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¹³Carbon and ¹⁵Nitrogen isotopes in autopsy liver tissue samples from Greenlandic Inuit and Danes: consumption of marine versus terrestrial food

Running title: Liver content of ¹³C and ¹⁵N in Inuit and Danes

Nils Milman¹, Jens Laursen², Gert Mulvad³, Henning Sloth Pedersen³, Agnes N. Pedersen⁴, Henrik Saaby²

¹Department of Clinical Biochemistry, Næstved Hospital, University of Copenhagen, Næstved, Denmark, ²Department of Basic Sciences and Environment, Faculty of Life Sciences, University of Copenhagen, Denmark

³Medical Clinic of Nuuk, Greenland

⁴Department of Nutrition, National Food Institute, Technical University of Denmark, Copenhagen, Denmark

Corresponding author: Nils Milman, MD
Department of Clinical Biochemistry
Næstved Hospital
DK-4700 Næstved
Denmark

Phone +45 56514107
Fax +45 56513730
E-mail nils.mil@dadlnet.dk

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Abstract

Background: The content of ^{13}C and ^{15}N isotopes is higher in marine than in terrestrial food. ^{13}C and ^{15}N in human tissue therefore reflects the relative proportions of marine and terrestrial food consumed by the individual. **Objective:** To measure ^{13}C and ^{15}N in liver tissue from Greenlandic Inuit and Danes

Methods: Normal liver tissue was obtained at autopsy in 1992-1994 from 60 Inuit with a median age of 61 years (range 25-83) and in 1986 from 15 ethnic Danes with a median age of 84 years (range 66-93). By sieving, liver tissue was separated in a “cellular fraction” and a “connective tissue fraction”. ^{13}C and ^{15}N in dry liver tissue was measured on a mass spectrometer. $\delta^{13}\text{C}$ indicates the ^{13}C content relative to the IAEA-CH-6 Reference Standard. $\delta^{15}\text{N}$ indicates ^{15}N content relative to the Atmospheric Nitrogen Reference Standard.

Results: Inuit: Median $\delta^{13}\text{C}$ was -21.2‰ in cellular and -20.0‰ in connective tissue fractions ($p=0.001$). Median $\delta^{15}\text{N}$ was 10.6‰ in both cellular and connective tissue fractions. Body mass index was negatively correlated with $\delta^{13}\text{C}$ in the connective tissue fraction ($r_s=-0.42$, $p=0.057$). Danes: Median $\delta^{13}\text{C}$ was -27.0‰ in cellular and -24.3‰ in connective tissue fractions ($p=0.11$). Median $\delta^{15}\text{N}$ was 9.5‰ in cellular and 8.9‰ in connective tissue fractions ($p=0.5$). Inuit had higher $\delta^{13}\text{C}$ than Danes in both cellular and connective tissue fractions ($p < 0.001$) as well as higher $\delta^{15}\text{N}$ in the cellular fraction ($p=0.01$).

Conclusions: Inuit displayed considerable variation in the ratio between marine/terrestrial food consumption, reflecting a vanishing hunter culture where elderly Inuit still adhere to the traditional hunters food with a high content of marine food, whereas the younger urbanized Inuit population consume food with a lower content of marine food and a higher content of terrestrial food. Danes consumed food of almost exclusively terrestrial origin. The present ^{13}C and ^{15}N analyses are in accordance with the dietary patterns obtained by dietary surveys.

Key words: ^{13}C Carbon; Denmark; Eskimos; Greenland; Inuit; liver; mass spectrometry; ^{15}N Nitrogen; nutrition

Introduction

You are what you eat! This slogan indicates that the composition of body tissues reflects the dietary intake of the individual. All atoms in human tissues, except hydrogen and oxygen, come from the foods we consume.

The distribution of the stable isotopes ^{13}C and ^{15}N differs in food elements derived from marine and terrestrial sources, i.e. the content of ^{13}C and ^{15}N is higher in the marine than in the terrestrial food chain (Hoering, 1955; Tauber, 1981; Tauber, 1986; Fischer *et al.*, 2007; Buchardt *et al.*, 2007). Therefore, the content of ^{13}C and ^{15}N in human tissue to a large extent reflects the dietary habits by indicating the relative proportions of marine and terrestrial food consumed by the individual (Hoering, 1955; Tauber, 1981; Tauber, 1986; Fischer *et al.*, 2007; Buchardt *et al.*, 2007).

In the marine milieu, the connecting links to the human food chain are marine fish, marine mammals (seal, walrus, whale) and marine birds, which constitute the main components in the traditional hunters food in Greenland (Buchardt *et al.*, 2007, Deutsch *et al.*, 2007).

