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ARTICLE *in* SCIENTIFIC REPORTS · JANUARY 2015

Impact Factor: 5.58 · DOI: 10.1038/srep07926 · Source: PubMed

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Received

2 May 2014

Accepted

15 December 2014

Published

21 January 2015

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J.L.G.-G. (jlguil@ual.  
es)

# The PUFA-Enriched Fatty Acid Profiles of some Frozen Bison from the Early Holocene found in the Siberian Permafrost

José L. Guil-Guerrero<sup>1</sup>, Ignacio Rodríguez-García<sup>3</sup>, Irina Kirillova<sup>4</sup>, Fedor Shidlovskiy<sup>4</sup>,  
Rebeca P. Ramos-Bueno<sup>1</sup>, Grigoriy Savvinov<sup>5</sup> & Alexei Tikhonov<sup>2</sup>

<sup>1</sup>Food Technology Division, CeIA3, University of Almería, Almería, Spain, <sup>2</sup>Zoological Institute, Russian Academy of Sciences, Saint-Petersburg, Russian Federation, <sup>3</sup>Organic Chemistry Division, CeIA3, University of Almería, Almería, Spain, <sup>4</sup>Ice Age Museum, All Russia Exhibition Centre 71, 129223 Moscow, Russia, <sup>5</sup>Institute of applied ecology of the North, North-eastern federal University, Yakutsk, Russia.

Knowledge concerning the availability of n-3 fatty acids for humans in prehistoric times is highly relevant in order to draw useful conclusions on the healthy dietary habits for present-day humans. To this end, we have analysed fat from several frozen bison found in the permafrost of Siberia (Russia). A total of 3 bison were included in this study, all them very close to the early Holocene (8,000; 8,200; and 9,300 years BP). All samples were analysed by gas-liquid chromatography-mass spectrometry (GLC-MS) and GLC flame-ionization detection (GLC-FID). Fat samples from two bison showed two well-differenced areas, i.e. brown and white, the latter being saturated fatty acid enriched, corresponding to an intermediate stage of adipocere formation, while the brown ones yielded  $\alpha$ -linolenic acid in higher percentages than found in present-day bison. As demonstrated in this work, the subcutaneous fat of bison consumed by Mesolithic hunters contained amounts of n-3 fatty acids in higher quantities than those found in current bison; thus, the subcutaneous fat of bison could have contributed to meet today's recommended daily intake of essential fatty acids for good health in the Mesolithic to a greater extent than previously thought.

In the late Pleistocene (120,000–10,000 years BP), the main part of Eurasia including eastern Siberia was inhabited by rich mammalian fauna, called the “Mammoth complex”. Typical components of this megafauna were mammoths (*Mammuthus primigenius* Blum.), woolly rhinoceros (*Coelodonta antiquitatis* Blum.), Lena horse (*Equus lenensis* Russ.), and Pleistocene bison (*Bison priscus* Bojanus), as the most prominent species<sup>1</sup>. It is assumed that this megafauna went extinct by the beginning of the Holocene; however, proof exists today that the Mammoth fauna existed in the early Holocene in eastern Siberia, including bison as typical species<sup>1,2</sup>.

Archaeological and paleontological data indicate that in the Upper Palaeolithic (35,000–10,500 years BP) and Mesolithic (10,500–6,000 years BP), Stone Age humans actively hunted mammoths, woolly rhinoceroses, horses, and bison. This latter species was widely consumed in Eurasia by Stone Age hunters: during the early Upper Palaeolithic in Siberia<sup>3</sup>; during the Aurignacian in Vogelherd, Germany<sup>4</sup>; during the Upper Palaeolithic in the British Isles<sup>5</sup>; from the Middle Palaeolithic to the early Upper Palaeolithic in Western Europe<sup>6</sup>; and so on.

Frozen animals contemporaneous with Upper Palaeolithic and Mesolithic hunters sporadically appear in the permafrost of north-eastern Siberia. Their frozen carcasses occasionally include tissue debris in relatively good condition, rich in subcutaneous fat. Recently, frozen bodies of steppe bison (*B. priscus*) have been found in northern Yakutia (north-eastern Siberia), which were recovered in an absolutely complete state<sup>1,7,8</sup>, and thus suitable to perform a detailed analysis of the fatty acid (FA) profile of their subcutaneous fat. The information derived from this analysis yielded information on the availability of essential FAs (EFAs) for hunters within that frame time, enabling differences to be established between Stone Age and present-day dietary FA profiles.

