THE INFLUENCE OF DISTRACTION OSTEOGENESIS ON DISTANT PARTS OF THE SKELETON
HISTOMORPHOMETRIC STUDY IN THE YUCATAN MINI PIG

INTRODUCTION:
The objective of this study was to evaluate the effect of distraction osteogenesis on the remodeling of distant parts of the skeleton. Evidence suggests that bone injury has growth promoting effects on distant uninjured bones through the release of local growth factors and to a lesser extent through hormonal changes occurring during the process of bone injury and healing (1). Impairment of the bone marrow is considered to be the primary trigger (2). The influence and duration of this effect on bone remodeling remain incompletely understood. There are no studies so far regarding the effect of distraction osteogenesis on distant bone remodeling. An increase of bone cell mitogenes during distraction has been described (3). Most studies evaluate the influence on cancellous bone or serum parameters for bone turnover. This study shows the systemic effect of distraction osteogenesis on cortical bone of the none weight bearing ribs of the axial skeleton.

METHODS:
All experiments were approved by the national authority for animal rights protection. Distraction osteogenesis was performed on the left tibiae of 24 mature female yucatan mini pigs with a mean age of 77 (range 32 to 116) weeks. They were osteotomized in mid diaphysis and stabilized using an external half ring fixator. Following a 4 day latency period, distraction of 2 mm per day was performed for 10 days. After 10 days of consolidation the animals were sacrificed.

During the period of this study three different fluorochrome marker substances were applied intravenously: calcein green at day two after surgery (15 mg/kg body weight), tetracycline hydrochloride one day after the end of the distraction period (25 mg/kg body weight), and xylenol orange two days before sacrifice (90 mg/kg body weight). The application of the fluorochrome marker substances was timed according to the assumption that the peek uptake into mineralizing bone takes place one day after intravenous injection.

RESULTS:
After the sacrifice the 9th right rib was excised and immersed in buffered formaline solution. The ribs were cut with a precision saw orthogonal to the bone, and 3 specimen per rib were obtained. A 3 mm section was taken 2 cm from the costovertebral joint, another one out of the middle of the rib, and the third 2 cm from the bone cartilage border. Specimen were embedded in methylmetacrylate and ground with a precision grinding machine (Exakt GmbH, Norderstedt, Germany) to a thickness of 80 µm.

The histomorphometric examination performed in this study included the evaluation of full size osteons marked with all three fluorochrome substances. Sections were examined using a microscope at a 40x10x0.55 magnification combined with a digital video camera and a computer equipped with the KS 400 image analysis software (Kontron Elektronik GmbH, Eching, Germany). With three filters integrated in the microscope the optimal enhancement of the three fluorescent markers xylenol orange, calcein green, and tetracycline hydrochloride was achieved (I3, D, N2,1, Leica, Bensheim, Germany). Each image was then contrast enhanced. All three images obtained from one osteon were combined into one image, so that the measurement of the mineral apposition rate (MAR) could be performed. Therefore, a corona of 72 rays was generated by the program originating from the center of gravity of the osteon. The distances intersected by the fluorescent marks were measured automatically. Each mean was calculated resulting in two values representing the mineral apposition during distraction and consolidation, respectively. The length divided by the days that passed between application of the markers yielded the MAR in µm/d. Statistical analysis was performed using the paired Wilcoxon test. The level of significance was set at p < 0.05. Shown are median values and the 25/75 percentile.

LITERATURE:
1. Einhorn, T; Simon, G; Devlin, V; Warman, J; Sidhu, S; Vigorita, V. The osteogenic response to distant skeletal injury. JBJS 72A (9): 1374-1378, 1990.