

THE INFLUENCE OF FREEZING ON THE MAXIMUM TORQUE CAPACITY OF LONG BONES

An Experimental Study on Dogs

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The strength of bone has often been measured in previously frozen samples and the freezing and thawing effect on the strength properties has been considered negligible. By means of a specially constructed torsion machine and a new method for fixing the test bone into the machine, previously frozen bones have been compared to fresh controls. The previously frozen samples were significantly weaker, with an average difference of - 4.6 per cent.

Key words: freezing; thawing; maximum torque capacity; bone; experimental measurement of bone strength

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In order to determine accurately the physical properties of long bones, preparation and storage effects must be kept to a minimum. Many investigators (Braden et al. 1973, Burstein et al. 1972, Engelbrecht 1970) have used previously frozen samples of bones, assuming any effect on the physical properties to be negligible. For a closer study of this assumption, we have used a new measuring apparatus to compare the maximum torque capacity of fresh bones with that of bones thawed after 48 hours of freezing.

MATERIAL

The test material consisted of 10 femora and 10 tibiae of five adult healthy male dogs (Swedish harriers, weight 14-16 kg). From each pair of bones from the same dog, one was selected at random for freezing and the other was tested fresh immediately after sacrifice.

Preparation, freezing and thawing procedure

All soft tissues were removed from the test bones after sacrifice of the dogs, leaving the periosteum intact. The samples to be frozen were wrapped in gauze saturated with physiological saline solution and put into plastic bags. The preparations were immediately placed in a freezer with a constant temperature of - 40° C.

Forty-eight hours later, the bones in their bags were removed from the freezer and kept in air, at + 37° C. After 5 hours the bones had a temperature of about + 35° C.

Measurement of the maximum torque capacity and maximum angle of torsion

The thawed bones were tested in torsion at about + 35° C. Within 10 minutes after sacrifice, the maximum torque capacity and the maximum angle of torsion of the contralateral fresh bone were measured. All bones were kept humid until the tests were completed. During testing the maximum torque capacity, all bones in this investigation were subjected to medial rotation.

The testing machine. In a previous paper we have described a specially constructed apparatus

for recording the torque of long bones as a function of torsion angle (Strömberg & Dalén 1976).

Sources of error. In a previous paper (Strömberg & Dalén 1976), we have calculated the experimental error (1. S.D.), including the error due to the difference between paired bones of the same dog, expressed as a percentage of the mean value of the maximum torque capacity of the paired bones, as 3.1 per cent. The error was determined by the comparative measurement of maximum torque capacity in paired bones from the same individual.

Maximum torque capacity is probably dependent upon the direction of the torque. All bones in this investigation were therefore subjected to medial rotation.

RESULTS

Compared with the fresh contralateral samples, the bones that had been frozen had a significantly ($P < 0.05$) reduced maximum torque capacity (Table 1) and a significantly ($P < 0.05$) reduced maximum angle of torsion (Table 2). In all the bones tested, a spiral fracture occurred at the diaphysis without any intermediary butterfly fragment being observed.

Table 1. Maximum torque capacity of the femora and tibiae of five dogs. One of each pair of bones was frozen, and the deviation for each frozen sample is expressed as a percentage of the value for the respective control. All the bones that had been frozen were weaker than the corresponding controls (mean = -4.6 per cent; S.D. = 2.3 per cent). Paired observation test: $t = 5.8$; $P < 0.05$.

Dog no.	Bone	Maximum torque capacity (Nm)		Percentage deviation
		fresh	defrosted	
1	femur	34.8	33.5	- 3.7
1	tibia	23.2	22.6	- 2.6
2	femur	30.9	30.1	- 2.6
2	tibia	22.1	21.1	- 4.5
3	femur	36.4	35.0	- 3.8
3	tibia	25.2	24.4	- 3.2
4	femur	27.7	25.0	- 9.7
4	tibia	19.7	18.2	- 7.6
5	femur	20.0	18.9	- 5.5
5	tibia	16.3	15.8	- 3.1

Table 2. Maximal angle of torsion for the femora and tibiae of five dogs. One of each pair of bones was frozen, and the deviation for each frozen sample is expressed as a percentage of the value for the corresponding control. Most of the bones that had been frozen had a smaller angle of torsion than the control (mean = -6.7 per cent; S.D. = 7.5 per cent). Paired observation test: $t = 2.8$; $P < 0.05$.

Dog no.	Bone	Maximum angle of torsion		Percentage deviation
		fresh	defrosted	
1	femur	36.0°	33.4°	- 7.2
1	tibia	45.8°	40.1°	- 12.4
2	femur	31.2°	30.0°	- 3.8
2	tibia	38.2°	37.9°	- 0.8
3	femur	43.4°	36.2°	- 16.6
3	tibia	39.8°	41.8°	+ 5.0
4	femur	39.6°	36.7°	- 7.3
4	tibia	35.5°	36.0°	+ 1.4
5	femur	36.5°	30.0°	- 17.8
5	tibia	41.3°	37.9°	- 8.2

DISCUSSION

Changes in humidity and postmortem changes of the organic compound in bones have a significant influence on maximum torque capacity (Sedlin & Hirsch 1966). Most investigators (Braden et al. 1973, Burstein et al. 1972, Engelbrecht 1970), in determining bone strength, have recognized these facts, but have considered the effects of freezing to be of negligible importance.

It is known that freezing and thawing have a destructive effect on the microscopic structures of tissues (Mazur 1970). This effect is caused by the formation of ice crystals. Prompt freezing in fluid nitrogen at about -195° C largely prevents the formation of ice crystals. The temperature of the preparation can be raised to about -30° C without any noticeable crystallizing occurring, but destructive crystallization will start on further warming.

Since freezing affects tissue structures in a very prominent way, it might be assumed that the mechanical properties

are also affected. Sedlin & Hirsch (1966), however, found a tendency towards increased strength in bones that had been submitted to freezing, as compared with fresh bones, although this difference was not significant. Engelbrecht (1970) found no influence on maximum torque capacity or maximum angle of torsion. We, on the contrary, have found that the maximum torque capacity and maximum angle of torsion of bones which have been frozen decrease significantly.

This discrepancy between our results and those of Sedlin & Hirsch, as well as those of Engelbrecht, is probably due to the fact that we used a more sensitive measuring procedure.

Our results indicate that the mechanical properties of previously frozen bones are significantly different from those of fresh bones. No conclusions regarding the maximum torque capacity of bones in the living body should therefore be drawn from measurements on bones that

have been frozen, without due consideration to possible errors incurred by the freezing and thawing processes.

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