanford & Bunn, 1999) that were either scavenged or hunted (Marean & Assefa 1999; Stanford & Bunn, 1999). Anthropological and ethnographic data indicate that modern day hunter-gatherers, as well as Stone Age humans, consumed not just muscle tissue, but relished certain fatty portions of the carcass including brain, marrow and depot fat (Defleur *et al*, 1999; Harako, 1981; McArthur, 1960; Silberbauer, 1981; Stefansson, 1960). Consequently, the lipid composition of these wild ruminant tissues provide insight into the qualitative range of dietary lipids that ancestral humans typically encountered and may be useful in the determination of present day dietary lipid recommendations for the prevention and treatment of chronic disease.

Several investigators have characterized the fatty acid composition of muscle (Crawford, 1968a; Crawford & Gale, 1969, 1970; Crawford *et al*, 1970; Crawford & Woodford, 1971; Miller *et al*, 1986; Moczygemba, 1991; Rule & McCormick, 1998) and adipose tissue (Booren *et al*, 1973; Crawford & Gale, 1970; Crawford *et al*, 1970; Duncan & Garton, 1968; Garton *et al*, 1971; Innis & Kuhnlein, 1987; Mattson *et al*, 1964; Rule & McCormick, 1998) in wild ungulates (mainly ruminants). However, there is scant information for marrow (Meng *et al*, 1969; Turner, 1979; West & Shaw, 1975) and brain (Crawford *et al*, 1976). The latter tissues would have comprised a substantial portion of the average dietary lipid intake in pre-agricultural humans (Defleur *et al*, 1999; Harako, 1981; McArthur, 1960; Silberbauer, 1981; Stefansson, 1960). Consequently, the purpose of the present study was to fully characterize the lipid composition of muscle, brain, marrow and adipose tissues in three North American ruminant species (*Cervus elaphus, Odocoileus hemionus* and *Antilicapra americana*) and contrast them to wild African ruminants and to pasture and grain-fed cattle. These data provide evolutionary insight into present-day human, dietary lipid recommendations for the treatment and prevention of chronic disease.

Materials and methods

Animals and tissue samples

Muscle, brain, marrow and adipose tissue samples were collected from 17 elk (*Cervus elaphus)*, 16 mule deer (*Odocoileus hemionus*), and 17 antelope (*Antilicapra americana*) during the fall of 1997 in northern Colorado. All animals were brought to a local game processor directly after slaughter (mean time post-mortem 14.2 h), and tissues were dissected and frozen (− 70°C) for later analysis. Muscle tissues were obtained from the right biceps femoris; marrow samples were taken from the right rear metatarsal bones at mid-shaft, and subcutaneous adipose tissue samples were obtained on the dorsal body surface directly above the sacrum or posterior neck. Following sagittal sectioning of the skull with a band saw, both halves of the brain were homogenized in a blender and then frozen (− 70°C) in glass test tubes with screw caps under nitrogen for later analysis.

Analysis of fatty acids

Lipids in the tissue samples were extracted with chloroform/methanol (2:1, vol/vol). Fatty acid methyl esters (FAME) were prepared from the tissue lipids using boron trifluoride (BF_3) in methanol (14%, w/w; Supelco Inc., Bellefonte, PA, USA) following the procedure of Watkins *et al*, (1997). For quantification of conjugated linoleic acid (CLA) isomers, lipids extracted from tissue samples were methylated (sodium methoxide) following the method of Li and Watkins (1998). Lipids were dissolved in dry toluene (1 ml) in a test tube with a teflon-lined screw cap, 0.5 M sodium methoxide in anhydrous methanol (2 ml) added, the solution maintained at 50°C for 10 min, and glacial acetic acid (0.1 ml) added followed by deionized water (5 ml). All FAME were extracted into hexane (2×3 ml), dried over anhydrous sodium sulfate, and filtered. The FAME were analyzed using a gas chromatograph (GC; HP 5890 Series II, autosampler 7673, HP 3365 ChemStation; Hewlett-Packard Co., Avondale, PA, USA) equipped with DB225 or DB23 columns (30 m, 0.53 mm i.d., 0.5μm film thickness; J&W Scientific Co., Folsom, CA, USA). The GC was operated at 140°C for 2 min, temperature programmed 1.5°C/min to 198°C and held for 7 min. In addition, CLA isomers were further characterized using an SP2560 capillary column (100 m, 0.25 mm i.d., 0.20μm film thickness, Supelco, Inc. Bellefonte, PA, USA). The GC was operated at 100°C for 4 min, temperature programed at 10°C/min to 175°C and held for 30 min, followed by 5°C/min to 220°C and held for a final 15.9 min. For all GC operations the injector and flame-ionization detector temperatures were 225 and 250°C, respectively. FAME were identified by comparison of their retention times with authentic standards (GLC-422, CLA (UC-59-A and UC-59-M), Nu-Check-Prep, Elysian, MN; CLA (catalog no. 1245, *c*-9, *t*-11 and 1181, *t*-9, *t*-11), Matreya Inc., Pleasant Gap, PA, USA) and FAME prepared from menhaden oil (Matreya Inc.).

Statistical analyses

Differences among the three species for fatty acid percentages in each of the four tissues analyzed were determined using a two-way analysis of variance. *Post-hoc*, analyses were done with Tukey's range test ($P < 0.05$). All statistical procedures were carried out with the SigmaStat® for Windows software package, version 2.03 (SPSS Inc., Chicago, IL, USA).

Results

The values for fatty acids found in marrow, muscle, brain and subcutaneous fat tissues for the three North American species are shown in Tables 1–4, respectively. Figures 1–3 contrast the differences in total saturated, monounsaturated and polyunsaturated fatty acids among the four tissues (marrow, muscle, brain, adipose) within each of the three species (*Cervus elaphus*, *Odocoileus hemionus* and *Antilicapra americana*), respectively.

