

## Effects of cigarette smoke inhalation and coffee consumption on bone formation and osseous integration of hydroxyapatite implant

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Received October 3, 2011 – Accepted April 4, 2012 – Distributed February 28, 2013

(With 2 figures)

### Abstract

The present study aims to assess the effects of cigarette smoke inhalation and/or coffee consumption on bone formation and osseous integration of a dense hydroxyapatite (DHA) implant in rats. For this study, 20 male rats were divided into four groups (n = 5): CT (control) group, CE (coffee) group, CI (cigarette) group and CC (coffee + cigarette) group. During 16 weeks, animals in the CI group were exposed to cigarette smoke inhalation equivalent to 6 cigarettes per day; specimens in the CE group drank coffee as liquid diet; and rats in the CC group were submitted to both substances. In the 6<sup>th</sup> week a 5 mm slit in the parietal bone and a 4 mm slit in the tibia were performed on the left side: the former was left open while the latter received a DHA implant. As soon as surgeries were finished, the animals returned to their original protocols and after 10 weeks of exposure they were euthanised (ethically sacrificed) and the mentioned bones collected for histological processing. Data showed that exposure to cigarette smoke inhalation and coffee consumption did not interfere in weight gain and that solid and liquid diet consumption was satisfactory. Rats in the CC group showed a decrease in bone neoformation around the tibial DHA implant ( $31.8 \pm 2.8$ ) as well as in bone formation in the parietal slit ( $28.6 \pm 2.2$ ). On their own, cigarette smoke inhalation or coffee consumption also led to diminished bone neoformation around the implant and delayed the bone repair process in relation to the CT group. However, reduction in the bone repair process was accentuated with exposure to both cigarette smoke inhalation and coffee consumption in this study.

*Keywords:* cigarette, bone, hydroxyapatite, bone formation, coffee.

### Os efeitos do cigarro e do consumo de café sobre a formação óssea e a integração óssea de implantes de hidroxiapatita

#### Resumo

O presente estudo teve como objetivo avaliar os efeitos do tabagismo e do consumo de café, isolada ou concomitantemente, sobre a formação óssea e a osseointegração de implantes hidroxiapatita densa. Foram utilizados 20 ratos machos, divididos em quatro grupos (n = 5): grupo CT (controle); grupo CA (café); grupo CI (cigarro), e grupo CC (cigarro + café). Durante 16 semanas, os animais do grupo CI foram expostos à fumaça de seis cigarros/dia; os animais do grupo CA consumiram café como dieta líquida, e os animais do grupo CC, ambas as substâncias. Após seis semanas de exposição, uma falha óssea de 5 mm foi produzida no osso parietal esquerdo e de 4 mm, na tibia esquerda dos animais. A falha do parietal foi mantida aberta, enquanto na tibia corpos cerâmicos de hidroxiapatita densa (HAD) foram implantados em cavidade produzida cirurgicamente. Após as cirurgias, os animais retornaram aos protocolos experimentais e, ao término de dez semanas, foram eutanasiados, sendo as tíbias e os parietais coletados para processamento histológico. A exposição à fumaça do cigarro e o consumo de café não interferiram no ganho de peso dos animais, e os consumos de dieta líquida e sólida foram satisfatórios entre os grupos. Os animais do grupo CC apresentaram menor volume de osso neoformado ao redor do implante de HAD na tibia ( $31,8 \pm 2,8$ ) e menor osteogênese na falha produzida no osso parietal ( $28,6 \pm 2,2$ ). O café e o cigarro consumidos isoladamente provocam a diminuição do volume de osso ao redor do implante e o atraso no processo de reparação óssea. Observou-se que o consumo de café associado à exposição à fumaça do cigarro reduziu de forma acentuada o processo de reparação óssea, no presente estudo.

*Palavras-chave:* cigarro, osso, hidroxiapatita, formação óssea, café.

