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Bone reactions to longstanding functional load at implants: an experimental study in dogs

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Abstract

Objectives: The aims of the present investigation were (i) to study marginal bone level alterations following implant installation, abutment connection and functional loading and (ii) to analyse bone tissue reactions to functional load.

Material and Methods: Six beagle dogs, about 1-year old, were used. All mandibular pre-molars were extracted. Three months later four implants of the Astra Tech Implants® Dental System were installed in one side of the mandible and four standard fixtures of the Brånemark System® were placed in the contralateral side of the mandible. Abutment connection was performed 3 months later and a plaque control programme was initiated. Three months after abutment connection fixed partial dentures (FPDs) made in gold were cemented to the maxillary canines and pre-molars. FPDs were also connected to the three posterior implants in each side of the mandible, while the mesial implant in each side was used as an unloaded control. Radiographs were obtained from all implant sites following implant installation, abutment connection and FPD placement. Ten months after the FPD placement the radiographic examination was repeated. The animals were sacrificed and biopsies from all implant sites were obtained and prepared for histological analysis.

Results: The radiographic analysis revealed that largest amount of bone loss occurred following implant installation and abutment connection and that this loss was more pronounced at Brånemark than at Astra implants. The bone level alterations that were observed at implants exposed to 10 months of functional load in both implant systems were small and did not differ from control sites. The histological analysis revealed that implants exposed to functional load exhibited a higher degree of bone-to-implant contact than control implants in both implant systems.

Conclusion: It is suggested that functional load at implants may enhance osseointegration and does not result in marginal bone loss.

Key words: bone loss; fixed partial denture; histology; radiographs; titanium

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Marginal bone loss assessed in radiographs is a critical outcome variable in implant dentistry and is included as one of the several proposed success criteria (Albrektsson et al. 1986, Albrektsson & Isidor 1994). It was recommended that a baseline radiographic examination of an implant site should be carried out in conjunction with the insertion of the prosthesis and be repeated at 1-, 3- and 5-year intervals (Wennström & Palmer 1999). Results from earlier clinical studies on implants indicated that marginal bone loss, as a result of bone re-modelling to functional load, was larger in the first year in function than during the subsequent years (Adell et al. 1981, 1986, Lindquist et al. 1988, 1996). It was suggested that the initial marginal bone level change occurred as an adaptation of the peri-implant bone to the load applied to the implants during function (Adell et al. 1981).

In a consensus report from the 3rd European Workshop on Periodontology it was stated, ''there is no evidence of loss of osseointegration due to occlusal loading in man manifested by progressive marginal bone loss'' (Nilner & Lundgren 1999). Such evidence, however, was provided in animal experiments examining the effect of different types of load on implants. Hoshaw et al. (1994) applied excessive cyclic axial load on implants placed in the tibiae of 10 dogs. Analysis performed 6 and 12 weeks following loading revealed that marginal bone loss had occurred at test but not at control implants. Duyck et al.

(2001) in a similar study in rabbits reported that dynamic load to implants resulted in the establishment of marginal crater formed defects, while no obvious effect on osseointegration could be identified in other parts of the implant.

It was reported that excessive occlusal load under certain conditions may result in loss of osseointegration along the entire implant (Isidor 1996, 1997), while there are conflicting data regarding marginal bone level alterations as result of occlusal load (Isidor 1996, Barbier & Schepers 1997, Miyata et al. 2000, Heitz-Mayfield et al. 2004). In experimental models employing static lateral load no or only a minimal influence was observed on the marginal bone support at implants (Gotfredsen et al. 2001a–c, 2002, Duyck et al. 2001).

The aims of the present investigation were (i) to study marginal bone level alterations that occur following implant installation, abutment connection and functional loading and (ii) to analyse bone tissue reactions to functional load.

