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Brief Communication

Effects of the freezing and thawing process on biomechanical properties of the human skull

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ABSTRACT

The aim of this study was to determine if biomechanical investigations of skull samples are reliable after skulls have been subjected to a freezing and thawing process. The skulls were obtained from 105 Japanese cadavers (66 males, 39 females) of known age that were autopsied in our department between October 2012 and June 2013. We obtained bone specimens from eight sites (four bilaterally symmetrical pairs) of each skull and measured the mass of each specimen. They were then classified into three groups (A, B, C) based on the duration of freezing of the experimental samples. The left-side samples were subjected to frozen storage (experimental group). The corresponding right-side samples were their controls. Bending tests were performed on the controls immediately after they were obtained. The experimental samples were preserved by refrigeration at −20 °C for 1 day (group A), 1 month (group B), or 3 months (group C). Following refrigeration, these samples were placed at 37 °C to thaw for 1 h and then were subjected to bending tests using a three-point-bending apparatus attached to a Handy force gauge. The device recorded the fracture load automatically when the specimen fractured. Statistical analyses revealed that there were no significant differences in sample fracture loads between the frozen preserved/thawed samples and the unfrozen controls for each of the cryopreservation intervals. We eliminated any possible sample mass bias by using controls from the same skull in each case. The results suggest that the freezing/thawing process has little effect on the mechanical properties of human skulls. Thus, frozen storage for up to 3 months is a good method for preserving human skulls.

1. Introduction

For forensic analysis of cases involving a fractured skull, it is important to estimate the applied external force. This is because skull fracture is an independent risk factor for intracranial life-threatening complications such as epidural hematoma, subdural hematoma, and cerebral hemorrhagic contusion [1]. It is also vital to have a sound understanding of the biomechanical properties of the skull so that accurate estimates can be provided. Previous engineering studies have assessed the mechanical properties of human cranial bones using a variety of methods, including compression, tension, and bending tests [2–10]. During the last four decades, biomechanical studies have been conducted in forensic research as well [11]. The importance of such research requires that the skull be preserved in the best possible manner to avoid adding complications to the already difficult determinations.

Some researchers have reported that for human long bones the freezing and thawing process does not significantly alter mechanical properties [12–18]. Hence, fresh human cadaver bones are usually preserved by refrigeration at −20 °C [12]. However, the effects of the freezing and thawing process on mechanical properties of human skull have not been previously studied. The aim of this study was to determine if biomechanical investigations are reliable after freezing and thawing skull samples.

2. Materials and methods

2.1. Subjects

The skulls used in this research were obtained from 105 Japanese cadavers of known age and sex that were autopsied at the Department of Legal Medicine, Chiba University, between October 2012 and June 2013. The estimated postmortem interval of each body was within 7 days. Cases were excluded if the history highlighted conditions or events that could have affected the skull.

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For example, skulls with a fracture, burns, obvious injury, or acquired or congenital abnormality were excluded from this study. The ethics committee of Chiba University approved the study. The skull samples were classified into three groups (A, B, C) as presented in Table 1.

2.2. Sampling

During autopsy, bone specimens were obtained from eight cranial sites (four bilaterally symmetrical pairs) from each cadaver skull (left frontal, right frontal, left medial parietal, right medial parietal, left superolateral parietal, right superolateral parietal, left inferolateral parietal, and right inferolateral parietal bones). Fig 1 shows the orientations of samples extracted from each of the cadaver skulls. The length of each specimen was fixed at 50 mm and the width at 10 mm. Left and right frontal specimens were tangential to the left and right side of the coronal suture and along the anterior extended line of the sagittal suture, respectively. Left and right medial parietal specimens were tangential to the left and right side of the coronal suture and along the sagittal suture, respectively. Left and right superolateral and inferolateral parietal specimens were obtained from the lateral portion of the parietal bone immediately superior and inferior to the left and right superior temporal line, respectively. Samples were obtained using an oscillating saw and washed with saline.

The mass (grams) of each sample was measured using an electronic scale that measured from 0.1 g to 600 g. A total of 840 samples (420 left-side and 420 right-side samples) were collected. Each of the three groups (A, B, C) comprised 280 samples (140 left-side and 140 right-side samples). The left-side (experimental) samples were subjected to frozen storage. The corresponding right-side samples were their controls in each case.

The biomechanical test was performed on the control samples immediately after they were obtained. In contrast, the experimental samples were preserved in a biomedical freezer (MDF-U443; Sanyo, Osaka, Japan) with automatic defrosting function at −20 °C for 1 day (group A), 1 month (group B), or 3 months (group C). Following refrigeration, samples were placed at 37 °C to thaw for 1 h and then underwent biomechanical testing.

