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ANALYSIS OF MECHANICAL SYMMETRY IN RABBIT LONG BONES

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It is common practice to use the unaltered member on the opposite side as a control in experimental designs using quadripeds. Implicit in such an approach in biomechanical studies is the assumption that in the same animal a pair of corresponding right and left bones have similar mechanical properties. There is not a good deal of experimental evidence, however, which either supports or denies this assumption. Bending tests performed by Mather (1967) on twenty-eight pairs of human femurs are probably the only experiments designed for the study of symmetry with respect to the mechanical properties of bone. His results support the above assumption. However, a series of experiments performed on human as well as animal limbs by Singh (1971), Chhibber & Singh (1971), and Dogra & Singh (1971) show that there is statistically significant asymmetry or one-sided dominance in the bone and muscle weight of paired limbs. This suggests that there may also be asymmetry of the mechanical properties of long bones. To resolve the above question satisfactorily, it was decided to perform experiments to determine the relative mechanical properties of paired rabbit limb bones.

This study was also conceived as a preliminary to gain some fundamental information essential for the designing of well structured, efficient biomechanical experiments. In this vein, we posed the question as to whether or not the use of a paired experimental design could be expected to yield statistically significant information using less animals than would be required to get the same significance using an unpaired

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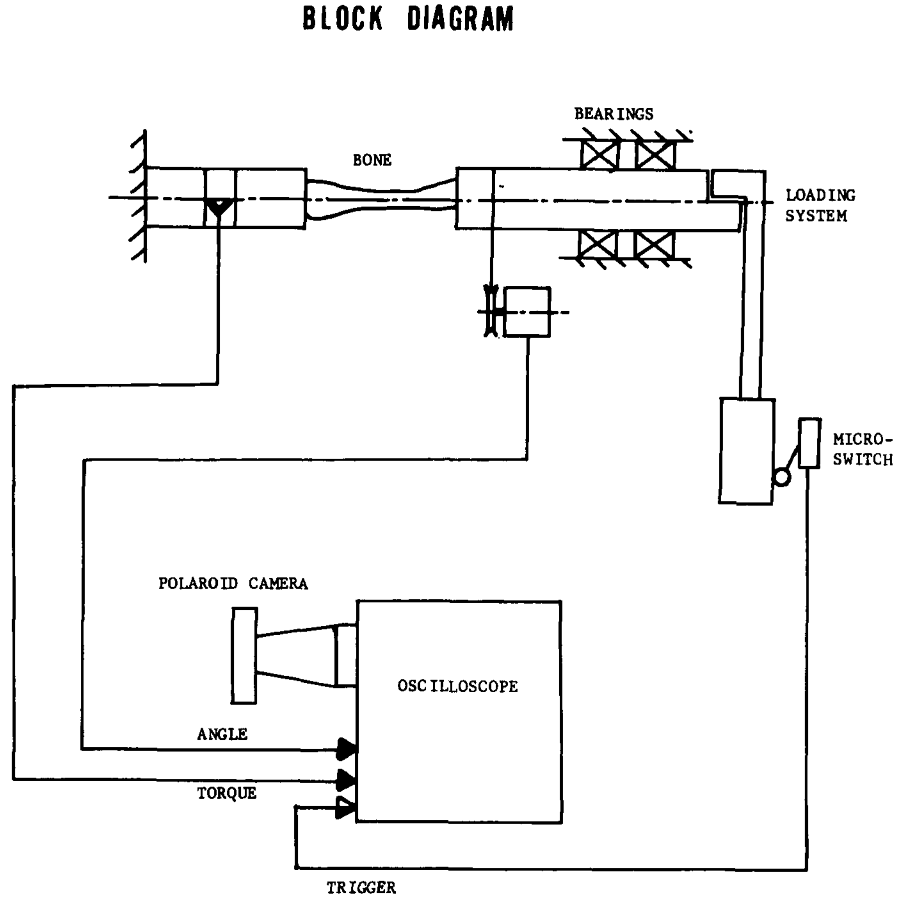

Figure 1. Block diagram of the experimental set-up. For description, see the text.

design. The answer has considerable practical importance in terms of time and money.