Material and Methods

Six beagle dogs, about 1 year old, were included (The protocol of the present study was approved by the regional Ethics Committee for Animal Research, Göteborg, Sweden). During all surgical procedures the animals were under general anaesthesia induced with propofol (10 mg/ml, 0.6 ml/kg) intravenously and sustained with N_2O:O_2 (1:1.5–2) and isoflurane employing endotracheal intubation. At the start of the experiment all mandibular pre-molars ($_4P_4$, $_3P_3$, $_2P_2$, $_1P_1$) were extracted. Three months later crestal incisions were made and mucoperiostal flaps were raised in the edentulous pre-molar regions. In one side of the mandible four implants of the Astra Tech Implants® Dental System (Astra Tech AB, Mölndal, Sweden) were installed (two ''standard'' TiOblast™ implants and two Fixture MicroThread™; 8 × 3.5 mm). In the contralateral side of the mandible four standard fixtures (SDCA 002; 375 × 7 mm) of the Brånemark System® (Nobel Biocare AB, Göteborg, Sweden) were placed.

The implants were placed according to the protocol given in the manual provided by the manufacturer. Thus, the Astra implants were placed in such a way that the implant margin coincided with the bone crest (Fig. 1) and the Brånemark implants were placed to the depth indicated by the reference mark on the fixture mount (i.e. the platform of the implant was located 1.4 mm below the bone crest) (Fig. 2). Radiographs were obtained immediately after fixture installation using a custom made film holder device (Abrahamsson et al. 1999) connected to the posterior implant. In the radiographs the distance between the abutment-fixture junction (A/F) and the marginal bone level (B) was determined at the mesial and distal aspect of each implant. The measurements were carried out using a Leica DM-RBE® microscope (Leica, Wetzlar, Germany) equipped with an image system (Q-500 MC®, Leica). Cover screws were placed and the flaps were sutured to cover the implants. The sutures were removed after 2 weeks.

Three months later all implants were uncovered and Uni abutments (1.5 mm/20°; Astra Tech Implants® Dental System) and standard abutments (4.0 mm; Brånemark System®, Nobel Biocare AB) were connected. The flaps were sutured and a new set of radiographs was obtained. Sutures were removed 2 weeks later and a plaque control program (daily cleaning of all exposed implant surfaces and neighbouring teeth using toothbrush and dentifrice) was initiated and maintained until the end of the experiment.

The maxillary canines and pre-molars were exposed to full-crown preparations. In the mandibular pre-molar regions impression pick-up copings compatible for each implant system were connected to the three posterior implants in each quadrant. Impressions from the maxillary and mandibular pre-molar segments were obtained using individual acrylic impression trays and polyether impression materials (Impregum®; ESPE, Seefeld, Germany and Permadyne®; ESPE). Three months after abutment connection fixed partial dentures (FPDs) made in gold (Fig. 3) were cemented to the maxillary canines and pre-molars using an adhesive resin-cement (Panavia® 21; Kuraray Co. Ltd., Osaka, Japan). FPDs were also connected to the three posterior implants (test implants) in each side of the mandible (Fig. 4), while the mesial implant in each side was used as an unloaded control. Occlusal contact between the maxillary and the mandibular pre-molar bridge segments was established and appropriate load distribution between the left and the right side was achieved (Fig. 5). Immediately after placement of the FPDs, a new set of radiographs from all implant sites was obtained using an individually prepared film holder device (Hawe-Super-Bite™, Hawe-Neos Dental, Bioggio, Switzerland) and an impression material (Impregum®). Ten months after the FPD placement the radiographic examination was repeated and a clinical examination including assessments of plaque and soft tissue inflammation was performed.

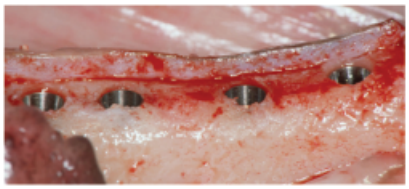

Fig. 1. Clinical photograph of four Astra fixtures immediately following implant installation. Note the position of the implant marginal in relation to the adjacent bone crest.

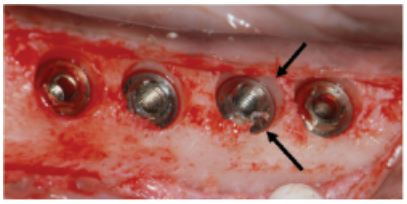

Fig. 2. Clinical photograph of four Brånemark fixtures immediately following implant installation. Arrows indicate the 1.4 mm distance between the rim of the implant and the adjacent bone crest.