The data in Table 1 reveal that the monounsaturated fatty acids (MUFA) were the predominant (59.3–67.0% of total) fatty acids (FA) in marrow of metatarsal bone for all three North American species of wild ruminants. Elk marrow was different from both deer and antelope in that it contained significantly ($P<0.05$) higher percentages of 16:1 n-7 and 18:1 n-7 and lower percentages of 18:1 n-9 and 18:0. Elk marrow also maintained a higher ratio of P/S (0.33) than the other two species because of a greater percentage of total polyunsaturated fatty acids (PUFA), primarily *trans*, *trans* (*t*,*t*) 18:2 (not a CLA isomer). Of all the four tissues analyzed, CLA isomers were found in the highest concentration in marrow (antelope, 1.5%; elk, 1.0%; and deer, 1.0%) followed by adipose tissue (deer, 0.3%; antelope, 0.3%). CLA isomers were also found in the muscle of antelope (0.4%) and in trace amounts in elk muscle, but were not detected in the brain of any species. The marrow of all species maintained a low ratio of P/S that ranged from 0.24 to 0.33, and a ratio of n-6/n-3 FA from 2.24 to 2.88. Further, marrow contained the lowest percentages of saturated fatty acids and the second lowest percentage of PUFA for all four tissues (Figures 1–3).

Table 1 Fatty acid composition (wt%) of rear metatarsal bone marrow. Values are mean ± s.e.m.

	Elk (n = *17*)	*Deer* (n = *12*)	*Antelope* (n = *17*)
14:0	1.82* (0.2l)	0.84*(0.07)	l.92* (0.49)
14:1 n-5	1.96* (0.16)	0.68† (0.08)	0.78* (0.15)
16:0	13.97† (0.61)	16.33† (0.59)	19.81* (1.10)
16:1 n-7	15.54* (0.74)	7.79† (0.66)	5.84† (0.34)
17:0	0.44‡ (0.03)	0.77† (0.05)	1.11* (0.06)
18:0	3.l7† (0.23)	4.66* (0.30)	4.90* (0.35)
18:1 n-9	35.35† (1.13)	54.09* (1.51)	49.49* (1.92)
18:1 n-7	14.l6* (0.96)	3.11† (0.16)	2.85† (0.15)
t,*t*18:2	1.43* (0.14)	0.87† (0.09)	1.04† (0.09)
18:2 n-6	2.38* (0.11)	2.35* (0.11)	2.41* (0.07)
18:3 n-3	1.44* (0.10)	1.49* (0.09)	1.23* (0.07)
20:1 n-9	0.96* (0.04)	0.39‡ (0.04)	0.55† (0.02)
18:2 (9,11)[a]	0.99† (0.07)	1.04† (0.09)	1.45* (0.13)
SAT[b]	19.37†(0.94)	22.60† (0.75)	27.73* (1.81)
MONO[c]	67.01* (0.98)	65.88* (1.18)	59.30† (l.74)
PUFA[d]	6.24* (0.21)	5.75† (0.32)	6.13† (0.31)
n-6/n-3 PUFA	2.86* (0.23)	2.24* (0.18)	2.88* (0.11)
PUFA/SAT	0.33* (0.02)	0.25† (0.0l)	0.24† (0.02)

*,†,‡Mean values having different superscripts are significantly different ($P<0.05$).
[a]*cis* 9, *trans* 11 and *trans* 9, *cis* 11.
[b]SAT, total saturated fatty acids.
[c]MONO, total monounsaturated fatty acids.
[d]PUFA, total polyunsaturated fatty acids.

Analysis of muscle tissues demonstrated that the general lipid characteristics(%PUFA, %MUFA, n-6/n-3 FA, and P/S) were similar within all three species (Table 2), except that elk maintained a lower percentage of saturated fats (SAT). Additionally, elk were different from the other two species in that values for 16:1 n-7 and 18:1 n-7 were significantly higher and 18:1 n-9 and 18:0 were lower, similar to the pattern detected in marrow. The percentage concentrations of individual PUFA among species were remarkably similar except for slightly higher ($P<0.05$) percentages of 20:3 n-6 and lower percentages of 22:6 n-3 in elk. Muscle tissue for all three species maintained values for the ratio of P/S ranging from 0.80 to 1.09 and ratio of n-6/n-3 FA from 2.32 to 2.60. Additionally, muscle contained the highest percentage of PUFA for all four tissues analyzed (Figures 12–3).

Similar to muscle, the values for fatty acids in lipids (%SAT, %PUFA, %MUFA, n-6/n-3 FA, and P/S) of brain were not different among the three species. Elk brain contained greater ($P<0.05$) percentages of 16:1 n-7 and 18:1 n-7 when compared to either deer or antelope. Of all the tissues, brain had the lowest ratio of n-6/n-3 FA (1.20–1.29), and the highest percentages of 22:6 n-3 (elk, 8.90%; deer, 9.62%; antelope, 9.25%) and 22:4 n-6. The mean value for the ratio

Table 2 Fatty acid composition (wt%) of biceps femoris muscle. Values are mean ± s.e.m.

	Elk (n = *14*)	*Deer* (n = *12*)	*Antelope* (n = *15*)
14:0	2.10* (0.29)	1.33† (0.18)	0.94† (0.11)
14:1 n-5	1.20* (0.25)	trace†e	trace†
15:0	0.40* (0.03)	0.32* (0.02)	0.38* (0.03)
16:0	16.19* (1.47)	17.75* (1.00)	15.21* (0.46)
16:1 n-7	7.81* (1.10)	1.33† (0.16)	1.22† (0.06)
17:0	0.46† (0.03)	1.02* (0.09)	1.21* (0.13)
18:0	11.59† (0.68)	17.73* (0.62)	18.28* (0.89)
18:1 n-9	10.42† (0.71)	20.97* (1.73)	20.66* (2.01)
18:1 n-7	6.06* (0.34)	1.23† (0.11)	2.48† (0.83)
t,*t*18:2	0.39* (0.03)	0.53* (0.06)	0.76* (0.16)
18:2 n-6	14.28* (1.07)	13.54* (1.30)	13.46* (0.95)
18:3 n-3	2.89* (0.26)	3.82* (0.32)	3.47* (0.32)
20:1 n-9	trace	ND [f]	ND
20:2 n-6	trace*	trace*	0.42* (0.04)
20:3 n-6	0.87* (0.09)	0.44† (0.05)	0.48† (0.04)
20:4 n-6	6.88* (0.62)	5.71* (0.72)	6.97* (0.58)
20:5 n-3	2.34* (0.20)	1.96* (0.20)	2.19* (0.25)
22:4 n-6	trace*	trace*	trace*
22:5 n-3	3.18* (0.25)	3.17* (0.46)	2.28* (0.22)
22:6 n-3	0.56† (0.05)	1.86* (0.30)	1.51* (0.28)
18:2 (9,11)[d]	trace*	ND *	0.28* (0.05)
SAT	30.49† (1.30)	38.02* (1.66)	35.82* (1.40)
MONO	25.33* (1.86)	23.55* (1.87)	24.38* (1.54)
PUFA	31.27* (2.24)	28.67* (2.55)	30.17* (2.09)
n-6/n-3 PUFA	2.59* (0.15)	2.32* (0.13)	2.60* (0.16)
PUFA/SAT	1.09* (0.13)	0.80* (0.11)	0.89* (0.09)