Previously, we have demonstrated that a relict species of horse from the Ice Ages contained in the subcutaneous fat omega-3 (n-3) FAs in higher percentages than other present-day species<sup>9</sup>, and later, after analysing the frozen fat of several Palaeolithic and Mesolithic mammals, we showed that the fat of single-stomached mammals often consumed by hunters at those times contained suitable amounts of n-3 and n-6 FAs, possibly in quantities



sufficient to meet the today's recommended daily intake for good health<sup>10</sup>. The output of the analyses indicated high levels of polyunsaturated FAs (PUFAs) in the fatty tissues of mammoths and horses, while for bison the fat was mainly saturated. This is so because bison are ruminants and therefore hydrogenate the carbon-carbon double bonds of the FAs during digestion and, hence, PUFAs are scarcely incorporated into the depot fat<sup>11</sup>. Conversely, single-stomached animals, such as elephants, rhinoceroses, and horses, are susceptible to changes in the FA composition in their adipose tissue as a result of the intake of fats having different FA profiles<sup>12</sup>. However, differences in the ability to store PUFAs in fat tissues among ruminants have been reported, e. g. bison being more effective than sheep in storing PUFAs in their depot fats<sup>13</sup>.

This paper reports on the FA profiles of the fat of some bison from the Preboreal and Boreal Holocene found in the permafrost of Siberia (Russian Federation), discussing the possibility of the use of this fat as a source of essential FAs for hunters in those times.

## Methods

**Samples.** A total of 3 specimens of frozen bison (*B. priscus*) from Siberia (Figure 1) were included in this study (Table 1), i.e. a baby bison from Batagay (bison Batagay), a complete body of an adult male from the Yuka region (bison Yukagir; Figure 2), and a bison from Rauchua river (bison Rauchua), which were very close in time, all from the early Holocene (8,200; 9,300; and 8,000 years BP, respectively<sup>7,8</sup>). Permission was received to examine the relevant specimens from museum collections. Samples from the frozen carcass of bison Rauchua were donated by the Ice Age museum in Moscow; baby bison Batagay by the Museum of Mammoth, Institute of applied ecology of the North, North-eastern federal University in Yakutsk; and bison Yukagir from by the Yakutian Academy of Sciences, Yakutsk (all them in Russian Federation).

The samples were kept in the freezers of Zoological Institute Russian Academy of Sciences in Saint Petersburg. Sterile conditions were maintained during the dissection procedures and a special drill was used on the frozen carcasses. The samples were taken from the layers under the skin in the best preserved areas (Table 1). Two bison, Yukagir and Rauchua, showed two different coloured areas, i.e. white and brown (Figure 3), which were separately analysed, yielding two well-differentiated FA profiles, as discussed below. However, in the baby bison Batagay only white fat was detected.

Samples from all frozen mammals are available for inspection upon request.

**Oil extraction and transesterification.** Simultaneous oil extraction and transesterification was performed according to previous works<sup>14</sup>. From each sample, 50 mg were weighed in test tubes and n-hexane (1 mL) was added to each one. FA methyl esters (FAMES) were obtained after adding 1 mL of the methylation mixture, which was composed of methanol:acetyl chloride (20:1 v/v), and then heated at 100°C for 10 min. After cooling at room temperature, 1 mL of distilled water was added in each tube, after which the tubes were centrifuged at 3500 rpm for 5 min. The Upper hexane layer was removed for gas-liquid chromatography (GLC) analyses.

**GLC Analyses.** Firstly, FAMES were analysed by using a Focus GLC (Thermo Electron, Cambridge, UK) equipped with Flame Injection Detector (FID) and an Omegawax 250 capillary column (30 m × 0.25 mm i.d. × 0.25 µm film thickness; Supelco, Bellefonte, PA, USA). The temperature programme was: 1 min at 90°C, heating to 200°C at a rate of 10°C/min, constant temperature at 200°C (3 min), heating to 260°C at a rate of 6°C/min, and constant temperature at 260°C (5 min). The injector temperature was 250°C with a split ratio 50:1. The injection volume was 4 µL and the detector temperature was 260°C. Nitrogen was used as the carrier gas (1 mL/min) and peaks were identified by retention times determined for known FAME standards (PUFAs No. 1 from Sigma, St. Louis, USA), while FA contents were estimated by using methyl pentadecanoate (15:0) as an internal standard.