2.3. Biomechanical tests

Bending tests, which have been used to determine the mechanical properties of various bones including human skull in previous studies [5,19,20], were performed on the skull samples using a three-point-bending apparatus (JSV-H1000; JISC, Nara, Japan) attached to a Handy force gauge (HF-100; JISC) as shown in Fig. 2.

Table 1
Descriptive factors for the skulls in groups A, B, and C.

Factor	Group A	Group B	Group C
Number of male skulls	22	22	22
Number of female skulls	13	13	13
Age range (years)	19–95	28–89	24–89
Mean ± SD age (years)	60.7 ± 20.5	66.5 ± 17.0	63.5 ± 17.0
Frozen interval of left-side samples	1 day	1 month	3 months
Number of all left-side samples	140	140	140
Left frontal	35	35	35
Left parietal	35	35	35
Left medial temporal	35	35	35
Left lateral temporal	35	35	35
Number of all right-side samples	140	140	140
Right frontal	35	35	35
Right parietal	35	35	35
Right medial temporal	35	35	35
Right lateral temporal	35	35	35

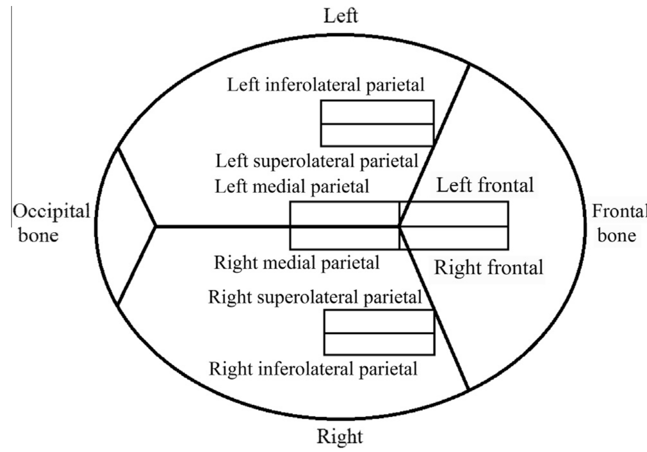

Fig. 1. Orientations of samples extracted from the cadavers skulls.

Two lower supports were set 40 mm apart, and the specimen being tested was placed on the two supports. Above this setup was a 1000-N load cell that applied pressure at the center of the specimen from the outer surface at a constant speed of 100 μm/s. When the specimen fractured, the device automatically recorded the fracture load (N).

2.4. Statistical analysis

The fracture load-to-sample mass ratio (FL/SM), in Newtons per gram, was determined by dividing the fracture load by the sample mass. Mann–Whitney U-tests were used to compare the fracture load, mass, and FL/SM between the experimental and control samples by site in each group. Differences of $P < 0.05$ were considered statistically significant. All statistical analyses were performed on a personal computer using Statistical Package for the Social Sciences (SPSS) version 21.0 software (IBM, Armonk, NY, USA).

3. Results and discussion

Table 2 shows the fracture load, mass, and FL/SM of the experimental (left side) and control (right side) samples by site in groups A, B, and C, respectively. The main finding of the present study was that there was no significant difference in sample fracture loads between the frozen-preserved experimental samples

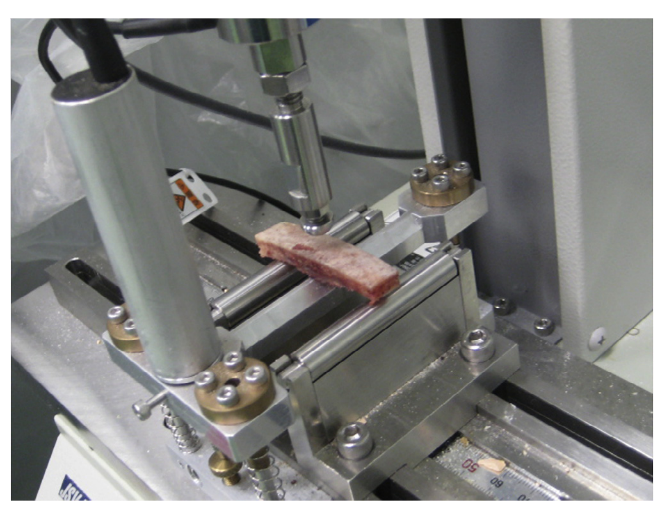

Fig. 2. Three-point-bending apparatus.