*,†,‡Mean values having different superscripts are significantly different ($P<0.05$).
cis 9, *trans* 11 and *trans* 9, *cis* 11.
SAT, total saturated fatty acids.
MONO, total monounsaturated fatty acids.
[d]PUFA, total polyunsaturated fatty acids.
[e]Trace, less than 0.01% of total fatty acids.
[f]ND, not detected (peak detection at 10 ng).

Table 3 Fatty acid composition (wt %) of homogenized brain. Values are mean ± s.e.m.

	Elk (n = *16*)	*Deer* (n = *15*)	*Antelope* (n = *15*)
14:0	0.32† (0.04)	0.34† (0.02)	0.42* (0.02)
15:0	0.09* (0.01)	trace^{*,e}	trace*
16:0	13.19* (0.56)	13.73* (0.55)	13.78†* (0.43)
16:1 n-7	0.61* (0.03)	0.53† (0.02)	0.48† (0.02)
17:0	0.28† (0.02)	0.30*† (0.01)	0.32* (0.01)
18:0	16.57* (0.58)	17.24* (0.43)	17.13* (0.30)
18:1 n-9	20.24†* (0.81)	22.14* (0.76)	19.92† (0.39)
18:1 n-7	4.04* (0.17)	3.37† (0.04)	3.58† (0.03)
*t,t*18:2	0.17* (0.03)	0.16* (0.03)	0.31* (0.06)
18:2 n-6	0.58†(0.05)	0.71*,† (0.04)	0.78* (0.05)
18:3 n-6	trace*	trace*	trace*
18:3 n-3	0.18* (0.03)	0.19* (0.03)	0.22* (0.03)
20:0	0.30* (0.03)	0.28* (0.02)	0.34* (0.02)
20:1 n-9	2.44* (0.37)	1.70† (0.21)	2.30†* (0.17)
20:2 n-6	0.65* (0.05)	0.59* (0.02)	0.47† (0.02)
20:3 n-6	0.45* (0.04)	0.57* (0.07)	0.47* (0.06)
20:4 n-6	5.58†,* (0.36)	5.24† (0.29)	6.39* (0.19)
20:5 n-3	0.10* (0.01)	trace*	trace*
22:0	0.33†,* (0.03)	0.29† (0.02)	0.38* (0.01)
22:1 n-9	0.65* (0.10)	0.49* (0.06)	0.62* (0.05)
22:4 n-6	4.11†,* (0.34)	4.47* (0.18)	3.57† (0.11)
22:5 n-6	0.36† (0.06)	0.50†,* (0.04)	0.54* (0.05)
22:5 n-3	0.61† (0.07)	0.76* (0.04)	0.53† (0.03)
22:6 n-3	8.90* (0.92)	9.62* (0.74)	9.25* (0.49)
18:2(9,11)	ND^{f}	ND	ND
SAT	31.00* (0.60)	31.97* (0.94)	32.36* (0.56)
MONO^{f}	27.98* (0.80)	28.10* (1.04)	26.82* (0.57)
PUFA	21.44* (0.82)	22.33* (1.02)	22.18* (0.57)
n-6/n-3 PUFA	1.29* (0.09)	1.20* (0.07)	1.29* (0.07)
PUFA/SAT	0.69* (0.02)	0.70* (0.02)	0.69* (0.02)

*,†,‡Mean values having different superscripts are significantly different ($P < 0.05$).
cis 9, *trans* 11 and *trans 9 cis* 11.
SAT total saturated fatty acids.
MONO, total monounsaturated fatty acids.
^{d}PUFA, total polyunsaturated fatty acids.
^{e}Trace, less than 0.01% of total fatty acids.
^{f}ND, not detected (peak detection at 10 ng).

Table 4 Fatty acid composition (wt %) of subcutaneous fat. Values are mean ± s.e.m.

	Elk (n = *15*)	*Deer* (n = *14*)	*Antelope* (n = *12*)
14:0	5.48* (0.65)	2.51† (0.15)	3.56† (0.68)
14:1 n-5	1.28* (0.29)	0.22† (0.01)	ND^{f}
15:0	0.84†,* (0.14)	0.57† (0.05)	1.03* (0.10)
16:0	34.66* (2.03)	23.06† (0.72)	23.96† (1.70)
16:1 n-7	7.33* (1.65)	1.27† (0.10)	1.09† (0.15)
17:0	1.17‡ (0.17)	2.00† (0.08)	3.19* (0.24)
18:0	23.30† (4.06)	31.16†,* (1.01)	34.25* (2.08)
18:1 n-9	17.50‡ (1.49)	30.08* (1.28)	24.07† (1.41)
18:1 n-7	3.59* (0.71)	0.73† (0.08)	1.06†*(0.12)
*t,t*18:2	0.90* (0.13)	1.01* (0.11)	1.12* (0.13)
18:2 n-6	1.61* (0.21)	2.20* (0.20)	1.73* (0.09)
18:3 n-6	0.19* (0.03)	trace*^{e}	0.22* (0.01)
18:3 n-3	1.12* (0.16)	1.56* (0.15)	1.14* (0.09)
20:0	0.50* (0.06)	0.26* (0.03)	0.29* (0.04)
20:1 n-9	trace	trace	ND
20:2 n-6	trace	trace	ND
20:3 n-6	trace	ND	ND
20:4 n-6	trace	trace	ND
22:5 n-3	trace	trace	ND
22:6 n-3	ND	trace	ND
18:2(9,11)^{d}	trace*	0.33* (0.03)	0.31* (0.04)
SAT	65.38* (3.05)	59.43* (1.45)	66.10* (1.59)
MONO	28.98* (3.42)	32.14* (1.33)	25.51* (1.47)
PUFA	3.11† (0.53)	4.83* (0.27)	3.93†,* (0.16)
n-6/n-3 PUFA	2.25* (0.19)	2.96* (0.77)	2.47* (0.24)
PUFA/SAT	0.05† (0.01)	0.09* (> 0.01)	0.06† (> 0.01)

