

## EFFECTS OF SALMON CALCITONIN ON MECHANICAL PROPERTIES OF HEALING AND INTACT BONE AND SKIN IN RATS

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Mechanical properties of healing femoral fractures, intact femora, healing skin wounds and intact skin, have been studied in young male rats during treatment with salmon calcitonin (CT) (3 MCR-U/kg body weight/day). Serum ionized calcium was reduced after each CT injection, and served as a control of the biological activity of the administered hormone.

CT did not influence mechanical properties of healing fractures, intact bones or skin wounds. Neither did it change bone growth or body weight of the treated rats. In contrast, injections of the hormone impaired mechanical properties of intact skin. Thus, the tensile strength and the ultimate elongation of skin specimens from CT treated rats were reduced by about 20 and 10 per cent respectively compared to controls after 30 days of treatment.

The results of the present study do not support the use of salmon CT in the treatment of fractures.

*Key words:* biomechanics; bone development; calcitonin; femur; fractures; growth; wound healing

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The thyroidal hormone calcitonin (CT) has been claimed to stimulate both fracture repair (Delling et al. 1970, Tarsoly & Bucher 1973, Khomullo et al. 1978) and bone formation (Wase et al. 1967, Matrajt et al. 1968) in rats. Following an experimental fracture in these animals, plasma levels of CT increased (Ekeland et al. 1981b), but it is still unknown whether the hormone is involved in the normal repair process. A substance able to promote fracture healing might have important clinical potential, and CT has lately been used in the therapy of human fractures (Kaplan et al. 1976, Rasskazov 1977). Several studies in rats have, however, failed to demonstrate any effect of CT either on fracture repair (Bright & Estep 1968, Lindgren et al. 1980, 1981) or on bone formation

(Russel et al. 1973). Similar findings have been reported in rabbits (Harris et al. 1975, Golan et al. 1976). The divergence of the results in these CT studies is probably due to differences in experimental design. Thus, while it is generally accepted that CT inhibits bone resorption (Martin et al. 1977, Talmage & Cooper 1979), further studies are required to decide whether the hormone also may enhance bone formation.

The recovery of mechanical strength is the main clinical requirement of a healing fracture. A substance able to promote fracture repair should therefore accelerate this recovery in order to have any functional significance. The purpose of the present study was to investigate whether salmon CT might increase the strength of healing

fractures and intact bones in young rats. Since collagen is the main organic component both in bone and skin, the influence of CT on the strength of healing wounds and intact skin was also studied.

## MATERIALS AND METHODS

### Experimental animals

A total of 120 male Wistar/Af/Han/Mol SPF rats were used. They were initially about 25 days old, weighing 58–64 g (median weight: 61 g). The rats were divided into two weight-matched groups of 60 animals each. One group was treated with salmon CT (Miacalcic®, Sandoz, Basel, Switzerland) 3 Medical Research Council Units (MRC-U) per kg body weight. The hormone was dissolved in 3 per cent gelatine, and injected subcutaneously once daily (at 10–11 a.m.) from the first postoperative day. The other group received corresponding injections of the vehicle. Four to five rats were kept in each cage, and they were given standard animal pellets containing 0.9 per cent calcium and 0.7 per cent phosphorus (Bl. nr. 3155, Møllecentralen i/s, Oslo, Norway) and water *ad libitum*.

### Experimental fracture and wound

With the animals under ether anaesthesia, a standardized, closed, transverse fracture was made in the left femora. The first two rats were killed immediately, and used to adjust the fracturing forceps to give fractures in the middle of the bone (Ekeland et al. 1981a). The rats walked on the non-immobilized fractured limb within a few days.

Under the same anaesthesia, a 4-cm-long incision was made in the midline along the spine in 74 of the rats (37 to be CT treated and 37 controls). The incision was made through the skin and down to the fascial layer. Each wound was closed by three interrupted sutures (3-0 Silk Skin Closure, Davis & Geck, American Cyanide Company, New York, USA). All wounds healed well without infection, and the sutures were removed after 3 days.