## 1. Introduction

The World Health Organization defines dependence as a state in which at least one out of three of these situations occurs: strong desire or compulsion to use a substance; difficulty in controlling its use; physiological abstinence; tolerance; progressive abandonment of alternate interests; persistent use, despite prejudicial consequences (WHO, 1993). Tobacco dependence (tabagism) and possible coffee consumption dependence have already been widely discussed (Planeta and Cruz, 2003; Nehlig, 2004; Alves et al., 2009), as well as their repercussions in the human body.

Due to its characteristic flavour and taste (Pinto, 2002), coffee is one of the most popular beverages and it is highly appreciated among smokers, who often drink a cup of coffee before smoking or vice-versa. Caffeine, the principal chemical substance found in coffee, and nicotine, the toxic addicting agent in cigarettes, trigger several cellular and pharmacological responses in many biological systems, such as central nervous system and cardiac muscle stimulation, increased diuresis, smooth muscle relaxation (Sakamoto et al., 2001), balance impairment (Felipe et al., 2005); caffeine and nicotine can also be associated to a rise of fracture risk and osteoporosis development (Lloyd et al., 1997; Bueno et al., 2011; Soares et al., 2010).

The effects of coffee consumption and cigarette smoke inhalation on the bone repair process and osseous integration of biomaterials have been little investigated. Considering the great number of smokers and coffee drinkers in the general population, the present study aims to evaluate the effects of cigarette smoke inhalation and/or coffee consumption on bone formation and osseous integration of a dense hydroxyapatite (DHA) implant in rats.

## 2. Material and Methods

### 2.1. Animal protocol

This study was performed in the Laboratory of Phytopharmacology of José do Rosário Vellano University, after being approved by its Ethics Committee for Laboratory Animals (protocol n° 19A/2007), according to the Brazilian Legislation on Experimental Animals (Federal Bill 6638/1979). This study also complied with the ethical principles outlined by the Brazilian College on Animal Experimentation (COBEA).

For this experiment, 20 albino male rats (*Rattus norvegicus* – Wistar), 40-days old, were provided by the University Biotery, based in the city of Alfenas, in the state of Minas Gerais, Brazil. The animals were divided into four groups (n = 5):

**CT group (Control):** all animals in this group were fed with Nuvilab CR-1 Autoclave ration for mice as solid diet and water *ad libitum* (at will) as liquid diet.

**CE group (Coffee):** all animals in this group received a coffee beverage as liquid diet. In order to standardise the amount of coffee to be offered, the daily consumption of five cups of 200 mL for a 70 kg person and the ABIC (2007) directions for coffee preparation were taken into

account: that is, 100 g of coffee powder diluted in one litre of boiling water, as proposed by Araujo (2007). In the first seven days, the animals drank a preparation of 25 g of coffee powder diluted in one litre of boiling water, followed by 4 days of a preparation of 50 g of coffee powder diluted in one litre of boiling water in order to adapt the animals to full protocol. The coffee powder used in this study came from a ground roasted *Coffea arabica* registered trademark from the south of the state of Minas Gerais, roasted at 160 °C for about 13 minutes and graded as 45 ideal for consumption.

**CI group (cigarettes):** all animals in this group were exposed daily in the morning to the smoke of 6 cigarettes for about 20-30 minutes with 1.3 mg nicotine, 16.5 mg tar and 15.2 mg carbon monoxide level concentrations. Initially, the animals were submitted to an adaptation period of 6 days, in which the number of burnt cigarettes was progressively raised from 1 to 6, according to the Bueno et al. (2011) and Nociti Junior et al. (2002) method. To accomplish cigarette smoke inhalation, animals were put in a 45 × 25 × 20 cm transparent acrylic chamber-box provided with large holes for ventilation at the bottom and small holes for smoke exposure at the top, where cigarettes were housed. After that, the animals were sent back to their conventional cages (Bueno et al., 2011).

**CC group (Coffee + Cigarettes):** all animals in this group were exposed to both experimental protocols of both the CE and CI groups.

All animals in the CE, CI and CC groups were fed with Nuvilab CR-1 Autoclave ration for mice as solid diet and rats in the CI group took water *ad libitum* (at will) as liquid diet. All specimens had their weight gain checked weekly and diet intakes measured.