*,†‡Mean values having different superscripts are significantly different ($P < 0.05$).
cis 9, *trans* 11 and *trans 9 cis* 11.
SAT, total saturated fatty acids.
MONO, total monounsaturated fatty acids.
PUFA, total polyunsaturated fatty acids.
^{e}Trace, less than 0.01% of total fatty acids.
^{f}ND, not detected (peak detection at 10 ng).

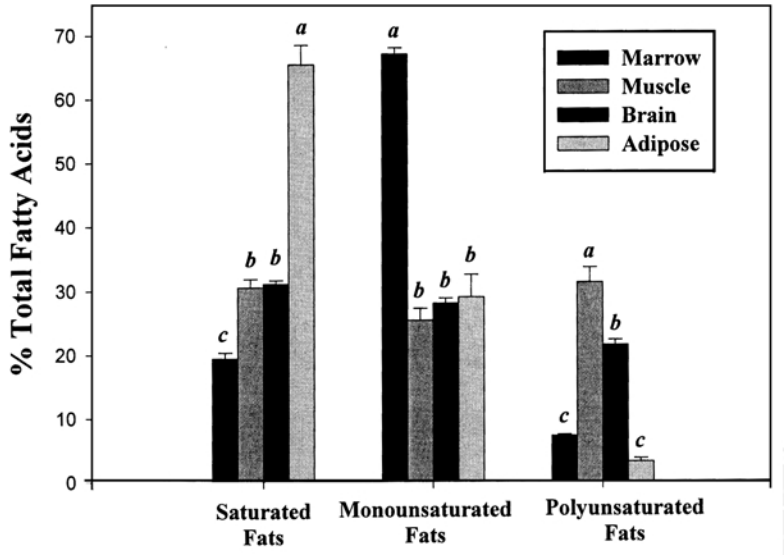

Figure 1 Fatty acid composition of elk (*Cervus elaphus*) tissues. Values are means ± s.e.m.; ^{a,b,c,d}mean values within specific fat categories having different superscripts are significantly different ($P < 0.05$).

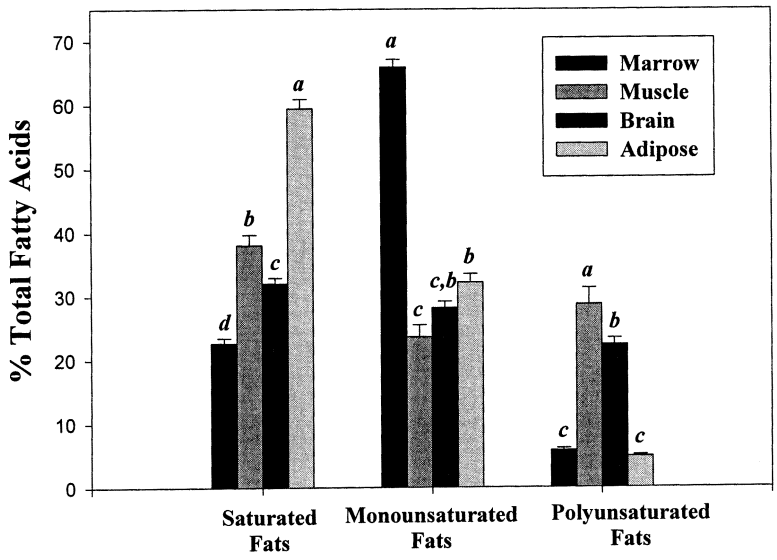

Figure 2 Fatty acid composition of deer (*Odocoileus hemionus*) tissues. Values are means ± s.e.m.; [a,b,c,d]mean values within specific fat categories having different superscripts are significantly different ($P < 0.05$).

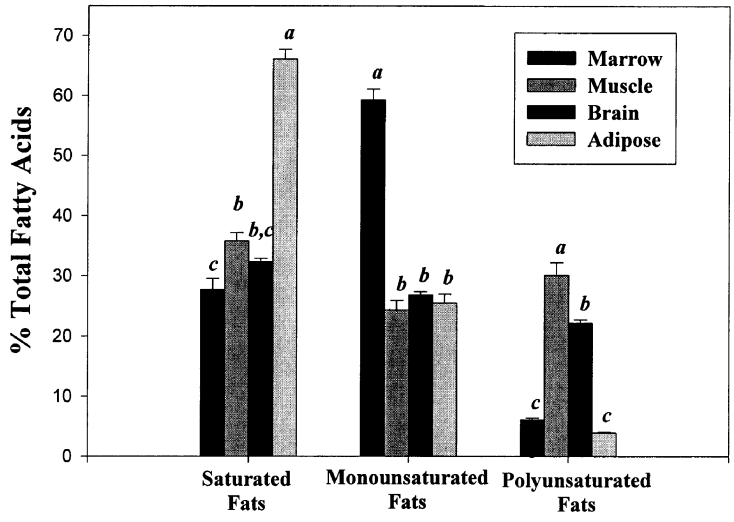

Figure 3 Fatty acid composition of antelope (*Antilicapra americana*) tissues. Values are means ± s.e.m.; [a,b,c,d]mean values within specific fat categories having different superscripts are significantly different ($P < 0.05$).

of P/S in brain for all three species (0.69) was lower than the values found in muscle (0.92), but much higher than values found in subcutaneous adipose (0.07) or marrow (0.27). When compared to the other three tissues, brain was relatively rich in all of the highly unsaturated long-chain fatty acids with carbon length $\geq$20C.