### Mechanical testing of bone

At 10, 20, 30 and 40 days after the fracture, 22–32 rats were weighed and anaesthetized with ether. Blood was collected anaerobically by aortic puncture. Death followed by exsanguination. The collected blood was centrifuged, and the serum analyzed for ionized calcium.

Both femora from the rats with skin wound were dissected free and stored in cooled, isotonic Ringer's solution. The length of the intact, right femora was measured with a sliding caliper (Ekeland et al. 1981a). Within 8 h, the healing fractures and the intact bones

were tested in torsion until failure, as previously described (Engesæter et al. 1978, Ekeland et al. 1981a). The strength, stiffness and deformation of the bones were defined as the *ultimate torsional moment*, the *torsional stiffness* and the *ultimate torsional angle*, respectively, and obtained from the load-deformation curves (Ekeland et al. 1981a, 1982). For healing fractures, also the torsional moment required to twist the bones 20° (0.35 rad) was read from the curves, as this torsional moment has proved to be a functional and reliable measure for the degree of fracture repair in rats (Ekeland et al. 1982).

### Mechanical testing of skin

Samples containing the healing incision of the wounded rats, and intact skin from the corresponding area of the rats without wound were removed. The samples were unfolded and distended to their original size before frozen in Petri dishes at  $-20^{\circ}\text{C}$ .

By means of a template, each sample was later cut into three standardized skin specimens. These specimens were tested in tension until failure (elongation rate 0.09 mm/mm/second), as previously described (Engesæter & Skar 1978, Wie et al. 1979).

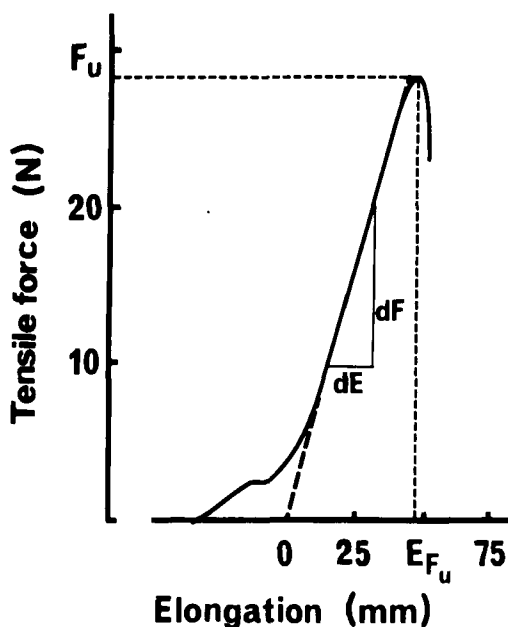


Figure 1. Load-deformation curve for an intact skin specimen of a control rat, 20 days after the start of the experiment. The ultimate tensile force ( $F_u$ ) and the ultimate elongation ( $E_{F_u}$ ) in the linear elastic and the plastic range of the skin are indicated. The tensile stiffness ( $dF/dE$ ) is determined from the slope of the linear portion of the curve. Corresponding curves were obtained for specimens of healing wounds.

Biomechanical parameters corresponding to those used in the previously described bone tests were also obtained from the load-deformation curves of skin (Figure 1). The strength was defined as the *ultimate tensile force* (maximal tensile force). The *tensile stiffness* (elastic stiffness) was measured from the slope of the linear portion of the curve (Viidik 1968, 1973, Vogel 1972). The *ultimate elongation* (ultimate deformation) in the linear elastic and the plastic range of the skin was measured as the x-coordinate of the ultimate tensile force (Figure 1).

Intact skin samples were not tested after 40 days of CT treatment due to the small number of experimental animals (4) in this group.

#### Serum analyses

The rats with skin wounds received their final CT injection 24 h before they were sacrificed, while the last injection to rats without skin wounds was given 3 h prior to sacrifice. Serum from all the rats was analyzed for ionized calcium using a Calcium Ion Selectrode® (Radiometer, Copenhagen, Denmark) in a special arrangement developed for serum analyses (Myhre 1980).