METHOD

It was decided to employ torsional loading. This was chosen because a constant load is applied to all sections of the bone, irrespective of bone length or dimensions. Bones are not symmetrical; the left is a mirror image of the right. Thus, two combinations of torsional loading are possible for a pair of bones. Both bones can either be loaded in external or internal rotation, or one bone may be loaded in external and the other in internal rotation. Two separate experiments were set up to test the two combinations. A constant fast rate of torsional deformation was used to simulate a fracture situation.

Limbs of thirty-four female adult New Zealand rabbits were collected within one hour of death. Soft tissue was removed, and the bones were labeled, paired, and deep frozen in plastic bags at -20° C. It has been established that freezing has no effect on the mechanical properties of bones (Evans 1957, Sedlin & Hirsch 1966). The bone to be tested was wrapped in a paper towel which had been soaked with Ringer's-Lactate solution. Its ends were molded in epoxy resin that has a curing time of ten to fifteen minutes (Plastic Padding, Göteborg, Sweden, Hirsch 1964).

The apparatus used for testing the bones consisted of a torsion testing machine and a dual beam oscilloscope. The testing machine is made by A. H. Burstein, Shaker Heights, Ohio, and the oscilloscope is Tektronix Type 561 B. A block diagram of the set-up is shown in Figure 1. One end of the bone was held rigidly while the other end was held in a rotatable fixture. The load was applied through a falling pendulum which engaged the rotatable fixture and transmitted the impact to the bone as it neared the bottom position. Two transducers were provided to measure the torque and torsional deformation of the bone. A micro-switch, activated by the falling pendulum, triggered the oscilloscope sweep just before loading of the bone. Both the torque and the angle were produced on the vertical axis of the oscilloscope screen. Time was produced on the horizontal axis. The recording was done with a Polaroid camera. An example of the record is shown in Figure 2.

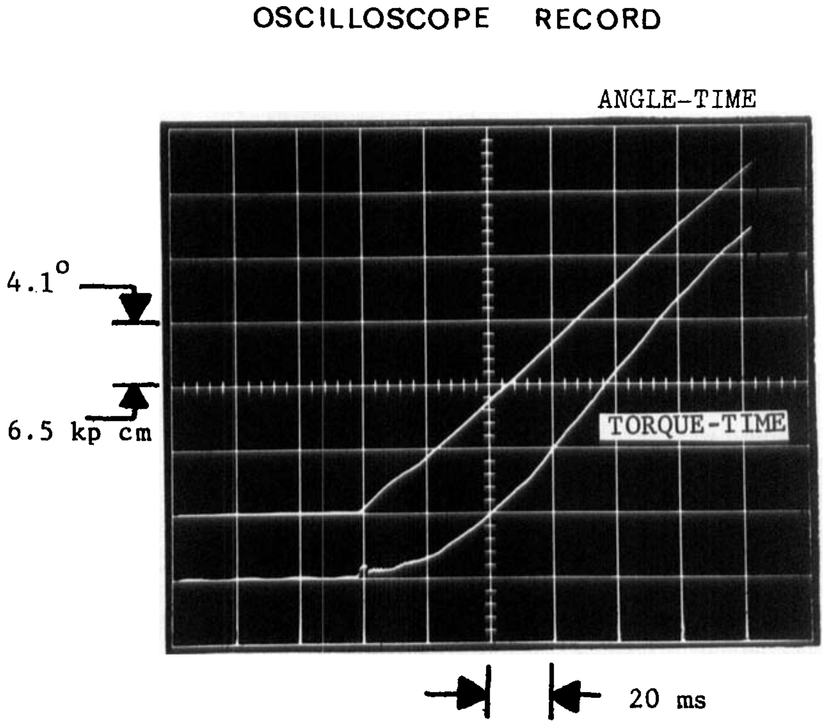

Figure 2. Oscilloscope record showing variation of torsional deformation and torque with time up to the point of fracture.