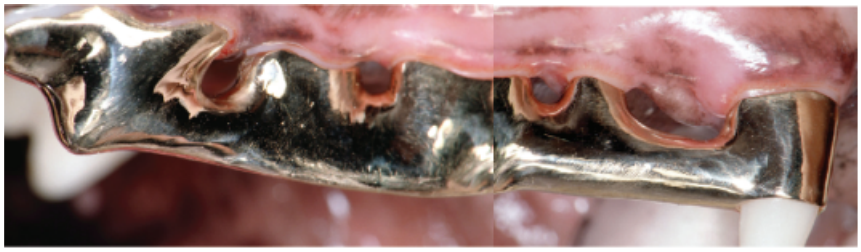

Fig. 3. Clinical photograph of a fixed partial denture supported by maxillary canine and premolars.

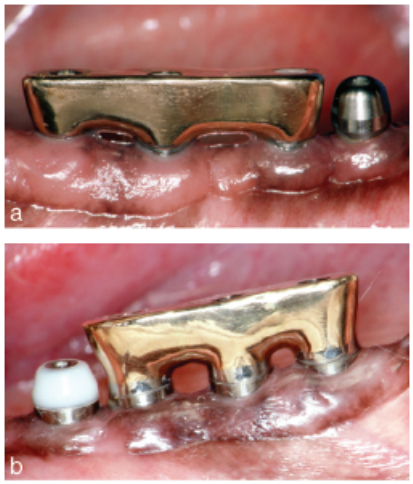

Fig. 4. Fixed partial dentures made of gold on three Astra (a) and three Brånemark (b) implants. The non-loaded control implant in the mesial position.

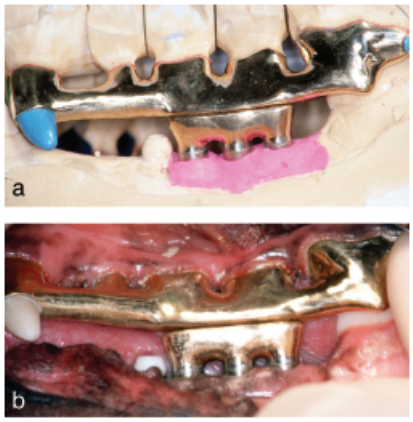

Fig. 5. Maxillary and mandibular fixed partial dentures in contact on a cast model (a) and following connection to teeth and implants (b).

The animals were sacrificed with an overdose of sodium pentothal and perfused with a fixative through the carotid arteries. The fixative consisted of a mixture of 5% glutaraldehyde and 4% formaldehyde buffered to pH 7.2 (Karnovsky 1965). The mandibles were removed and placed in the fixative. Each implant region was dissected using a diamond saw (Exakt®, Kulzer, Friedrichsdorf, Germany) and further processed for ground sectioning. The tissue blocks were dehydrated in serial steps of alcohol concentrations and subsequently embedded in a methyl–methacrylate resin (Technovit® 7200 VLC, Exakt®, Kulzer). Using a cutting-grinding unit and a micro-grinding system (Exakt®, Apparatebau, Norderstedt, Germany) the blocks were cut in a mesio-distal plane and two central sections were obtained. From the buccal part of the tissue block (containing 40–45% of the implant and the surrounding tissues) two central sections in a buccal–lingual plane were prepared. All sections were reduced to a final thickness of approximately 20 μm. Thus, from each implant block two mesio-distal and two buccal–lingual ground sections were obtained. The sections were stained in toluidine blue (Donath 1993).

