Lipid oxidation may reduce the quality of a fresh-frozen bone allograft. Is the approved storage temperature too high?

Minna Laitinen[1], Riitta Kivikari[2] and Martti Hirn[1]

Departments of [1]Orthopedics and Traumatology, University Hospital of Tampere and Medical School, University of Tampere, FI-33521Tampere, [2]Applied Chemistry and Microbiology, University of Helsinki, FI-00014 Helsinki, Finland
Correspondence ML: minna.laitinen@pshp.fi
Submitted 05-05-06. Accepted 05-10-30

Background **International standards indicate that bone allografts for human use should be stored in a –40°C or colder environment and the storage time should be up to 5 years. Bone allografts contain lipids which oxidate and become toxic with time.**

Material and methods **We determined lipid oxidation in femoral head bone allografts by headspace gas chromatography at different storage temperatures and storage times.**

Results **We found that lipid oxidation in fresh-frozen bone allograft was influenced by storage temperature and storage time. Lipid oxidation was significantly more rapid at –30°C than at –70°C. Even at –70°C, however, some oxidation occurred but it was quite minimal after 3 years.**

Interpretation **Because of the negative effects of lipid oxidation, we recommend a storage temperature of –70°C or lower for bone allografts.**

Fresh-frozen bone includes unnecessary components such as fat and blood, which can even have some harmful effect on the recipient. Blood increases the risk of viral transmission and lipids are a known reservoir of oxidation (Aspenberg 1998, Moreau et al. 2000). In morcelized bone, the amounts of fat and blood can be reduced by washing (Hirn et al. 2001). Washing will not remove the organic components present in the cavities, however, and it is not as effective in structural bone blocks. Under these circumstances, lipid oxidation may be a problem.

Oxidation of lipids has long been recognized to be a leading measure of deterioration in quality of foods, and is often the decisive factor in determining food product storage life (Frankel 1993). From studies in food, it is known that free radicals generated from hydroperoxides can abstract H-atoms from protein. Protein radicals are formed and they, in turn, combine with each other, resulting in the formation of a protein network. Also, monocarbonyl compounds derived from auto-oxidation of unsaturated fatty acids readily condense with the free amino groups of proteins (Belitz and Grosch 1999). If a sample contains volatile oxidation products derived from the oxidation of lipids, it is likely that reactions between these compounds and proteins will occur, resulting in destruction of the biological functionality of the proteins.

In bone, peroxidated lipids have been reported to induce cell apoptosis (Girotti 1998, Hogg and Kalyanaram 1999), inhibition of osteoblastic differentiation (Parhami et al. 1997, 1999, Jackson and Demer 2000), and release of toxic compounds for osteoblast-like cells (Moreau et al. 2000). To date, there has been no research on the rate of lipid oxidation in bone lipids of bone allografts. Thus, in the present study we wanted to find out the extent of lipid oxidation in fresh-frozen femoral head allografts held at different storage temperatures.

DOI 10.1080/17453670610046343

Material and methods

Bone allograft preparation

At our hospital, femoral heads are harvested from primary hip arthroplasty operations under aseptic conditions. The living donors are selected according to EAMST criteria (EATB and EAMST 1997) and screened before donation to the bone bank. During a 3-month period, we selected 21 femoral heads for the study. These were split into 4 pieces and washed with low-pressure pulse lavage (Micro-Air Pulse Lavage 4740) using sterile physiological saline, dried and stored as two different groups at –30ºC and at –70ºC. The measurements for lipid oxidation were done after 1 year and at 3 years.

Measurment of lipid oxidation using head-space gas chromatography

Volatile oxidation products were analyzed using static headspace gas chromatography. From the oxidation products, hexanal was chosen for closer documentation. It has been shown to be a rapid and reliable tool for measurement of lipid oxidation in the food industry (Goodridge et al. 2003). 1,000 mg of morselized bone sample was placed in a headspace vial and sealed with PTFE-coated septa and aluminium caps. Samples were injected with an automatic sampler (Perkin Elmer HS 40XL). Vials were thermostated for 20 min at 80°C. The gas chromatograph was equipped with a capillary column (Nordion NB-54) and an FID-detector. The figures of hexanal peak areas (µVsec) were analyzed by comparing the results of samples from the same donor stored at different temperatures and for different times. Student's t-test was used for the statistical analysis.