Subcutaneous adipose tissue for all three species had the lowest value for the ratio of P/S (0.05 – 0.09) of any of the four tissues, primarily because of both high SAT percentages (59.4 – 66.1) and low PUFA percentages (3.1 – 4.8; Figures 1 – 3). The values for the ratio of n-6/n-3 FA ranged from 2.25 to 2.96 for the three species studied. Interspecies comparisons again revealed that elk maintained higher percentages of 16:1 n-7 and 18:1 n-7, but lower percentages of 18:0 and 18:1 n-9 in adipose tissue when compared to the other two species. In deer and antelope, the predominant saturated fat was 18:0, whereas in elk it was 16:0. Elk subcutaneous fat tissue also contained higher percentages of 14:0 when com-

pared to either deer or antelope. Despite interspecies differences in specific SAT and MUFA, the general subcutaneous adipose tissue lipid characteristics (%SAT, %MUFA, %PUFA, n-6/n-3 FA, and P/S) for the three species were not significantly different from one another (Table 4).

Review

Crawford's seminal work (Crawford, 1968a; Crawford & Gale, 1969, 1970; Crawford *et al*, 1970, 1976; Crawford & Woodford, 1971) demonstrated that there were significant differences between the fatty acid composition of tissues from wild and domesticated animals that had potentially important dietary ramifications for human health and freedom from coronary heart disease (CHD; Crawford, 1968a, b). In the ensuing 25 – 30 y, there has been increasing recognition that the fatty acid composition of lipids in wild animal tissues may provide important insight into the composition and range of dietary fats to which humans are genetically adapted (Eaton, 1992; Eaton *et al*, 1998; Simopoulos, 1999; Simopoulos *et al*, 1999). The results of the present study add to the growing body of evidence supporting Crawford's original hypothesis and provide novel information regarding the lipid composition in the tissues of three species of North American ruminants.

Fatty acid comparisons among elk, deer and antelope

From earlier studies of wild ruminants in Africa (Crawford & Gale, 1969, 1970) and in North America (Garton *et al*, 1971; Miller *et al*, 1986), it is apparent that inter-species differences exist in tissue lipid composition that probably result from interactions among differences in season and locale (Crawford *et al*, 1970; Rule & McCormick, 1998), gender (Garton *et al*, 1971), forage consumed (Crawford *et al*, 1970; Garton *et al*, 1971; Miller *et al*, 1986) and genetic constitution (Rule & McCormick 1998). Data from the present study indicated that the fatty acid composition of deer and antelope were generally more similar to one another for all four tissues studied than when compared to elk. The GC analysis revealed that elk tissue contained higher percentages of 16:1 n-7 and 18:1 n-7 and lower percentages of 18:1 n-9 and 18:0 in muscle, marrow and subcutaneous fat relative to deer and antelope. Other studies have also reported higher concentrations of 16:1 and lower concentrations of 18:1 in elk muscle (Miller *et al*, 1986) and subcutaneous fat (Garton *et al*, 1971) when compared to other species of North American ruminants. The reason for these differences is not entirely clear, but perhaps they are due to the fatty acid composition of preferred forage as well as genetic factors controlling lipogenic pathways in both liver and adipose tissue of elk. In ruminant adipose tissue, synthesis of 16:0 is followed by elongation to 18:0 and then desaturation to 18:1 (Smith, 1995). The lower concentrations of 18:0 and 18:1 n-9 in elk muscle, marrow and adipose tissue are suggestive of reduced elongase activity both peripheral and hepatic. The higher concentrations of 16:1 n-7 and 18:1 n-7 in elk may result directly from consumption of this fatty acid in a preferred food, elevated endogenous delta-9 desaturase activity (16:0 to yield 16:1 n-7) in liver, or perhaps via bacterial isomeration of 18:1 n-9 in the rumen.

It is difficult to directly compare our muscle and subcutaneous fat tissue fatty acid compositions to previous studies of elk (Garton *et al*, 1971; Miller *et al*, 1986), mule deer (Miller *et al*, 1986) and antelope (Booren *et al*, 1973; Miller *et al*, 1986) because many of the earlier studies did not report certain fatty acids (particularly the long-chain PUFA). In addition, the previous GC analyses were unable to adequately resolve positional and geometric unsaturated FA isomers that we have reported. However, there are many typical fatty acids characterized among our three wild ruminant species that conform to a more universal pattern for all wild ruminants and which, from the perspective of human consumption, have important health implications, particularly when compared to domestic meats.

Fatty acid comparisons between wild and domesticated ruminants

Comparisons of wild ruminant and cattle muscle tissue show that the percentage of PUFA in wild ruminants is substantially higher than that in domesticated animals (Crawford *et al*, 1970; Miller *et al*, 1986). Because the two major acyl lipids in muscle (phospholipids and triacylglycerols) contain quite different PUFA proportions (Sinclair & O'Dea, 1987; Sinclair *et al*, 1982) and since the phospholipid content of muscle is relatively constant (Sinclair & O'Dea, 1987), as the muscle lipid content increases due to triacylglycerol infiltration, the meat fatty acid proportions change to reflect the major lipid, triacylglycerols (Sinclair & O'Dea, 1987; Sinclair *et al*, 1982). The present data show that, in all three North American species, muscle contained the highest percentage of total PUFA (28.7 – 31.3% of total FA) for the four tissues we examined (Table 2, Figures 1 – 3). Additionally, muscle was particularly rich in long-chain PUFA, including all three PUFA (20:3 n-6, 20:4 n-6, 20:5 n-3) that serve as eicosanoid substrates. Table 5 shows that high relative percentages of PUFA are characteristic of all wild ruminant muscle tissue, whereas the relative percentage of PUFA in the muscle tissue of pasture-fed steers and grain-fed steers is considerably lower than that found in wild ruminants and results in a characteristically low ratio of P/S.