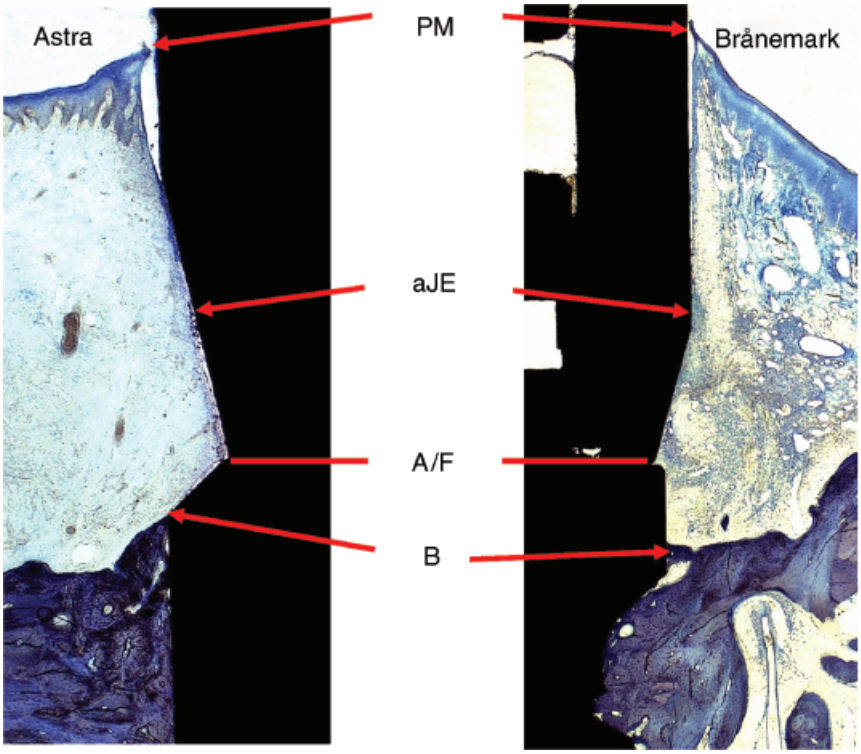

Fig. 6. Cross-sections of the marginal portion of the periimplant tissues at one Astra (left) and one Brånemark (right) implant. The landmarks indicate the marginal position of the peri-implant mucosa, the apical termination of the barrier (junctional) epithelium (aJE), the level of the abutment/fixture border and the marginal level of bone- to- implant contact (B).

Histological analysis

The histometric and morphometric measurements were performed in a Leica DM-RBE® microscope (Leica) equipped with an image system Q-500 MC® (Leica). The following landmarks were used for the linear measurements (Fig. 6); the marginal position of the peri-implant mucosa (PM), the apical termination of the barrier (junctional) epithelium (aJE), the marginal level of bone to implant contact (BIC) (B) and the level of the (A/F) border. The distances between the various landmarks were determined.

Measurements describing BIC%, i.e. the fraction (%) of mineralized bone that was in direct contact with the implant surface, were performed at a magnification $\times$ 100. Bone density (proportion of mineralized bone) analysis was carried out using a point counting procedure at a magnification $\times$ 200. A lattice comprising 100 light points was superimposed over the area to be examined and the mineralized and non-mineralized structures were identified using a mouse cursor. The analysis was confined to the bone tissue located between the threads of the implant and a 200 μm wide zone lateral to the threads of the entire implant.

Statistical analysis

Differences between unloaded implant units and sites exposed to functional load within each implant system were analysed using the Student's *t*-test for paired comparisons ($n = 6$). A similar test was applied to analyse differences between the implant systems. *p*-values <0.05 were considered as significant.

Results

Clinical observations

Healing following implant placement and subsequent abutment connection

was uneventful in all but one implant site. A minor abscess formation that resulted in a circumferential 3 mm deep angular bony defect occurred in one of the Astra implant sites after fixture installation. This site was excluded from the radiographic and histological examination. The peri-implant mucosa at all remaining sites was found to be clinically healthy from the time of abutment connection and throughout the study period. No technical problems related to the tooth- or the implant-supported FPDs were observed during the 10 months of functional load.

Radiographic measurements

Radiographs obtained from the implant sites at different time intervals are illustrated in Fig. 7 and the results from the radiographic measurements are presented in Table 1. Marginal bone loss occurred during the 3-month healing period between implant installation and abutment connection (Phase 1) and amounted to 0.12 ± 0.19 mm at Astra implants and 0.53 ± 0.18 mm at Brånemark implants. This difference was statistically significant. Continuous loss of marginal bone support was detected at Brånemark implants (0.27 ± 0.18 mm) between abutment connection and bridge connection (Phase 2; 3 months), while a small gain (0.05 ± 0.13 mm) was observed at Astra implants during the same period. Also this difference was statistically significant. During the course of the 10 months of functional load (Phase 3) the marginal bone level remained virtually unchanged at all sites and no differences were found between sites exposed to functional load and control units. The overall mean changes in marginal bone level throughout the entire study period were significantly larger at Brånemark implants than at Astra implants at both control sites and sites exposed to functional load (0.74 ± 0.32 *versus* 0.13 ± 0.43 mm and 0.77 ± 0.42 *versus* 0.09 ± 0.16 mm, respectively).

