^{14}C BOMB-PULSE DATING AND STABLE ISOTOPE ANALYSIS FOR GROWTH RATE AND DIETARY INFORMATION IN BREAST CANCER?

K. Lång[1],*,†, K. Eriksson Stenström[2],†, A. Rosso[3], M. Bech[4], S. Zackrisson[1], D. Graubau[5] and S. Mattsson[6]
[1]Department of Translational Medicine, Division of Diagnostic Radiology, Lund University, Malmö, Sweden
[2]Department of Physics, Division of Nuclear Physics, Lund University, Lund, Sweden
[3]Epidemiology and Register Centre South, Skåne University Hospital, Lund, Sweden
[4]Department of Clinical Sciences, Division of Medical Radiation Physics, Lund University, Lund, Sweden
[5]Department of Clinical Sciences, Division of Pathology, Lund University, Lund, Sweden
[6]Department of Translational Medicine, Division of Medical Radiation Physics, Lund University, Malmö, Sweden

*Corresponding author: kristina.lang@med.lu.se

The purpose of this study was to perform an initial investigation of the possibility to determine breast cancer growth rate with ^{14}C bomb-pulse dating. Tissues from 11 breast cancers, diagnosed in 1983, were retrieved from a regional biobank. The estimated average age of the majority of the samples overlapped the year of collection (1983) within 3σ. Thus, this first study of tumour tissue has not yet demonstrated that ^{14}C bomb-pulse dating can obtain information on the growth of breast cancer. However, with further refinement, involving extraction of cell types and components, there is a possibility that fundamental knowledge of tumour biology might still be gained by the bomb-pulse technique. Additionally, δ^{13}C and δ^{15}N analyses were performed to obtain dietary and metabolic information, and to serve as a base for improvement of the age determination.

INTRODUCTION

Large amounts of the radioactive carbon isotope ^{14}C were produced during atmospheric testing of nuclear weapons in the late 1950s and early 1960s. As a consequence, the concentration of ^{14}C in air was almost doubled by 1963. Since the ^{14}C produced was incorporated in atmospheric CO_2 and introduced into the global carbon cycle, all organisms living during the bomb-pulse era, including humans, have been labelled with bomb-^{14}C[1–3]. When the Limited Test Ban Treaty from 1963 was implemented, the atmospheric ^{14}C concentration commenced decreasing mainly due to uptake of $^{14}CO_2$ into the oceans and also into the biosphere (see Figure 1).

Already in the early 1970s, Harkness and Walton[8] realised the potential of using bomb-^{14}C as a kinetic tracer in humans. Since then, the well-known decreasing atmospheric ^{14}C concentration has provided useful information in several fields in the medical sciences, see e.g. Falso and Buchholz[9] and Spalding et al.[10]. For instance, this so called ^{14}C bomb pulse has served in studies of turnover rates in human cells and tissues (e.g. in eye lenses[11], fat cells[12] and Achilles tendon[13]). The development of various diseases has also been investigated

using the ^{14}C bomb-pulse technique, with ^{14}C measurements on e.g. gallstones[14] and Alzheimer plaques[15]. A common denominator for these studies is slow turnover (years). Another example is Gonçalves et al.[16], who demonstrated that human atherosclerotic plaques, known to cause e.g. heart attacks and strokes, develop slowly (mean biological ages 5–15 years). Quantification of the age of various structures in the plaque has gained a better understanding of the development of the plaque and opened for improved treatment methods.

The bomb-pulse technique has to the present authors' knowledge not yet been used to study cancer. Understanding of the time cause for the development of human cancer tissue is of utmost importance for improved prevention and treatment strategies. So far, certain assumptions have had to be made in order to estimate how long a cancer has grown. There are theoretical models to describe tumour growth rates, such as an exponential growth model or the Gompertz Model, where the latter takes into consideration a slower growth rate as the tumour size increases. These models have been applied to cancer in general[17] and to breast cancer in particular[18–20]. These models have been shown to fit experimental and clinical data, such as observations of the doubling of tumour volume on serial mammograms. From these studies, the average

---

†These authors contributed equally to this work.

tumour volume doubling time for human breast cancer has been estimated to be between 105 and 327 days[21–29]. Theoretically, this means that a breast cancer that is 10-mm large at detection (30 volume doublings) has been growing for 9–27 years. However, these studies are limited due to the lack of direct observations of tumour age.

The main purpose of this study was to perform an initial investigation of the possibility to determine the growth rate of breast cancer with ^{14}C bomb-pulse dating. An additional aim was to highlight the limitations of the ^{14}C bomb-pulse technique of human tissues due to dietary variations. Furthermore, the paper pays attention to how dietary information can be obtained from stable isotope ratio analysis of carbon and nitrogen, also providing means to increase the accuracy of bomb-pulse dating. Stable isotope ratio analysis may also give important information relating cancer development to diet.