In the terrestrial milieu, the connecting links to the human food chain consist of plants (grasses, cereals, vegetables, fruits) (Fischer *et al.*, 2007; Buchardt *et al.*, 2007). Many plants and fruits are ingested by man without any processing and plant-derived foods are eaten by domestic animals, used in the meat-producing industry and subsequently consumed by man.

The purpose of the present study was to assess the content of ^{13}C and ^{15}N in autopsy liver tissue samples from Greenlandic Inuit and Danes in order to establish reference materials from populations consuming either a diet, which is rich in products of marine origin or a diet, which is almost entirely of terrestrial origin.

Material and methods

Subjects

Inuit: The series comprised 60 Inuit (31 men, 29 women) with a median age of 61 years (range 25-83) (Laursen *et al.*, 1998). The autopsies of these Inuit were performed in 1992-1994 within 12 hours after death at the hospitals in the two largest towns in Greenland, i.e. the capital Nuuk (n = 53) and Ilulissat (n = 7). Causes of death are shown

in Table 1. All subjects had normal liver tissue by macroscopic and microscopic examination. Body mass index (BMI) was calculated as body weight in kg divided by body height in m². Food protocols were not available.

Danes: The series comprised 15 ethnic Danes (7 men, 8 women) from Copenhagen with a median age of 84 years (range 66-93). The autopsies were performed in 1989 (Laursen *et al.*, 1990). All subjects were deceased from cardiovascular or cerebrovascular disease and had normal liver tissue by macroscopic and microscopic examination. BMI and food protocols were not available.

Preparation of liver specimens

Liver tissue samples weighing ~45 g were excised with a plastic knife from beneath the capsule on the anterior surface of the right lobe and kept frozen at -20C° until preparation (Laursen *et al.*, 1990). After thawing, the samples were blended with 200 ml demineralized water for 2 min at 1100 rotations/min in a Sorvall 17106 Omni Mixer with blades of stainless steel (Sorvall Inc., Newton, Connecticut, USA). The blended tissue was strained through a sieve with a quadratic mesh size of 1.1 mm². The material retained in the sieve being designated the “connective tissue fraction”, was flushed with 50 ml demineralized water, freeze-dried to constant weight and homogenised. The material, which had passed through the sieve being designated the “cellular fraction” was freeze-dried to constant weight and homogenised (Laursen *et al.*, 1990). Utensils were stored in citric acid solution and rinsed in demineralized water prior to use. All reagents were analytical grade (Suprapur®, Merck, Darmstadt, Germany).

Analysis of ¹³C and ¹⁵N

The content of ¹³C and ¹⁵N was measured on an automated nitrogen carbon analysis-mass spectrometer (Europa Scientific Ltd., Crewe, UK) (Barrie *et al.*, 1989). The analyses were run in duplicate on 10 mg (range 9.0-10.1) samples of dry tissue, and the result was expressed as the mean of the two values. The IAEA-CH-6 Reference Standard (former Sucrose ANU Reference Standard) approved by the International Atomic Energy Agency, was used for ¹³C analysis (Coplen TB, *et al.*, 2006). The results of the ¹³C-isotope analysis are expressed as the δ¹³C value, designating the content of ¹³C in the

liver sample relative to the IAEA-CH-6 Reference Standard. For example, a $\delta^{13}\text{C}$ value of -25‰ indicates that the content of ^{13}C in the analysed sample is 25‰ lower than in the reference standard. The analysis of variance (standard deviation) was 5%.

The standard for ^{15}N analysis consists of atmospheric nitrogen. The results of the ^{15}N -isotope analysis are expressed as the $\delta^{15}\text{N}$ value, indicating the content of ^{15}N in the sample relative to the reference standard. For example, a $\delta^{15}\text{N}$ of 10‰ indicates that the content of ^{15}N in the analysed liver sample is 10‰ higher than in the reference standard. The analysis of variance (standard deviation) was 5%.

Statistics

Differences were evaluated with non-parametric statistical methods, Willcoxon's signed rank sum test for paired values corrected for ties, Mann-Whitney's test for unpaired values and χ^2 -test with Yates' correction. Correlations were assessed by Spearman's rank correlation coefficient (r_s) corrected for ties.

Results

General results are shown in Table 2. The $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values are shown in Figs. 1-2 and individual values in Fig. 3.

Inuit: Median age was 63 years in men and 59 years in women. The difference was not statistically significant (Mann-Whitney's test $p=0.8$); 11 subjects were 25-40, 19 were 41-60 and 30 were 61-83 years of age.

The $\delta^{13}\text{C}$ value in the cellular fraction was higher in men, median -20.9‰, than in women, median -21.4‰ ($p<0.02$).

The total carbon and nitrogen content was significantly higher in the cellular than in the connective tissue fraction (Table 2). However, C/N ratio was not significantly different between the cellular and connective tissue fraction.