All samples were subjected to a second round of analyses by GLC-mass spectrometry (GLC-MS) at the Scientific Instrumentation Centre of the University of Granada (Spain). Samples were injected (2 µl) into an Agilent 7890A gas chromatographer with an apolar column in split mode, coupled with a Quattro micro GLC mass spectrophotometer (Waters, UK), with a positive electron impact source (70 eV) and full scan spectra acquisition. All FAs were detected and quantified by comparison of retention times and mass spectra with external standards, which were run at three different concentrations. The full dataset of the GLC-MS analysis is available upon request.

The map and drawings in Figure 1 were generated using Corel Draw X3 (Corel Corporation, Ottawa, Ontario, Canada).

Experiments for all samples were conducted at least in triplicate. Results are expressed as mean value ± S.D in Tables 2 and 3.

## Results

A chromatogram of the FAs taken from the brown fat under the skin of bison Rauchua (BRb) is plotted in Figure 4, while the mass spectra of a selection of PUFA showing their characteristic fragmentation patterns are displayed in Figure 5. The FA profiles of the animals analysed are shown in Tables 2 and 3.

The subcutaneous fat of all samples, either white or brown, contains noticeable amounts of saturated even-chain FAs: myristic acid



**Figure 1** | Major Late Pleistocene/early Holocene archaeological sites of north-eastern Siberia. The human remains are marked with the image of an archer, while the localities of the bison found frozen in the permafrost are marked with bison petroglyphs<sup>19</sup>. Map and drawings created with Corel Draw X3 software.



Table 1 | Sample characteristics of frozen bison

Sample code	Animal	Organ	Years BP	Epoch
BYw	Bison "Yukagir" white	White fat under the skin on the belly	9,300 <sup>8</sup>	Preboreal
BYb	Bison "Yukagir" brown	Brown fat under the skin on the belly	9,300 <sup>8</sup>	Preboreal
BBw	Baby bison "Batagay"	White fat under the skin on the belly	8,200 <sup>8</sup>	Boreal
BRw	Bison "Rauchua" white	White fat under the skin, near the anus	8,000 <sup>7</sup>	Boreal
BRb	Bison "Rauchua" brown	Brown fat under the skin, near the anus	8,000 <sup>7</sup>	Boreal

(MA, 14:0), palmitic acid (PA, 16:0), and stearic acid (SA, 18:0). Meanwhile minor and variable amounts of saturated FAs (SFAs) were detected in some samples: C12, 13, 15, 17, 21, 22. In addition, quantifiable amounts of oleic acid (OA, 18:1n-9) were present in all samples, while linoleic acid (LA, 18:2n-6) was found in the brown fat of bison Yukagir (BYb), and in both fat types of bison Rauchua (BRw and BRb). With regard to the n-3 and n-6 series, it is noticeable that  $\alpha$ -linolenic acid (ALA, 18:3n-3) was found in variable amounts in all fat types, except in bison Batagay (BBw) samples; eicosatrienoic acid (ETE, 20:3n-3) was present in bison Batagay and bison Rauchua (BRb fats; while arachidonic acid (AA, 20:4n-6) was detected in bison Rauchua brown fat (BRb). In addition, other unsaturated FAs (UFAs) were detected in variable amounts in some fatty tissues: myristoleic acid (MOA, 14:1), palmitoleic acid (POA, 16:1n-7), heptadecenoic acid (HDA, 17:1), and gondoic acid (GOA, 20:1n-9).

A significant difference was found in the colour of some fat samples, which has well-differentiated brown and white areas (Figure 3). Independent analyses of these two areas for both Yukagir (BYb and BYw) and Rauchua fats (BRb and BRw) yielded different FA profiles, indicating that brown fats (BYb and BRb) contained lower amounts of PA and SA than did white fats (BYw and BRw), and conversely registered higher amounts of OA, LA, and ALA than did white fat. However, no presence of brown fat was detected in the bison Batagay (BBw) (Table 2), a sample for which we previously reported the FA profile<sup>10</sup>. The FA profiles from brown areas of both bison are very similar to others commonly found in the subcutaneous fat of present-day grass-feeding bison<sup>13</sup>. However, some differences in the FA percentages were found, as discussed below.