Bomb-pulse dating and stable isotope analysis

The ^{14}C bomb-pulse technique uses ^{14}C data from atmospheric clean-air CO_2 to translate the ^{14}C specific activity of the sample into a calendar date (so called CaliBomb dates, see 'Material and Methods' section). For humans, carbon enters the body mainly through the diet. Thus, ^{14}C data from clean-air CO_2 may not be fully representative for humans.

A previous study by Georgiadou *et al.*[30] used human blood serum samples from a biobank to estimate the accuracy of bomb-pulse dating on human material. Blood serum samples collected from residents of Malmö (Sweden) in 1978 exhibited CaliBomb dates between 3.0 ± 0.4 years before the collection date and 0.2 ± 0.5 years after the collection date (the average deviation from collection date was -1.5 ± 0.7 years). Two major effects associated with the age deviation, competing in opposite directions, were identified: (1) delay time between production and consumption of foodstuffs, which can explain CaliBomb dates obtained before the collection date and (2) excessive consumption of marine food products, which has the potential of producing CaliBomb dates after the collection date.

As discussed in the study by Georgiadou *et al.*[30], there are also other factors that have the possibility to influence the obtained CaliBomb date, however, for the majority of the population probably to a more limited extent than delay time and marine food consumption. Anthropogenic ^{14}C released from nuclear power plants or from research laboratories, industry or hospitals using ^{14}C as a tracer has the potential of producing too old CaliBomb dates (at the declining bomb-pulse curve)[31, 32]. On the other hand, fossil fuel-based products in food industry (e.g. in CO_2 used in cultivation in greenhouses) as well as food grown in heavily industrialised areas may lead to foodstuffs
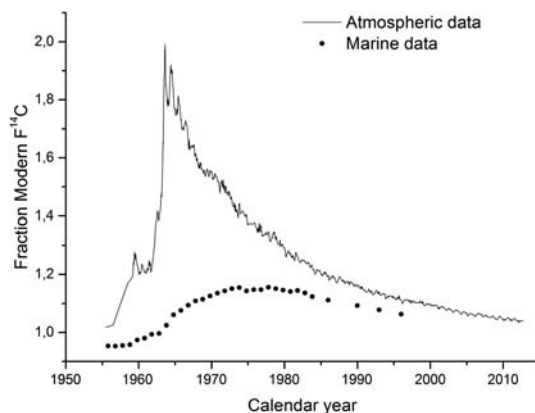

Figure 1. ^{14}C specific activity in atmospheric CO_2 and oceans representative for the northern hemisphere (expressed in units of Fraction Modern, $F^{14}C$[4]; $F^{14}C$ is ~1.0 before the nuclear weapons tests, corresponding to the natural $^{14}C/^{12}C$ ratio of about $10^{-10}\%$[5–7]).

having lower ^{14}C specific activity than clean air, thus contributing to producing too young CaliBomb dates (at the declining bomb-pulse curve)[33].

^{14}C bomb-pulse dates reported in the literature usually do not take into account that there is a delay time between production and consumption of the food[33, 34]. Neither is it commonly considered that marine foodstuffs can influence the accuracy of the calibration (see the difference in marine and atmospheric calibration curve in Figure 1). However, since a large consumption of marine foodstuffs has the potential to affect the calibration curve (see Figure 1) on an individual basis, information about the diet may be important in bomb-pulse dating of human tissue samples. Additionally, the individual diet is of interest for studies of the development of various diseases, including breast cancer[35].