Median $\delta^{13}\text{C}$ value was significantly higher in the cellular (-21.2‰) than in the connective tissue fraction (-20.0‰). Median $\delta^{15}\text{N}$ was 10.6‰ both in the cellular and connective tissue fraction.

There were no associations between the age of the subjects and total carbon or total nitrogen content in either the cellular or connective tissue fraction.

The $\delta^{13}\text{C}$ values in the cellular fraction were not correlated with age ($r_s=0.21$ $p=0.11$), whereas $\delta^{13}\text{C}$ values in the connective tissue fraction displayed a positive correlation with age ($r_s=0.69$, $p=0.001$) as shown in Fig 3.

The $\delta^{15}\text{N}$ values were not correlated with age either in the cellular or the connective tissue fraction ($r_s=0.17$, $p=0.2$ and $r_s=0.18$, $p=0.5$, respectively).

BMI was similar in men and women with a median of 22.9 in both genders (Mann-Whitney's test $p=0.97$). In the entire series, BMI was median 22.9, mean 23.7 ± 5.0 SD (standard deviation) with an observed range of 13.9-37.5. In the connective tissue fraction, BMI showed a significant positive correlation with the percent carbon content ($r_s=0.54$, $p=0.15$) and an almost significant negative correlation with $\delta^{13}\text{C}$ values ($r_s=-0.42$, $p=0.057$).

Danes: The cellular fraction constituted median 96.7% and the connective tissue fraction median 3.5% of the total dry weight liver sample (Table 2).

Median $\delta^{13}\text{C}$ value was -27.0‰ in the cellular and -24.3‰ in the connective tissue fraction; the difference was not statistically significant. Median $\delta^{15}\text{N}$ value was 9.5‰ in the cellular and 8.9‰ in the connective tissue fraction; the difference was not statistically significant.

There were no significant gender differences concerning $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values and no significant correlations between the age of the subjects and any of the variables in either the cellular or connective tissue fraction.

Inuit vs. Danes: Median C/N ratio was significantly higher in Inuit than in Danes both in the cellular and connective tissue fraction (Table2).

Inuit had significantly higher $\delta^{13}\text{C}$ values than Danes both in the cellular and connective tissue fraction. Likewise, Inuit had significantly higher $\delta^{15}\text{N}$ values than Danes in the cellular fraction. When subjects above 65 years of age were compared (Inuit $n=24$, Danes $n=15$), we found similar results with the same level of significance as in the entire series.

Discussion

In the nature, ^{13}C constitutes 1.108% and ^{12}C 98.892% of the carbon atoms (Lederer *et al.*, 1968). By photosynthesis, marine and terrestrial plants produce carbohydrates using

marine hydrocarbonate and atmospheric CO₂, respectively. The content of ¹³C is ~7‰ higher in marine hydrocarbonate than in atmospheric CO₂. Consequently, marine plants contain more ¹³C than terrestrial plants and this difference is maintained throughout the marine and terrestrial food chains.

In the atmosphere, ¹⁵N constitutes 0.365% and ¹⁴N 99.635% of the nitrogen atoms (Lederer *et al.*, 1968). Marine plants, which use marine nitrogen for amino acid synthesis, contain more ¹⁵N than terrestrial plants, which use atmospheric nitrogen. Therefore, proteins from the marine food chain contain more ¹⁵N than proteins from the terrestrial food chain (Fischer *et al.*, 2007; Buchardt *et al.*, 2007).

Animals and humans consuming food of marine origin have a higher content of ¹³C and ¹⁵N in the tissues than those consuming food of terrestrial origin. The mean δ¹³C value in marine plants is -13‰ and in bone collagen from marine mammals -14.1‰ (Hoering, 1955; Tauber, 1981; Tauber, 1986; Fischer *et al.*, 2007; Buchardt *et al.*, 2007). The mean δ¹³C value in terrestrial C₃ plants is -25‰ and in bone collagen from terrestrial herbivorous animals -21.6‰ (Hoering, 1955; Tauber, 1981; Tauber, 1986; Fischer *et al.*, 2007; Buchardt *et al.*, 2007).

The δ¹⁵N value in flesh collagen from marine fish is ~13‰, in terrestrial plants ~3‰, in tissue from terrestrial herbivorous animals ~9‰ and in bloodflour from domestic pigs ~9‰ (Fischer *et al.*, 2007).

We have performed isotope analyses on liver tissue, which had been separated into a cellular and a connective tissue fraction. The C/N ratio in liver tissue with a total range of 3.4 to 6.4 appears to be significantly higher than the ratio in bone collagen ranging from 2.9 to 3.6 (DeNiro, 1985).