As has been previously pointed out (Crawford, 1968a; Crawford *et al*, 1970; Eaton, 1992; Miller *et al*, 1986), the major difference between domestic meat (muscle tissue) and game meat is in the total amount of fat. Figure 4 shows that the total fat (5.63 g) in 100 g of grain-fed beef trimmed of all visible fat is more than double that in the muscle of antelope, deer, elk, cape buffalo and eland (mean = 2.2 g per 100 g tissue). The relatively higher total fat content in beef muscle meat (trimmed of surrounding fat) results from lipid accumulation in interfascicular adipocytes, a trait known as

Table 5 Comparison of muscle tissue lipid compositions (wt%) in wild ruminants, pasture-fed cattle and grain-fed cattle. Data adapted from Crawford *et al* (1970); Miller *et al*, (1986); Marmer *et al*, (1984); Rule and McCormick (1998)

Species	*SAT*[a]	*MUFA*[b]	*PUFA*[c]	*n-6/n-3*	*PUFA/SAT*
Giraffe (*Giraffa camelopardalis*)	36.2	16.3	40.7	4.00	1.12
Eland (*Taurotragus oryx*)	47.5	19.8	34.3	3.22	0.72
Hartebeest (*Acephalus buselaphus*)	48.1	17.9	32.2	2.50	0.67
Topi (*Damaliscus korrigum*)	55.5	15.0	37.6	3.44	0.68
Cape buffalo (*Syncerus caffer*)	39.1	27.7	32.4	3.44	0.83
White-tailed deer (*Odocoileus virginianus*)	49.0	34.6	16.4	2.91	0.33
Pronghorn antelope (*Antilocapra americana*)	43.4	29.7	26.9	5.04	0.62
Mule deer (*Odocoileus hemionus*)	45.2	35.1	22.2	3.46	0.49
Elk (*Cervus elaphus*)	41.0	33.0	26.0	6.20	0.65
Brangus cross (*Bos taurus*) (pasture-fed steer)	43.5	40.3	10.3	2.22	0.24
Brangus cross (*Bos taurus*) (grain-fed steer)	44.7	45.2	7.1	5.19	0.16

[a]SAT, percentage total saturated fatty acids.
[b]MONO, percentage total monounsaturated fatty acids.
[c]PUFA, percentage total polyunsaturated fatty acids.

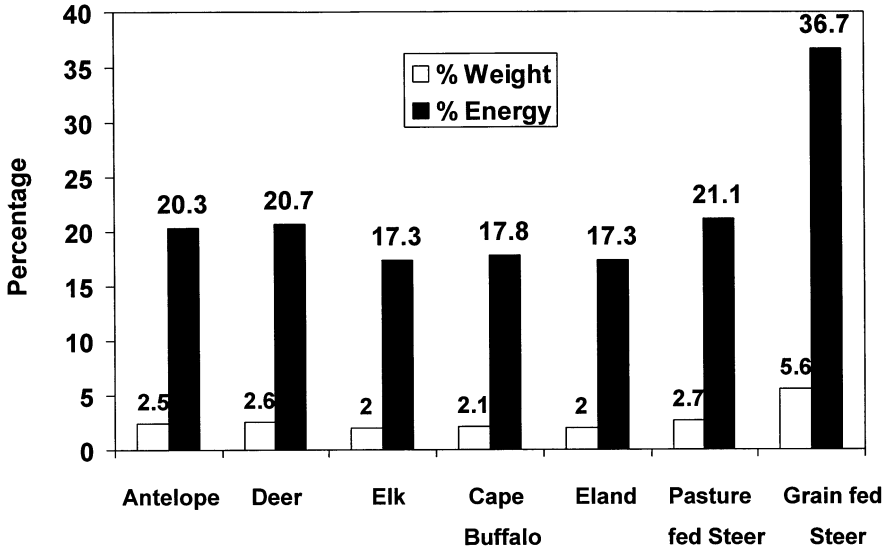

Figure 4 Total fat percentage in the muscle tissue of antelope (*Antilicapra americana*), deer (*Odocoileus hemionus*), elk (*Cervus elaphus*), cape buffalo (*Syncerus caffer*), eland (*Taurotragus oryx*), pasture and grain-fed steers (*Bos taurus*). Steer muscle was trimmed of all visible, peripheral fat.

marbling (Lin *et al*, 1992; Sweeten *et al*, 1990a). Hence, triacylglycerol rich adipocytes located between muscle fiber bundles are largely responsible for the quantitative differences in fat content between grain-fed domestic meat and wild ruminant meat. The fatty acids accumulated in the interfascicular adipocytes of feed-lot produced beef are similar to those found in beef subcutaneous fat (Sweeten *et al*, 1990b). Consequently, the mixture of triacylglycerol-rich interfascicular adipocytes with muscle tissue (marbled meat), produces a lipid profile that (except for the residual muscle PUFA) varies little from beef subcutaneous fat (Marmer *et al*, 1984; Sweeten *et al*, 1990b, Table 6).

Table 7 shows the concentrations (g fatty acid/100 g muscle) of various fatty acids in wild ruminants, pasture-fed cattle and grain-fed cattle. The data illustrate that there are a number of important characteristics that differentiate wild ruminant muscle meat from both pasture and grain-fed beef. Grain-fed beef contains 2–3 times more absolute saturated fat and 3–4 times less n-3 PUFA than does game meat. Game meat is particularly high in 18:3 n-3 when compared to pasture and grain-fed beef. Further the absolute PUFA content of grain-fed beef is approximately half that of game meat while the ratio of n6/n3 PUFA (5.28) is more than twice that in our samples of elk, antelope and deer. Pasture-fed beef resembles game meat more closely than does grain-fed beef, however there are a number of critical traits in which it also varies from wild ruminant meat. Pasture-fed beef maintains relatively low concentrations of saturated fat

Table 6 Fatty acid composition (percentage total fatty acids) of muscle, subcutaneous fat, interfascicular fat and subcellular fat in a feedlot steer (adapted from Sweeten *et al*, 1990b)

	Tissues				
Fatty acid	*Muscle*[a]	*Subcutaneous fat*	*Interfascicular fat*	*Muscle cytoplasm*	*Muscle membrane*
10:0	0	0.6	0.1	0	0
12:0	0	0.1	0.1	0	0
14:0	2.6	3.6	3.5	3.4	1.6
16:0	26.4	25.3	24.2	29.3	23.3
18:0	12.0	11.9	13.5	11.1	12.8
20:0	0.1	0.1	0.2	0	0.1
14:1	0.2	0.9	2.1	0.2	0.4
16:1	4.4	5.1	5.6	4.7	4.0
18:1	37.9	38.4	37.7	42.7	33.1
20:1	1.1	0.3	0.1	0	2.2
18:2	10.4	1.9	2.7	6.0	14.8
18:3	0.8	2.6	2.4	0.9	0.7
20:2	0.2	1.7	0.9	0	0.5
20:3	0.9	1.3	0.2	0.1	1.7
20:4	0	0.9	0.7	0	0.1
SAT[b]	41.0	41.6	41.6	43.9	37.9
MONO[c]	43.6	44.7	45.5	47.7	39.6
PUFA[c]	12.4	8.4	6.9	21.0	31.33
n-6/n-3 PUFA	13.92	2.23	1.88	6.46	25.8
PUFA/SAT	0.30	0.20	0.17	0.48	0.83