#### Statistical analyses

Median with 0.25- and 0.75-fractiles were used to express the average and the dispersion of the measured values. As three skin specimens from each rat were tested, three values for each biomechanical parameter were obtained in the same animal. Only the median of these three values was used in the further calculations.

The statistical significance probability was calculated by Wilcoxon's two-tailed test for two samples (Diem &

Lentner 1975). Differences were considered significant when  $2P \leq 0.05$ . The coefficient of variance (V) was calculated from the formula (Diem & Lentner 1975, Høyland & Walløe 1977):

$$V = \frac{(0.75 \text{ fractile} - 0.25 \text{ fractile}) \times 0.74}{\text{median}}$$

## RESULTS

### Growth of the animals

The body weight of the rats increased by about 130 per cent, and the length of intact femora by about 30 per cent from the 10th to the 40th day after the fracture. There were no significant differences between CT treated and control rats (Table 1). Only data from rats with skin wounds are shown, but the results from rats without wounds were almost identical.

### Serum analyses

Serum ionized calcium was significantly reduced at 3 h after each CT injection throughout the experiment (Figure 2). This CT effect was transient, and 24 h after the injection there were no significant differences between ionized calcium in sera from CT treated and control rats (data not shown).

Table 1. Growth of the rats with skin wound during 40 days of calcitonin (CT) treatment. No significant differences between CT treated and control rats

Days after fracture	n	Body weight (g)		Length of intact femora (mm)	
		CT treated rats	Controls	CT treated rats	Controls
10	10	110 (106–116)	118 (115–119)	23.93 (23.70–24.04)	24.11 (23.83–24.53)
20	10	170 (160–171)	168 (159–175)	27.10 (26.94–27.44)	27.44 (27.10–27.58)
30	10	226 (218–233)	223 (212–228)	29.80 (29.73–30.40)	29.88 (29.81–30.02)
40	7	264 (255–267)	257 (254–274)	31.80 (31.53–32.34)	31.65 (31.25–31.98)

n = Number of animals. Median with 0.25- and 0.75-fractiles.

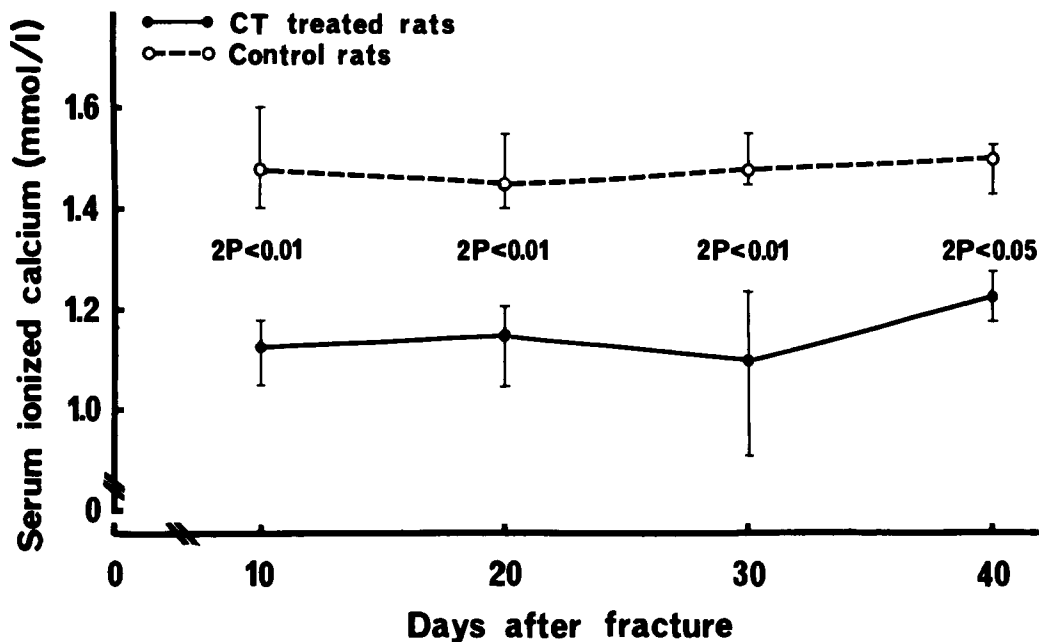


Figure 2. Serum ionized calcium in rats without skin wounds, 3 h after injection of salmon calcitonin (CT), 3 MRC-U/kg body weight. There were 4–6 animals in each group. Significant differences between CT treated and control rats are indicated by P-values. (Median with 0.25- and 0.75-fractiles).