The ¹³C and ¹⁵N isotope content should preferably be assessed in tissues with a slow metabolic turnover, which is assumed to reflect long-term dietary habits (Fischer *et al.*, 2007). This would tend to reduce confounding by nutritional changes in the period prior to death, e.g. during hospital admission. The connective tissue in bone (Fischer *et al.*, 2007), and assumingly also in liver, has a slow turnover, and would presumably be the most suitable tissue to assess long-term dietary patterns. However, recent studies have shown that even in the case of a constant diet, constancy of isotopic values between

different types of tissues within one and the same individual cannot be taken for granted (O'Connell *et al.*, 2001; Fischer *et al.* 2007).

In Inuit, we demonstrated a positive correlation between age and $\delta^{13}\text{C}$ values in the connective tissue fraction. This finding is consistent with the fact that the elderly Inuit population during their lifetime consume more of the traditional Greenlandic diet (hunters food) than the younger urbanized Inuit population who consumes a western-style diet (Deutsch *et al.*, 2007). Men displayed a significantly higher $\delta^{13}\text{C}$ value in the cellular fraction than women, probably due to a higher consumption of marine food.

The hepatocytes constitute ~75% of the cytoplasmic mass of the liver and have an average lifespan of ~5 months (<http://en.wikipedia.org/wiki/Hepatocyte>). Consequently, the isotope content in the cellular fraction would tend to reflect the short-term nutritional pattern prior to death. The differences in isotope composition between the cellular and connective tissue fraction could be due to differences in the amino acid composition of the two tissues (O'Connell *et al.*, 2001).

Our results confirm the well-known fact that Danes consume a diet of almost exclusively terrestrial origin (Haraldsdóttir *et al.*, 1987). The median $\delta^{13}\text{C}$ value in liver tissue was approximately -26‰, i.e. similar to the value of about -25‰ in terrestrial plants and herbivorous mammals (cow, horse, sheep) (Fischer *et al.*, 2007). A model based on bone collagen, which adjusts for the “reservoir effect” (Fischer *et al.*, 2007), showed that $\delta^{13}\text{C}$ values of ≥ -10 ‰ are predominantly found in individuals consuming an entirely marine diet, and values of ≤ -21.6 ‰ are predominantly found in individuals consuming a purely terrestrial diet. In the present study, 10% of Inuit vs. 93% of Danes had $\delta^{13}\text{C}$ values of ≤ -21.6 ‰ in the connective tissue fraction ($p < 0.001$).

In Inuit, the median $\delta^{15}\text{N}$ value in liver tissue was ~11‰, i.e. close to the 12-15‰ found in marine mammals and marine fish (Fischer *et al.*, 2007) and significantly higher than the value of ~9‰ found in Danes, which actually is similar to the value of 9‰ in terrestrial herbivorous mammals and fresh water fish (Fischer *et al.*, 2007). Our results are thus consistent with a higher consumption of marine foods in Inuit than in Danes (Deutsch *et al.*, 2007).

In Danes, marine food consists exclusively of fish and fish products, predominantly from marine species. Fish products such as fish oil are used in the food industry to

produce e.g. margarine and are also used in the feeding of domestic meat-producing animals. In the Danish Nutrition Survey in 1985, the consumption of fish and fish products was low, median 20 g/day (Haraldsdóttir *et al.*, 1987) and 25% of the 2242 participants had a negligible consumption of fish. The subsequent dietary survey in Denmark 1995 (Andersen *et al.*, 1996) showed that the consumption of fish and fish products had decreased to median 15 g/day. When stratified for age, the consumption was highest in 65- to 74-year-old subjects. The findings of these nutritional surveys are thus in accordance with the results of our isotope analyses.

The variation in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values was more pronounced in Inuit (Fig. 3) than in Danes, indicating greater individual differences in the ratio between marine/terrestrial food consumption among Inuit compared to Danes, who appeared to have a more uniform marine/terrestrial food composition ratio. Interestingly, among Inuit, the negative correlation between BMI and $\delta^{13}\text{C}$ values in the connective tissue fraction suggests that a higher BMI is associated with a lower intake of marine food and a higher intake of terrestrial western fare.

Concerning marine vs. terrestrial food consumption, analyses of ^{13}C and ^{15}N in body tissue enable us to compare nutrition nowadays with prehistoric dietary habits (Hoering, 1955; Tauber, 1981; Tauber, 1986; Fischer *et al.*, 2007). In the Mesolithic, settlements in Denmark were predominantly located at the seaside and the median $\delta^{13}\text{C}$ value in human bone collagen was -13‰, being consistent with a diet of predominantly marine origin (Table 4) (Fischer *et al.*, 2007). Similar high $\delta^{13}\text{C}$ values were found in the Greenlandic Qilakitsoq Inuit mummies dated ~1450 AD (Hansen *et al.*, 1989). Following the introduction of agriculture in Denmark in the Neolithic around 3900 BC, human bone collagen displayed a sudden decline in $\delta^{13}\text{C}$ values to -20‰ indicating the profound change from marine to terrestrial food (Fischer *et al.*, 2007).

