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Prenatally administered HMB modifies the enamel surface roughness in spiny mice offspring: An atomic force microscopy study

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ABSTRACT

Objective: The aim of this research was to check the effect of the prenatally administered β-hydroxy β-methylbutyrate (HMB) on the development of enamel surface of the spiny mice offspring.
Design: The spiny mice dams were randomly assigned into three groups: control group (not supplemented with HMB) and two experimental groups in which powdered HMB was given at the daily dosage of 0.2 g/kg of body weight (group I) and 0.02 g/kg of body weight (group II) during the last period of gestation. Newborn pups were euthanized by CO_2 inhalation. The morphology of incisor teeth was analysed using atomic force microscopy (AFM) in semi-contact mode in the height, magnitude and phase domains. Height images became a basis for determination of surface roughness parameters.
Results: Conducted study indicated that maternal HMB administration markedly influences enamel development. Enamel of offspring's teeth in both experimental groups was characterized by significantly smaller values of indices describing surface roughness and profile. HMB supplementation influenced the calculated parameters regardless of the diet type and offspring sex, however higher dose of HMB caused stronger changes in enamel surface's physical properties and could be observed in higher intensity in the male group.
Conclusions: HMB administration caused reduction in the irregularities of enamel surface, thereby possibly reducing the probability of bacteria adhesion and caries development. These observations may serve to improve nutrition and supplementation of animals and could be a lead for further research.

1. Introduction

Tooth development is a complex process involving a series of epithelial–mesenchymal interactions and coordination between the crown and the root with its associated periodontium. Each tooth passes through four morphological stages: initiation, bud, cap, and cell (Marson et al., 2008; Tucker & Sharpe, 2004). Mineralized structures characteristic for teeth, that is, dentin and enamel, are formed by specialized cells, the odontoblasts and ameloblasts, differentiating from the mesenchyme and epithelium, respectively (Thesleff, 2006). A consequence of ameloblast cell differentiation is the sequential expression of tissue-specific genes whose products form the enamel extracellular matrix. This process, known as amelogenesis, ends by the formation of calcium hydroxyapatite crystals resulting in complete mineralization of the enamel extracellular matrix (Zeichner-David et al., 1997). Enamel, the hardest material of the body and translucent substance composed of parallel rods or prisms of highly calcified material cemented together by an almost equally calcified interprismatic material (Thesleff, 2006). The enamel is mainly responsible for overcoming the mechanical resistance of food, resisting crushing stresses and reducing wear. It protects the underlying dentin and pulp against mechanical wear, acidic erosion and bacteria attack (Sa et al., 2014).

Experimental research on tooth development is based very largely on the teeth of murine rodents. Mice have highly

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specialized incisors (large and constantly growing) and a small number of teeth (Butler, 1967). Spiny mouse (*Acomys cahirinus*) is a small rodent species currently being characterized and used as a model for foetal and neonatal studies owing to its long gestation (38–40 days), few offspring (1–5, usually 2–3), and advanced stage of development of principal organ systems (Dickinson, Walker, Wintour, & Moritz, 2007; Dickinson, Griffiths, Walker, & Jenkin, 2008; Hułas-Stasiak, Dobrowolski, & Tomaszewska, 2015). It could be said that the spiny mouse is relatively mature at birth so we hypothesized that like other organs, the teeth may be more developed in the spiny mouse at birth, than in other rodents.