## Discussion

It has been reported that frozen fat changes into deposits during the decomposition of tissue remains, becoming a structure called "adipocere". Such structure formation is due to the conversion of the soft tissues into a greyish-white, wax-like substance, which over time can become an armour-like solid mass<sup>15</sup>. Under favourable environmental conditions, UFAs undergo hydrogenation by bacterial



**Figure 2** | Bison Yukagir (*Bison priscus* Bojanus, 1827). This frozen bison was found along the eastern shore of Chukchalakh Lake at an unnamed hill (558 m a.s.l.). The carcass is complete, including snout, ears, tail and genitalia. All organs are fully preserved, including the contents of the digestive tract, as well as the rumen, stomach and intestines.

enzymes to saturated ones. Thus, hydrogenation of OA will yield SA. In addition, UFAs are transformed by single step  $\beta$ -oxidation during the hydrogenation process in favour of two units of shorter SFAs<sup>16</sup>. As PA is found in much higher percentages than those usually shown by adipose tissues from ruminant animals, an intense  $\beta$ -oxidation of OA and C18 PUFAs is assumed<sup>17</sup>.

The FA profiles found in white and brown fat are consistent with what is known about FA degradation in frozen samples. That is, the brown fat of bison Yukagir (BYb) and bison Rauchua (BRb) have more PUFA than the corresponding white fats; thus, the white fat of both bison seem to have the nature of an early stage of adipocere formation, while the brown fat corresponds to unaltered fat. Therefore, the FA profiles of the samples shown in Tables 2 and 3 would be due to a combination of certain factors: the foods consumed by the animals, the physiology of their digestive system, and the extent of the FA post-mortem transformation. The absolute variations for the main FAs of the white fat with respect to brown fat for both bison, BYw and BRw, are presented in Table 4. Note that the white fat corresponds to a relatively early stage of adipocere formation, in which OA, LA, and ALA are diminished in favour of saturated FAs, which are PA and SA, thus corroborating the above-discussed mechanism of adipocere formation. Moreover, the differences detected between the percentages of MA, SA and PA showed by brown fats (BYb and BRb) are striking (Table 2). However, the total percentages for these three FAs are similar in both cases (43.9% and 48.3%, respectively). Probably, such differences reflect feed availability for both bison, which is also corroborated by considering differences in ALA percentages that are found in both samples (Table 3).

In a previous article<sup>10</sup>, we have reported on the FA profiles of several frozen mammals found in the permafrost of Siberia, i.e.



**Figure 3** | Fragments of the subcutaneous fat of bison Rauchua. There are light and dark areas, which correspond to different stages of fat conservation. The clear area termed "white", corresponds to an intermediate state of formation of a structure called "adipocere", which corresponds to the result of saturation of unsaturated fatty acids. The dark "brown" area is the primeval fat, which contains hairs (H), and the unchanged fatty acids.