**Bone tests**

CT did not influence the mechanical properties of healing femoral fractures (Table 2) or intact fem-

ora (Figure 3). The fractures were consolidated as evaluated manually at 20 days, and had regained the mechanical properties of the intact,

Table 2. Mechanical properties of healing femoral fractures during 40 days of calcitonin (CT) treatment. No significant differences between CT treated and control rats

Days after fracture	n	Ultimate torsional moment (10 <sup>-2</sup> Nm)		Max. torsional moment during 20° torsion (10 <sup>-2</sup> Nm)		Torsional stiffness (10 <sup>-3</sup> Nm/degrees)		Ultimate torsional angle (degrees)	
		CT treated rats	Controls	CT treated rats	Controls	CT treated rats	Controls	CT treated rats	Controls
10	10	6.1 (5.6–6.6)	5.7 (5.3–6.5)	3.0 (2.5–3.3)	3.0 (2.8–3.6)	1.5 (1.3–1.7)	1.5 (1.4–1.8)	50 (44–58)	55 (39–59)
20	10	12.6 (12.0–13.4)	12.5 (10.7–15.1)	9.4 (7.1–12.4)	8.7 (6.6–11.3)	6.2 (5.2–9.8)	6.4 (4.5–8.5)	38 (32–53)	37 (33–47)
30	10	16.5 (15.0–19.1)	17.3 (13.9–21.2)	13.3 (10.0–16.5)	14.6 (11.9–17.0)	14.8 (12.7–17.0)	14.1 (11.2–18.7)	25 (22–26)	24 (18–31)
40	5-6	21.2 (19.1–24.8)	21.7 (18.6–25.6)	21.2 (19.1–24.3)	21.7 (18.5–25.6)	22.3 (17.9–24.0)	23.5 (18.3–24.0)	16 (15–18)	17 (16–18)

n = Number of animals. Median with 0.25- and 0.75-fractiles.

Table 3. Mechanical properties of healing skin wounds during 40 days of calcitonin (CT) treatment. No significant differences between CT treated and control rats

Days after wound	n	Ultimate tensile force (N)		Tensile stiffness (N/mm)		Ultimate elongation (mm)	
		CT treated rats	Controls	CT treated rats	Controls	CT treated rats	Controls
10	10	5.4 (5.0–5.7)	5.7 (5.2–6.0)	2.2 (1.8–2.3)	2.1 (2.0–2.6)	3.4 (2.6–3.6)	3.0 (2.8–3.3)
20	10	23.5 (21.6–24.8)	21.8 (21.3–28.9)	4.7 (4.4–4.9)	4.6 (4.0–5.6)	5.0 (4.9–5.7)	5.0 (4.4–5.9)
30	10	69.1 (61.3–76.0)	65.2 (53.5–68.2)	9.4 (8.9–10.3)	8.6 (8.1–10.6)	7.5 (6.7–8.0)	7.1 (6.4–8.8)
40	5-6	90.2 (83.9–96.6)	84.3 (77.0–90.7)	10.0 (7.7–10.9)	9.7 (8.6–11.3)	10.1 (8.5–11.6)	8.1 (7.4–10.4)

n = Number of animals. Median with 0.25- and 0.75-fractiles.