Histologic observations

In the ground sections produced from the tissue blocks representing control sites and sites exposed to functional load of both implant systems, the implants were surrounded by a dense, mature bone tissue that was comprised of lamellar bone and bone marrow (Figs 8–11). The implant units subjected to 10 months of functional load in both the Astra and Brånemark system frequently exhibited the presence of large amounts of bone multi-cellular units (BMUs) in the bone–implant interface (Fig. 12). Re-modelling areas were less frequent in the control sites.

Soft tissue dimensions

The results from the linear measurements are presented in Table 2. The height of the peri-implant mucosa (PM-B) at control sites and sites exposed to functional load varied between 3.62 and 3.32 mm for the Astra system and between 4.28 and 3.84 mm for the Brånemark system. The barrier epithelium (PM-aJE) at Astra control and functional load sites was 2.07 and 2.02 mm long, while the corresponding dimension assessed at Brånemark

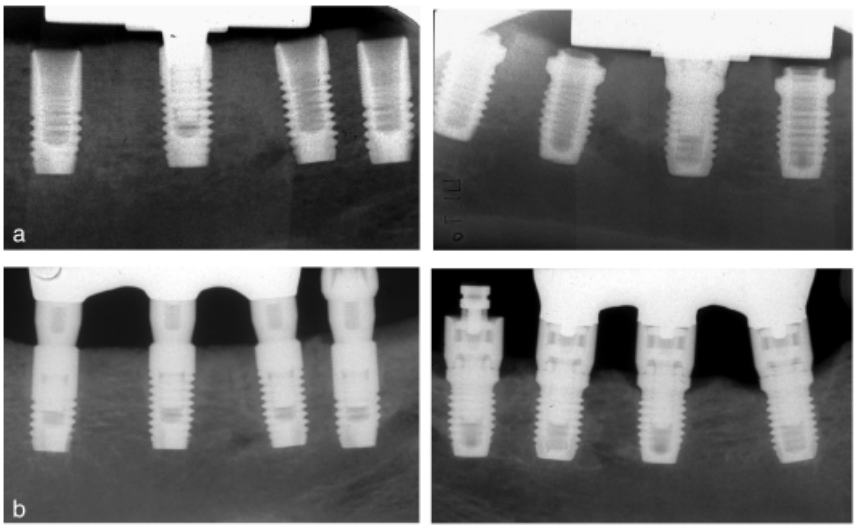

Fig. 7. Radiographs obtained from the Astra (left) and Brånemark (right) implants immediately following implant installation (a) and following 10 months of functional load (b).

Table 1. Results from the radiographic measurements

	Astra			Brånemark
Phase 1	−0.12 (0.19)	*		−0.53 (0.18)
Phase 2	+0.05 (0.13)	*		−0.27 (0.18)
	Control	Functional load	Control	Functional load
Phase 3	−0.06 (0.36)	−0.02 (0.20)	+0.07 (0.35)	+0.03 (0.32)
Total	−0.13 (0.43)	−0.09 (0.16)	−0.74 (0.32)	−0.77 (0.42)
	*		*	

Bone level alterations (in millimetres) during three phases. Phase 1 (3 months): fixture installation - abutment connection, Phase 2 (3 months): abutment connection–bridge connection, Phase 3 (10 months): bridge connection–biopsy. Mean values and standard deviation (SD).
*$p < 0.05$.

Fig. 8. Mesio-distal cross-section of a non-loaded control implant unit of the Brånemark system. Original magnification × 16. Toluidine blue.