For Experiment 1, all the bones were tested in external rotation by dropping the pendulum clockwise for the left bones and counter-clockwise for the right bones. Experiment 2 required testing the right bones in internal rotation while their paired counterparts were tested in external rotation. This was accomplished by dropping the pendulum in the clockwise direction for all the bones. For both of the experiments, constant rate of torsional deformation of 3.3 rad/s (190°/s) was used.

From the photographic record of each bone, five quantities were calculated: maximum torque, torsional deformation, energy absorbed to fracture, torsional stiffness, and rate of torsional deformation of the bone. The traditional method of recording torque versus torsional deformation or angle gives directly the energy to fracture but gives no information regarding the rate of torsional deformation. The present method, i.e., torque and angle versus time, gives both. While the rate of torsional deformation is given by the slope of the angle-time curve, the energy to fracture is obtained by

$$\begin{aligned}\text{Energy} &= \int T\, d\theta \\ &= \frac{d\theta}{dt}\int T dt \qquad (1)\end{aligned}$$

where T is the torque, θ is the torsional deformation or angle, and t is the time. Thus, energy equals the area under the torque-time curve multiplied by the rate of torsional deformation. The above formula is valid under the assumption that the rate of torsional deformation $d\theta/dt$ is constant. This is true in our study, as seen in Figure 2. Finally, torsional stiffness is measured on the major portion of the torque-time curve and is given by

$$\begin{aligned}\text{Torsional Stiffness} &= dT/d\theta \\ &= (dT/dt)/(d\theta/dt) \qquad (2)\end{aligned}$$

Thus, torsional stiffness of the bone is given by the slope of the torque-time curve divided by the slope of the angle-time curve.

RESULTS

Experiment 1

Rabbit humeri were used for this study. Of the thirty-four pairs tested, twenty-nine pairs are reported here. The rest were discarded due to technical difficulties at some point in the testing procedure. Right and left bones were tested in external rotation. Variables studied were the same as mentioned earlier. Experimental data for all pairs of bones are given in Table 1.

Means and standard deviations of these variables for the right and left bones are shown in Table 2. Also shown are the means and standard deviation of the 'difference' given by the formula:

Table 1. Data of Experiment 1. Twenty-nine pairs of rabbit humeri were tested. The bones were loaded in external rotation. Constant rate of torsional deformation 3.3 rad/s (190°/s) was used.

Bone	Torque kp cm		Angle degrees		Energy kp cm		Stiffness kp cm/rad	
	right	left	right	left	right	left	right	left
5	26.2	53.7	18.1	18.1	2.80	10.75	127.7	167.0
8	34.1	26.2	20.6	8.6	4.72	2.38	137.5	157.2
10	40.6	34.1	27.2	16.5	6.94	3.86	127.7	127.7
11	36.7	24.9	19.4	9.9	4.52	2.01	137.5	147.3
13	12.4	20.9	14.8	13.1	0.76	2.11	117.9	117.9
14	30.8	30.8	14.4	10.7	3.05	3.21	196.5	167.0
16	19.6	5.2	15.2	10.3	1.78	0.63	121.1	58.9
17	22.9	22.3	20.6	12.4	2.41	2.11	108.0	137.5
18	24.9	3.9	16.4	14.8	2.30	0.63	124.4	12.7
19	35.4	32.1	22.6	14.4	6.14	4.18	108.0	127.7
20	24.9	19.6	11.9	7.4	2.70	1.35	137.5	147.3
21	29.5	27.5	22.6	16.9	3.93	3.33	117.9	108.0
22	41.9	38.6	21.4	15.6	7.85	5.71	127.7	137.5
23	7.8	28.2	9.9	13.6	0.53	3.53	98.2	124.4
24	24.9	33.4	16.5	20.2	2.24	5.54	117.9	124.4
25	39.3	35.4	16.5	15.2	5.52	5.32	147.3	147.3
26	36.7	36.0	20.6	18.9	5.42	4.52	137.5	147.3
27	27.5	27.5	14.4	14.0	3.40	3.33	98.2	124.4
28	26.2	26.2	19.0	14.4	4.18	3.70	81.8	98.2
29	22.9	22.9	13.1	8.2	2.01	1.91	153.9	153.9
31	28.8	27.5	19.0	19.3	4.26	3.53	98.2	108.0
33	28.8	24.9	16.1	11.5	3.33	2.58	124.4	117.9
34	34.1	22.9	18.1	11.5	5.18	2.21	117.9	117.9
35	30.8	36.7	15.4	16.9	4.35	5.40	117.9	124.4
36	6.5	7.8	2.0	5.3	0.19	0.36	186.6	85.1
37	34.7	32.8	16.1	11.5	4.98	3.60	117.9	157.2
38	22.3	23.6	16.5	13.2	2.69	2.77	104.8	104.8
39	17.6	22.9	10.7	9.0	1.98	2.11	140.8	127.7
40	28.2	23.6	9.4	9.0	2.44	1.65	167.0	167.0