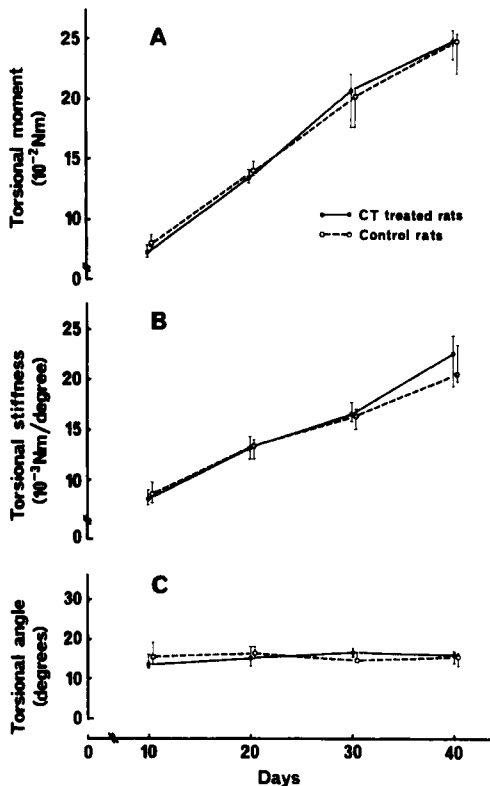


Figure 3. Ultimate torsional moment (A), torsional stiffness (B) and ultimate torsional angle (C) for intact rat femora during 40 days of calcitonin (CT) treatment. There were 7–10 animals in each group. No significant differences between CT treated and control rats. (Median with 0.25- and 0.75-fractiles).

contralateral bones 40 days after the fracture (Table 2, Figure 3).

#### Skin tests

CT treatment did not influence the mechanical properties of healing skin wounds either (Table 3). All wounds failed along the incisional line, except at 40 days, when some failed outside the line.

The strength of intact skin from CT treated animals was, however, significantly reduced compared to that from controls both after 20 and 30 days of treatment (Figure 4A). Similar but less pronounced findings were recorded for the tensile stiffness (Figure 4B) and the ultimate elongation (Figure 4C) of the skin specimens.

#### Dispersion of the data

The coefficient of variance for body weight and length of intact femora were small, being about 0.05 and 0.02 respectively. The data obtained during the mechanical tests showed a wider dispersion, as the median coefficient of variance for the strength of healing fractures and intact bones was 0.18 and 0.10 respectively. The corresponding coefficient for strength of wounds (0.11) and intact skin (0.17) were of similar magnitude.

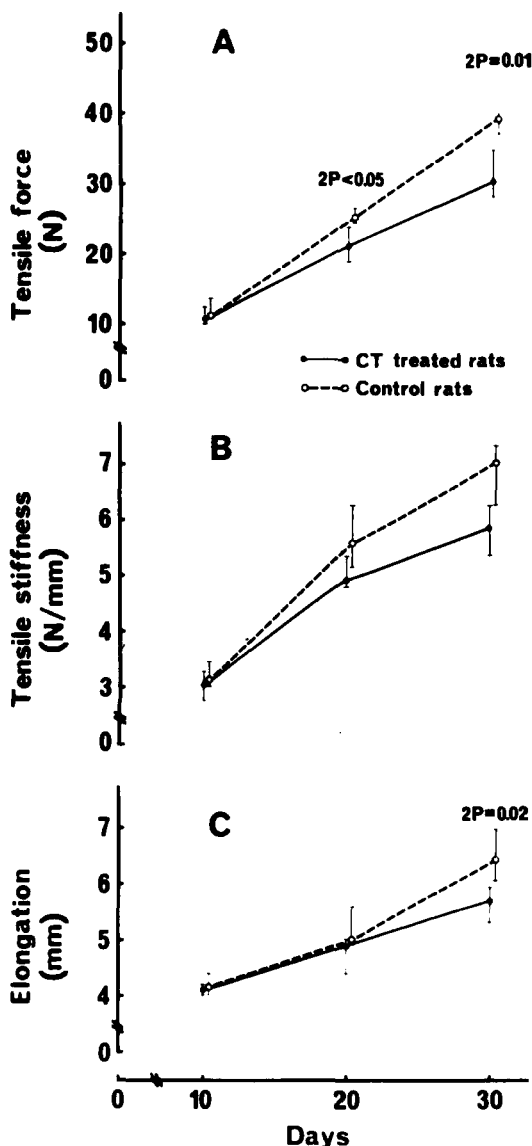


Figure 4. Ultimate tensile force (A), tensile stiffness (B) and ultimate elongation (C) for intact skin specimens of rats during 30 days of calcitonin (CT) treatment. There were 6 animals in each group. Significant differences between CT treated and control rats are indicated by P-values. (Median with 0.25- and 0.75-fractiles).