### 2.2. Surgical procedure

In order to assess the effect of coffee consumption and/or cigarette smoke inhalation on the bone repair process, a 4 mm-width circular slit in the proximal epiphysis (lateral condylus) of the left tibia and a 5 mm-width slit in the left parietal bone were carried out in all animals in CE, CI, and CC groups, after 6 weeks of exposure to cigarette smoke inhalation and/or coffee consumption.

The animals were anaesthetised with a 1:1 ketamine (Francotar®) and xylazine chloride (Virbaxyl 2%®) solution, 0.10 mL/100 g dose given IM, and trichotomised in the area to be evaluated; then the skin was cut and turned back and the bone surface was exposed. On the left side, the parietal slit was left open and the tibial one received a 3 mm diameter-3 mm long DHA biomaterial, manufactured by UNICAMP Chemical Institute. The periosteum was replaced and sutured with 8.0 silk thread and the skin sutured with 4.0 cotton thread. At the end of the 16<sup>th</sup> week (6 weeks pre-operative and 10 weeks post-operative) all animals were euthanised with an anesthetic overdose.

### 2.3. Histomorphometric processing

The left tibia and parietal bone were collected and fixed in a 10% formaldehyde solution for 48 hours and then

decalcified in a solution of formic acid, formaldehyde and sodium citrate for 15 days. After that, bones were reduced and included in paraffin for histological processing. Semi-serial 6  $\mu$ m-wide transverse cuts were made, hematoxylin/eosin (HE) stained and glass-mounted for morphological analysis (Soares et al., 2010). The volume of bone neoformation was assessed with the aid of a 100-point quadrilateral reticule put into the microscope eyepiece.

After point-counting according to the Delesse principle as proposed by Mandarim-de-Lacerda (1999), the following formula was used:  $Vv = Pp/Pt$  (%), where  $Vv$  = volume density or relative volume;  $Pp$  = amount of points counted over bone neoformation;  $Pt$  = total amount of points of the reticule.

#### 2.4. Statistical analysis

Results were reported as the mean  $\pm$  standard deviation, and statistical comparison among all groups was performed by ANOVA and *Tukey-Kramer* tests. Results were considered significant for  $p \leq 0.05$ .

### 3. Results

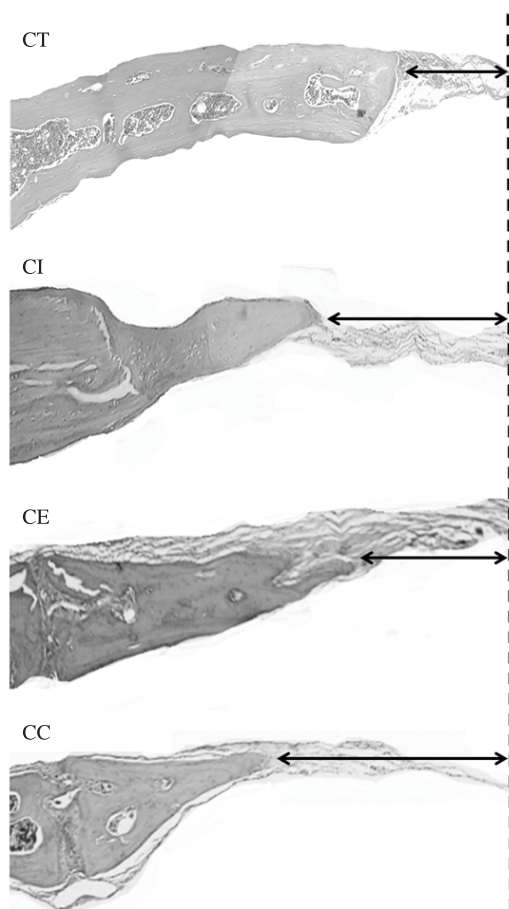
Solid and liquid diet consumption was considered satisfactory in all groups and no statistical significant differences were observed among them (Table 1). During the experiment all animals experienced weight gain (Table 1).

