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## 9. Manuscript



### **The preventive effects of systemic casein phosphopeptides on the resorption of roots in rats**

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The preventive effects of systemic casein phosphopeptides on the resorption

of roots in rats

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## 9.1 Abstract

**Introduction:** Root resorption is a well documented side effect of orthodontic treatment. The aim of this study is to assess the potential for casein phosphopeptides (CPPs) to create an environment in which teeth are less susceptible to root resorption following orthodontic tooth movement.

**Method:** Thirty one eleven week old, Wistar rats, were used in this experiment. Experimental and control animals were given the same diet of nutrigel (Troy Laboratories Pty. Ltd, NSW, Australia) mixed with rat chow with the same frequency and had free access to water throughout the whole experimental period. The 14 experimental rats received a diet supplemented with 40mg CPP per 100g of rat while the 17 control rats did not receive any supplements in their diet. The rats underwent a 2-week period of acclimatization with their respective diets followed by a two week experimental period after which the animals were euthanized and the samples were harvested. The tooth movement appliance was a 150cN sentalloy<sup>®</sup> (GAC Australia) closed coil spring (wire diameter 0.22mm, eyelet diameter 0.56 mm) applied from the maxillary incisor randomly to the right and left first maxillary molar. A volumetric analysis of the extent of root resorption on the mesial root of the first maxillary molar was examined using a micro CT scan.

**Results:** The rats which received dietary CPP did not have a significant reduction in overall root resorption ( $p=0.28$ ) although the results did reveal that dietary CPP appeared to lower the average root resorption by about a 15%. Dietary CPP was also found to have no statistically significant effect on decreasing tooth movement ( $p=0.42$ ) although the average tooth movement was reduced by about 15%.

**Conclusions:** CPP seems to have a variable effect on the volumetric quantification of root resorption. While on average, there was less root resorption observed in rats fed dietary CPP, individual variability makes this effect statistically insignificant. Similarly, CPP did not have a statistically significant effect on reducing tooth movement. Although it is possible that CPP may have a beneficial effect on reducing cementum solubility, this may be counteracted by its anabolic effect on bone mass which explains the variability observed in this study.

Key words: Casein Phosphopeptides, Root resorption, 3D Micro CT, Volumetric analysis, Orthodontic tooth movement, Wistar rats

## 9.2 Introduction

Casein Phosphopeptides (CPPs) are multi-phosphorylated peptides from an enzymatic digest of the bovine milk protein casein.<sup>1</sup> In milk, casein stabilizes the structure of the liquid in order for it to maintain its high calcium phosphate concentration without allowing precipitation.<sup>2</sup>

Bioactivity of phosphopeptides yielded after tryptic hydrolysis of casein was reported more than 50 years ago by Mellander who discovered that CPPs were found to improve calcium balance in rachitic newborns.<sup>3</sup>

CPPs have been shown to enhance calcium absorption by increasing calcium solubility *in vitro* and *in situ*.<sup>4</sup> CPPs have been shown to enhance paracellular transport of calcium in the distal small intestine in elderly female rats at least under conditions of marginal Calcium levels.<sup>5</sup> It has been found that rats fed a diet supplemented with CPP have less bone loss than rats given Ca and P as pure minerals.<sup>4</sup>

The positive effects of CPP supplementation on the skeleton has also been demonstrated in humans. Mellander demonstrated that calcium bound to phosphopeptides could be absorbed from the digestive tract and promote bone calcification in rachitic children.<sup>6</sup> Heaney *et al*<sup>7</sup> studied the effects of administering 87.5mg of CPP to 35 normal post-menopausal women as a part of a standard test meal containing a calcium load of 250mg. Their findings suggested that casein phosphopeptide supplementation is particularly useful for persons with low basal absorptive performance.

In recent years there has been significant interest in the potential for casein phosphopeptides to prevent and remineralise carious lesions. Casein Phosphopeptides (CPP) stabilize amorphous calcium phosphate (ACP), localize ACP in dental plaque and are anticariogenic in animal and *in vivo* human caries models.<sup>1</sup>

The use of a remineralizing solution containing calcium and phosphate ions have not been clinically successful due to the low solubility of calcium and phosphates.<sup>1</sup> Calcium and phosphate ions at low concentrations do not incorporate to any significant degree into plaque or localize at the tooth surface.<sup>1</sup> In contrast, dairy products are a food group which are widely recognized to exhibit anti-caries activity.<sup>8,9</sup> Animal and *in situ* caries models have demonstrated that the components responsible for the anticariogenic nature of dairy products are the casein, calcium and phosphate.<sup>1</sup>

Reynolds has proposed that the anticariogenic mechanism for CPP-ACP is the localization of ACP at the tooth surface which buffers the free calcium and phosphate ion activities, thereby helping to maintain a state of supersaturation with respect to tooth enamel depressing demineralization and enhancing remineralization.<sup>1</sup> CPP-ACP has proved to be easy to deliver in the diets of both humans and rats. Reynolds *et al*<sup>1,10</sup> have exposed the rats to CPP-ACP solution in their drinking water while in humans the CPP-ACP complex has been delivered in solution,<sup>1</sup> in sugar-free gum<sup>11,12</sup> and it has also been incorporated into a self-cured glass-ionomer cement (GIC).<sup>13</sup> CPP is not considered to be a hazardous product.<sup>14</sup> Toxicological tests on GC tooth mousse which is a CPP



containing product showed no death in rats following oral consumption at a concentration of 200mg/kg.<sup>14</sup>

Root resorption is a physiologic or pathologic process that results in a loss of substance from dentine or cementum.<sup>15</sup> Physiological root resorption occurs during the exfoliation of deciduous teeth. Pathological root resorption may be either internal or external in origin. Internal root resorption is initiated from within the pulp while external root resorption arises from the periodontium affecting the external surface of the tooth.

The mineralized tissues of the permanent dentition are normally protected in the root canal by dentine and osteoblasts, and on the root surface by cementum and cementoblasts.<sup>16,17</sup> Resorption of the root surface is not a normal process.<sup>18</sup> However if the cementum is mechanically damaged, multi nucleated cells will arrive near the surface and resorption will occur.<sup>19</sup> In the field of orthodontics, this process has been termed, Orthodontically Induced Inflammatory Root Resorption (OIIRR).<sup>20</sup> OIIRR occurs on the cemental surface of the tooth root. Although cementum is more resistant to resorption relative to bone it is still possible for both the cementum and dentine to resorb as a result of this inflammatory process. This process is an unavoidable consequence of orthodontic tooth movement.

Risk factors for OIIRR may be classified as either systemic, local or treatment related.

Systemic factors include genetic predisposition,<sup>21-24</sup> ethnicity,<sup>25,26</sup> chronological age,<sup>27</sup> dental age,<sup>28</sup> endocrine imbalance,<sup>29-32</sup> nutrition,<sup>28,33</sup> alveolar bone density and

turnover.<sup>33,34</sup> Local factors include habits,<sup>28,35</sup> history of trauma,<sup>28,36</sup> root resorption prior to orthodontic treatment,<sup>37,38</sup> abnormal or dilacerated roots,<sup>35,39</sup> a hypofunctional periodontium<sup>40,41</sup> and increased overjet and openbite.<sup>25,28,38,42</sup> Treatment related factors included extended duration of treatment,<sup>28,43-48</sup> the use of rapid maxillary expanders,<sup>49,50</sup> heavy orthodontic forces,<sup>50-53</sup> orthodontic intrusion,<sup>54,55</sup> contact with the cortical plate<sup>56</sup> and the distance moved by the tooth roots.<sup>25,26</sup>

Like bone, teeth also require a balance in mineral content in order to prevent resorption. The ability for CPP to effectively provide bioavailable calcium for maintaining mineralization of both the skeleton and the dental tissues has been extensively demonstrated in both rat and human models.

