inserted in sham-operated rats. The immobilized rats and sham-operated rats made up the immobilized-remobilized (Im-Re) group and control group, respectively. Five-mm sections at the medial midcondylar region in sagittal plane were obtained and stained with H-E and Safranin-O (S-O). Six areas (NC area, T area, C area in the femur and tibia) were set and modified Mankin’s score, thickness of the articular cartilage, and number of chondrocytes were evaluated at each area. Mechanical properties of the articular cartilage were assessed by the scanning acoustic microscope (SAM).

Results: [C Area] Chondrocytes decreased and disappeared after 1W Im-Re group (Figs. 1, 2). A marked reduction of S-O staining was observed (Figs. 4, 5). Mankin’s score was significantly higher after 1W Im-Re group (Fig. 7). Number of chondrocytes was significantly smaller after 2W Im-Re group (Fig. 9). The articular cartilage in the Im-Re group was almost blue (low sound speed) compared to the control (Figs. 10, 11). [T Area] Hypertrophy and cloning of chondrocytes were observed after 2W Im-Re group (Fig. 3). Thickness of the cartilage was significantly higher at 4W Im-Re group (Figs. 8, NC Area) Reduction of S-O staining in the non-calcified cartilage was almost restored but it was not restored around the tidemark (Fig. 6).

Conclusions: These results have indicated that atrophic changes (decrease of proteoglycans) through decreased mechanical stress in the NC area were reversible, but chondrocytes death and hypertrophy of chondrocytes in the C and T areas through increased mechanical stress by rigid immobilization were irreversible after remobilization. Clinicians should be aware that even a short-term rigid immobilization could cause irreversible articular cartilage damage.

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ROLE OF HIGH EXTRACELLULAR GLUCOSE CONCENTRATIONS IN MODULATING ANABOLIC AND CATABOLIC GENE EXPRESSION IN NORMAL AND OSTEOARTHRITIC HUMAN CHONDROCYTES

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Purpose: Articular chondrocytes require a steady supply of glucose for optimal energy production and cell homeostasis, as well as for anabolic functions, namely the synthesis of cartilage matrix molecules. As such, articular chondrocytes may be especially sensitive to alterations in the synovial fluid glucose concentration due to disorders that affect glucose metabolism, namely Diabetes Mellitus (DM). Our previous study showed that hyperglycemia-like glucose concentrations reduce glucose transport into normal but not into osteoarthritis (OA) chondrocytes, in which it leads to intracellular glucose accumulation with prolonged production of Reactive Oxygen Species (ROS) and oxidative stress. Since ROS are known to contribute to OA pathogenesis, this study aimed at elucidating the role of high extracellular glucose in modulating anabolic and catabolic gene expression in normal and OA human chondrocytes. For this, we examined the effects of culturing normal and OA human chondrocytes under elevated glucose concentrations, using real time RT-PCR (qRT-PCR) to evaluate the expression of several genes important in cartilage homeostasis and OA pathogenesis, namely TIMPs-1 and -2, MMPs-1 and -13 and collagen types I and II.

Methods: Normal (N=7) and OA (N=11) human chondrocytes were obtained from multi-organ donors or patients undergoing total knee replacement surgery, respectively, at the University Hospitals of Coimbra. Non-proliferating non-pooled chondrocyte cultures were maintained for 24, 48 or 72 h in Ham’s F-12 containing 10 (regular glucose) or 30 mM (high glucose) D-glucose. Gene expression was assessed by qRT-PCR.

Results: Basal MMP-1 and -13 mRNA levels were approximately 5 and 8 fold higher in OA than in normal chondrocytes, respectively. A trend towards increased expression of TIMP-1 and collagen I and decreased expression of collagen II was found in OA relative to normal chondrocytes, although it didn’t reach statistical significance. Culture of OA chondrocytes in high glucose for 24 or 48 h increased MMP-1 (1.5±0.2 and 1.4±0.3) and -13 (1.7±0.2 and 1.4±0.2) mRNA levels relative to cells maintained in regular glucose, whereas no changes were observed in normal chondrocytes. TIMP-1 and -2 gene expression was not affected by culture of either normal or OA chondrocytes in high glucose. Culture in high glucose for 24 h modest and similarly increased collagen II expression in normal (1.28±0.07) and OA chondrocyte cultures (1.34±0.13).

Conclusions: Acute exposure of OA, but not normal chondrocytes to high glucose increased MMP gene expression which was not compensated by concurrent increases in the expression of their tissue inhibitors, but was accompanied by a modest transient increase in collagen II expression. These results indicate that OA chondrocytes are more sensitive to high glucose-induced deleterious effects than normal ones. This may constitute a pathogenic mechanism by which conditions characterized by hyperglycaemia, like DM, can promote changes in chondrocytes that facilitate the development and/or progression of OA. Besides, the possibility that more prolonged and/or repeated exposure to high glucose can also induce uncompensated catabolic gene expression in normal chondrocytes, favoring OA development, deserves to be further investigated.

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METHYLATION SPECIFIC MICROARRAY ON HUMAN CHONDROCYTES

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Purpose: Osteoarthritis (OA) is an increasing, multifactorial disease affecting millions of people worldwide. Methylation, the most common eukaryotic DNA modification, is an epigenetic event and has been intensively studied in embryogenesis, aging and carcinogenesis. Up to now very few studies have examined the DNA methylation status of human cartilage genes. The aim of our study was to perform a whole genome methylation specific array on human chondrocytes for the first time to display molecular patterns of OA that may lead to a better understanding of the disease.