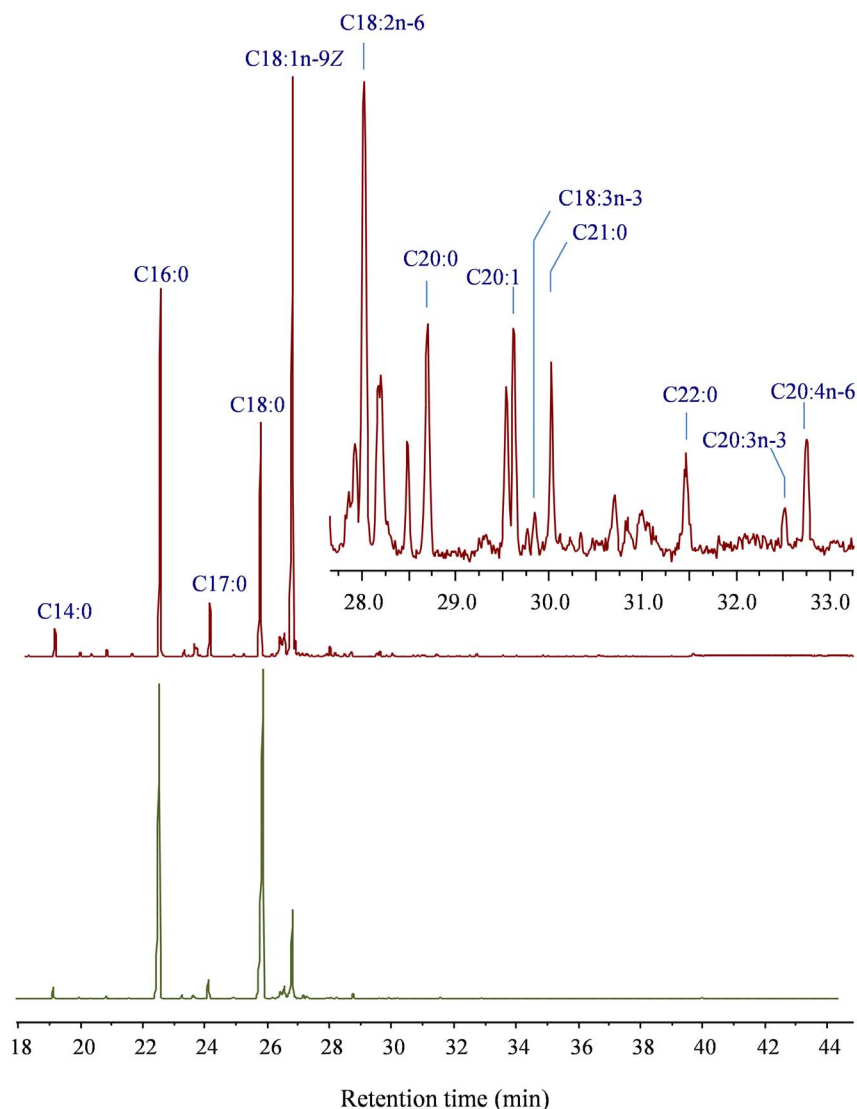
Table 2 | Total fatty acids and saturated fatty acids composition of the fat from frozen bison<sup>a</sup>

Samples	Total FAs g/100 g tissue	FAs % of total FAs										
		12:0	13:0	MA 14:0	15:0	PA 16:0	17:0	SA 18:0	20:0	21:0	22:0	
BYw	16.3 ± 1.2	-	-	8.0 ± 0.2	-	48.0 ± 2.8	0.1 ± 0.0	28.0 ± 1.7	-	-	-	
BYb	12.7 ± 1.2	0.1 ± 0.0	-	3.2 ± 0.2	0.2 ± 0.0	36.9 ± 2.8	0.1 ± 0.0	3.8 ± 1.7	-	-	-	
BBw	15.7 ± 0.9	0.4 ± 0.1	-	5.7 ± 0.3	0.6 ± 0.0	48.7 ± 3.2	0.2 ± 0.0	37.4 ± 2.0	1.1 ± 0.1	-	0.1 ± 0.0	
BRw	17.3 ± 0.4	0.1 ± 0.0	0.1 ± 0.0	1.2 ± 0.1	0.4 ± 0.1	35.4 ± 0.9	2.0 ± 0.2	50.6 ± 1.0	-	0.1 ± 0.0	-	
BRb	9.3 ± 0.8	0.4 ± 0.1	0.2 ± 0.0	2.2 ± 0.1	-	25.8 ± 0.7	3.8 ± 0.3	20.3 ± 0.5	0.8 ± 0.1	0.7 ± 0.1	0.5 ± 0.1	

<sup>a</sup>Mean ± SD of three independent determinations performed by GLC-MS.Table 3 | Unsaturated fatty acids composition of the fat from frozen bison<sup>a</sup>

Samples	FAs % of total FAs											
	MOA 14:1	POA 16:1	HDA 17:1	n-9E	n-7	n-9Z	Total 18:1	LA 18:2n-6	GOA 20:1n-9	ALA 18:3n-3	ETE 20:3n-3	AA 20:4n-6
BYw	-	-	-	-	-	16.0 ± 1.3	16.0 ± 0.9	-	0.1 ± 0.1	0.9 ± 0.1	-	-
BYb	0.2 ± 0.0	7.1 ± 0.2	0.4 ± 0.1	-	1.5 ± 0.1	40.1 ± 1.3	41.6 ± 0.9	1.8 ± 0.1	0.0 ± 0.1	4.2 ± 0.1	-	-
BBw	-	-	-	0.7 ± 0.2	-	4.6 ± 0.5	5.3 ± 0.4	-	-	-	0.1 ± 0.0	-
BRw	-	0.2 ± 0.0	-	0.2 ± 0.0	-	9.1 ± 0.2	9.3 ± 0.3	0.4 ± 0.1	-	0.1 ± 0.0	-	-
BRb	-	0.7 ± 0.1	0.3 ± 0.0	0.2 ± 0.0	-	41.7 ± 1.1	41.9 ± 1.7	0.9 ± 0.2	0.5 ± 0.1	0.2 ± 0.0	0.3 ± 0.1	0.6 ± 0.1

<sup>a</sup>Mean ± SD of three independent determinations performed by GLC-MS.