The association between bone metabolism and root resorption has also been demonstrated in studies which have looked at the effects of medication which increase the amount of bone resorption. A study by Kalia *et al*<sup>57</sup> on rats demonstrated that subjects which received long term corticosteroids had a significant increase in the relative extent of root resorption in rats who received both chronic and acute corticosteroid treatment. This can be explained by the evidence that long term side effects of steroid therapy which include disturbances in mineralized tissue metabolism and wound healing, discrepancies in chondrogenesis and osteogenesis, bone loss and osteoporosis.<sup>58</sup> This lends support to the theory that mineralization of the bones and the teeth have an effect on the levels of root resorption during orthodontic tooth movement.

Based on the association between bone resorption, tooth movement and root resorption it follows that if the mineralization of the bone and dental tissues were to be increased, then there is a potential to protect teeth against orthodontically induced inflammatory root resorption. The importance of minerals with respect to root resorption has been demonstrated by Rex *et al*<sup>59</sup> who performed an electron probe micoranalysis (EPMA) on the concentrations of calcium (Ca), phosphorous (P), and fluoride (F) concentrations in human first premolar cementum after the application of light and heavy orthodontic forces. The application of heavy forces caused a significant decrease in the calcium concentration of cementum at certain areas of periodontal ligament tension which demonstrates the dematerializing nature of orthodontic force.

Seifi *et al*<sup>60</sup> demonstrated how the provision of calcium can potentially protect against root resorption. They studied the effect of submucosal injections of Prostaglandins in association with intraperitoneal injections of Calcium Gluconate on root resorption in rats. They showed that while the prostaglandins increased tooth movement, the combination with Calcium Gluconate led to a decrease in root resorption.<sup>60</sup> Goldie and King<sup>33</sup> studied the association of parathyroid hormone with root resorption. The main action of parathyroid hormone is to mobilize calcium from bone. They showed that there is an increased risk of root resorption associated with hyperparathyroidism.<sup>33</sup> These studies lend support to the suggestion that protection against root resorption may be achieved if calcium can be made available to the periodontium during orthodontic tooth movement.

The ability for CPP to effectively provide bioavailable calcium for maintaining the mineralization of both the skeleton and the dental tissue<sup>4,5,61</sup> is of significance due to the importance of calcium in the maintenance of tooth structure. The influence of diet on the structure of teeth has been studied in depth. Of particular interest to this study are the factors which influence the hardness of the teeth. Bielacyc and Golebiewska in 1997 demonstrated the importance of calcium and vitamin D in roots of teeth in rats.<sup>62</sup> Scanning microscope observations showed the increased cementolysis and decreased mineralization of cementum and dentin in rats fed a low calcium and vitamin D-deficient diet. Some of the earliest studies in this field were conducted by Mellanby.<sup>63,64</sup> Mellanby concluded that a low dietary calcium content may result in teeth being soft to the point that they can be cut with a scalpel.<sup>63</sup> Mellanby demonstrated that well and badly calcified teeth can be produced at will by altering in the diet the relative amounts of calcifying vitamin, found in milk, egg-yolk, cod-liver oil, etc., and anti-calcifying substances found chiefly in cereals.<sup>64</sup> These studies demonstrate how dietary CPP, which increases bioavailable calcium, may be of benefit in increasing the hardness of teeth which may help to decreasing their susceptibility to root resorption.

Dairy products are particularly effective in storing calcium because the milk protein casein stabilizes the structure of the liquid which allows it to maintain its high calcium phosphate concentration without allowing precipitation.<sup>2</sup> It follows that the provision of dietary casein would be of potential benefit in making calcium available for the protection of tooth roots against orthodontically induced inflammatory root resorption.

The question therefore follows as to whether or not CPP can offer potential benefits to teeth which are at risk of losing mineral content as is the case in teeth which are loaded with orthodontic force. CPP has been demonstrated to be a safe, natural substance when delivered topically or systemically in both rat and human models.<sup>7,10-14</sup> CPP could therefore be a potentially effective and safe way of preventing root resorption. This study was undertaken to determine whether or not casein phosphopeptides have the potential to protect teeth against orthodontically induced inflammatory root resorption in a rat experimental model.

### 9.3 Materials and Methods

Ethical approval was sought and approved by the University of New South Wales (ACEC Number: 07/32A). Forty, 11 week old female Wistar rats were used in this study. The rats were allowed 2 weeks to acclimatise to their new laboratory environment and were housed with two animals per cage.

#### *CPP delivery*

Casein Phosphopeptides can be obtained in a powdered form and this can be easily mixed in the diet.<sup>5</sup> Both the experimental and control rats received water, *ad libitum* and a diet of nutrigel (Troy Laboratories Pty. Ltd, NSW, Australia) mixed with rat chow. Nutrigel is an oral supplement which is used in veterinary medicine to provide partial or full nutritional support for mammals.

The rats received 5ml of nutrigel per day mixed with 5g of ground rat chow on a daily basis. The rat chow was mixed with the nutrigel to provide a source of fibre in the diet. The rat chow was ground so as not to pose a risk of damage to the appliances. The experimental rats were given 40mg CPP/100g body weight on a daily basis for 29 days.<sup>5</sup> A visual estimation of the consumption of the nutrigel was made on a daily basis.

#### *Orthodontic tooth movement*

Orthodontic tooth movement was implemented by use of a Nickel Titanium closed coil (GAC USA), with a force of 150g. The NiTi coils were assigned randomly to the left and right rat maxillary first molars. The coils were ligated from the first maxillary molar to

both maxillary incisors with steel ligatures. **Figure 1** The wires were then bonded to both the incisor anchor unit and the first maxillary molar with composite resin. **Figure 2** Attachment of the nickel titanium coils to the teeth was performed under general anaesthesia. The rats were weighed, and they were constantly monitored for changes in vital areas (e.g. breathing) during surgery. The animals were induced and maintained using isoflurane (2%) and oxygen (2%) inhalation during surgery. The rats were given the analgesic Temgesic (Buprenorphine) at a dose of 0.01mg/kg post-operatively.

The rats were divided into 2 groups **Figure 3**

#### **Group 1 Control**

Group 1 (n=20) had orthodontic tooth movement but did not receive any CPP supplements

#### **Group 2 Experimental**

Group 2 (n=20) had orthodontic tooth movement and received a dietary supplement of 40mg CPP /100g body weight.

Two experimental rats were lost due to anaesthetic deaths and seven rats were eliminated from the study due to appliance failure. 17 control rats and 14 experimental rats were left at the completion of the study.

The acclimatisation period was 2 weeks in which the rats received their diet with or without supplements. The experimentation period during which the tooth movement appliances were active was 2 weeks. **Figure 3** After this period, the rats were euthanized

using carbon dioxide. The maxillae were then dissected and sectioned to include only the segment of the palate with the three molars on the side where the orthodontic force was applied. The samples were then stored in formalin.

#### *SkyScan 1172 Micro CT*

The maxillae were scanned with a SkyScan 1172 Micro CT which is a compact, desktop x-ray system used for the non destructive three dimensional reconstructions of samples.