In conclusion, the present ^{13}C and ^{15}N analyses in Inuit and Danes are by and large in accordance with the dietary patterns assessed by dietary surveys (Deutsch *et al.*, 2007; Haraldsdóttir *et al.*, 1987) Our results show that Inuit displayed considerable variation in the ratio between the consumption of marine/terrestrial diet. This variation reflects a vanishing hunter culture where elderly Inuit still adhere to the traditional Greenlandic hunters food with a high content of food of marine origin, whereas the younger urbanized

Inuit population during the past four decades to an increasingly extent consume western fare with a low content of marine and a high content of terrestrial food (Deutsch *et al.*, 2007). Danes consumed food of almost exclusively terrestrial origin.

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Legends for figures

Fig. 1A. Box plots of $\delta^{13}\text{C}$ values in cellular and connective tissue fractions of autopsy liver samples from Greenlandic Inuit and Danes

Fig. 1B. Box plots of $\delta^{15}\text{N}$ values in cellular and connective tissue fractions of autopsy liver samples from Greenlandic Inuit and Danes

Fig. 2. Individual $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values in autopsy liver samples from Greenlandic Inuit and Danes. One Inuit having a $\delta^{15}\text{N}$ outlier value of 28.2‰ in the connective tissue fraction was excluded from the figure. The discriminatory $\delta^{13}\text{C}$ level of -20‰ between a terrestrial and a mixed terrestrial/marine diet is adapted from studies on bone collagen (Fischer *et al.*, 2007)

Fig. 3. Correlation between age and $\delta^{13}\text{C}$ values in the connective tissue fraction from autopsy liver samples in Greenlandic Inuit (● men, ○ women)

Table 1. Causes of death in 60 Greenlandic Inuit

Cause of death	Inuit	
	n	%
Cancer	14	23
Cardio- and cerebrovascular disease	14	23
Other natural causes	18	30
Unnatural causes (accidents, suicide, homicide ect.)	14	23

Table 2. The content of carbon (C), nitrogen (N) and the relative content of ^{13}C ($\delta^{13}\text{C}$) and ^{15}N ($\delta^{15}\text{N}$) in cellular and connective tissue fractions of autopsy liver samples from Greenlandic Inuit and ethnic Danes

		Fraction of liver sample (%)	Carbon (%)	Nitrogen (%)	C/N ratio	$\delta^{13}\text{C}^*$ (‰)	$\delta^{15}\text{N}^{**}$ (‰)
INUIT							
Cellular fraction n = 60	median		48.5	11.0	4.4 ^c	-21.2 ^a	10.6 ^c
	range		45.0-52.4	8.2-12.3	3.9-6.4	-23.0 to -18.7	-5.5 to 19.0
	mean \pm SD		48.9 \pm 1.6	10.9 \pm 0.9	4.5 \pm 0.5	-21.0 \pm 1.0	9.5 \pm 5.0
	95%CI for mean		48.5-49.3	10.6-11.1		-21.3 to -20.8	8.2-10.8
P-value***			0.0001	0.04	0.8	0.001	0.7
Connective tissue fraction n = 20	median		46.4	10.3	4.5 ^a	-20.0 ^b	10.6 ^d
	range		43.8-48.2	8.8-12.2	3.8-5.3	-21.6 to -17.0	-0.9 to 28.2
	mean \pm SD		46.4 \pm 1.1	10.5 \pm 0.9	4.5 \pm 0.4	-19.8 \pm 1.4	11.0 \pm 6.7
	95%CI for mean		45.9-46.9	10.1-10.9		-20.4 to -19.1	7.8-14.3
DANES							
Cellular fraction n = 15	median	96.7	49.8	12.1	4.1 ^c	-27.0 ^a	9.5 ^c
	range	92.3-98.3	46.0-52.4	9.3-13.4	3.6-5.4	-31.2 to -20.9	3.3-11.0
	mean \pm SD		49.5 \pm 1.7	11.8 \pm 1.1	4.2 \pm 0.5	-26.3 \pm 3.2	8.1 \pm 2.6
	95%CI for mean		48.5-50.4	11.2-12.4		-28.1 to -24.5	6.7-9.6
P-value***			0.02	0.04	0.04	0.11	0.5
Connective tissue fraction n = 15	median	3.5	48.7	12.8	3.8 ^a	-24.2 ^b	8.9 ^d
	range	1.7-7.7	46.3-50.7	10.5-13.7	3.4-4.7	-29.1 to -20.5	6.2-10.9
	mean \pm SD		48.4 \pm 1.3	12.6 \pm 0.9	3.8 \pm 0.4	-24.6 \pm 2.3	7.8 \pm 1.5
	95%CI for mean		47.7-49.1	12.1-13.1		-25.9 to -23.3	8.0-9.7