**Figure 4** | Gas-liquid chromatogram of fatty acid methyl esters from under skin fat from the belly of bison *Rauchua*. Red GLC from brown fat; green GLC from white lumps. As noted in the chromatogram, oleic acid (C18:1n-9Z) is the main FA component for brown fat, while palmitic acid (C16:0) and stearic acid (C18:0) are the main component for white fat.

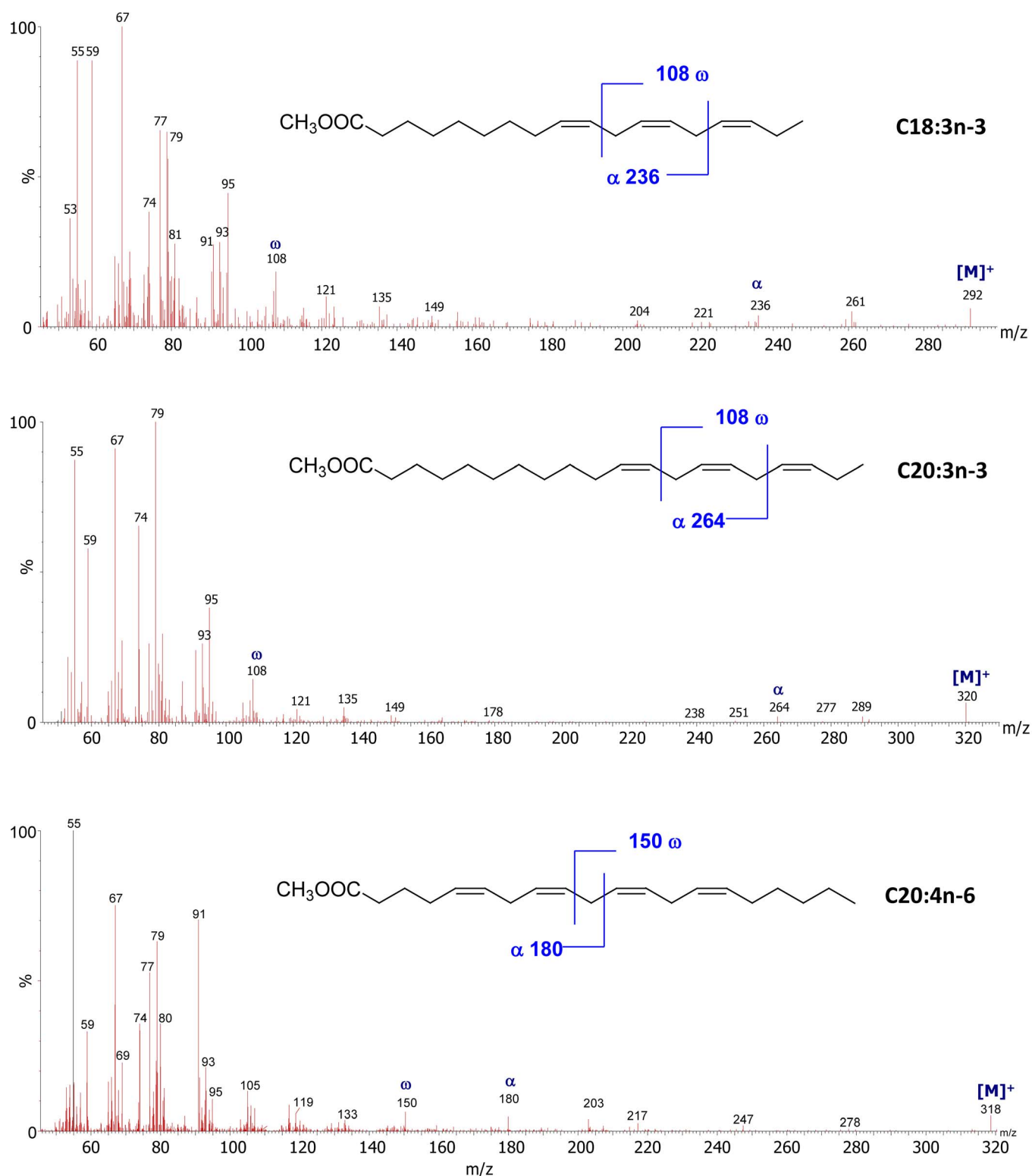
two mammoths, two horses, and two bison. Most PUFA were found to be transformed into saturated FAs, presumably following the aforementioned mechanism. However, the remaining PUFAs indicated the ability of this tissue to store them in all animals analysed, thus the subcutaneous fat of this bison species could be a multi-depot organ apt to serve as PUFA reservoir. For this last species, we reported two FA profiles, Bison Yukagir and Batagay, both corresponding to white fat taken from under the skin on the belly. However, later on, a detailed examination of the fat of bison Yukagir revealed the presence of dark areas, and subsequent analyses of the FAs taken from both areas, white and brown, provided two clearly differentiated FA profiles (Table 2 and 3). In addition, the subcutaneous fat of a new bison is here reported (bison *Rauchua*, Table 1), which also yielded differently coloured areas (Fig. 3).