[a]Muscle, muscle cytoplasm and muscle membrane values represent the mean for three muscles (longissimus dorsi, psoas major and semitendinosus).
[b]SAT, percentage total saturated fatty acids.
[c]MONO, percentage total monounsaturated fatty acids.
[d]PUFA, percentage total polyunsaturated fatty acids.

Table 7 Comparison of muscle tissue lipid concentrations (mg fatty acids/100 g sample) in elk (*Cervus elaphus*), deer (*Odocoileus hemionus*), antelope (*Antilicapra americana*), pasture and grain-fed cattle (*Bos taurus*). Data adapted from the present study; Marmer *et al* (1984); and Miller *et al* (1986)

Fatty acid	*Elk*	*Deer*	*Antelope*	*Pasture-fed steer*	*Grain-fed steer*
SAT[a]	610	989	895	910	1909
MUFA[b]	507	612	610	793	1856
PUFA[c]	625	746	754	262	341
n-3 PUFA	178	225	216	61	46
n-6 PUFA	448	524	536	138	243
18:2 n-6	286	352	336	86	155
18:3 n-3	58	99	87	24	11
Long chain PUFA	281	295	331	152	175

[a]SAT, total saturated fatty acids.
[b]MONO, monounsaturated fatty acids.
[c]PUFA, total polyunsaturated fatty acids.

similar to wild game, however it contains 2–3 times less total PUFA and 2–3 times less n-3 PUFA. Muscle meat from both pasture and grain-fed beef has lower concentrations of long chain PUFA when contrasted to game meat.

The practice of feeding cattle grain during the feedlot fattening process is directly responsible for the differences in fatty acid composition between grain and pasture-fed cattle. Grain feeding increases the absolute saturated and monounsaturated fat content of beef while it concurrently lowers the absolute n-3 PUFA content (Table 7). Corn and sorghum are frequently the predominant cereal grains fed to cattle in the feedlots, hence the inherently high ratio of n-6/n-3 FA (70.7 and 16.2 respectively) of these grains (Cordain, 1999), along with the dilution of the phospholipid fraction by increases in triacylglycerols, is responsible for the higher n-6/n-3 FA ratio in grain-fed beef (Table 5).

Nutritional and health considerations

When contrasting the lipid characteristics of muscle tissue in wild ruminants to cattle, there are four inter-related factors that have important health ramifications: (1) the total fat content; (2) distribution of specific FA; (3) the ratio of P/S; and (4) the ratio of n-6/n-3 FA. Each of these dietary lipid elements has been shown to influence the development of CHD, and in all cases each element in wild ruminant muscle tissue is superior to that in grain-fed beef.

The muscle tissue of wild ruminants contains approximately half the total fat of grain-fed beef trimmed of visible fat, whereas the fat content of free ranging beef is slightly higher or similar to wild ruminant meat (Figure 4). Table 7 shows that the absolute saturated fat content of grain-fed beef (1909 mg/100 g sample) is 2–3 times greater than that of game meat. Increases in dietary levels of saturated fat, particularly 12:0, 14:0 and 16:0 have been identified as the major dietary factor responsible for raising total and LDL serum cholesterol concentrations (Hegsted *et al*, 1965; Howell *et al*, 1997). Elevations in total and LDL cholesterol are major risk factors for CHD (National Cholesterol Education Program, 1991). Hence, the increased saturated fat in grain-fed beef substantially contributes to the total saturated fat in the American diet (Bloch *et al*, 1985) and would cause elevations in both total and LDL cholesterol (O'Dea *et al*, 1990), which may in turn lead to an increased risk for CHD. However, it should be noted that consumption of fat trimmed lean beef (2–5% fat) was associated with reduced LDL cholesterol, and only when beef fat itself was added to the diet was there a rise in LDL cholesterol (Morgan *et al*, 1997; O'Dea *et al*, 1990).

In addition to its greater total fat content, grain-fed beef differs significantly from wild ruminant muscle in its lower content of n-3 PUFA (both 18:3 n-3 and n-3 long-chain PUFA). There is substantial evidence to show that increased consumption of n-3 fatty acids, particularly from marine sources, provide protection from CHD in a variety of ways (Leaf & Weber, 1988; Simopoulos, 1997) and that excessive consumption of n-6 fatty acids at the expense of n-3 fatty acids may promote CHD and other chronic diseases (Simopoulos, 1999). Increased consumption of n-3 fatty acids have been shown to beneficially affect the function of cells involved in atherothrombosis in many ways. These include the modification of eicosanoid products in the cyclooxygenase and lipoxygenase pathways, the reduced synthesis of cytokines and platelet derived growth factor, and alterations

of leukocyte and endothelial cell properties (Simopoulos, 1997). In some studies, n-3 fatty acids decrease serum triacylglycerols, while lowering thrombic tendencies and reducing the incidence of ventricular arrhythmias (Leaf & Weber, 1988; Leaf *et al*, 1999; Simopoulos, 1997). The net result of increased n-3 fatty acid consumption suggests that total CHD mortality and morbidity can be reduced (de Lorgeril *et al*, 1999).

In addition to their beneficial influence upon CHD, n-3 fatty acids are also known to have a positive influence on many inflammatory diseases, including rheumatoid arthritis (James & Cleland, 1997) and inflammatory bowel disease (Endres *et al*, 1999). Omega 3 fatty acids (particularly 22:6 n-3) may also be of benefit in psychological disorders (Hibbeln & Salem, 1995). The practice of feeding linoleic acid containing grain to cattle not only increases the total amount of atherogenic saturated fats in the meat, but it also substantially reduces the concentrations of n-3 PUFA, while simultaneously increasing the concentrations n-6 PUFA (Table 7). Consequently, feed-lot produced beef does not contain an optimal fatty acid profile that would help to reduce the incidence of several chronic diseases.