One technique to assess the diet of an organism is analysis of the ratios of stable isotopes of carbon and nitrogen (expressed as $\delta^{13}C$ and $\delta^{15}N$ in ‰, where $\delta X = [(R_{sample}/R_{standard}) - 1] \times 1000$, and R is the ratio of heavy to light isotope, i.e.$^{13}C/^{12}C$ or $^{15}N/^{14}N$) (see e.g. Schoeller[36]). $\delta^{13}C$ and $\delta^{15}N$ provide dietary information since the stable isotope ratios in tissues and organs of the consumer reflect those of the diet with a small shift. This discrimination of one of the stable isotopes occurs in each step of the food chain. The difference in δ-value between an organism and its diet, referred to as the discrimination factor, is generally about 1‰ for $\delta^{13}C$ and 3‰ for $\delta^{15}N$[37]. The discrimination factor, however, also depends on various factors such as species, age, metabolic processes and environmental conditions (see e.g. Caut *et al.*[37] and O'Connell *et al.*[38]). Thus, different trophic levels (positions in
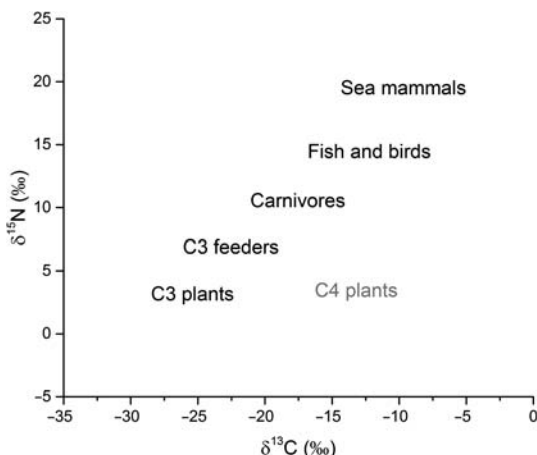
Figure 2. Generalised isotopic trophic level diagram for terrestrial and marine food webs (values from Georgiadou *et al.*[30]). The $\delta^{13}C$ values of C3 plants (e.g. wheat and potatoes) differ from those of C4 plants (e.g. maize and sugar cane) due to photosynthetic pathways. For the Swedish diet, of relevance for this study, C3 plants dominate over C4 plants.

the food chain) are characterised by different ranges of δ values (see Figure 2).

Despite the complexity and variation of discrimination factors, several studies have shown that $\delta^{13}C$ and $\delta^{15}N$ are valuable biomarkers of dietary intake (see e.g. Georgiadou *et al.*[30] and Patel *et al.*[39] and references therein). The approach can additionally be used as a tool to identify samples where extreme dietary conditions (high intake of marine products) might influence the ^{14}C bomb-pulse date (decreasing the applicability of the atmospheric calibration curve).

The serum study[30] showed that the age deviation (difference between CaliBomb date and actual sampling date) correlated strongly with $\delta^{13}C$, which was interpreted as influence from marine dietary components. $\delta^{15}N$, however, did not show any correlation with age deviation in that study. $\delta^{13}C$ data from the serum study have also been used to demonstrate the possibility to develop general methods increasing accuracy as well as precision of the age determination using the ^{14}C bomb-pulse technique[40]. However, more data are needed to produce a general correction model.

MATERIAL AND METHODS

Frozen breast cancer tissues from 11 women collected in 1983 were retrieved from the South Swedish Breast Cancer Group Biobank. In three cases, the samples were large and sub-samples from each tumour could be taken (maximum of three sub-samples per cancer).

Prior to ^{14}C analysis, parts of each tissue sample were graphitised according to the procedures described by Andersson Georgiadou *et al.*[41] and Genberg *et al.*[42]. Total carbon in the graphite samples ranged between 32 and 124 µg. The $^{14}C/C$ ratio, expressed as $F^{14}C$[4], was analysed with accelerator mass spectrometry (AMS) at the Lund Single Stage AMS facility at Lund University[43, 44]. The analytical precision of the $F^{14}C$ measurements for human tissue samples is usually 0.5–1% for this instrument[40]. The obtained $F^{14}C$ values were translated into calendar years using the CaliBomb software (http://calib.qub.ac.uk/CALIBomb/), choosing the calibration dataset 'Levin' (representative for European clean-air CO_2) and 'smoothing' (averaging the dataset) of 0.5 years. A given analytical uncertainty in $F^{14}C$ results in various uncertainties in the CaliBomb date depending e.g. on the steepness of the declining bomb-pulse curve and on seasonal variations (see Figure 1).

$\delta^{13}C$ and $\delta^{15}N$ were analysed using isotope ratio mass spectrometry (IRMS) at the Department of Biology, Lund University, using between 0.28 and 0.40 mg of dried tumour material for each sample. The analytical precision obtained for standards at this instrument is usually $<0.4‰$ for carbon and $<0.12‰$ for nitrogen (1σ). Further information about the IRMS analysis can be found in Andersson Georgiadou[40].

RESULTS AND DISCUSSION

The results of the ^{14}C measurements and the IRMS analysis are shown in Table 1. A few samples lack IRMS data due to insufficient amount of sample material. The analytical uncertainties of the ^{14}C measurements were higher than normally because of the difficulty of accurately weighing the samples and due to smaller and more varying sample sizes than usually.

Table 1 only includes CaliBomb dates after the peak in 1963 (all calibrations also return a result between the mid-1950s and beginning of the 1960s). It is however not likely that the results from the rising part of the bomb-pulse curve are relevant in this study. A result from the rise of the bomb pulse would imply that the carbon in the analysed tissue was about 20 years old at the time of collection for all the subjects (not likely, the subjects were between about 30 and 60 years of age in the late 1950s). Another alternative would be that the turnover of cells is very slow resulting in an average $F^{14}C$ value corresponding to the rise of the bomb pulse. Neither this explanation appears likely. The most plausible explanation seems to be that the carbon in the breast tumour samples mainly originates from recently consumed food.

