INTRODUCTION
During maturation bone undergoes changes in its composition, organization and mechanical properties [1-2]. However, the contribution of the collagen framework on the properties of maturing bone is not well understood. Previous studies in mice and horses showed that the collagen network experiences significant structural modifications during development and maturation [2-3].

Our objective was to assess the biochemical and mechanical development of the collagen matrix in rabbit bone from birth to adult mature age. Mechanical properties of decalcified bone tissue were investigated by tensile mechanical testing and correlated with biochemical analysis of the collagen matrix. These structure-composition-function interactions are crucial for understanding the development of the organic matrix in bone from young age to adulthood. We hypothesized that the mechanical strength of the collagen network is highly related to its chemical composition, including its enzymatic and non-enzymatic cross-links.

METHODS
Experimental conditions: Decalcified left tibiae from female New Zealand white rabbits of different ages were used. The rabbits were sacrificed at following ages and experimental time points; at birth (NB), and at 1, 3, 6, 9 and 18 months of age (n=14 per group). The weight and the bone length of rabbits were recorded. The study was approved by the local animal care and use committee.

Tensile testing: The left tibia was decalcified in 0.5M EDTA for 14 days, and thereafter stored in PBS at -20°C until tensile testing (Newport material testing device). The bone was centered between two clamps to ensure that the breaking point occurred at the mid-diaphysis of the bone. Due to the large variation in bone size between experimental groups, the distance between the clamps was adapted so that the anatomical position was identical during the tests. Time, force, and actuator displacement were recorded during the test and the maximal force, strain at failure, strain at failure, tensile stiffness, absorbed energy and Young’s modulus were calculated. The bone was assumed ellipsoidal, and the cross-sectional area of the breaking point was calculated using the mean semi-minor and semi-major axis distances from both breaking ends.

Biochemical analysis: High-pressure liquid chromatography (HPLC) was used for assessment of collagen content and cross-links. Mid-diaphyseal samples were cut after the tensile testing. Tissue collagen per wet weight (Col), as well as hydroxypyrolidine (HP), hydroxylysylpyridinoline (LP) and pentosidine (PEN) cross-linking were measured using reversed phase HPLC analysis [4].

Statistics: Mann-Whitney U-test was used to study differences in measured parameters between the consecutive age groups. Pearson’s correlation test was used to test associations between the mechanical and biochemical parameters using SPSS 16 (SPSS Inc., Chicago, IL).

RESULTS
The weight of the animal and the length of the tibiae increased similarly and significantly between each age group until 6 months (p<0.001), and was thereafter constant.

Tensile testing: Parameters related to the mechanical strength of the collagen network revealed significant changes during maturation of the animals (Fig 1). Maximum force, stored energy and Young’s modulus increased most significantly up to 3 months of age (p<0.001). Maximum force and energy also increased between 3 to 6 months of age (p<0.05), and thereafter decreased until 18 months of age. The maximal strain was the highest in the newborn bone. It decreased rapidly until 3 months of age (p<0.05) to thereafter continue to decrease with a slower rate.

Biochemical analysis: The collagen content in the bone also underwent the most changes during the early time points, i.e. from newborn until 3 months of age (p<0.05), to thereafter level out (Fig 1). On the other hand, the enzymatic cross-links (HP and LP) increased more steadily throughout life, and only small differences were found between the consecutive time points. The non-enzymatic cross-link PEN increased during aging (between 9 and 18 months) (Fig 1).

Correlation analysis between the mechanical properties and the biochemical evaluation showed that the Young’s modulus of the collagen network was highly associated with the collagen content in the tissue (p<0.01, r = 0.617), whereas the HP and LP cross-links correlated with the strain of the tissue at rupture (p<0.01, r = -0.536, -0.432) (Fig 2).

DISCUSSION
Bone tissue is most often assessed using information on its strength and mineral content. However, much less is known about its collagen matrix during maturation, adult age and aging. In this study, the collagen network was assessed by tensile testing of decalcified bone as well as by biochemical evaluation of collagen content and the amounts of cross-links. Most properties were changing drastically until the rabbit was 3 months of age to thereafter level out during adult age. During the last time points, the mechanical characteristics, such as the ability of bones to store energy, started to decrease, as a sign of aging. This coincided with an increase in the non-enzymatic cross-link PEN. This matches with previous studies, suggesting that PEN increases during aging, or with reduced or slow collagen turnover [5]. To conclude, the comprehensive assessment of bone properties during maturation and aging needs improved knowledge of the development of the collagen framework, in addition to information about the mineralized phase of the tissue.

ACKNOWLEDGEMENTS
This study was funded by the Ministry of Education, Finland, Academy of Finland (128863) and the European Commission (219980).

REFERENCES