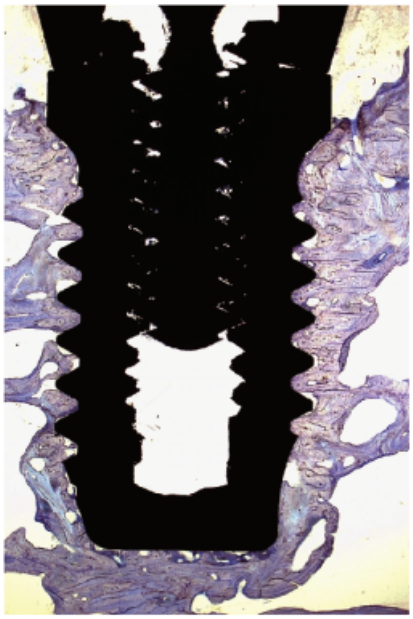

Fig. 9. Mesio-distal cross-section of a test implant site of the Brånemark system. Original magnification × 16. Toluidine blue.

Fig. 11. Mesio-distal cross section of a test implant site of the Astra system. Original magnification X 16. Toluidine blue.

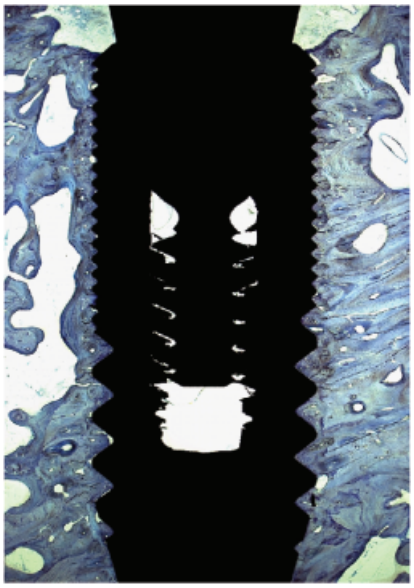

Fig. 10. Mesio-distal cross-section of a non-loaded control implant unit of the Astra system. Original magnification × 16. Toluidine blue.

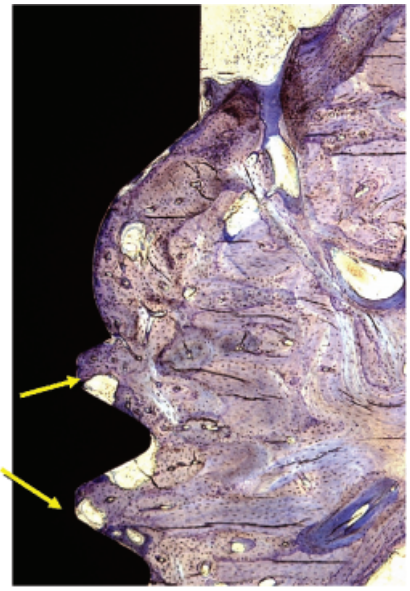

Fig. 12. A larger magnification of the marginal portion of the Brånemark implant unit representing the test group illustrated in Fig. 9. Yellow arrows indicate bone multi-cellular units.

implants was 2.35 and 2.27 mm, respectively.

The marginal level of BIC (B) at Astra implants was located 0.52 and 0.37 mm ''apical'' of the (A/F) junction at control and test sites. The corresponding figures for the Brånemark implants were 0.75 and 0.74 mm, respectively. The differences between Astra and Brånemark implants for control sites and sites exposed to functional load were statistically significant.

Bone tissue analysis

The results from the assessments of the degree of BIC% are presented in Table 3. A significantly higher percentage of BIC was found in sites exposed to functional load than in control units for both implant systems. Furthermore, the BIC levels were significantly higher for Astra implants than Brånemark implants in both types of sites.

The data from the determination of bone density between threads and in a 200 μm wide zone lateral to the implant are reported in Table 4. The mean density varied between 73.5% (Astra) and 82.1% (Brånemark) in control units. In the sections representing functional load the bone density amounted to 79.5% for Astra and 81.2% for Brånemark implants.

Discussion

In the present animal experiment, marginal bone level alterations that occurred following implant installation and abutment connection and during functional loading were analysed. It was demonstrated that largest amount of bone loss occurred following implant installation and abutment connection and that this loss was more pronounced at Brånemark than at Astra implants. The bone level alterations that were observed at implants exposed to 10 months of functional load in both implant systems were small and did not differ from control (unloaded) sites. Furthermore, the histological analysis revealed that implants exposed to functional load exhibited a higher degree of bone-to-implant contact than control implants in both implant systems. It is suggested that functional load at implants may enhance osseointegration and does not result in marginal bone loss.