*) Relative to the IAEA-CH-6 Reference Standard

**) Relative to the Atmospheric Air Standard

***) Willcoxon's test

Mann-Whitney's test between Inuit and Danes:

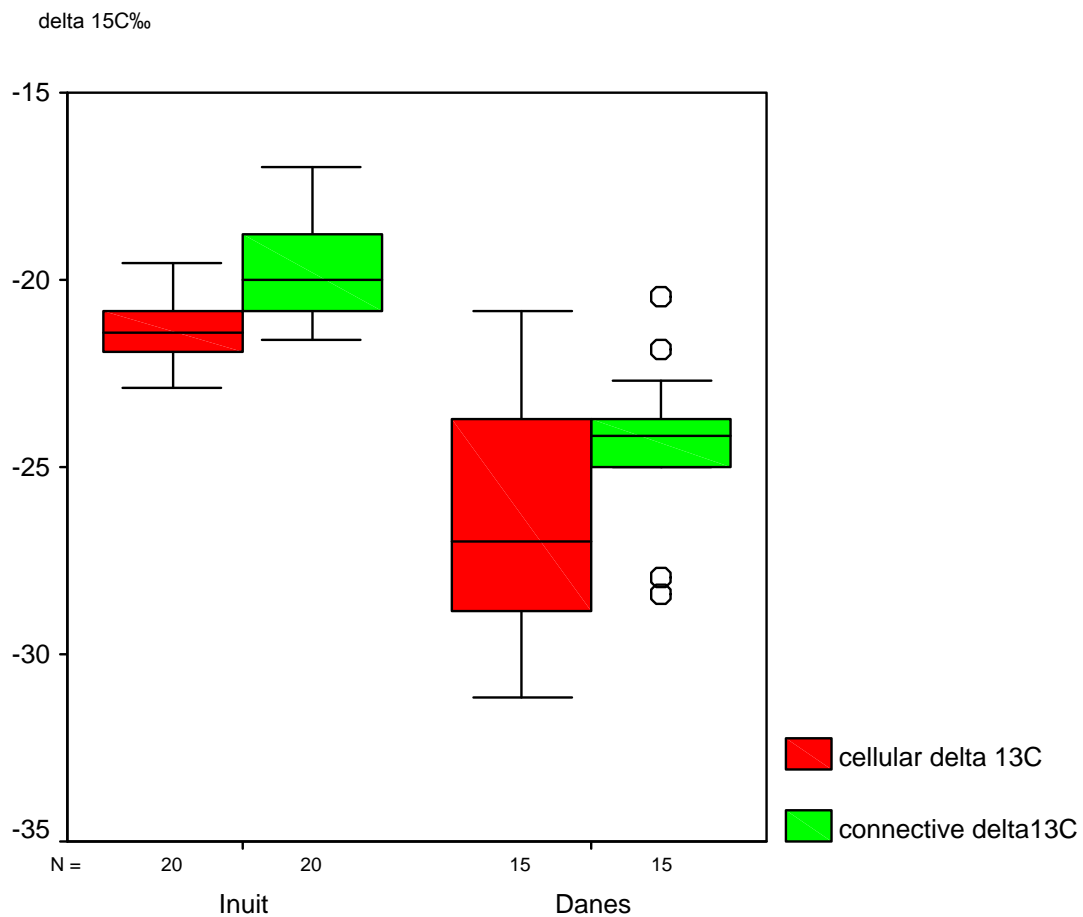
a) p=0.0001 b) p=0.001 c) p=0.01 d) p=0.2

Table 3. $\delta^{13}\text{C}$ values in human tissues from various periods in Denmark Greenland and U.K.

Period	Location	Tissue	Subjects (n)	$\delta^{13}\text{C}^*$ (‰)	Reference
8000-7500 BC	Denmark	bone collagen	5	-18	Fischer <i>et al.</i> , 2007
6000-4000 BC	Denmark	bone collagen	23	-13	Fischer <i>et al.</i> , 2007
3800 BC-1000	Denmark	bone collagen	60	-19	Fischer <i>et al.</i> , 2007
1450	Greenland, Quilakitsoq	bone collagen	5	-14	Hansen <i>et al.</i> , 1989
1989	Denmark, Copenhagen	liver collagen	15	-24	present study
1998-2006	Denmark, Copenhagen	nail keratin	29	-21	Buchardt <i>et al.</i> , 2007
1993	Greenland, Nuuk	liver collagen	20	-20	present study
1997	Greenland, Uummannaq	nail keratin	82	-18	Buchardt <i>et al.</i> , 2007
2000	U.K., Oxford	bone collagen	8	-19	O'Connel <i>et al.</i> , 2001
2000	U.K., Oxford	nail keratin	12	-21	O'Connel <i>et al.</i> , 2001
2000	U.K., Oxford	hair keratin	20	-21	O'Connel <i>et al.</i> , 2001

