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

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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-differented 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 α-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 rhinoceroses (Coelodonta antiquitatis Blum.), Lena horse (Equus lenensis Russ.), and Pleistocene bison (Bison priscus Bojanus), as the most prominent species¹. It is assumed that this megafauna went extinct by the beginning of the Holocene; however, proof exits today that the Mammoth fauna existed in the early Holocene in eastern Siberia, including bison as typical species¹².

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 Siberia⁵; during the Aurignacian in Vogelherd, Germany⁴; during the Upper Palaeolithic in the British Isles⁶; from the Middle Palaeolithic to the early Upper Palaeolithic in Western Europe⁶; 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. Recently, frozen bodies of steppe bison (B. priscus) have been found in northern Yakutia (north-eastern Siberia), which were recovered in an absolutely complete state¹²⁸, 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 species⁹, 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...
PUFAs are scarcely incorporated into the depot fat. Conversely, the bon-carbon double bonds of the FAs during digestion and, hence, because bison are ruminants and therefore hydrogenate the car-horses, while for bison the fat was mainly saturated. This is so unsaturated FAs (PUFAs) in the fatty tissues of mammoths and SCIENTIFIC

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. Map and drawings created with Corel Draw X3 software.
enlarges 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), a sample for which we previously reported the FA profile10. 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 bison13. 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 mass13. Under favourable environmental conditions, UFAs undergo hydrogenation by bacterial enzymes to saturated ones. Thus, hydrogenation of OA will yield SA. In addition, UFAs are transformed by single step β-oxidation during the hydrogenation process in favour of two units of shorter SFAs16. As PA is found in much higher percentages than those usually shown by adipose tissues from ruminant animals, an intense β-oxidation of OA and C18 UFAs is assumed17.

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 PUFAs 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 variances 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).

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 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>a</sup> | Preboreal |
| BYb | Bison “Yukagir” brown | Brown fat under the skin on the belly | 9,300<sup>a</sup> | Preboreal |
| BBw | Baby bison “Batagay” | White fat under the skin on the belly | 8,200<sup>a</sup> | Boreal |
| BRw | Bison “Rauchua” white | White fat under the skin, near the anus | 8,000<sup>a</sup> | Boreal |
| BRb | Bison “Rauchua” brown | Brown fat under the skin, near the anus | 8,000<sup>a</sup> | Boreal |

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.

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**

| Samples | Total FAs g/100 g tissue | 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 |

*Mean ± SD of three independent determinations performed by GLC-MS.

**Table 3 | Unsaturated fatty acids composition of the fat from frozen bison**

| OA 18:1 | 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 |

*Mean ± SD of three independent determinations performed by GLC-MS.
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 bison13, 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, Aulacomnium, and Hylocomium; meadow grasses; as well as fragments of vascular tissues and residues of herbal epidermis7. All mosses are usually cited as good sources of C18, 20, and 22 PUFAs18; 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 change19. 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 hunting20.

The known human sites for this period21 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 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.
bison Yukagir (BYb) (4.2%), and a 90% estimated total FA content in it\textsuperscript{22}, an average of ALA content of \texttimes{} 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\textsuperscript{13}. From this percentage, it is difficult to know the exact amount of both eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3) that can be biosynthesised in the human body, because on average, only \texttimes{} 1% is metabolized to DHA\textsuperscript{23}. However, considerable variability in the conversion rates among individuals have been reported, and the indicated percentage varies widely depending on several factors, such

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**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 C180 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.
as intake of n-6 PUFA, age, gender, and so on. 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, ~650 g of SF of bison appear to have been necessary. However, given that this organ provides ~900 kcal/100 g, the amount of fat of bison required to fulfill 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.

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. 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. 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. 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.

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% 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. 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 PUFA 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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