Marginal bone loss

In the present study bone level change over time was analysed using radiographs obtained immediately after implant installation as the day 0 reference. A similar design was applied in a clinical study on Astra and Brånemark implants evaluated at 1, 3 and 5 years of function (Åstrand et al. 1999, 2004, Engquist et al. 2002). Åstrand et al. (1999) placed 184 Astra implants and 187 Brånemark implants in edentulous jaws of 68 patients. It was reported that the mean bone loss that occurred between fixture installation and abutment connection was somewhat larger at Astra than at Brånemark implants,

Table 2. Results from the histometric measurements (millimetres)

	Astra		Brånemark	
	Control	functional load	control	functional load
PM-B	3.62 (0.53)	3.52 (0.56)	4.28 (0.86)	3.84 (0.56)
PM-aJE	2.07 (0.15)	2.02 (0.32)	2.35 (0.38)	2.27 (0.27)
A/F-B	0.52 (0.60)	0.37 (0.16)	0.75 (0.35)	0.74 (0.28)
		*		
				*

The landmarks are described in Fig. 10. Mean values and standard deviation (SD).
*$p < 0.05$.

Table 3. The degree of bone to implant contact (BIC) (%)

	Control		Functional load
Astra	77.4 (8.0)	*	83.6 (4.6)
	*		*
Brånemark	61.0 (10.5)	*	67.0 (10.4)

Mean values and standard deviation (SD).
*$p < 0.05$.

Table 4. Bone density between threads and in a 200 micrometres wide zone lateral to the implant (%)

	Control	Functional load
Astra	73.5 (8.4)	79.5 (4.8)
Brånemark	82.1 (4.7)	81.2 (3.7)

Mean values and standard deviation (SD).

while an opposite relationship was found regarding bone loss in the interval between abutment connection and insertion of the prosthesis. These findings are not entirely consistent with data from the current animal experiment. Thus, during Phase 1 and 2 in the present study bone loss was significantly larger at Brånemark than at Astra implants. Hence, while in both studies bone remodelling following fixture installation resulted in different amounts of bone loss at the two implant systems, the ensuing bone loss after abutment connection was consistently larger at Brånemark than at Astra implants. This difference between the two systems may be related to differences regarding implant design and access to the fixture at abutment connection.

Åstrand et al. (1999) reported that the average marginal bone loss that took place between baseline (bridge insertion) and the 1-year re-examination in both implant systems was considerably smaller than the bone loss that occurred between fixture installation and baseline. This observation is in agreement with findings of the current experiment and suggests that bone re-modelling is more pronounced after the surgical trauma, including implant installation and abutment connection, than during the period of functional load. It is also evident that differences in design and geometry between the Astra and the Brånemark implant systems in the present study influenced the bone re-modelling following the surgical therapy, while no such effects were identified during the period of functional load.

In this context it should be realized that during implant installation of the Brånemark implants a countersink preparation is made in the coronal part of the canal to provide space for the neck portion of the implant. Following implant placement the rim of the neck portion will thus be located 1.4 mm apical of the adjacent bone crest (Fig. 2). During healing following implant installation and abutment connection there was in the current study an apical shift of radiographic BIC that for the Brånemark system amounted to 0.8 mm. In other words, prior to placement of the FPD the marginal bone level at these types of implants was located about 2 mm apical of the original bone crest. Such a relation between the original bone crest and the resulting marginal bone level was not observed at Astra implants. Thus, the margin of the Astra fixtures coincided with the bone crest following installation and the bone loss that occurred during healing following implant installation and abutment connection was only about 0.1 mm. Obviously, this pronounced difference regarding bone tissue reaction to the implant installation and abutment connection procedures between the two systems will not be detected if the radiograph obtained immediately following FPD placement is used as the baseline for bone level alterations that may occur during the follow-up period.