The fats analysed here notably differed among them in terms of FA preservation, and considering the high amounts of PUFA detected in brown samples, these were well preserved. However, all samples contained percentages of total FAs in their subcutaneous fat much lower than those shown by present-day bison<sup>13</sup>, which could be due to an effect of the degradation of the fats and/or a consequence of the contamination of the original tissues by foreign substances, such as hair and fur.

In bison *Rauchua*, a biomorphic study of the gastric content showed abundant residues of mosses, belonging to the genus *Polytrichum*, *Drepanocladus*, *Aula-comnium*, and *Hylocomium*; meadow grasses; as well as fragments of vascular tissues and residues of herbal epidermis<sup>7</sup>. All mosses are usually cited as good sources of C18, 20, and 22 PUFAs<sup>18</sup>; thus, the high content of ALA and LA in the subcutaneous fat of the bison analysed here was due to moss consumption, which provided a PUFA-enriched profile to these bison to a greater extent than they show today.

Nowadays, there is no doubt that bison hunting by humans was responsible for its declining into the Holocene, period commensurate with the climate warming and vegetation change<sup>19</sup>. In this regard, the bison analysed in this work were contemporaries of humans in north-eastern Siberia and, thus, this mammal provided nourishment to our ancestors in those times. Moreover, in areas surrounding the Siberian permafrost, the human subsistence in the Upper Palaeolithic and Mesolithic was, in a large extent, based on the bison hunting<sup>20</sup>.

The known human sites for this period<sup>21</sup> and the localities where the bison analysed here were found are shown in Figure 1. The FAs detected may have been appropriate nutrients for these humans; that is, considering both the ALA percentage of total FAs in the SF of



**Figure 5** | Selection of mass spectra of polyunsaturated fatty acids methyl esters. The typical  $\omega$  fragmentation peaks at m/z 108 due to  $n-3$  terminal groups, as well as the  $\alpha$  fragments (at m/z 236 for C18:3n-3, m/z 264 for C20:3n-3, and m/z 180 for C20:4n-6) are clearly visible. Note that the methyl group is a consequence of the derivatization, and was not present in the original sample. Waters MassLynx V 4.1 software was used for processing spectral data.

bison Yukagir (BYb) (4.2%), and a 90% estimated total FA content in it<sup>22</sup>, an average of ALA content of  $\sim 4\%$  could be expected in this organ. This figure is approximately four times higher to that shown by current free-range bison in the same organ<sup>13</sup>. From this percentage, it is difficult to know the exact amount of both eicosapentanoic

acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3) that can be biosynthesised in the human body, because on average, only  $\sim 1\%$  is metabolized to DHA<sup>23</sup>. However, considerable variability in the conversion rates among individuals have been reported, and the indicated percentage varies widely depending on several factors, such



Table 4 | Absolute variation in the percentages of the major FAs of the white fat of two frozen bison with respect to their contents in brown fats