## DISCUSSION

This study revealed a negative influence of CT treatment on the mechanical properties of intact

skin from growing rats. Corresponding properties of healing fractures, intact bones and skin wounds were not significantly influenced by the hormone. These findings are probably not due to different precision of the mechanical test methods employed. Thus, the coefficient of variance of the tested strength was higher for intact skin than for intact bones and wounded skin, and similar to that of healing fractures.

Salmon CT was used in the present study as it is more stable and potent (Copp 1979), and has a longer biological half life (Martin et al. 1977) than mammalian CT. A dose comparable to the CT doses recommended in human medicine (Hamdy 1977, Pezeshki & Brooker 1977) was employed. The biological activity of this CT dosage (3MRC-U/kg body weight/day) was investigated in a preliminary study, where a subcutaneous injection of the drug in 3 per cent gelatine reduced serum ionized calcium in young, male rats for at least 8 h. In the present study, the drug was given only once daily, since continuous CT administration may reduce osteoblast population (Rasmussen & Bordier 1974), and increase the risk of inducing desensitization to the hormone (Martin et al. 1977). Serum ionized calcium is very discriminative for changes in serum levels of calcium (Bringham & Potts 1979, Myhre 1980), and the reduced concentration observed after each CT injection (Figure 2) proved that the rats were under effective CT influence several hours each day throughout the experimental period.

In agreement with our results, Bright & Estep (1968) and Lindgren et al. (1980, 1981) found no effect of CT on fracture repair in rats. In all these experiments, the mechanical strength of the healing fractures was tested. In contrast, Delling et al. (1970), Tarsoly & Bucher (1973), Rolevich et al. (1975), Kolář et al. (1976), and Khomullo et al. (1978) have reported a stimulating effect of CT on fracture repair in rats. None of these authors, however, tested the mechanical strength of the healing fractures. Since doses and species of CT and animal age varied both in the mechanical and in the non-mechanical studies cited above, the incompatible results concerning the effects of CT on fracture repair in rats do not seem to be exclusively dose dependent, related to CT species or age of the experimental animals. They are

probably also related to the method of evaluation and interpretation of the experimental data.

We did not find any effect of CT on bone growth. This is in accordance with the observations of Russel et al. (1973), but in contrast to those of Wase et al. (1967) who found a stimulating effect in similar experimental animals. Unlike our results, Lupulescu & Habowsky (1978) found salmon CT to accelerate wound repair in a morphological and autoradiographic study in rabbits. The lack of uniformity in these results illustrates again the problems discussed above.

Collagen is responsible for the tensile strength of skin (Barbenel et al. 1973, Viidik 1973, Vogel 1974). The mechanical properties of skin were therefore measured in the linear elastic and the plastic range of the load-deformation curve, as this represents the deformation of collagen (Barbenel et al. 1973, Vogel & Hilgner 1977). The negative effects of salmon CT administration on mechanical properties of intact skin suggest that the hormone influences collagen metabolism. Healing and intact bone and skin contain different types and combinations of collagen (Prockop et al. 1979). This may possibly explain why CT influenced the strength of intact skin alone.

In conclusion, administration of salmon CT to rats reduced the strength of intact skin by about 20 per cent after 30 days of treatment. No effects were observed on the strength of healing fractures, intact bones or skin wounds. CT has been proposed in the treatment of fractures (Duriez 1979). Evidence for a stimulating effect of functional significance has, however, not been presented, and the proposal is not supported by the results of the present study.

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