Morphometric results showed that rats in CE, CI, and CC groups had a decreased volume of bone neoformation around the DHA implant and a diminished bone repair process in the parietal slit in comparison to those of the CT group. The volume of bone neoformation in the CE group was greater than in the CI group which, in turn, was greater than in the CC group. Rats in the CC group exhibited the least degree of bone formation in the bone slit (Figure 1) and osseous integration of the DHA implant (Figure 2 and Table 1).

### 4. Discussion

Bone repair depends on migration, proliferation and differentiation of osteogenic cells into osteoblastic ones (Marks and Popoff, 1988) and this process can be altered by some patients' life habits such as alcoholism (Lima et al., 2011), tabagism (Bueno et al., 2011) and coffee consumption

(Tsuang et al., 2006; Lacerda et al., 2010). The present study shows that cigarette smoke inhalation and coffee consumption interfered substantially negatively on the bone formation and osseous integration of the DHA implant in the CC group. These alterations are due to cigarette and/or coffee effects and not to any state of under-nourishment, because all animals experienced weight gain during the

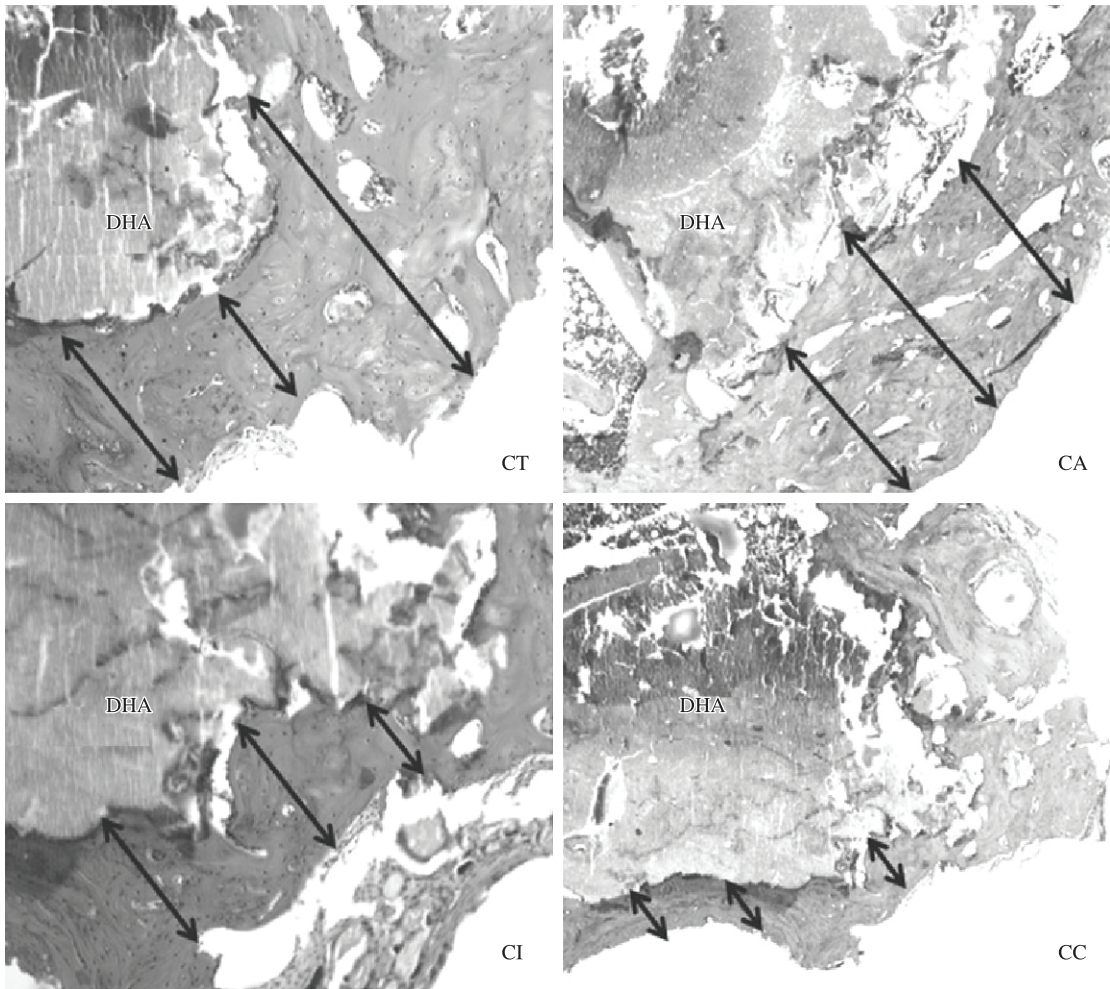


**Figure 1.** Microphotography of a transverse section of the parietal slit area in CT, CE, CI and CC groups. Comparison of lesser volume of bone neoformation ( $\downarrow$ ) in CC, CI and CE groups in relation to CT group (400 $\times$  – HE staining).

**Table 1.** Comparison of weight gain ( $\Delta W$ ); mean of daily solid and liquid diet intake; bone neoformation volume (BNV) around the DHA implant and in the parietal slit in CT (Control), CI (cigarette), CE (Coffee) and CC (cigarette + coffee) groups.

	CT	CA	CI	CC