Table 1. Results from the ¹⁴C and IRMS analyses of the breast tumour samples.

Sample	Year of birth	Lab code	¹⁴C analysis			IRMS analysis		
			m C (µg)	$F^{14}C \pm 1\sigma$	CaliBomb date $\pm 1\sigma$	m (mg)	$\delta^{13}C$ (‰)	$\delta^{15}N$ (‰)
T1	1924	GEO_1511	45	1.211 ± 0.014	1984.8 ± 1.1	0.30	−22.3	11.0
T2	1901	GEO_1512	56	1.193 ± 0.013	1986.2 ± 1.9	0.30	−22.0	11.7
T3A	1928	GEO_1513	130	1.309 ± 0.015	1979.3 ± 0.6	0.35	−22.5	13.3
T3B		GEO_1514	63	1.241 ± 0.014	1982.8 ± 0.9	0.28	−22.7	12.8
T3C		GEO_1515	88	1.240 ± 0.014	1982.9 ± 0.9			
T4	1919	GEO_1516	54	1.239 ± 0.014	1982.9 ± 0.9			
T5	1922	GEO_1517	53	1.222 ± 0.014	1984.1 ± 0.8	0.39	−21.2	13.2
T6	1901	GEO_1518	124	1.244 ± 0.014	1982.7 ± 0.9	0.37	−21.0	13.0
T6:2		GEO_1519	103	1.232 ± 0.014	1983.1 ± 0.8			
T7	1898	GEO_1520	83	1.274 ± 0.014	1980.9 ± 1.0	0.32	−27.3	9.3
T8	1932	GEO_1521	101	1.247 ± 0.014	1982.3 ± 1.3	0.40	−23.3	11.0
T9A	1902	GEO_1522	52	1.184 ± 0.013	1987.5 ± 1.7	0.35	−22.8	9.4
T9B		GEO_1523	37	1.190 ± 0.013	1986.7 ± 1.7	0.28	−24.7	7.8
T9C		GEO_1524	32	1.157 ± 0.013	1990.2 ± 1.7			
T10	1915	GEO_1525	98	1.137 ± 0.013	1992.8 ± 3.3	0.37	−20.6	13.7
T11	1911	GEO_1526	79	1.223 ± 0.014	1984.1 ± 0.7	0.32	−21.9	10.3

All samples were collected in 1983. Only results from the time after the bomb-pulse peak in 1963 are shown. A few samples lack IRMS data due to insufficient amount of sample material (m means mass of carbon (m C (ug)) and sample (m (mg)), respectively).
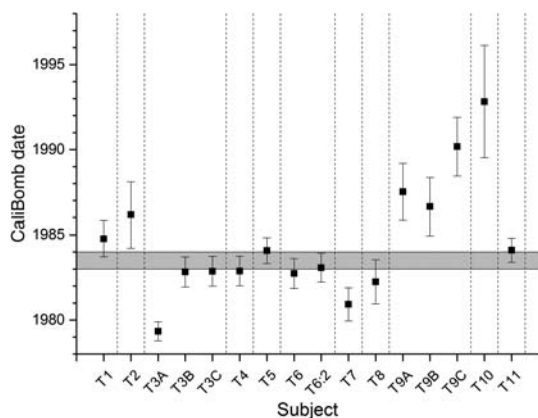

Figure 3. Results from the ¹⁴C measurements (±1σ) for each subject (the year of collection, 1983, is indicated in grey).

Figure 4. $\delta^{13}C$ and $\delta^{15}N$ obtained for the tumour samples (compare with Figure 2). A few tumour samples lack IRMS data due to insufficient amount of sample material.

Figure 3 shows the results from the ¹⁴C measurements (±1σ, results only for the declining part of the bomb-pulse curve) for each subject (the year of collection was 1983). The CaliBomb date of all samples except T3A and T9C overlaps with the year of collection 1983 within 3σ.