$$\text{Difference } (X) = \text{Right } (X) - \text{Left } (X) \qquad (3)$$

where X stands for the variable under consideration. The last column contains the probability as calculated by the Student's t test.

Experiment 2

Rabbit tibiofibulae were used for this study. Out of the thirty-four pairs tested, twenty-two were available for complete analysis because

Table 2. Computed results of Experiment 1.

Variable	Right		Left		Difference			Relative efficiency
	Mean	S.D.	Mean	S.D.	Mean	S.D.	P (*t* test)	
Torque kp cm	27.5	8.75	26.6	9.87	.9	9.26	0.6	0.51
Angle degrees	16.5	4.81	13.1	3.74	3.4	4.03	0.01	0.58
Energy kp cm	3.54	1.83	3.25	2.02	.29	2.14	0.4	0.39
Stiffness kp cm/rad	127.6	24.9	125.7	32.7	1.9	34.6	0.7	0.30

Table 3. Data of Experiment 2. Twenty-two pairs of rabbit tibiofibulae were tested. Right bones were tested in internal rotation and left bones in external rotation. Constant rate of torsional deformation 3.3 rad/s (190°/s) was used.

Bone	Torque kp cm		Angle degrees		Stiffness kp cm/rad		Energy kp cm	
	right	left	right	left	right	left	right	left
8	30.8	23.6	13.4	9.9	3.53	2.02	68.7	65.5
10	26.4	20.5	21.8	10.7	3.70	2.00	94.9	101.5
12	12.5	23.6	7.4	14.7	0.79	2.60	49.1	52.4
13	6.5	14.4	3.3	7.8	0.26	0.99	49.1	55.6
14	31.8	33.7	21.4	17.3	6.10	5.70	81.8	108.0
16	24.2	21.0	11.9	10.3	2.60	1.94	58.9	55.6
17	15.1	11.8	7.8	7.0	1.18	0.80	52.4	58.9
22	20.6	17.2	12.0	10.0	2.18	1.40	52.4	45.8
23	18.7	14.0	7.9	6.1	1.18	0.76	72.0	58.9
24	27.8	17.1	12.8	7.4	3.36	1.18	65.5	65.5
25	19.4	25.6	7.5	9.1	1.18	2.19	68.7	78.6
26	19.7	15.1	11.9	7.8	1.98	1.06	49.1	55.6
27	23.6	24.9	9.9	11.1	2.28	2.54	65.5	65.5
29	17.1	19.7	9.5	11.6	1.60	1.79	52.4	52.4
30	17.9	22.5	10.7	12.4	1.63	2.45	88.4	94.9
33	14.0	16.0	6.4	7.1	0.83	0.99	65.5	65.5
34	16.8	14.0	8.1	6.1	1.18	0.86	62.2	58.9
35	30.0	20.2	12.4	11.1	3.80	1.60	127.7	147.3
36	22.3	13.2	11.9	5.8	2.38	0.79	85.1	58.9
37	14.0	14.8	4.9	5.4	0.73	0.76	72.0	81.8
38	17.7	24.2	14.0	19.0	2.16	3.90	72.0	78.6
39	25.6	9.2	16.2	5.8	3.76	0.42	49.1	45.8

Table 4. Computed results on Experiment 2.