*) Median or mean content relative to the IAEA-CH-6 Reference Standard

Figure 1a



1b

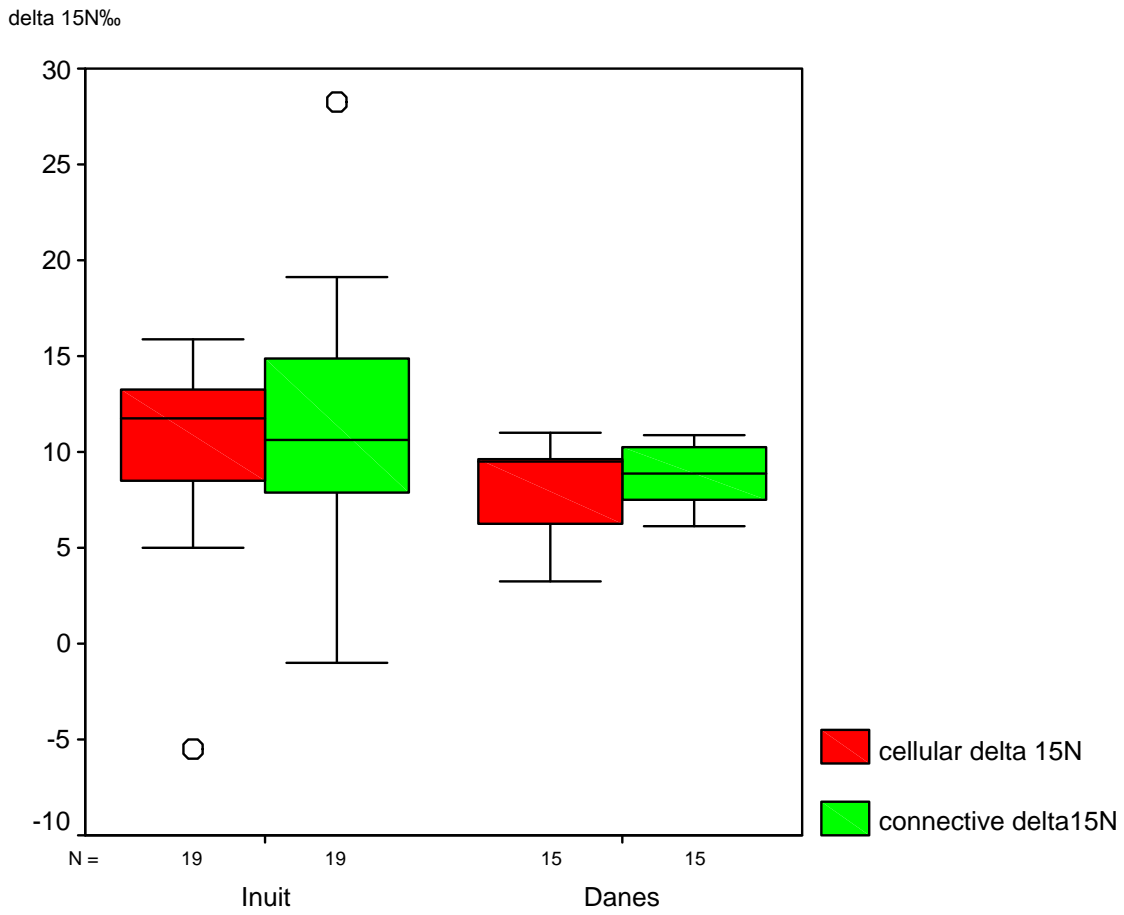
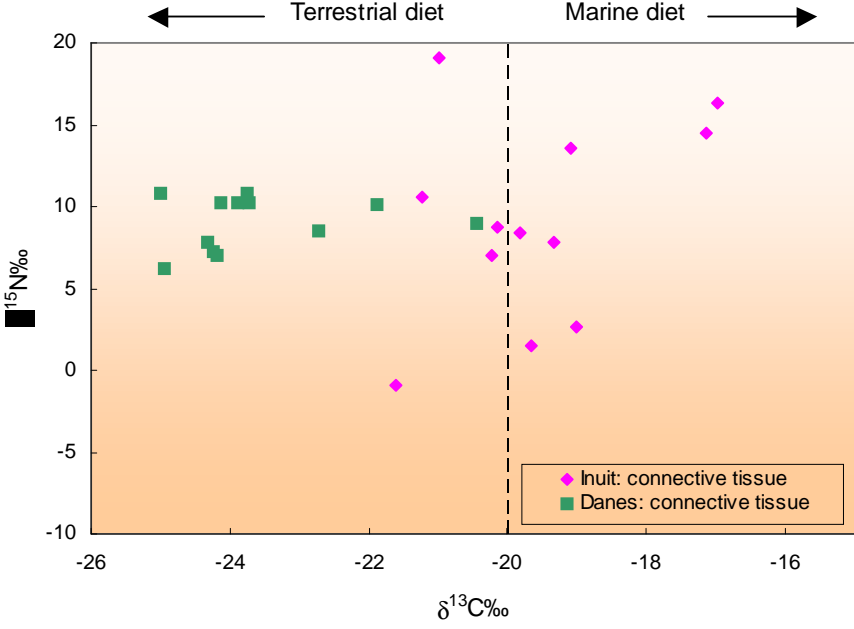
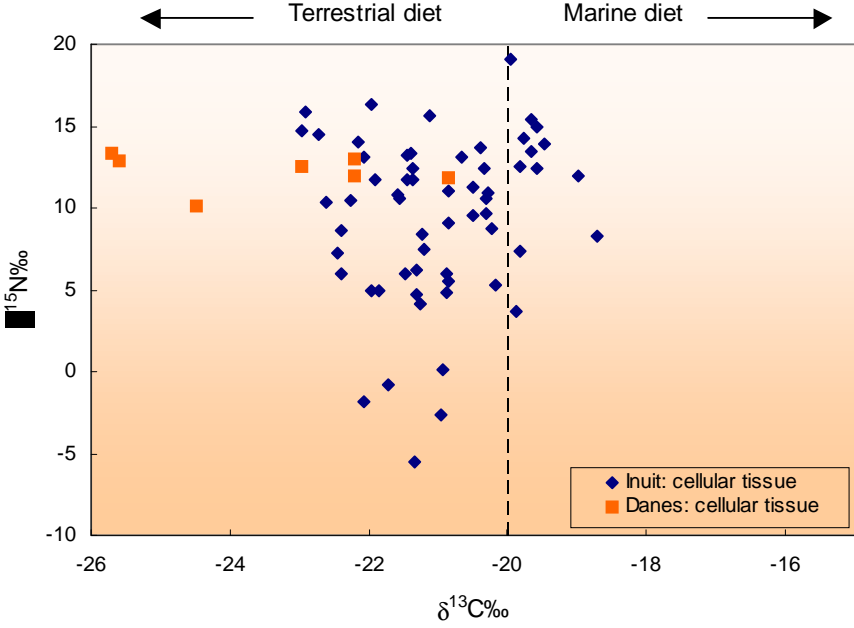