Figure 4 shows the $\delta^{13}C$ and $\delta^{15}N$ values obtained for the tumour samples. Sample T7 seems to lie apart from rest of the samples. In contrast to the other samples, T7 contained mainly fat tissue, alternatively necrosis, which might explain the separate location of the samples in the diagram. In stable isotope analysis
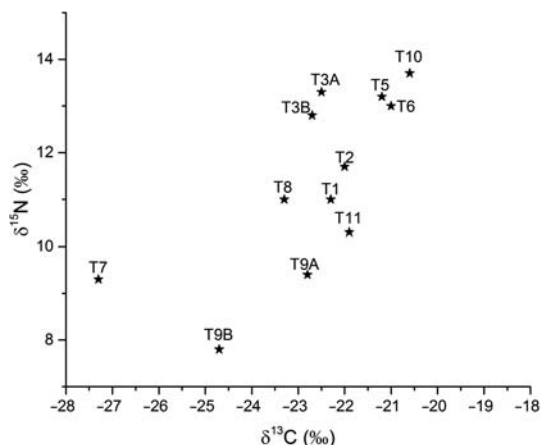
of human tissues, it is also known that different tissues do not have identical $\delta^{13}C$ and $\delta^{15}N$ values (see e.g. Caut *et al.*[37] and Tieszen *et al.*[45]). Fatty acids, e.g., are known to be depleted in $\delta^{13}C$ (having a negative discrimination factor) compared to other tissues as well as diet. Schoeller *et al.*[46] found that $\delta^{13}C$ (plasma lipid) < $\delta^{13}C$(plasma protein) in 10 American subjects. Tieszen *et al.*[45] report that $\delta^{13}C$ values in gerbil were ranked as fat < liver < muscle < brain < hair (the discrimination factor between diet and fat being about −3‰). The present results for sample T7 follow
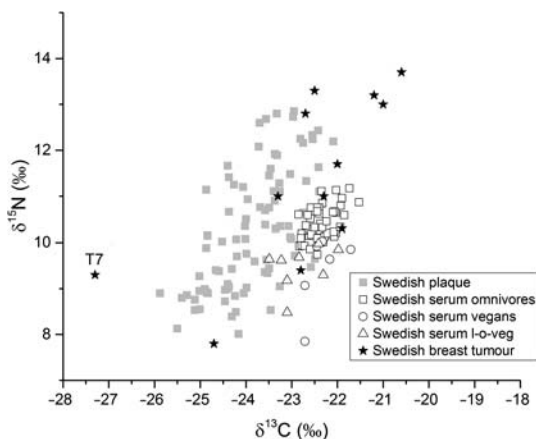
Figure 5. δ^{13}C and δ^{15}N for the tumour samples and previous measurements on Swedish plaque[41] and serum[30]. Note that serum for the omnivores, in general, has higher δ^{13}C and δ^{15}N than vegans and lacto-ovo vegetarians (l-o-veg), in concordance with the generalised isotopic trophic diagram in Figure 2.

the same trend as these observations and indicate that δ^{13}C(fat cells/fat tissue) $< \delta^{13}$C(cancer cells).

Figure 5 shows the δ^{13}C and δ^{15}N values obtained for the tumour samples including previous measurements of serum and atherosclerotic plaques from Swedish patients (see Gonçalves *et al.*[47]). It should be noted that the subjects are not the same, neither is the year of collection (serum from 1978 and plaque from 2007–2009). However, in this diagram sample T7 still lies apart from the rest of all sample types. Figure 5 also indicates linear correlations between δ^{13}C and δ^{15}N for the various sample types. This can be expected considering the tropic level diagram in Figure 2.

The main purpose of the δ^{13}C and δ^{15}N measurements of the breast tumour samples was to evaluate if the diet of any of the subjects contained a large proportion of fish products, which could influence the ^{14}C value (according to Figure 1). High values of both δ^{13}C and δ^{15}N (upper right corner of Figure 2) correspond to more fish products in the diet than low values (lower left corner of Figure 2). According to Figures 4 and 5, tumour samples with high δ^{13}C and δ^{15}N values (upper right corner) could indicate that these subjects consume more marine foodstuffs than the others (e.g. the diet of subjects 5, 6 and 10 contains more fish products than others). The excess marine influence could subsequently affect the bomb-pulse dating (see Figure 1), and possibly give dates after the collection date (above the grey area in Figure 3). This is not observed (see Figure 3). In view of the limited amount of samples, the magnitude of the uncertainty of the measurements, the

variability of discrimination factors and probably variability in growth rate of breast tumours, it is difficult to draw any general conclusions from the findings.