**Figure 4** There is a significant risk of root fracture and damage to tooth roots when extracting rat molars and therefore the ability to digitally remove the bone from the teeth is of advantage because it eliminates their need for their extraction. The software package enables non destructive evaluation of the molar root surfaces and digital measurements of the molar movements.<sup>65</sup>

The scanner is a cone beam x-ray source with a spatial resolution of between 2 and 5 micrometres. The recommended sample size is a diameter of 1.5 cm and a height of 3 cm. The sample is placed on a rotating platform, and depending on its proximity to the x-ray beam, there is a magnification factor. A high resolution Charged Coupled Device with a resolution of 1024 x 1024 pixels detected the incoming x-rays. **Figure 5** The scanned segments of the maxillae included the three molars on the side of the palate on which the orthodontic force was applied. **Figure 6** The samples were scanned at a resolution of 6  $\mu\text{m}$ . Throughout the scanning procedure, the samples were rotated 360 degrees with a scanning period of 2 seconds per degree of rotation. The average scanning time per sample was around 1 hours and 15 minutes.



Non destructive three dimensional reconstruction of the objects' inner structure from two dimensional x-ray shadow projections were achieved with the software program NRecon version 1.4.2. Approximately 1800 cross sections were collected per sample and the reconstructions took approximately 6 hours to complete. Once this was completed, the raw data was converted to 8 bit, Bit Mapped Picture files, with a resolution of 1024x1024 pixels. The software package, VGStudioMax v1.2 was then used to collate all the axial slices to form a three dimensional reconstruction of the scanned images.

#### *VGStudioMax 1.2*

VGStudioMax v1.2 was used to collect the data to create viewable three dimensional reconstructions of the samples with 256 shades of gray. Only the first maxillary molar and the mesial portion of the second molar were reconstructed for the purposes of data collection. **Figure 7** The bone was not removed from the images for the purposes of data collection. However for the purposes of visualisation for the sample, the bone can be digitally removed and the image of the tooth **Figure 8** can be manipulated to allow for visualisation of resorption craters. **Figure 9**

#### *VGStudioMax v1.2 crater isolation*

Due to the variability and size of the molar roots it was decided that only the mesial root of the maxillary molar was large enough to ensure consistent recording of the location of the root resorption craters. Furthermore it was found that the apical section of the molar roots was too porous to ensure a clear differentiation between normal tooth anatomy and

root resorption craters. **Figure 10** It was found that there was a definite delineation of the porous section of the molar root and this could be delineated accurately to within a few axial sections. It was therefore decided that for the purposes of data collection we would only use the section of the most mesial, first maxillary molar tooth between the cementoenamel junction and the start of the porous apex. **Figure 10**

For the purposes of collecting root resorption data, the images were viewed in axial slices. A ‘mask’ was created for the volumetric analysis of the crater images. The procedure was as follows. **Figure 11**

1. The section of the mesial molar root to be analysed between the CEJ and the porous apex was defined.
2. A map was created of the crater locations and coordinates.
3. A ‘mask’ was created for each crater. The mask was created as follows:
  - a. The axial section at which the crater began was located
  - b. An outline of the crater was drawn using the VGstudiosMax segmentation tool. The outline was drawn to follow the internal contours of the crater while the external margin of the crater was an estimate of the continuation of the convexity of the root surface. The continuation in convexity was estimated by connecting the 2 points at the edge of the break in convexity in a straight line.
  - c. The mask was then propagated through the axial section of the tooth for no more than 6 slices.

- d. The propagation of the slice was discontinued if the limits of the crater were reached or if the mask no longer followed the outline of the crater. If the mask ceased to resemble the crater a new outline was drawn.
4. The masks for all of the craters in the tooth were cumulated in a single segmentation. **Figure 12**
5. VG StudioMax then calculated the volume of the segmented mask which was recorded as the amount of root resorption.

### *Tooth Movement*

Tooth movement was measured digitally utilizing the 3D micro-CT images. It was assumed that the first and second molars were in contact prior to appliance placement and therefore the tooth movement was measured as the distance between the first and second molars at the end of the experimental period. The measurement was taken with a software tool that is designed to identify and measure the closest distance between two parallel or nearly parallel surfaces or lines. **Figure 7**

Due to variations in the angles at which the samples were scanned, there was occasionally a difference in the axial and sagittal measurements of tooth movement which was due to parallax error. **Figure 13** The measurements were therefore taken in a stepwise fashion. Firstly the observer scrolled down through the axial sections of the contact area between the first and second molars and visually identified the axial section that appeared to show the shortest distance between the two surfaces. This axial section was used as a starting point. The tool was then employed to identify the line of shortest

distance between the distal surface of the first molar contact area to the second molar contact area in the axial plane. **Figure 14** The sagittal sections were then viewed and the same software tool was used to identify the narrowest point between the heights of contour of the proximal surfaces of the first and second molars in the occluso-lingual dimension. **Figure 15** These measurements were repeated until the measurements of the axial and sagittal sections corresponded to the same position between the molar surfaces. The axial and sagittal tooth movement measurements were not always identical due to parallax errors associated with variations in the angle at which the samples were scanned. In these cases the sagittal measurements and the last axial measurements were averaged.

## 9.4 Results

### *Weight*

The average weight of rats which were included in the scanned samples at the start of force application for group 1 control was  $204 \pm 26\text{g}$  and group 2 experimental was  $213 \pm 21\text{g}$ . The average finish weight for group 1 control was  $243 \pm 46\text{g}$  and group 2 experimental was  $249 \pm 19\text{g}$ . The average weight gained for the control group was  $39\text{g}$  and the average weight gained for the experimental group was  $35.5\text{g}$ . Only one rat experienced severe weight loss. This weight loss was attributed to appliance failure and the rat was eliminated from the experiment.

### *CPP intake*

It was anticipated that the average weight of the rats would reach  $250\text{g}$ . This proved to be a fair assumption as the average weight of the control group reached  $243\text{g}$  while the average weight of the experimental group reached  $249\text{g}$ . The experimental rats were therefore supplied with  $100\text{mg}$  of CPP per day in accordance with the regime of administering  $40\text{mg}$  CPP/ $100\text{g}$  of rat.<sup>5</sup> ie.  $100\text{mg}$  of CPP mixed with  $5\text{ml}$  of nutrigel per day.

### *Nutrigel and Rat Chow consumed*

The water consumed by the rats was given *ad libitum* and was not measured. Both the experimental and control rats consumed the nutrigel/rat chow blend in entirety throughout the duration of the experiment.

### *Accuracy of Measurements*

#### Root Resorption

An intraoperator study was conducted whereby the same operator measured the same 10 samples on two separate occasions. The coefficient of variation (CV) was found to be 7.2%. **Table I**

#### Tooth Movement

The intraoperator error associated with the tooth movement measurement tool had a CV of 1.5% in the sagittal sections and 2.4% in the axial sections. **Table I**

### *Distribution of root resorption cratering*

Root resorption was only measured for the cervical portion of the most mesial root of the first maxillary molar. **Figure 10** The distribution of cratering in this section of the tooth was almost entirely localised to the mesial aspect of the teeth.

