can be affected by some environmental conditions such as temperature, ultraviolet radiation or visible light, modifying the final concentration of this compound. The aim of our study is to assess a method for determine the concentration of HP in commercial bleaching preparations when this solutions are applied on the teeth.

**Material and Methods**

We selected three commercial preparations: Opalescence® Endo® (35%PH), Pola Office +® (37.5 %PH) and DayWhite® (37 %) carbamide peroxide, All of them were preserved at 4ºC in darkness. In order to quantify the amount of HP, the different compounds were dissolved in 6% bidestilated water (weight / volume) and 1 ml of this solution was placed in a special quartz cuvette and introduced into a fluorometer. Subsequently, the light emitted by the fluorometer (λ=420 nm) passed through the sample and the associated software provided the fluorescence emitted by the HP. Using this data and line pattern, the concentration of HP in the samples was obtained.

**Results**

The concentration of HP was similar in the Endo Opalescence® and Pola Office +® samples, 26.09 M and 26.68 M, respectively. On the other hand, the concentration of HP in DayWhite® sample was 5.28 M.

**Conclusions**

The method used in this study allows to calculate the concentration of HP (active agent) in the bleaching products analyzed. However, future studies should be developed in order to compare the final concentration with the desired product concentration.

- Oral Presentation 47

**TITLE:** Stimulatory effect of leptin in the dentin sialophosphoprotein (DSPP) production in human dental pulp

**AUTHORS:** Martín González J, Sánchez Domínguez B, Crespo Gallardo I, Martín Jiménez M, Segura Egea JJ.


* doi:10.4317/jced.17643831
http://dx.doi.org/10.4317/jced.17643830

**Introduction**

Leptin, a mediator of the inflammatory response, and its receptor (LEPR) are expressed in the human dental pulp. Sialophosphoprotein dentin (DSPP) is a protein involved in odontogenesis and the dentin-pulp reparative response. This research aims to describe the im-
munohistochemical localization of LEPR and study the effect of leptin on the expression of dentin sialophosphoprotein (DSPP) by human pulp cells.

Materials and Methods
Twenty-five dental pulp samples were obtained from freshly caries- and restoration-free extracted human third molars. The pulp samples were processed and mineralization produced by odontoblasts in response to leptin was determined analyzing the expression of DSPP by immunoblot and by real time PCR (qRT-PCR). LEPR localization was examined by immunohistochemistry using anti-human LEPR monoclonal antibody.

Results
The immunoreactivity for antibodies anti-LEPR was localized in the odontoblastic layer and the predentine. Leptin dose-dependently stimulated dentin sialophosphoprotein expression in human dental pulp. Western blot analysis of leptin-stimulated human dental pulp samples revealed the presence of a protein with an apparent molecular weight of approximately 100 kDa, which corresponds to the estimated molecular weight of DSPP. The expression of DSPP mRNA was confirmed by qRT-PCR analysis, and the size of the amplified fragments was confirmed by agarose gel electrophoresis.

Conclusions
For the first time it has been demonstrated that human odontoblasts express the leptin receptor (LEPR), and the binding of leptin to LEPR results by DSPP production by odontoblasts. These findings suggest that leptin plays a role in the defensive response pulp and dentino-genesis.

- Oral Presentation 48
TITLE: Activation of PKB Pathway signaling by leptin in human dental pulp

AUTHORS: Martín Jiménez M, Martín González J, Crespo Gallardo I, Sánchez Domínguez B, Segura Egea JJ.

* doi:10.4317/jced.17643832
http://dx.doi.org/10.4317/jced.17643832

Objectives
Leptin, initially described as an adipocyte-derived hormone to regulate weight control, as well as its receptor (LEPR), are expressed in human dental pulp. Both leptin and LEPR are up-regulated during pulp experimental inflammation. This study aims to assess if leptin signal transduction in human dental pulp involves PKB phosphorylation.

Materials and Methods
Fifteen dental pulp samples were obtained from freshly caries- and restoration-free extracted human third molars. Pulp samples were processed and leptin signaling was determined analyzing PKB phosphorylation by immunoblot. To measure activation of PI3K pathway in human dental pulp in response to human leptin, the activation of the central kinase of this pathway, i.e. PKB, was measured using antibodies that specifically recognize the phosphorylated form of PKB (P-PKB). Anti-β-tubulin antibodies were used for the control of the immunoblot.

Results
Leptin stimulated PKB phosphorylation. The phosphorylated band corresponded with an apparent molecular mass of about 60 kDa, which corresponds to the estimated molecular weight of P-PKB. An increase in phosphorylation was observed at 0.1 nM leptin, maintaining the effect at 1 and 10 nM leptin. The relative amount of PKB in stimulated pulps was significantly higher than in unstimulated pulps (p < 0.05).

Conclusions
PKB is involved in leptin signalling pathways in human dental pulp.

- Oral Presentation 49
TITLE: Root perforations in central incisors: 12 years of evolution

AUTHORS: Martínez Osorio J, Canalda Salhi C, Berástegui Jimeno E.

* doi:10.4317/jced.17643833
http://dx.doi.org/10.4317/jced.17643833

Introduction
In the endodontics field, iatrogenesis is a common and very complex issue, we had the chance to perform a re-treatment in a patient with two root perforations performed while trying to access the pulp chamber of 1.1 and 1.2, due to lack of direction in the access opening.

Case report
A 65 years-old patient was referred to our clinic to retreat endodontically both central incisors after a previous failed attempt to in which the deviation of the axis to access cavity did a root perforation iatrogenically in