It is evident that further development of the application of ^{14}C bomb-pulse dating technique for breast cancer research is needed before it can be of use. A main drawback of the technique is that the ^{14}C content in the tumour tissue originating from the time when the tumour was in its initial stage is small relative to the ^{14}C content from the later created part of the tumour. Analysis of extracted tumour DNA may be a way to obtain more relevant growth rate information, since 50% of carbon in DNA originates from the latest cell division[9]. A better approach to estimate the onset of the tumour growth would be to isolate and analyse cancer stem cell DNA. The main challenge of this approach will be to obtain samples large enough for the ^{14}C analysis (at least 10 µg of carbon is required for the system used). An associated difficulty is that the slope of the bomb pulse nowadays is approaching zero. This means that the resulting uncertainty in the age determination increases to inapplicability when approaching present days. Thus bomb-pulse dating of fresh material is only possible for cells or tissues with a slow turnover (several years). For materials with shorter turnover, relevant age information can only be obtained from the steeper part of the bomb-pulse curve, and biological material from biobanks must be used. The available amounts of sample material in the biobanks are however often very limited.

If the estimate of the ^{14}C turnover rate can be more exact, the information gained can be of value, primarily in the understanding of tumour biology: which is the tumour growth rate? Also, the clinical implications of the dating of breast cancer could be the revision or validation of existing tumour growth models. Furthermore, in breast cancer screening one major drawback is the overdiagnosis of slow-growing indolent tumours. Overdiagnosis occurs when women without symptoms are diagnosed with a disease that will not cause them to experience symptoms or lead to early death[48]. It has been estimated that the overdiagnosis in breast cancer screening is 10–20%[49, 50]. It is not possible to incorporate ^{14}C bomb-pulse dating in clinical practice since the method is both laborious and expensive and most importantly the atmospheric levels of ^{14}C are now down at a low level. That is why the ^{14}C bomb-pulse dating technique applied on breast cancer combined with an analysis of micro- and macro-structural changes in the tumour, in order to find a signature of a slow growing cancer, could be of interest. For example, in parallel to the efforts of obtaining relevant tumour growth rate information, samples of the same breast tumour could be investigated with a high-resolution imaging technique, i.e. synchrotron

radiation-based small angle X-ray scattering to detect changes in the collagen structure of the tumour tissue[51]. If structural changes in a breast tumour can be correlated to growth rate, this could provide means to find new biomarkers or a morphological signature that can be used to further refine breast cancer diagnosis and ultimately optimise the treatment, e.g. less aggressive treatment of indolent tumours.

CONCLUSIONS

This is a first pilot study assessing the potential of carbon and nitrogen isotope analysis for obtaining growth rate ($^{14}C/^{12}C$) and dietary ($^{13}C/^{12}C$ and $^{15}N/^{14}N$) information for breast cancer research. Stable carbon and nitrogen isotope analysis may give information about the relation between diet and breast cancer development and progression.

The measurements in this limited, initial study could not demonstrate the possibility to obtain age information on the onset of the breast cancer. However, analysis of total tumour material may primarily reflect the current turnover of the carbon in the various components of the metabolically active tumour tissue. Analysis of extracted tumour DNA, or the isolation and analysis of cancer stem cells, may be a way to obtain more relevant growth rate and age information. Furthermore, $^{13}C/^{12}C$ and $^{5}N/^{14}N$ analysis may provide important dietary information of value for studies of possible correlations between diet and tumour development.