Fats	Fatty Acids							SUM
	14:0 MA	16:0 PA	16:1n-7 POA	18:0 SA	18:1 OA	18:2n-6 LA	18:3n-3 ALA	
BYw	+4.8	+11.1	-7.1	+24.2	-25.1	-1.8	-4.2	+1.9
BRw	-1.0	+9.6	-0.5	+30.3	-32.6	-0.5	-0.3	+5.0

as intake of n-6 PUFA, age, gender, and so on<sup>24,25</sup>. In any case, considering the established average value of ~1%, we would expect a human to obtain ~40 mg of EPA+DHA by consuming 100 g of the subcutaneous fat of bison Yukagir. Thus, for the minimal recommended daily allowance of 250 mg of EPA+DHA, which is needed to maintain good health<sup>26</sup>, ~650 g of SF of bison appear to have been necessary. However, given that this organ provides ~900 kcal/100 g<sup>20</sup>, the amount of fat of bison required to fulfil the daily requirements of essential FAs seems to be excessive for any human hunter, i.e. providing ~6000 kcal. In this sense, calculations of energy requirements made for early anatomically modern humans indicated that the energy expenditure at that time would be ~4000 kcal<sup>27</sup>. Therefore, with so low amounts of ALA in bison fat, for maintaining good health, the hunters at those times might have also had to eat lean meat and visceral tissues; however, there are rather narrow limits for acquiring PUFAs from proteinaceous foods, due to their potential toxicity<sup>9,10</sup>. This means that it is doubtful that these sources could provide the n-3 PUFAs amounts necessary for good health, taking into account that most of these organs contain small percentages of EPA and DHA, as a result of both, their usually very low intramuscular fat content (~1–2% in lean meat) and their low n-3 PUFA percentage<sup>9,28</sup>. This indicates that the use of other fats with higher amounts of ALA would have been necessary in such period, as ingesting the fat of single-stomached mammals such as horses and bears, as well as mammoths and woolly rhinos in some areas where they could occasionally persist<sup>10</sup>. Of course, in the selected epoch, the hunter-gatherers populations would consume marine or other terrestrial n-3 PUFA resources in appropriate areas, alternatively or complementarily to those considered here; nevertheless, hunting remained as the most important food strategy for these societies, though gradually declining in importance<sup>29</sup>.

The estimation of the needs of n-3 PUFAs for Stone Age humans could be approached from another point of view; i.e. considering that the available evidence indicates that >0.5%E ALA per day corresponds to the prevention of deficiency symptoms, and also that the energy derived from a gram of fat catabolism is approximately 9 kcal<sup>26</sup>. Accordingly, for Mesolithic humans, an amount of ~2 g ALA, which would be supplied by ~50 g of bison Yukagir fat, yielding ~500 kcal, would need to be ingested to avoid n-3 PUFA deficiency symptoms. It is reasonable to suppose that such amount could be consumed, as the energy presumably produced would be under this ceiling. However, taking into account the variations in the percentage of ALA in the subcutaneous fat of bison detected at such period (Table 3), it is likely that the intake of n-3 were much more effective through the ingest of the subcutaneous fat of monogastric mammals, whose intake might have provided to early anatomically modern humans hunters the n-3 PUFAs needed for the maintenance of long-term health and prevention of specific chronic diseases.

In conclusion, this study presents the FA profiles of several bison fats found in the Siberian permafrost, all of them from the early Holocene, and thus contemporaries of Mesolithic hunters in the considered area. The coexistence of more or less transformed fatty areas confirms the mechanism of adipocere formation. The best-preserved areas indicate significant concentrations of n-3 PUFAs, in quantities greater than those displayed by bison today, although probably in insufficient concentrations to provide the daily needs of n-3 PUFAs for maintaining good health during that period.

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### Author contributions

A.T., I.K., F.S. and G.S. collected the samples, J.L.G.G. designed the study, wrote the paper, created Figure 1 and drawings in the figure legend, and discussed the results, J.L.G.G., I.R.G. and R.P.R.B. performed experiments, all authors commented on the manuscript.

### Additional information

**Competing financial interests:** The authors declare no competing financial interests.

**How to cite this article:** Guil-Guerrero, J.L. *et al.* The PUFA-Enriched Fatty Acid Profiles of some Frozen Bison from the Early Holocene found in the Siberian Permafrost. *Sci. Rep.* **5**, 7926; DOI:10.1038/srep07926 (2015).



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