

Correction

Correction: Catabolic stress induces expression of hypoxia-inducible factor (HIF)-1 α in articular chondrocytes: involvement of HIF-1 α in the pathogenesis of osteoarthritis

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In our recent article [1], we did not identify three paragraphs in the Material and Methods section that were reproduced from an article by Pfander *et al.* [2]. This omission has been corrected by the addition of a reference to the Pfander *et al.* paper as follows:

Measurement of lactic acid in cultured chondrocytes

To examine the role of HIF-1 α in the glycolysis in human OA chondrocytes, the level of lactic acid was measured in culture chondrocytes by the method reported by Pfander *et al.* [21]. Supernatants from chondrocyte cultures were collected after 24 hours under normoxic or hypoxic conditions. Lactic acid was determined by a colorimetric assay (Sigma) at 540 nm in accordance with the manufacturer's instructions. Lactic acid levels were normalized to total protein content as measured by the Bradford assay (Bio-Rad, Hercules, CA, USA)

ATP levels in cultured chondrocytes

To study the role of HIF-1 α in energy generation in human OA chondrocytes, the ATP level was analyzed in cultured chondrocytes by the method reported by Pfander *et al.* [21]. Chondrocytes were collected after a 24-hour incubation under normoxic or hypoxic conditions. The ATP Bioluminescence assay kit CLS II (Roche, Heidelberg, Germany) was used. The assay is based on the light-emitting oxidation of luciferin by luciferase in the presence of extremely low levels of ATP. After collecting the chondrocytes by scraping, cells were centrifuged for 10 min at 500 \times g in the cold. Chondrocyte pellets were extracted by boiling 100 mM Tris (tris(hydroxymethyl)aminomethane) buffer containing 4 mM EDTA (ethylenediaminetetraacetic acid) for 2 min in order to inactivate NTPases. Cell remnants were removed by centrifugation at 1000 \times g. Supernatants were removed and placed on ice. Determination of free ATP was as outlined in the manufacturer's protocol. Light emission was measured at 562 nm using a luminometer. ATP levels were normalized to protein content as measured by the Bradford assay (Bio-Rad) [19].

Real-time PCR

To compare the expression levels of mRNA for HIF-1 α we performed real-time PCR according to the method reported by Pfander *et al.* [21]. For PCR analyses, cDNA from triplicate dishes from four independent experiments (24 hours of hypoxia or normoxia) were diluted to a final concentration of 10 ng/ μ l. Quantitative real-time RT-PCR was performed with a TaqMan Universal Mastermix (Biosystems Inc, Foster City, CA). cDNA (50 ng) was used as template to determine the relative amounts of mRNA by real-time PCR (ABI 7700 sequence detection system) using specific primers and probes (Table 2). The reaction was conducted as follows: 95°C for 4 min, and 40 cycles of 15 s at 95°C and 1 min at 60°C [21]. To standardize mRNA levels, we amplified 18S rRNA as an internal control and calculated using Microsoft Excel.

We regret any inconvenience caused by our failure to fully acknowledge the work of Pfander *et al.*

References

1. Yudoh K, Nakamura H, Masuko-Hongo K, Kato T, Nishioka K: **Catabolic stress induces expression of hypoxia-inducible factor (HIF)-1 α in articular chondrocytes: involvement of HIF-1 α in the pathogenesis of osteoarthritis.** *Arthritis Research & Therapy* 2005, **7**:R904-R914.
2. Pfander D, Cramer T, Schipani E, Johnson RS: **HIF-1 α controls extracellular matrix synthesis by epiphyseal chondrocytes.** *J Cell Sci* 2003, **116**:1819-1826.