### *Effects of CPP on the overall volume of root resorption*

A comparison of the orthodontically loaded molars in the control group (Group 1) which did not receive CPP with the orthodontically loaded molars in the experimental group (Group 2) that received dietary CPP revealed that there was no significance in the decrease of overall root resorption in the experimental group. (p=0.28) **Table II,**

**Table III, Figure 16**

Although the results did not reveal enough evidence to suggest that CPP had a statistically significant effect on root resorption, it did appear to lower the average volume of root resorption by about a 15%. **Table II, Table III, Figure 16**

*Effect of CPP on Tooth Movement*

A comparison of the orthodontically loaded molars in the control group (Group 1) which did not receive CPP with the orthodontically loaded molars in the experimental group (Group 2) that received dietary CPP revealed that there was no significant decrease in the overall amount of tooth movement in the experimental group.( $p=0.42$ ) **Table IV, Table V, Figure 17**

Although the results did not reveal enough evidence to suggest that CPP had a statistically significant effect on tooth movement, it did appear to lower the average amount of tooth movement by about a 15%. **Table IV, Table V, Figure 17**

## 9.5 Discussion

### *Methodology*

This study aimed to investigate the influence of CPP on root resorption. An animal model was chosen so it would be possible to control the level of dietary CPP. Wistar rats were chosen because they have been successful subjects for root resorption experiments using similar methodologies to those used in this experiment<sup>65,66</sup> and they have also been used in experiments involving CPP.<sup>5</sup> All samples used were of the same age at the start of the experiment (11 weeks), and therefore the development of their teeth was the same. Matias *et al*<sup>67</sup> indicated that by 8 weeks of age, there is complete development of root dentine, cementum, periodontal ligaments and alveolar bone and for this reason mature 11 week old rats were chosen for this experiment.

The samples were divided into two groups. Both the control and experimental groups received orthodontic force. It has been shown that orthodontically loaded teeth show more root resorption than unloaded teeth and therefore the study was limited to evaluating the effect of the CPP dietary supplement.<sup>65</sup>

The experimental and control groups had orthodontic force applied to the maxillary first molars to initiate root resorption using a continuous force implemented by use of a Nickel Titanium closed coil (GAC) spring.<sup>65,66,68-74</sup> A heavy force of 150g was used because it was thought that if a reduction in root resorption was to be seen with dietary CPP, it would be more likely to be clearly seen with a heavy force. **Figure 2** The aim of the study was to analyze the maximum effect of the resorptive defects before any



reparative processes occurred. Hellsing and Hammarstrom<sup>75</sup> looked at resorption sites using a scanning electron microscope and found definitive resorption sites forming within a week of appliance placement while Owman Moll *et al*<sup>76</sup> showed cementum repair as soon as 2 weeks after stopping orthodontic tooth movement. It was therefore decided that a two week experimental period would be sufficient to produce resorption sites for analysis. This methodology allowed us to study the maximum resorptive sites after the 2 week experimentation period, without the possibility of the reparative phase.

While CPP has been proven to be effective in remineralising early carious lesions as a topical agent<sup>10</sup> it is not practical in either an animal or human model to administer CPP to root surfaces. CPP has been demonstrated to be safe when administered in the diet of both human and rat studies.<sup>4,5,61</sup> 40mg of CPP per 100g of rat has proven to be effective in preventing bone loss in aged rats and therefore this dosage of dietary CPP was chosen.<sup>5</sup> It would be of interest to look at the effect of different doses of CPPs on root resorption and different durations of supplementation prior to orthodontic tooth movement. This would give some insight as to when the effects of CPPs start becoming manifest.

The root resorption sites were measured using images from the SkyScan 1172 micro CT scanner. The non-destructive nature of these scans facilitated the avoidance of the physical extraction of the rat molars which carries the risk of creating artifacts on the root surface being analyzed. The scanned images were three dimensional in nature and therefore a volumetric analysis of the resorption craters could be quantified. **Figure 12** Previous volumetric analyses using scanning electron microscopy have been limited by

the two dimensional nature of the images which require extensive manipulation in order to achieve a volumetric quantification of root resorption.<sup>77</sup> The three dimensional reproduction of the micro CT images can be manipulated to visualise the location of the craters. **Figure 9** Once localised, software analysis can be undertaken to calculate the volume of the craters. **Figure 11**

In this experiment, all measurements were taken by the same operator in order to eliminate the interoperator variability. The analysis of the volume of root resorption was performed using a new method which was a modification of the method used by Foo *et al.*<sup>65</sup> It was therefore felt that it would be appropriate to determine the error of the measurement. The intraoperator error of the method used for quantification of root resorption was found to have a coefficient of variation (CV) of 7.2% which was considered to be acceptable. **Table I** The tool used to measure tooth movement in this study also involved a new protocol. The error of this tool was again determined using 10 samples which were measured on two separate occasions. The error in this measurement was lower than the root resorption measurement with a CV of 1.5% in the sagittal sections and 2.4% in the axial sections. **Table I** The use of the digital tool for the measurement of tooth movement proved to be useful in reducing the parallax error associated with measuring tooth movement in one plane as is the case when measuring tooth movement on a lateral cephalogram. **Figure 1, Figure 13**

### *Root Resorption*

Although the results did not reveal enough evidence to suggest that dietary supplements of casein phosphopeptides had a statistically significant effect on root resorption ( $p=0.28$ ) it did appear to reduce the average amounts of root resorption in orthodontically loaded teeth by about 15%. **Table II, Table III, Figure 16** While this trend in reduction of root resorption is encouraging, the trend was not statistically significant due to individual variability.

The effect of CPP on orthodontic tooth movement, root resorption and the periodontium is unclear as there are no previous investigations. Studies on the provision of dietary CPP to osteoporotic rats and calcium deficient humans give some insight into its effects on bone metabolism.<sup>4,5</sup>

Although cementum is more resistant to resorption relative to bone it is still possible for both the cementum and dentine to resorb as a result of inflammation associated with orthodontic tooth movement. This process is an unavoidable consequence of orthodontic tooth movement. The mineralized tissues of the permanent dentition are normally protected in the root canal by dentine and osteoblasts, and on the root surface by cementum and cementoblasts.<sup>16,17</sup>

A number of studies have shown that medications which prevent resorption of bone are associated with a reduction in both tooth movement and root resorption. Non-steroidal anti-inflammatory drugs (NSAIDs), have been shown to affect the sequence of tooth

movement by reducing the associated inflammatory and bone resorptive process.<sup>78</sup> This results in a decrease in tooth movement which in turn translated into a decrease in root resorption.<sup>79</sup> Mavragani *et al*<sup>80</sup> showed a similar association in a study which looked at the effect of low-dose systemic administration of the tetracycline, doxycycline (DC). The results revealed a significant reduction in root and bone resorption for the DC-administered group.<sup>80</sup> Similarly, Liu *et al*<sup>81</sup> showed this association between bone and root resorption in a study which looked at the effects of the local administration of the bisphosphonate clodronate on orthodontic tooth movement and root resorption in rats. Clodronate strongly inhibits bone resorption and consequently they found that there was a dose-dependent reduction in tooth movement in the rats and again there was found to be a reduction in root resorption. It is possible that the trend of decreased tooth movement in the rats which received dietary CPP in this study may be related to a decrease in the amount of bone resorption which ultimately may have contributed to the trend in decreased root resorption seen in the experimental animals.

### *Tooth Movement*

With respect to tooth movement, the results revealed that CPP did not have a statistically significant effect on the reduction in the average tooth movement between the experimental and control groups.( $p=0.42$ ) **Table IV, Table V, Figure 17** However there was a reduction in the average tooth movement by about 15%.