Comparison of pasture-fed and grain-fed cattle (Figure 4) shows that the total fat content in the muscle tissues of pasture-fed steers are similar to or slightly higher than values in wild ruminants. However, the absolute PUFA and n-3 PUFA content of pasture-fed beef remains lower when contrasted to elk, deer or antelope. Despite these differences, the overall lipid characteristics of pasture-fed cattle are closer to values found in wild ruminants, and from a health perspective, the meat from these animals would probably be superior to meat from grain-fed cattle.

Although the brain and marrow of both wild and domestic ruminants are infrequently eaten in modern, Westernized societies, there is substantial evidence from ethnographic observations in hunter-gatherers (Defleur *et al*, 1999; Harako, 1981; McArthur, 1960; Silberbauer, 1981; Stefansson, 1960) and from the ancestral, human fossil record (Binford, 1984; Bunn, 1986; Bunn & Kroll, 1986; Defleur *et al*, 1999; Marean & Assefa, 1999; Speth, 1983; Stiner, 1991; Stringer & Gamble, 1993) that these items were a preferred food that was frequently consumed. The present data shows that MUFA was the predominant FA in elk (67.0% of total FA), deer (65.9% of total FA) and antelope (59.3% of total FA) marrow. Additionally, marrow maintained a moderate P/S ranging from 0.24 to 0.33 while yielding a ratio of n-6/n-3 ranging from 2.24 to 2.88. Hence, the lipid profile of dietary marrow would be less hyperlipemic and consequently less atherogenic than subcutaneous fat, but probably more hyperlipemic than muscle tissue because of the lower ratio of P/S in marrow (Table 1) relative to muscle (Table 2). Marrow also contained the highest percentages of CLA, ranging from 1.0 to 1.5% of total FA. These values ($\sim$20–30 mg CLA/g fat) are comparable to those found in dairy products and beef, the highest modern day food sources of CLA (Chin *et al*, 1992). In several animal models of carcinogenesis, CLA has been shown to be a potent cancer inhibitor that provides significant protection at dietary concentrations (0.1–1% of total FA; Ip, 1997) that would be found in marrow. CLA may also beneficially influence the progression of atherosclerosis via its potential antioxidant property (Rudel, 1999).

The fatty acid composition of marrow in wild ruminants has not been extensively studied; however, data from North American animals shows that the relative degree of saturation decreases distally in both the front and rear legs (Meng *et al*, 1969; Turner, 1979; West & Shaw, 1975). The double bond index (summation of the weight percentage of each FA in a mixture multiplied by the number of double bonds it contains per molecule divided by 100) has been shown to increase as marrow is sampled from proximal to distal leg bones. Furthermore, the increase in the double bond index correlates most closely with 18:1 n-9 (Meng *et al*, 1969; Turner, 1979; West & Shaw, 1975). MUFA percentages in the more proximal humerus and femur of three North American ruminants ranged from 40 to 45% of total FA, whereas in the more distal metacarpus and metatarsus, MUFA increased to 70–75% of total FA (Meng *et al*, 1969; Turner, 1979; West & Shaw, 1975). Because we sampled marrow from the metatarsus at mid-shaft, our fatty acid composition values are consistent with previous values at similar anatomical sites, but not with values measured more proximally (Meng *et al*, 1969; Turner, 1979; West & Shaw, 1975). The functional utility of this graded fatty acid deposition pattern in the legs of ruminants is unclear, however, it has been suggested that it would help to maintain leg mobility at low temperatures (Turner, 1979; West & Shaw, 1975). This gradational (proximal to distal) fatty acid composition pattern also occurs in more tropical animals (Turner, 1979), consequently it is possible that the African ruminants which early humans preyed upon also maintained similar marrow fatty acid compositions.

In wild ruminants, the total fat content of marrow varies seasonally primarily as a function of the animal's environment (Franzmann & Arneson, 1976; Shackleton & Granger, 1989). Healthy, normal animals typically maintain total marrow fat percentages (percentage weight) between 50 and 90% (Franzmann & Arneson, 1976); hence wild ruminant marrow represents a high MUFA food source available throughout the year that would have comprised a substantial percentage of the diet of ancestral humans (Binford, 1984; Bunn, 1986; Bunn & Kroll, 1986, 1988; Speth, 1983; Stiner, 1991; Stringer & Gamble, 1993). A meta analysis of dietary MUFA has shown that substitution of saturated fat with MUFA lowers total and LDL cholesterol (Gardner & Draemer, 1995) and may substantially reduce the risk for CHD (Hu *et al*, 1997).

The skull contents of both scavenged and hunted ruminants would have provided pre-agricultural humans with a highly concentrated, terrestrial source of 22:6 n-3. Our data shows that of total fatty acids, homogenized ruminant brain contains between 8.9 and 9.6% 22:6 n-3. Consequently, the average DHA content of whole brain (8.6 mg/g) is higher

than the mean value (6.7 mg/g) for seven species of salmon (Hepburn *et al*, 1986). Consumption of 200 mg of DHA from fish is associated with a 50% reduction in risk of sudden death from myocardial infarction (Horrocks & Yeo, 1999; Siscovick *et al*, 1995) and may be more effective than 20:5 n-3 in providing protection from CHD via numerous mechanisms (McLennan *et al*, 1996). Further, 22:6 n-3 is required for normal brain growth and function in humans (Mitchell *et al*, 1998) and may be useful in the prevention and treatment of certain inflammatory disorders and cancers (Horrocks & Yeo, 1999; James *et al*, 2000).

In summary, there are both quantitative and qualitative differences among the tissues of wild and domesticated ruminants that have important implications for the prevention of certain chronic diseases in modern, western populations. Examination of the range of dietary lipids that humans encountered during the evolution of our species is useful in understanding the discordance between present-day, lipid consumption patterns and those for which our species is genetically adapted.

Acknowledgements

Supported in part by a Pope and Young Conservation grant, NSF 9630683 and Purdue University Experiment Station Hatch project (IND060037-2).

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