Liquid (mL)	43.7 $\pm$ 1.4 <sup>a</sup>	43.1 $\pm$ 0.7 <sup>a</sup>	42.6 $\pm$ 0.5 <sup>a</sup>	43.5 $\pm$ 1.8 <sup>a</sup>
Solid (g)	54.2 $\pm$ 2.3 <sup>a</sup>	53.2 $\pm$ 3.7 <sup>a</sup>	53.8 $\pm$ 2.4 <sup>a</sup>	52 $\pm$ 4.0 <sup>a</sup>
$\Delta P$ (g)	256.4 $\pm$ 4 <sup>a</sup>	259.4 $\pm$ 2 <sup>a</sup>	257 $\pm$ 3 <sup>a</sup>	257.2 $\pm$ 3.2 <sup>a</sup>
BNV (%) DHA tibial	50.8 $\pm$ 3.3 <sup>a</sup>	45 $\pm$ 3.1 <sup>b</sup>	38.4 $\pm$ 2.3 <sup>c</sup>	31.8 $\pm$ 2.8 <sup>d</sup>
BNV (%) parietal	45 $\pm$ 3 <sup>a</sup>	38.4 $\pm$ 1.6 <sup>b</sup>	33.6 $\pm$ 1.8 <sup>c</sup>	28 $\pm$ 2.2 <sup>d</sup>

Two averages followed by the same small letter are not different from each other ( $p > 0.05$ ). Tukey test. Results are reported as the mean  $\pm$  standard deviation.



**Figure 2.** Microphotography of a transverse section of the DHA tibial implant site in CT, CE, CI and CC groups. Comparison of lesser volume of bone neoformation (↓) in CC, CI and CE groups in relation to CT group (400× – HE staining).

experiment, their solid and liquid diet consumption being within the standard patterns recommended by Svendsen and Hau (1984).

In the present study, one cannot observe chronic inflammatory response or fibrous tissue formation in the neoformed bone tissue/DHA implant interface, for in all groups, bone neoformation in direct contact with the DHA implant was found.

Froes et al. (2002) demonstrated that caffeine lessens bone mineral density, alters calcium metabolism, increases diuresis and the plasmatic calcium level (Heaney, 2002; Lacerda et al., 2010) and causes osteoporosis development (Lloyd et al., 1997). These findings can explain the lesser volume of bone neoformation around the DHA implant and of the bone repair process in the CE group.