The trend towards a decrease in tooth movement associated with dietary supplements of CPP may be attributed to the mineralizing effect that it has on the skeleton.<sup>4,5,7,61</sup> It is

possible that the increase in bone retention observed in subjects fed CPP may have an inhibitory effect on orthodontic tooth movement because tooth movement relies on the removal of bone.<sup>82,83</sup>

Increases in tooth movement have been demonstrated in studies which have reduced bone mineralisation. Kale *et al*<sup>84</sup> looked at the effect of 1,25 dihydroxycholecalciferol on orthodontic tooth movement and found that it increased tooth movement. 1,25 dihydroxycholecalciferol is a biologically active Vitamin D derivative which induces the differentiation of osteoclasts, and increases the activity of existing osteoclasts. Kale *et al* suggested that the increase in the resorption of bone promotes tooth movement.

At present the interaction between bone metabolism and cementum remodelling is unclear. While there is evidence to suggest that CPP has a positive effect on bone retention, there is only anecdotal evidence to suggest that it could possibly have a positive effect on inhibiting cementum resorption. The variability of the results in this study can be explained in part by the complex nature of the interaction between bone and cementum remodelling and resorption. While the results were not statistically significant, the general trend in reduction of root resorption suggests that further investigation is warranted to clarify what effect casein phosphopeptides may have on the reduction in the solubility of the alveolar bone and the dental tissues.

Previous research on aged ovariectomized rats<sup>4</sup> and post-menopausal women<sup>7</sup> has demonstrated the anabolic effect that CPPs have on bone retention in both rats and

humans with low basal absorptive performance. This suggests that CPPs may have a more significant effect on subjects with low basal absorptive performance.

Due to the lack of statistical significance found in this study it is not possible to say that dietary CPP provides significant protection against root resorption. However the results lend some support for the argument that dairy products, from which CPPs are derived, may have a role in protecting against root resorption due to their ability to make calcium available to the mineralized tissues of the body.

## **9.6 Conclusions**

The results of this study show that

1. CPP seems to have a variable effect on the volumetric quantification of root resorption. While on average, the amount of resorption observed in rats fed dietary CPP was less, individual variability makes this effect statistically insignificant.
2. CPP did not have a statistically significant effect on reducing tooth movement however there was an overall decrease in the average tooth movement.
3. Although it is possible that CPP may have a beneficial effect on reducing cementum solubility it may be counteracted by its anabolic effect on bone mass which explains the variability observed in this study.

## **9.7 Acknowledgement**

This study was supported by the Australian Society of Orthodontics Foundation for Research and Education Inc, The Australian Dental Research Foundation and GAC Australia Pty Ltd.

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Table I: Intraoperator error for the measurements of the total volume of root resorption, tooth movement in the sagittal plane and tooth movement in the axial plane

	RR	TMs	TMa
mean	12106.95	38.3105	36.6145
mse	757759	0.337815	0.773585
<b>SE mt</b>	<b>870</b>	<b>0.581</b>	<b>0.880</b>
<b>CV(%)</b>	<b>7.19</b>	<b>1.52</b>	<b>2.40</b>

Table II: Univariate analysis of variance on the effect of CPP on the total volume of root resorption craters

**Tests of Between-Subjects Effects**

Dependent Variable: RRvol

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	64577948.843(a)	1	64577948.843	1.207	.281
Intercept	3209088090.263	1	3209088090.263	59.975	.000
CPP	64577948.843	1	64577948.843	1.207	.281
Error	1551697189.093	29	53506799.624		
Total	4945254171.000	31			
Corrected Total	1616275137.936	30			

a. R Squared = .040 (Adjusted R Squared = .007)

Table III: Estimated Marginal Means

**CPP**

Dependent Variable: RRvol

CPP	Mean	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound
no	11672.529	1774.108	8044.071	15300.987
yes	8772.286	1954.972	4773.920	12770.652

Table IV: Univariate analysis of variance on the effect of CPP on tooth movement

**Tests of Between-Subjects Effects**

Dependent Variable: TMav

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	175.700(a)	1	175.700	.677	.418
Intercept	26222.201	1	26222.201	100.987	.000
CPP	175.700	1	175.700	.677	.418
Error	7270.441	28	259.659		
Total	34728.877	30			
Corrected Total	7446.141	29			

a R Squared = .024 (Adjusted R Squared = -.011)

Table V: Estimated marginal means

**CPP**

Dependent Variable: TMav

CPP	Mean	Std. Error	95% Confidence Interval	
			Lower Bound	Upper Bound
no	32.273	3.908	24.267	40.279
yes	27.389	4.469	18.234	36.544

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Figure 1: Lateral Cephalogram showing a 150g Nickel Titanium coil attached from the maxillary incisors to the first molar

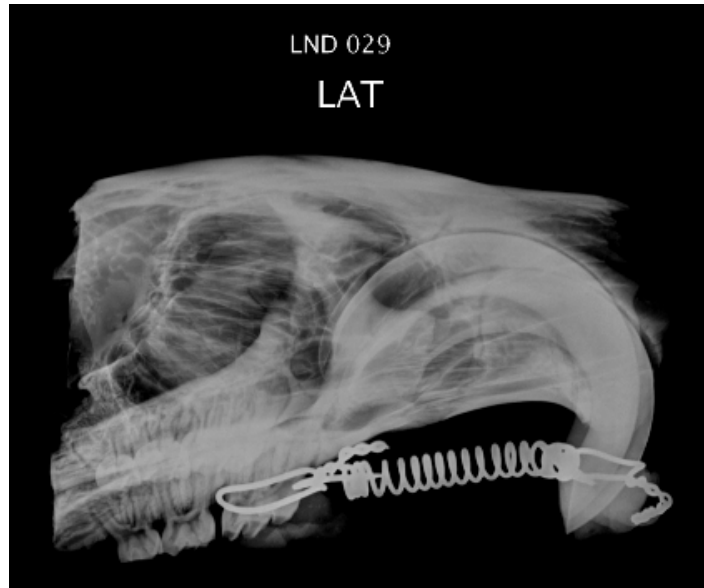


Figure 2: Nickel Titanium coil attached from the maxillary incisors to the first molar and bonded with composite resin.



Figure 3: Experiment Flow Chart

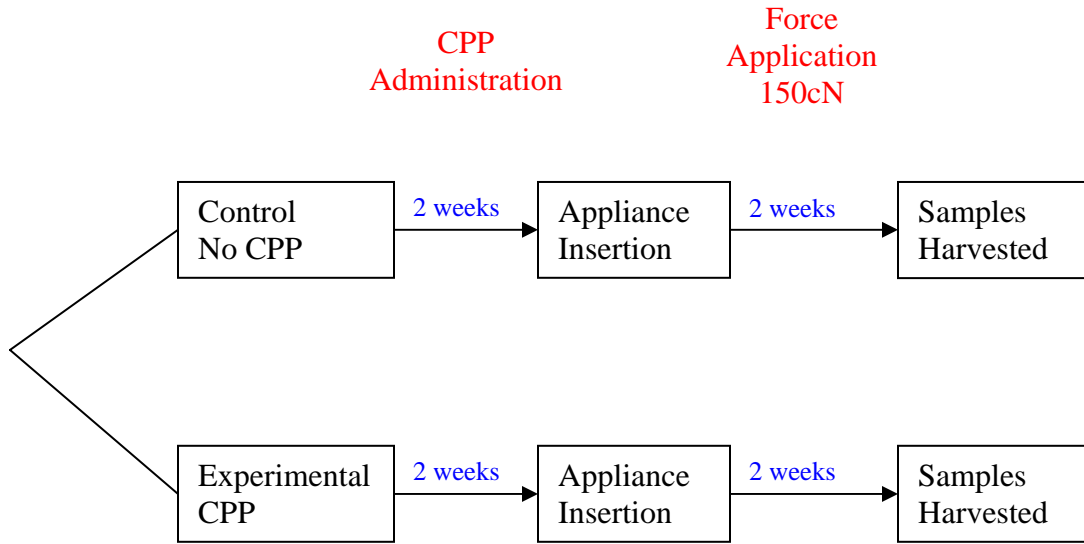


Figure 4: SkyScan 1172 **Desk Top microCT X-ray scanner** (SkyScan, Aartsellaar, Belgium)