Experimental model

In the current experiment FPDs were used to evaluate the effect of functional load on the bone tissue at implants. The FPDs were connected to three of the four implants in each side of the mandible and FPDs were also produced for the opposing tooth segments in the maxilla. The design of the prosthetic device including horizontal occlusal planes and the preserved vertical dimension provided an axial load to the implants. This model therefore differs from models used in similar experiments on occlusal load to implants in monkeys (Ogiso et al. 1994, Isidor 1996, 1997, Miyata et al. 1998, 2000) and dogs (Barbier & Schepers 1997, Heitz-Mayfield et al. 2004). Ogiso et al. (1994) analysed the effect of enhanced occlusal, axial loading to dense apatite implants in six monkeys. It was reported that implants exposed to load did not loose osseointegration but that re-modelling and thickening of the peri-implant bone occurred at such sites. Isidor (1996, 1997) evaluated bone reactions to excessive load. In each animal an FPD was connected to two implants on one side of the mandible. The prosthetic device was designed with an oblique plane that made early contact with a splint in the opposing maxilla and, hence, a lateral displacement of the mandible occurred during function. It was demonstrated that in this model, that created oblique excessive load during an 18-month period, loss of osseointegration occurred in five out of eight implants. In further experiments Miyata et al. (1998, 2000) utilized models with implant supported FPDs with enhanced vertical dimensions varying between 100 and 250 μm. Marginal bone loss occurred at implants that supported bridges with the most pronounced ''supra-occlusion'' (180–250 μm).

Barbier & Schepers (1997) analysed bone tissue reaction to axial and non-axial load in beagle dogs. In one side of the mandible a conventional three-unit FPD with a central pontic was connected to two implants, while in the other side of the mandible a three-unit FPD with a distal cantilever was placed on two implants. Bone re-modelling was modest at the implants supporting the conventional FPD, while the non-axial load introduced by the cantilever design elicited a more pronounced response with increased osteoclast activity in the peri-implant bone. In a recent study in

beagle dogs, Heitz-Mayfield et al. (2004) examined the effect of excessive occlusal load on osseointegration. In this experiment single crowns were connected to four implants in one side of the mandible, while no crowns were placed on the implants in the contra-lateral side. The restorations in the test side were designed with oblique occlusal planes in ''supra-occlusal'' contact with the opposing maxillary teeth yielding an increased vertical dimension of about 3 mm. The authors reported that no differences were found regarding clinical variables and radiographic bone loss between test and control implants.

In the studies referred to attempts were made to achieve loading situations that exceeded normal, functional conditions. This was not the purpose of the present experiment. On the contrary, in order to evaluate the possible influence of functional load on the marginal bone level at implants, the occlusal surfaces of the FPDs established a ''flat-to-flat'' surface contact between the mandibular and maxillary units (Fig. 5). This variation in design of the present experiment and the studies referred to may also explain the different outcome in terms of bone response to load.

Effect of load

The histological analysis of the peri-implant bone in the present study revealed that signs of bone re-modelling including presence of BMUs were more pronounced at implants subjected to load during 10 months than at ''non-loaded'' control implants. It was also demonstrated that the degree of BIC was larger at test than at control implants, while no differences were found regarding the density of mineralized bone neither in the area between threads nor in a 200 μm wide zone lateral to the implant. These observations are consistent with findings reported in similar experiments (Ogiso et al. 1994, Barbier & Schepers 1997, Heitz-Mayfield et al. 2004). Gotfredsen et al., (2001) in an experimental study in dogs found larger fractions of BIC to occur at implants exposed to lateral, static load than at non-loaded implants. This finding is also in agreement with data presented in the current study and indicates that load applied to implants, under certain limits, may induce bone re-modelling in the peri-implant bone that results in enhanced levels of BIC.

The absence of signs of reduced marginal bone levels at the test sites in the present study indicates that when this type of bone loss occurs it may not be associated with load applied to implants. This conclusion is in agreement with Assenza et al. (2003) who in an experimental study in beagle dogs evaluated osteoclast activity around loaded and unloaded implants. It was reported that no differences were found between test and control sites. Also in animal models employing excessive occlusal-oblique load to implants the marginal bone level remained unaffected (Isidor 1996, 1997, Heitz-Mayfield et al. 2004).

Acknowledgement

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