All of essential external and internal structures of teeth are formed during the prenatal period which is of great importance for human teeth development and growth. Because of the growth pattern and stability of the structure, primary enamel supplies a unique opportunity of recording metabolic changes occurring during development, from the latter half of the gestation period to the end of the first year of life (Teivens, Mörnstad, Norén, & Gidlund, 1996). As enamel is a bioceramic which is created as a result of complex protein biosynthesis it may be possible to influence the process of enamel creation on the prenatal stage of growth e.g. by proper nutrition conditions. β-hydroxy β-methylbutyrate (HMB) influence on protein metabolism has been confirmed in numerous research (Ostaszewski et al., 2000; Smith, Murkeji, & Tisdale, 2005; Wilkinson et al., 2013) which proved HMB activity in increasing body mass and muscle strength and its influence on physical, mechanical and physiological features of bones and internal organs (Bertman & Hanson, 2002; Flummer and Theil, 2012; Flummer, Kristensen, & Theil, 2012; Fowden, Giussani, & Forhead, 2005; Seckl, 2004; Tatara, Śliwa, & Krupski, 2007). As a metabolite of the ketogenic amino acid leucine, HMB is produced endogenously through leucine metabolism. This two steps process starts with leucine transamination to α-ketoisocaproate (KIC). Then KIC is metabolized to isovaleryl coenzyme A by the mitochondrial enzyme branched chain α-keto-acid dehydrogenase and ultimately enters the citric acid cycle. However, 2–10% of ketoisocaproate is converted to HMB by α ketoisocaproate dioxygenase in the cytoplasm (Fitschen, Wilson, Wilson, & Wilund, 2013; Shreeram et al., 2014). According to conducted research on animals and humans it can be concluded that HMB supplementation is safe and does not result in any major side effects (EFSA, 2011; Wilson et al., 2014). Enamel development and functional properties can be characterized by i.a. analyses of its texture (Cerci, Roman, Guariza-Filho, Camargo, & Tanaka, 2012; Meia, Busscher, Mei, & Ren, 2011; Stavrianos et al., 2010; Szcześniak, Ostaszewski, Fuller, Ciecierska, & Sadkowski, 2015). Hard tissue surface texture is an important issue when the interest is focused on understanding the nature of tissue surface and may play essential functional role in the tooth performance. One of the most popular variables characterizing surface texture is roughness. According to Thomas (1999) roughness is often described as closely spaced irregularities or using terms such as 'uneven', 'irregular', 'coarse in texture' or 'broken by prominences'. Roughness of biomaterial surface can be determined by various qualitative and quantitative techniques. Typically, roughness is quantified using a contact technique such as stylus instruments, scanning tunnelling microscopy, profilometer, scanning probe microscopy or atomic force microscopy (AFM) (Brinksmeier & Riemer, 1998; Kakaboura, Fragouli, Rahiotis, & Silikas, 2007). Atomic force microscopy, as an example of scanning probes, is capable of providing three-dimensional detailed topographical images of surface roughness at a nanometre resolution. These features make an AFM authoritative technique for measuring surface finish and evaluation of quality of dental materials (Cortés-Sandoval, Martínez-Castañón, Patiño-Marín, Martínez-Rodríguez, & Loyola-Rodríguez, 2015; De-Deus, Paciornik, Mauricio, & Prioli, 2006; Finke, Jandt, & Parker, 2000; Lombardini, Ceci, Colombo, Bianchi, & Poggio, 2014; Marshall, Chang, Saeky, Gansky, & Marshall, 2000). Surface roughness measured by AFM is often expressed by statistical parameters, such as the R_q (root mean square of the heights), which represents the average of the square height difference between surface peaks and valleys and the R_a (average roughness), which represents the average distance between peaks and valleys of the surface (Freitas et al., 2010). Both parameters are suitable tools for the comparison of different surface morphologies.

Due to the fact, that neonatal teeth surface has been recognized as a structural response to disturbances in the environment and nutrition at the gestation the objective of this experiment was to study the effect of HMB administration to the pregnant spiny mice during the last period of gestation on enamel surface development of the offspring using AFM.

2. Materials and methods

The experiment was approved by The Local Ethics Committee on Animal Experimentation of University of Life Sciences in Lublin, Poland (reference number 8/2014).

Research material was three groups of teeth from one-day spiny mice (*Acomys cahirinus*), whose mothers were subjected to the special diet during last stage of pregnancy or belong to the control. Each group counted six teeth: three from female (f) and three from male (m) specimens. After preparation, teeth enamel was subjected to the AFM measurements on the basis of which a number of surface roughness parameters was determined. Obtained results were analysed statistically with STATISTICA 10.0 application. The whole procedure is described in details below.

2.1. Pregnant dams

To investigate detrimental effects of maternal nutrition treatment on teeth development, β-hydroxy β-methylbutyrate (HMB) administration was performed on dams in two different dozes. The 4-month-old dams were randomised into control (n = 12) and experimental groups (n = 24) on the basis of bodyweight (40–50 g). All females were paired at a ratio of 1 male to 1 female and allowed to conceive naturally and deliver their first litter with no human intervention. The first 24 h period after the birth of the first litter is deemed to be the day of conception of the second litter and the next day as the first day of gestation. Pregnant spiny mice were housed singly in standard rodent cages under constant conditions with a 12 h light:dark cycle at 22 °C and 55–60% humidity. The experimental (HMB-treated) groups were fed with a daily dose of HMB (Lonza, Basel, Switzerland) of 0.2 g/kg of body weight (I) and 0.02 g/kg of body weight (II) from 26 day of gestation until parturition (26–39 day of the gestation). HMB doses were matched on the basis of literature and previous experience (EFSA, 2011; Flummer & Theil, 2012; Flummer et al., 2012; Ostaszewski et al., 2000; Tatara et al., 2007; Tatara, Krupski, Tymczyna, & Studziński, 2012; Tomaszewska et al., 2015; Wilkinson et al., 2013; Wilson et al., 2014). Animals were fed twice a day (at 08:00 and 12:00 o'clock) with a commercial diet (LSM, Agropol S.J., Motycz, Poland) and had unlimited access to fresh water. The total amount of food consumed by the females was estimated before the start of the experiment and was calculated as 15 g of feeding stuff per day (three pellets). In the morning, the experimental groups received one pellet with HMB while the control females received feed alone. At 12:00, the remaining part of the feed (two pellets) was given to the control and HMB-treated females. The gestation length (39–40 days) and the number of newborn pups (two to five) did not differ between the HMB and the control group. Newborn pups from the control (n = 20) and experimental groups (n = 24) were euthanized by CO_2 inhalation.