The lesser volume of bone neoformation observed in the CI and CC groups can be explained by nicotine effects: reduced mineral level in the osseous matrix, delayed repair process, decreased amount of neutrophiles and macrophages, raised platelet aggregation, lowered blood

microperfusion, microclot and thrombus formation, which associated to vessel constriction induces tissue ischemia (Sakakura et al., 2001; Soares et al., 2010). Bueno et al. (2011) demonstrated that the bone repair process in the slit and the osseous integration of DHA implant are expected to occur even in the presence of cigarette smoke inhalation, but to a lesser extent. Nicotine alters the normal histology of bone tissue by inhibiting osteoblastic cells and parathyroid hormone levels (Kapoor and Jones, 2005), and decreases bone density (Benatti et al., 2005), predisposing it to be easily fractured (Soares et al., 2010).

Coffee consumption and cigarette smoke inhalation, when considered singly, led to a lesser bone volume around DHA implant and delay in the bone repair process in the parietal slit. However, coffee consumption associated with cigarette smoke inhalation strongly reduced bone repair process in the present study.

In conclusion, the habits of coffee consumption or cigarette smoke inhalation (tabagism) have to be investigated in the patient's history in order not to compromise the

stability and maintenance of biomaterial implant. It is worth emphasizing that health professionals must pay attention to patients' life habits for accurate pre-operative planning and secure post-operative follow-up.

## References

Associação Brasileira da Indústria do Café - ABIC. *Tipo de preparo de café*. Available from: <<http://www.abic.com.br>>. Access in: 10 June 2007.

ALVES, RC., CASAL, S. and OLIVEIRA, B., 2009. Benefícios do café na saúde: mito ou realidade? *Química Nova*, vol. 32, no. 8, p. 2169-2180. <http://dx.doi.org/10.1590/S0100-40422009000800031>

ARAÚJO, FA., 2007. Café (*Coffea arabica* L.) submetido a diferentes torrefações: caracterização química e avaliação da atividade antioxidante e sensorial. São Paulo: Universidade de São Paulo. 157 p. Tese de Doutorado em Nutrição Experimental.

BENATTI, BB., CÉSAR-NETO, JB., GONÇALVES, PF., SALLUM, EA. and NOCITI, FH., 2005. Smoking affects the self-healing capacity of periodontal tissues. A histological study in the rat. *European Journal of Oral Science*, vol. 113, no. 5, p. 400-403. <http://dx.doi.org/10.1111/j.1600-0722.2005.00240.x>

BUENO, AC., RIBEIRO, KG., CRUZ, TM., IVO, TPF., SANTOS, AMM., CAMILLI, JA., GARCIA, JAD., CHAVASCO, JK. and SOARES, EA., 2011. Efeitos da fumaça de cigarro sobre a osteogênese e a resistência mecânica óssea em ratos. *Revista de Ciência Farmacêutica Básica Aplicada*, vol. 32, no. 1.

FELIPE, L., SIMÕES, LC., GONÇALVES, DU. and MANCINI, PC., 2005. Avaliação do efeito da cafeína no teste vestibular. *Revista Brasileira Otorrinolaringologia*, vol. 71, no. 6, p. 758-62. <http://dx.doi.org/10.1590/S0034-72992005000600012>

FROES, NDT., PEREIRA, ES. and NEGRELLI, WF., 2002. Fatores de risco da osteoporose: prevenção e detecção através do monitoramento clínico e genético. *Acta Ortopédica Brasileira*, vol. 10, no. 1, p. 52-56.

HEANEY, RP., 2002. Effects of caffeine on bone and the calcium economy. *Food and Chemical Toxicology*, vol. 40, no. 9, p. 1263-70. [http://dx.doi.org/10.1016/S0278-6915\(02\)00094-7](http://dx.doi.org/10.1016/S0278-6915(02)00094-7)

KAPOOR, D. and JONES, TH., 2005. Smoking and hormones in health and endocrine disorders. *European Journal of Endocrinology*, vol. 152, no. 4, p. 491-9. <http://dx.doi.org/10.1530/eje.1.01867>

LACERDA, SA., MATUOKA, RI., MACEDO, RM., PETENUSCI, SO., CAMPOS, AA. and BRENTGANI, LG., 2010. Bone quality associated with daily consumption of coffee: a biochemical, radiographic and histometric study. *Brazilian Dental Journal*, vol. 21, no. 3, p. 199-204. <http://dx.doi.org/10.1590/S0103-64402010000300004>