Figure 2



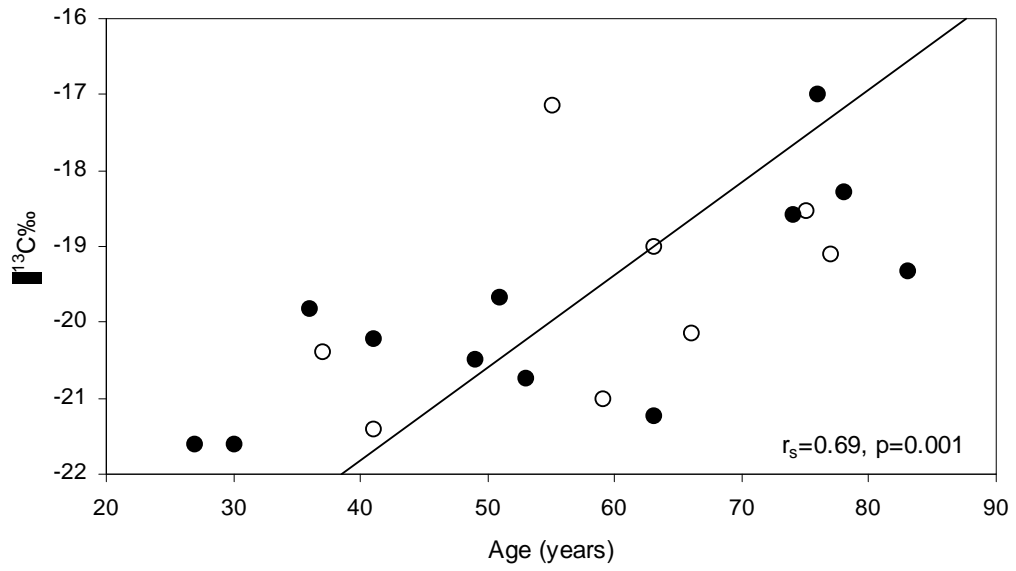


Figure 3