Figure 5: Diagram of the Micro CT Scanner

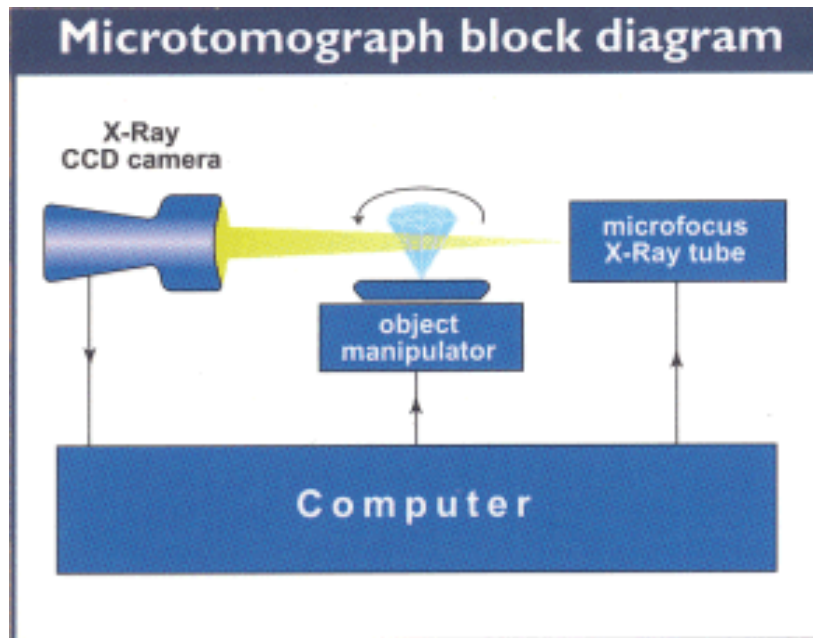


Figure 6: Scanned segment of the maxilla including three maxillary molars. The first maxillary molar is highlighted in yellow.

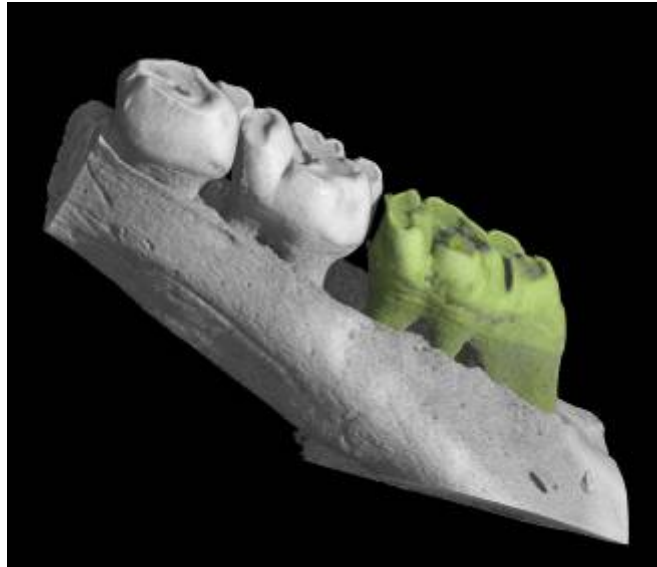


Figure 7: First maxillary molar and the mesial portion of the second maxillary molar. The thin red line between the contacts of the first and second molars represents the shortest distance between the two surfaces as detected by the software tool.

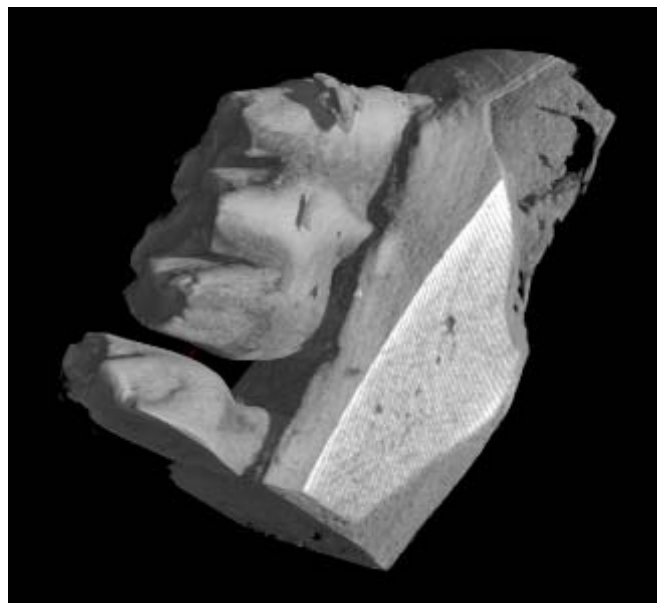




Figure 8: Digital Extraction of Rat Molar



Figure 9: Manipulation of the three dimensional image of the first maxillary rat molar