LIMA, CC., SILVA, TD., SANTOS, L., NAKAGAKI, WR., LOYOLA, YCS., RESCK, MCC., CAMILLI, JA., SOARES, EA. and GARCIA, JAD., 2011. Effects of ethanol on the osteogenesis around porous hydroxyapatite implants. *Brazilian Journal of*

*Biology*, vol. 71, no. 1, p. 115-119. <http://dx.doi.org/10.1590/S1519-69842011000100017>

LLOYD, T., ROLLINGS, N., EGGI, DF., KIESELHORST, K. and CHINCHILLI, VM., 1997. Dietary caffeine consumption and bone status of postmenopausal women. *American Journal of Clinical Nutrition*, vol. 65, p. 1826-1830.

MANDARIM-DE-LACERDA, C.A., 1999. What is the interest of normal and pathological morphological research to be quantitative? The example of the stereology. *Brazilian Journal of Morphological*, vol. 16, no. 2, p. 131-139.

MARKS, SC. and POPOFF, SN., 1988. Bone cell biology: the regulation of development, structure, and function in the skeleton. *American Journal of Anatomy*, vol. 183, no. 1, p. 2-44.

NEHLIG, A., 2004. *Coffee, Tea, Chocolate, and the Brain*. Boca Raton: CRC Press LLC. <http://dx.doi.org/10.1201/9780203618851>

NOCITI-JUNIOR, FH., CÉSAR, NJ., CARVALHO, MD. and SALLUM, EA., 2002. Bone density around titanium implants may be influenced by intermittent cigarette smoke inhalation – A histometric study in rats. *International Journal of Oral and Maxillofacial Implants*, vol. 17, no. 3, p. 347-352.

PINTO, NAVD., 2002. Avaliação química e sensorial de diferentes padrões de bebida do café arábica cru e torrado. Lavras: Universidade Federal de Lavras. 92 p. Tese de doutorado em Ciência dos Alimentos.

PLANETA, CS. and CRUZ, FC., 2005. Bases neurofisiológicas da dependência do tabaco. *Revista de Psiquiatria Clínica*, vol. 32, no. 5, p. 251-258.

SAKAKURA, CE., MARCANTONIO JUNIOR, E. and REZENDE, MLR., 2001. O efeito do tabagismo na implantodontia. *BCI*, vol. 8, no. 32, p. 276-280.

SAKAMOTO, W., NISHIHARA, J., FUJIE, K., IIZUKA, T., HANDA, H., OZAKI, M. and YUKAWA, S., 2001. Effect of coffee consumption on bone metabolism. *Bone*, vol. 28, no. 3, p. 332-6. [http://dx.doi.org/10.1016/S8756-3282\(00\)00444-0](http://dx.doi.org/10.1016/S8756-3282(00)00444-0)

SOARES, EA., FAVARO, WJ., CAGNON, VHA., BERTRAN, CA. and CAMILLI, JA., 2010. Effects of alcohol and nicotine on the mechanical resistance of bone and bone neoformation around hydroxyapatite implants. *Journal of Bone Mineral Metabolism*, vol. 28, no. 1, p. 101-107. <http://dx.doi.org/10.1007/s00774-009-0115-1>

SVENDSEN, P. and HAU, J., 1984. *Handbook of laboratory animal science*. Boca Raton: CRC Press. vol. 1.

TSUANG, YH., SUN, JS., CHEN, LT., SUN, SC. and CHEN, SC., 2006. Direct effects of caffeine on osteoblastic cells metabolism: the possible causal effect of caffeine on the formation of osteoporosis. *Journal of Orthopaedic Surgery*, vol. 1, p. 7. <http://dx.doi.org/10.1186/1749-799X-1-7>

World Health Organization – WHO, 1993. *The ICD-10 classification of mental and behavioural disorders: diagnostic criteria for research*. Geneva: WHO.