Results

When samples were analyzed after 1 year, it was apparent that lipid oxidation had proceeded significantly faster at –30°C than at –70°C ($p < 0.01$). At 1 year, the amount of hexanal produced at –30°C was so high that it was quite likely that lipids and proteins had already become oxidized at that time.

Some oxidation could be detected in samples stored at –70°C for 1 year and 3 years, but the hexanal peak area increased only in 1 sample during the study period. In the other 20 samples, the amount of hexanal had even diminished during the time. All the concentrations of hexanal in this group were very low, i.e. close to the detection limit. However, in samples stored at –30°C, very high concentrations of hexanal could also be detected after 3 years of storage. In this group, the figures increased in every graft from 1 to 3 years. This high concentration of secondary oxidation products of lipids indicated massive breakdown of lipids in the sample. The results are summarized in the Table.

Hexanal peak areas (µVsec) × 10^3 of allograft samples as a function of storage temperature and time

Sample number	Absolute hexanal level 1 year		3 years		Proportional increase between 1 and 3 years	
	–70°C	–30°C	–70°C	–30°C	–70°C	–30°C
1	0.2	0.4	0.5	3.7	2.5	9.3
2	0.8	2.2	0.4	18.4	0.5	8.4
3	1	0.8	nd	2.9	nd	3.6
4	0.4	3.3	nd	6.3	nd	1.9
5	0.7	1	nd	10	nd	10
6	1	5.7	nd	18.4	nd	3.2
7	1.9	10.9	nd	17.9	nd	1.6
8	0.5	1	0.4	5.6	0.8	5.6
9	0.5	1.3	nd	8.3	nd	6.4
10	0.2	1.7	nd	2.1	nd	1.2
11	2.4	18.6	0.3	20.6	0.1	1.1
12	6.5	6.4	nd	7.2	nd	1.1
13	0.3	2.2	nd	2.9	nd	1.3
14	0.4	0.9	nd	7.1	nd	7.9
15	4.8	7.4	0.4	10	0.1	1.4
16	2	2.4	nd	3.1	nd	1.3
17	0.3	5.5	nd	5.8	nd	1.1
18	0.1	1.2	nd	1.7	nd	1.4
19	0.6	0.8	nd	3.3	nd	4.1
20	2.3	8.9	nd	35.9	nd	4.0
21	2.6	2.5	nd	13.3	nd	5.3

nd: not detected.

Discussion

There is a large amount of lipid in bone; 60–70% of the weight of the femoral head is lipid (Moreau et al. 2000). The oxidative deterioration of lipids involves auto-oxidative reactions of unsaturated fatty acids, accompanied by various secondary reactions (Kehrer 1993). Lipids in biological systems can undergo oxidation through two differ-

ent pathways: hydrolytic rancidity or oxidative rancidity caused by oxygen. In allograft bone, both of these chemical processes take place. Once oxidized, the by-products of lipids and oxidated lipids can interact with proteins and carbohydrates to cause changes in biochemical stability (Frankel 1993, Goodridge et al. 2003). Hexanal is a volatile oxidation product originating from linoleic acid. Linoleic acid occurs in all fats and oils. In food samples, it is well established that hexanal correlates well with other assays that measure the state of oxidation, and also with sensory evaluation of rancidity in food products (Goodridge et al. 2003).

We found increased oxidation of bone allografts at –30°C, which is quite near the European recommendation of –40°C (EATB and EAMST 1997). Although there have been no clinical reports of disadvantageous effects of grafts stored at –40°C, our findings are still alarming because we know that the temperature in the freezer can easily rise during loading and unloading.

As expected, the speed of oxidation was higher at higher temperature, but oxidation took place at the lower temperature also. The concentrations at the lower temperature were, however, near the detection limit. In bone cells, oxidized lipids may inhibit the differentiation and activity of osteogenic cells, and also induce bone resorption (Parhami et al. 1997). Oxidated lipids have also been reported to induce cell apoptosis and inhibited osteogenesis, but the exact cellular mechanism is not fully understood (Frye et al. 1992, Parhami et al. 1997, Moreau et al. 2000). The real actions of the by-products of oxidation in allograft bone still require to be examined, but they may lower the quality of bone allografts by inducing bone resorption and depressing new bone formation. Could this phenomenon explain part of the problems we so often experience with massive allografts?