ACKNOWLEDGEMENTS

FUNDING

REFERENCES

1. Libby, W. F., Berger, R., Mead, J. F., Alexander, G. V. and Ross, J. F. *Replacement rates for human tissue from atmospheric radiocarbon*. Science **146**, 1170–1172 (1964).
2. Broecker, W. S., Schulert, A. and Olson, E. A. *Bomb carbon-14 in human beings*. Science **130**, 331–332 (1959).
3. Harkness, D. D. and Walton, A. *Carbon-14 in the biosphere and humans*. Nature **223**, 1216–1218 (1969).
4. Reimer, P. J., Brown, T. A. and Reimer, R. W. *Discussion: reporting and calibration of post-bomb ^{14}C data*. Radiocarbon **46**, 1299–1304 (2004).
5. Levin, I. and Kromer, B. *The tropospheric $^{14}CO_2$ level in mid latitudes of the northern hemisphere (1959–2003)*. Radiocarbon **46**, 1261–1271 (2004).
6. Levin, I., Kromer, B. and Hammer, S. *Atmospheric $\Delta^{14}CO_2$ trend in Western European background air from 2000 to 2012*. Tellus B **65**, 20092 (2013).
7. Kalish, J., Nydal, R., Nedreaas, K., Burr, G. and Eine, G. *A time history of pre- and post-bomb radiocarbon in the Barents sea derived from Arcto-Norwegian cod otoliths*. Radiocarbon **43**, 843–845 (2001).
8. Harkness, D. D. and Walton, A. *Further investigations of the transfer of bomb ^{14}C to man*. Nature **240**, 302–303 (1972).
9. Falso, M. J. S. and Buchholz, B. A. *Bomb pulse biology*. Nucl. Instr. Meth. B **294**, 666–670 (2013).
10. Spalding, K. L., Bhardwaj, R. D., Buchholz, B. A., Druid, H. and Frisen, J. *Retrospective birth dating of cells in humans*. Cell **122**, 133–143 (2005).
11. Lynnerup, N., Kjeldsen, H., Heegaard, S., Jacobsen, C. and Heinemeier, J. *Radiocarbon dating of the human eye lens crystallines reveal proteins without carbon turnover throughout life*. PLoS One **3**, e1529 (2008).
12. Spalding, K. L. *et al. Dynamics of fat cell turnover in humans*. Nature **453**, 783–787 (2008).
13. Heinemeier, K. M., Schjerling, P., Heinemeier, J., Magnusson, S. P. and Kjaer, M. *Lack of tissue renewal in human adult achilles tendon is revealed by nuclear bomb ^{14}C*. FASEB J. **27**, 2074–2079 (2013).
14. Druffel, E. M. and Mok, H. I. *Time history of human gallstones: application of the post-bomb radiocarbon signal*. Radiocarbon **25**, 629–636 (1983).
15. Lovell, M. A., Robertson, J. D., Buchholz, B. A., Xie, C. and Markesbery, W. R. *Use of bomb pulse carbon-14 to age senile plaques and neurofibrillary tangles in Alzheimer's disease*. Neurobiol. Aging **23**, 179–186 (2002).
16. Gonçalves, I., Stenström, K., Skog, G., Mattsson, S., Nitulescu, M. and Nilsson, J. *Short communication: dating components of human atherosclerotic plaques*. Circ. Res. **106**, 1174–1177 (2010).
17. Laird, A. K. *Dynamics of tumour growth*. Br. J. Cancer **18**, 490–502 (1964).
18. Hart, D., Shochat, E. and Agur, Z. *The growth law of primary breast cancer as inferred from mammography screening trials data*. Br. J. Cancer **78**, 382–387 (1998).
19. Spratt, J. A., von Fournier, D., Spratt, J. S. and Weber, E. E. *Decelerating growth and human breast cancer*. Cancer **71**, 2013–2019 (1993).
20. Norton, L. *A Gompertzian model of human breast cancer growth*. Cancer Res. **48**, 7067–7071 (1988).
21. Schwartz, M. *A biomathematical approach to clinical tumor growth*. Cancer **14**, 1272–1294 (1961).
22. Fournier, D. V., Weber, E., Hoeffken, W., Bauer, M., Kubli, F. and Barth, V. *Growth rate of 147 mammary carcinomas*. Cancer **45**, 2198–2207 (1980).
23. Spratt, J. A., von Fournier, D., Spratt, J. S. and Weber, E. E. *Mammographic assessment of human breast cancer growth and duration*. Cancer **71**, 2020–2026 (1993).
24. Lundgren, B. *Observations on growth rate of breast carcinomas and its possible implications for lead time*. Cancer **40**, 1722–1725 (1977).

25. Heuser, L., Spratt, J. S. and Polk, H. C. *Growth rates of primary breast cancers*. Cancer **43**, 1888–1894 (1979).

26. Peer, P. G. M., van Dijck, J. A. A. M., Hendriks, J. H. C. L., Holland, R. and Verbeek, A. L. M. *Age-dependent growth rate of primary breast cancer*. Cancer **71**, 3547–3551 (1993).

27. Kusama, S., Spratt, J. S., Donegan, W. L., Watson, F. R. and Cunningham, C. *The gross rates of growth of human mammary carcinoma*. Cancer **30**, 594–599 (1972).

28. Spratt, J. S., Heuser, L., Kuhns, J. G., Reiman, H. M., Buchanan, J. B., Polk, H. C. and Sandoz, J. *Association between the actual doubling times of primary breast cancer with histopathologic characteristics and Wolfe's parenchymal mammographic patterns*. Cancer **47**, 2265–2268 (1981).

29. Kuroishi, T. *et al. Tumor growth rate and prognosis of breast cancer mainly detected by mass screening*. Jpn J. Cancer Res. **81**, 454–462 (1990).

30. Georgiadou, E., Stenstrom, K. E., Uvo, C. B., Nilsson, P., Skog, G. and Mattsson, S. *Bomb-pulse ^{14}C analysis combined with ^{13}C and ^{15}N measurements in blood serum from residents of Malmö, Sweden*. Radiat. Environ. Biophys. **52**, 175–187 (2013).

31. Stenström, K., Leide-Svegborn, S. and Mattsson, S. *Low-level occupational ^{14}C contamination – results from a pilot study*. Radiat. Prot. Dosim. **130**, 337–342 (2008).