Variable	Right		Left		Difference			Relative efficiency
	Mean	S.D.	Mean	S.D.	Mean	S.D.	P (*t* test)	
Torque kp cm	20.6	6.34	18.9	5.58	1.7	6.67	0.2	0.38
Angle degrees	11.1	4.52	9.71	3.61	1.4	4.42	0.1	0.43
Energy kp cm	2.20	1.36	1.76	1.19	.44	1.27	0.1	0.51
Stiffness kp cm/rad	68.3	18.5	70.6	23.7	−2.3	10.4	0.3	0.91

of the type of losses mentioned above. Right bones were tested in internal rotation and left bones were tested in external rotation. Experimental data for the variables are given in Table 3. The computed means, the standard deviations, and probability are given in Table 4.

DISCUSSION

Results show that there is much inherent variation present in bones. The variation seems to be similar in the right and left bones so that it tends to cancel out. This is shown by the high values of probability noted in the calculation of the Student's *t* test applied to the 'differences'. Thus, the observed average difference between right bone and left bone can be attributed to normal biological variation. In other words, our hypothesis of symmetry between the paired right and left limbs of the same animal is not disproved by our data.

Results of the second experiment are similar to those of Experiment 1. All the variables have high probability for the differences present in the bones to be due to normal biological variation. Thus, there is no significant difference in the mechanical properties of the bones twisted internally or externally.

This information is crucial to experimental designs to study the effects of a particular variable on one of a pair of long bones in rabbits. Our findings have shown no evidence of a pattern of right or left dominance as regards these physical properties. This suggestion, now supported with experimental evidence, provides a sound basis for a useful experimental design. If a given variable can be shown to substantially alter mechanical properties of a group of randomly selected right or left bones, as compared with a group of their fellows from the opposite side, then this difference may be considered a reliable experimental effect of the given variable.

This study provides an additional important consideration from the standpoint of experimental design. We have shown that the variation in the physical properties of a given long bone of different animals is a good deal greater than between paired bones of the same animal. This fact has been proven statistically by the figures for relative efficiency given in Tables 2 and 4. Relative efficiency is defined as the correlation coefficient which measures the linear relationship between the right and left paired bones. The value of relative efficiency varies directly with the correlation coefficient between right and left bones. One can make use of this quality of symmetry in planning biomechanical experiments where the variable to be studied can be introduced on one long bone while the paired bone on the other side is used as a control. Our results show that relative efficiency is greater than zero. Therefore, for a given statistical significance level, lesser number of animals is required if a symmetry criterium is employed. Let us assume that 100 rabbits are required for an unpaired study for observing the effects of a certain fracture healing treatment. Further, let the criterium for healing be the maximum energy absorbing capacity of the bone. Then according to our results, for a paired study, for obtaining the same level of statistical significance, we will need $100\ (1-0.45) = 55$ rabbits. (Relative efficiency for energy for the two bones in Tables 2 and 4 is 0.39 and 0.51 respectively giving an average of 0.45). the reverse is also true, i.e., a higher level of statistical significance can be obtained from the same group of rabbits if paired experimental design is used as compared to unpaired design.

SUMMARY

Several properties of paired rabbit bones were analyzed to investigate their relative mechanical properties. The following quantities were observed: maximal torque, torsional deformation, energy absorbed to fracture, and torsional stiffness. One group of the pairs was tested by rotating each member of the pair in the same direction and another group was tested by rotating each member in the opposite direction.

In both the designs for torsional loading, there was considerable variation within the pairs. No pattern of right or left dominance emerged. Statistical analysis showed that the observed differences were

probably due to biologically normal variation. Thus, the assumption of mechanical symmetry in long bones was compatible with our observations in these animals. It has also been demonstrated that the use of a paired experimental design is more efficient than an unpaired design in terms of the numbers of animals required to achieve a given level of statistical significance. This information tends to confirm the validity of experimental designs which introduce a particular variable to one of a pair of long bones for the purpose of studying the effect on the mechanical properties of bone.

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