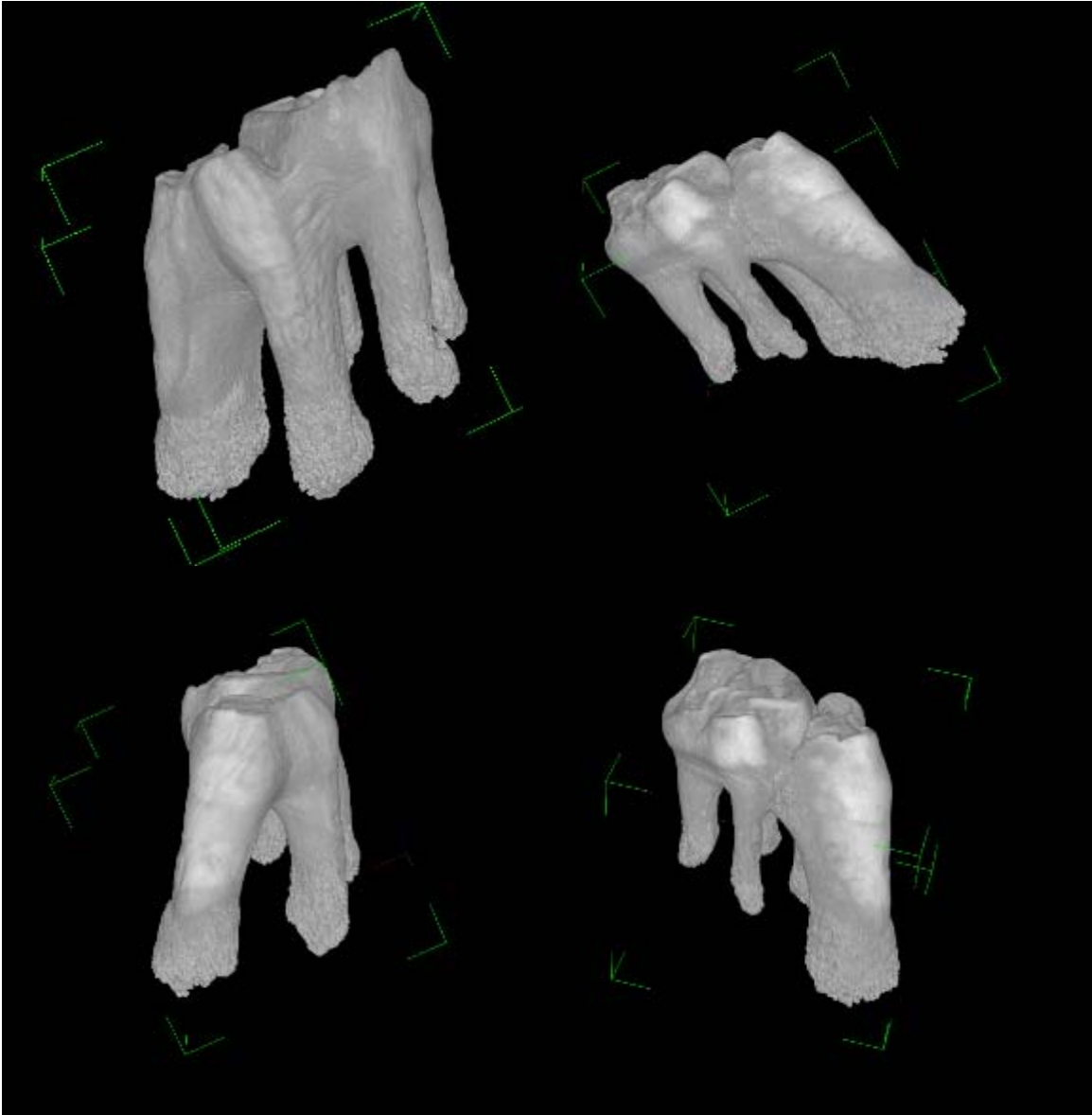


Figure 10: The mesial root of the maxillary first molar. The area defined by the red lines is the segment of the root which was analysed for root resorption. The porous apical region can be seen in this view.

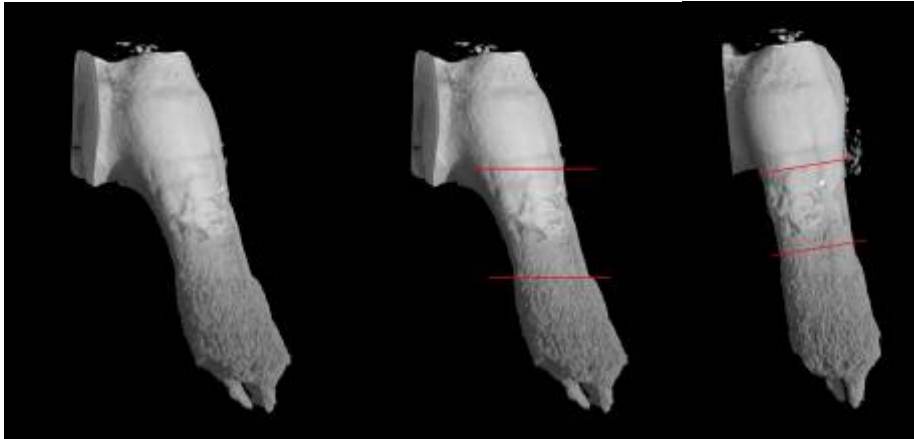


Figure 11: Crater isolation method

- a) Location of the craters in cross section
- b) Tracing of the crater outline
- c) Isolated craters. Isolated craters for each axial section are accumulated to achieve an overall calculation of the total volume of root resorption.

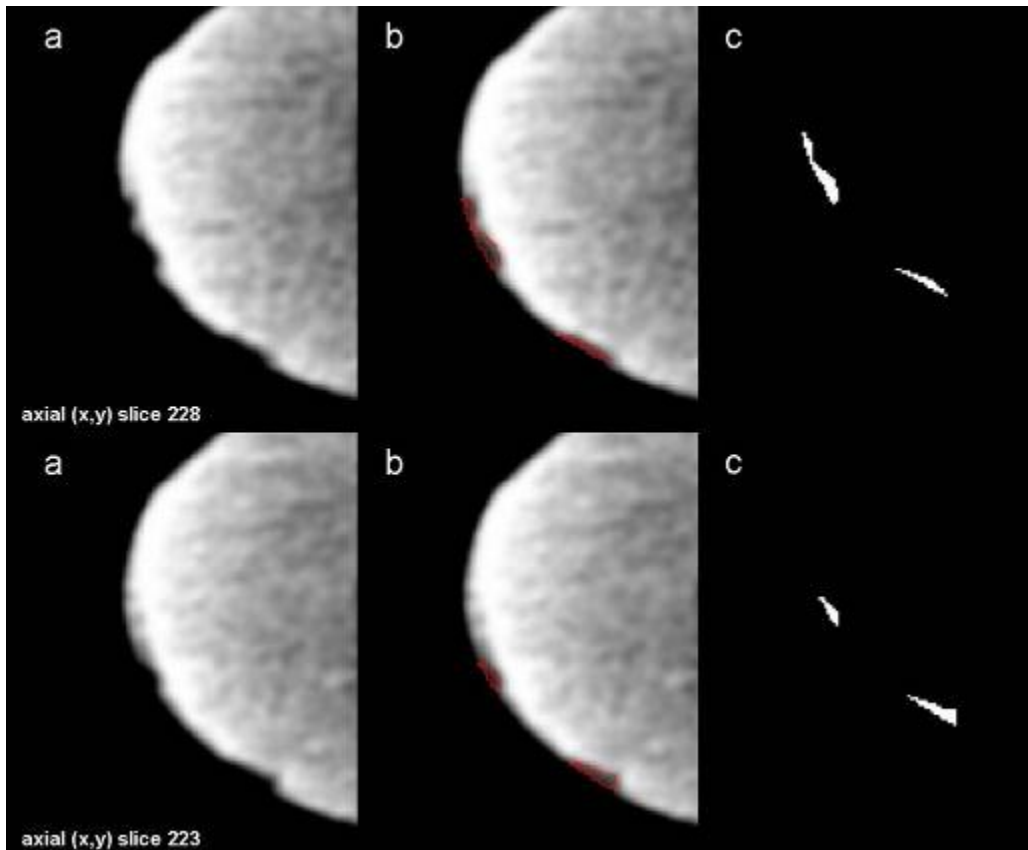


Figure 12: Total volume of root resorption. These images demonstrate the sequence of crater identification and isolation. The volume highlighted in green represents the total volume of root resorption which be calculated using the software.