The lower the temperature, the slower is the rate of lipid oxidation also. But the speed and extent of oxidation at each temperature can only be approximated, based our results. Our findings show clearly that –30ºC should not be used even for short-term storage, and all temperatures close to –30°C can be considered to involve risk of a high oxidation rate, as some oxidation was also seen at the lower temperature of –70°C. The degree of oxidation was low at 1 and 3 years at –70°C. This temperature can be recommended for storage of fresh-frozen bone allografts for at least 3 years. Allografts stored at –40°C have been used without reports of complications caused by oxidation. However, the higher the temperature, the more oxidation we will most likely detect. Thus, we do not recommend temperatures higher than –70°C.

Although the exact role of lipid and protein oxidation in fresh-frozen allografts remains unclear, the amount of oxidation at –30°C is so high that allografts stored for one year at this temperature should be regarded as spoiled—at least according to the criteria used in the food industry (EC No 852/2004). Even lower temperatures may involve a risk of allograft deterioration if there are long storage times. Extraction of lipid before storage may be one solution to avoid oxygenation. In experimental studies, this has been shown to enhance bank bone incorporation (Aspenberg and Thoren 1990). However, the controversy remains because there have also been other studies in which chemical processing has been shown to affect incorporation, and especially the structural properties of the graft (Boyce et al. 1999).

Contributions of authors

ML concept of the study, data handling, discussion, writing. RK measurements, data handling, discussion. MH concept of the study, supervision, discussion.

No competing interests declared.

Aspenberg P. Bank bone, infections and HIV. Acta Orthop Scand 1998; 69: 557-8.

Aspenberg P, Thoren K. Lipid extraction enhances bank bone incorporation. An experiment in rabbits. Acta Orthop Scand 1990; 61: 546-8.

Belitz H D, Grosch W. Food Chemistry, sec. ed. Springer-Verlag, Berlin 1999.

EATB (European Association of Tissue Banks) and EAMST (European Association of Musculo Skeletal Transplantation) Common Standards For Musculo Skeletal Tissue Banking, Vienna 1997.

Boyce T, Edwards J, Scarborough N. Allograft bone. The influence of processing on safety and performance. Orthop Clin North Am 1999; 30: 571-81.

EC No 852/2004. Regualtion (EC) No 842/2004 of the European Parliament and of the Council on the hygiene of foodstuff.

Frankel E N. In search for better methods to evaluate natural antioxidants and oxidative stability in food lipids. Trenads Food Sci Technol 1993; 4: 220-442.

Frye M A, Melton L J, Bryant L A, Fitzpatrick H W, Wahner R S, Schwartz B L. Osteoporosis and calcification of the aorta. Bone Miner 1992; 19 (2): 185-94.

Girotti A W. Lipid hydroperoxide generation turnover effector action in biological system. J Lipid Res 1998; 39 (8): 1529-42.

Goodridge C F, Beaudry R M, Pestka J J, Smith D M. Solid phase microextraction-gas chromatography for quatifying headspace hexanal above freeze-dried chicken myofibrils. J Agric Food Chem 2003: 51 (15); 4185-90.

Hirn M Y J, Salmela P M, Vuento R E. High-pressure saline washing of allografts reduces bacterial contamination. Acta Orthop Scand 2001; 72 (1): 83-5.

Hogg N, Kalyanaraman B. Nitric oxid and lipid peroxidation. Biochem Biophys Acta 1999; 1411 (2-3): 378-84.

Jackson S M, Demer L L. Peroxisome proliferators-activated receptor activators modulate the osteoblast maturation of MC3T3-E1 preosteoblasts. FEBS 2000; 471 (1): 119-24.

Kehrer J P. Free radicals as mediators of tissue injury and disease. Crit Rev Toxicol 1993; 23 (1): 21-48.

Moreau M F, Gallois Y, Basle M F, Chappard D. Gamma irradiation of human bone allografts alters medullary lipids and release toxic compounds for osteoblast-like cells. Biomaterials 2000; 2 (4) 1: 369-76.

Parhami F, Morrow A D, Balucan J, Leitinger N, Watson A D, Tintut Y, Berliner J A, Demer L L. Lipid oxidation products have opposite effects on calcifying vascular cell and bone vell differentiation. Arterioscler Thromb Vasc Biol 1997; 17 (4): 680-7.

Parhami F, Jackson S M, Tintut Y, Le V, Balucan J P, Territo M, Demer L L. Atherogenic diet and minimally oxidized low density lipoprotein inhibit osteogenic and promote adipogenic differentiation of marrow stromal cells. J Bone Miner Res 1999; 14 (12): 2067-78.