32. Stenström, K., Unkel, I., Nilsson, C. M., Rääf, C. and Mattsson, S. *The use of hair as an indicator of occupational ^{14}C contamination*. Radiat. Environ. Biophys. **49**, 97–107 (2010).

33. Stenström, K., Skog, G., Nilsson, C. M., Hellborg, R., Svegborn, S. L., Georgiadou, E. and Mattsson, S. *Local variations in ^{14}C – how is bomb-pulse dating of human tissues and cells affected?* Nucl. Instr. Meth. B **268**, 1299–1302 (2010).

34. Georgiadou, E. and Stenström, K. *Bomb-pulse dating of human material – modelling the influence of diet*. Radiocarbon **52**, 1351–1357 (2010).

35. Rossi, R. E., Pericleous, M., Mandair, D., Whyand, T. and Caplin, M. E. *The role of dietary factors in prevention and progression of breast cancer*. Anticancer Res. **34**, 6861–6875 (2014).

36. Schoeller, D. A. *Isotope fractionation: Why aren't we what we eat?* J. Arch. Sci. **26**, 667–673 (1999).

37. Caut, S., Angulo, E. and Courchamp, F. *Discrimination factors ($\delta^{15}N$ and $\delta^{13}C$) in an omnivorous consumer: effect of diet isotopic ratio*. Funct. Ecol. **22**, 255–263 (2008).

38. O'Connell, T. C., Kneale, C. J., Tasevska, N. and Kuhnle, G. G. C. *The diet-body offset in human nitrogen isotopic values: a controlled dietary study*. Am. J. Phys. Anthr. **149**, 426–434 (2012).

39. Patel, P. S. *et al. Serum carbon and nitrogen stable isotopes as potential biomarkers of dietary intake and their relation with incident type 2 diabetes: the Epic-Norfolk study*. Am. J. Clin. Nutr. **100**, 708–718 (2014).

40. Andersson Georgiadou, E. *Exploring the possibilities of ^{14}C bomb-pulse dating of human tissue samples*. Ph.D. thesis, Lund University (2014). ISBN 978-91-7623-023-0.

41. Andersson Georgiadou, E., Gonçalves, I., Bertacchi Uvo, C., Nilsson, J., Skog, G., Mattsson, S., Mendes Pedro, L., Fernades e Fernandes, J. and Eriksson Stenström, K. *Potential influence of diet on bomb-pulse dating of human plaque samples*. Radiocarbon **55**, 874–884 (2013).

42. Genberg, J., Stenström, K., Elfman, M. and Olsson, M. *Development of graphitization of μg-sized samples at Lund University*. Radiocarbon **52**, 1270–1276 (2010).

43. Skog, G. *The single stage AMS machine at Lund University: status report*. Nucl. Instr. Meth. B **259**, 1–6 (2007).

44. Skog, G., Rundgren, M. and Sköld, P. *Status of the single stage AMS machine at Lund University after 4 years of operation*. Nucl. Instr. Meth. B **268**, 895–897 (2010).

45. Tieszen, L. L., Boutton, T. W., Tesdahl, K. G. and Slade, N. A. *Fractionation and turnover of stable carbon isotopes in animal tissues: implications for $\delta^{13}C$ analysis of diet*. Oecologia **57**, 32–37 (1983).

46. Schoeller, D. A., Minagawa, M., Slater, R. and Kaplan, I. R. *Stable isotopes of carbon, nitrogen and hydrogen in the contemporary North American human food web*. Ecol. Food Nutr. **18**, 159–170 (1986).

47. Gonçalves, I., Andersson Georgiadou, E., Mattsson, S., Skog, G., Pedro, L., Fernandes e Fernandes, J., Dias, N., Engström, G., Nilsson, J. and Stenström, K. *Direct association between diet and the stability of human atherosclerotic plaque*. Scientific Reports 5, 15524 (2015).

48. Moynihan, R., Doust, J. and Henry, D. *Preventing overdiagnosis: how to stop harming the healthy*. Br. Med. J. **344**, e3502 (2012).

49. Zackrisson, S., Andersson, I., Janzon, L., Manjer, J. and Garne, J. P. *Rate of over-diagnosis of breast cancer 15 years after end of Malmö mammographic screening trial: follow-up study*. Br. Med. J. **332**, 689–692 (2006).

50. Independent UK Panel on Breast Cancer Screening. *The benefits and harms of breast cancer screening: an independent review*. Lancet **380**(9855), 1778–1786 (2012).

51. Keyriläinen, J., Bravin, A., Fernández, M., Tenhunen, M., Virkkunen, P. and Suortti, P. *Phase-contrast X-ray imaging of breast*. Acta Radiol. **51**, 866–884 (2010).