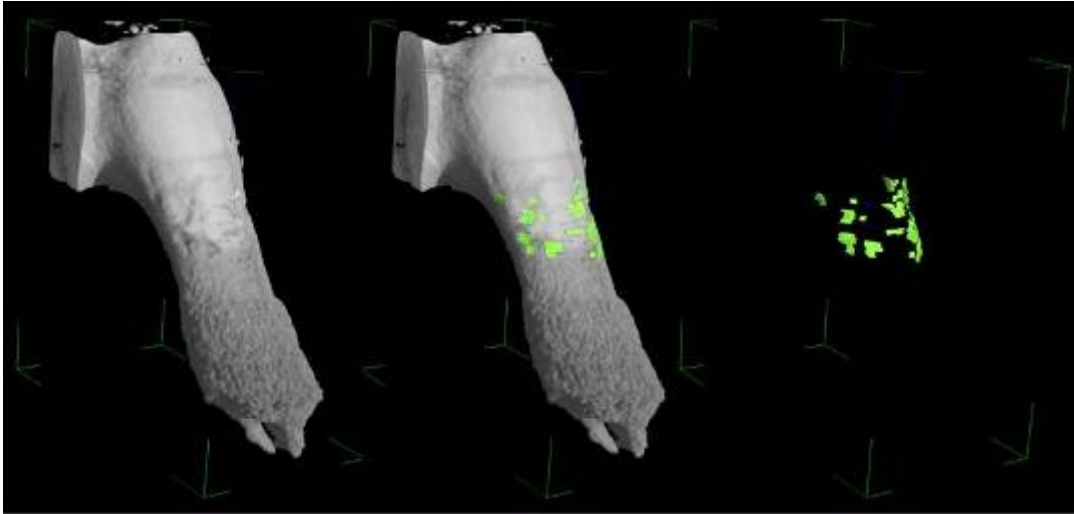


Figure 13: Parallax error when viewing the distance that the tooth has moved in one plane

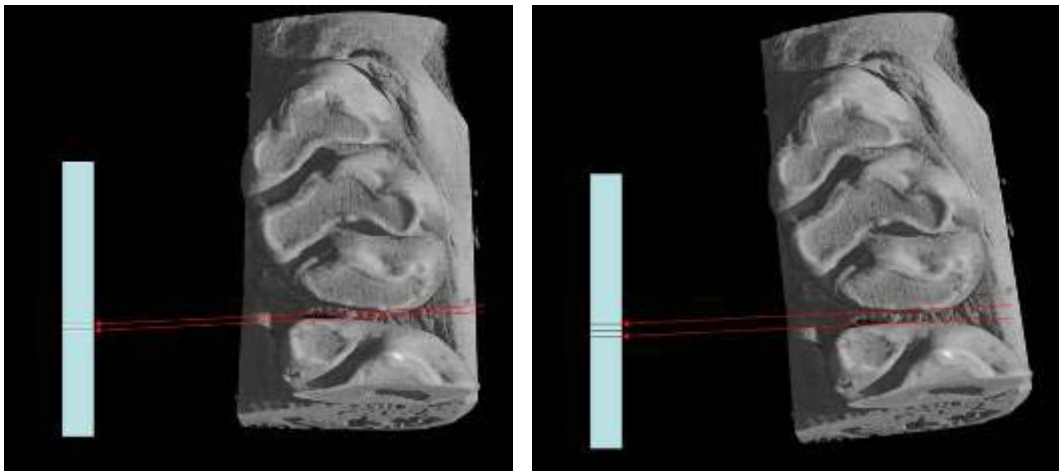


Figure 14: Tooth movement measured in the axial plane.



Figure 15: Tooth movement measured in the sagittal plane

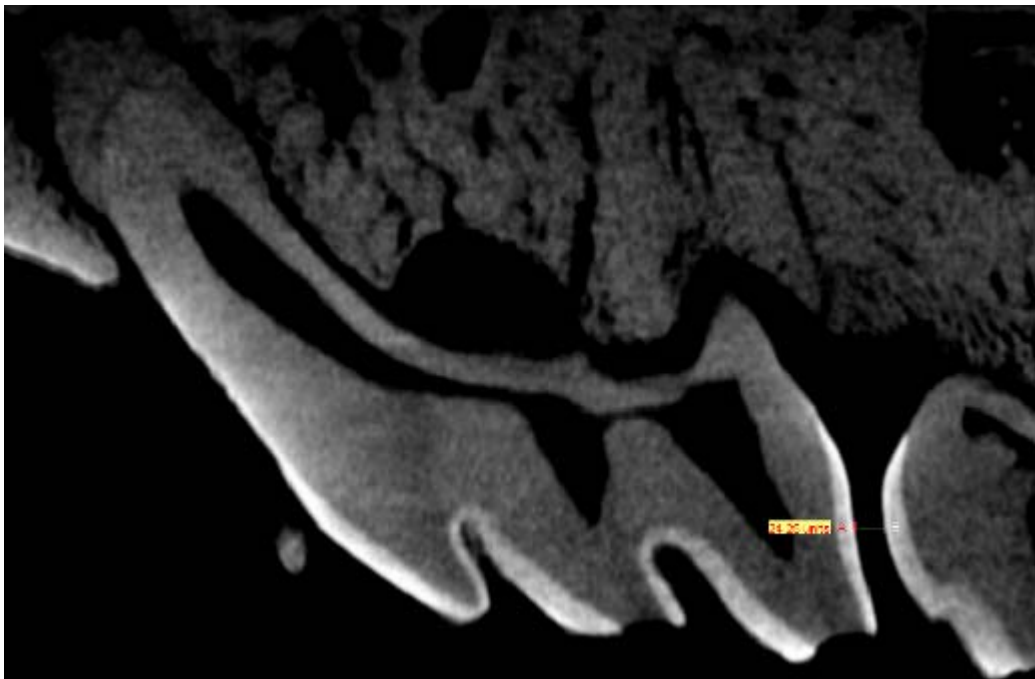


Figure 16: Box-plot of the total volume of root resorption for no dietary supplements and dietary supplements of CPP.

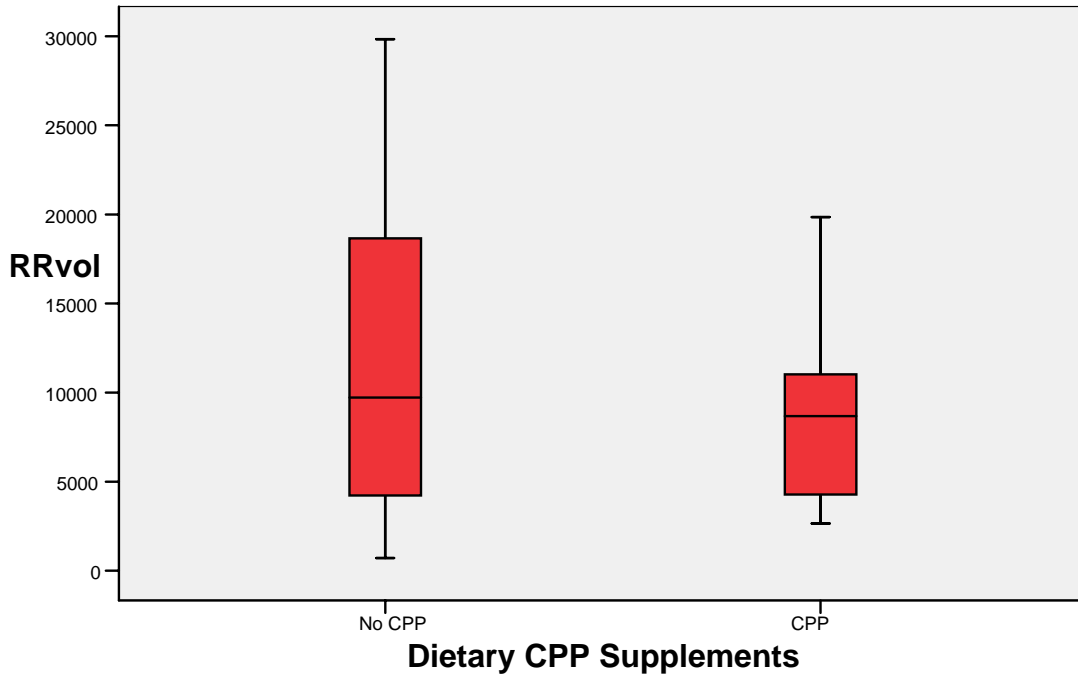


Figure 17: Box-plot for the amount of tooth movement for no dietary supplements and dietary supplements of CPP.

