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Age-related salivary polyamine increase in adolescents wearing orthodontic Ni-Ti archwires

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Summary. Until now information about the influence of puberty on gingival tissue responses to Ni-Ti alloy haven't been available. Since our previous researches have demonstrated that Ni-Ti appliances have an influence on hyperplastic gingivopathy and data has pointed out a possible hormonal influence on the susceptibility of gingival tissue to mechanical stress, we have attempted to study the relationship between fertility hormones and the periodontal response to Ni-Ti appliances. Three groups, ranging from 6 to 17 years old, were tested for salivary polyamine concentrations and for fertility hormone levels 12 months after Ni-Ti application. Results obtained from Pearson's correlation coefficient between polyamine and sexual hormone concentrations, as well as gingival and plaque indexes, suggest that the adolescent gingival tissue undergoes an hyperplastic process after long-term use of Ni-Ti appliances in relation to the puberty age-restricted peak of fertility hormones.

Keywords: Amino acids – Hyperplastic gingivitis – Orthodontic wires – Polyamines – Saliva

Introduction

The aliphatic polyamines spermine (SP), spermidine (SPD) and putrescine (PUT) play an important role in cell growth and division. In fact, these cofactors mediate normal, as well as pathological processes, since higher levels occur in tissues with increased metabolic activity (Goyns, 1982; Tabor and Tabor, 1984). The presence of polyamines in buccodental fluids and their role in both physiological and biochemical processes have been established (Perez, 1990). Recently, a sensible as well as specific method based on high performance liquid chromatography (HPLC) allowed to determine

quantitatively these proliferation mediators in human saliva (Venza et al., 2001).

Periodontal complications characterized by a variation of cell growth and division, such as gingivitis (Grimsdottir et al., 1992; Grimsdottir and Hensten-Pettersen, 1993), gingival hypertrophy (Creekmore, 1989), hyperplastic gingivopathy (Kocsis and Kocsis, 1997; Kamin, 1991; Scaramella and Quaranta, 1984), peripheral giant-cell granuloma (Schields, 1994; Wolfson et al., 1989) and hyperplastic soft tissue lesions (Barack et al., 1985) may arise during orthodontic treatment and lead to its interruption.

The nearly equiatomic nickel-titanium alloy, due to its exceptional physical, chemical and mechanical properties, has been employed worldwide. Nitinol based shape memory alloys possess a unique combination of properties including superelasticity, great workability in the martensitic state, resistance to fatigue and corrosion (Rondelli and Vicentini, 1998; Duerig et al., 1996), but its bio-compatibility remains controversial (Berger-Gorbert et al., 1996; Oshida and Miyazaki, 1991). Although *in vitro* studies have revealed the absence of any toxic effect for Ni-Ti alloys and have shown no decrease in cell proliferation (Rhalmi et al., 1999; Ryhanen et al., 1997; Wever et al., 1997), other recent data has demonstrated that corrosion products of nitinol altered cell morphology, induced cell necrosis and decreased cell numbers (Shin et al., 2000; Wataha et al., 2000).

Results from our laboratory demonstrated that the prolonged use of Ni-Ti appliances may contribute to local gingivitis hyperplastica. Early detection is possible only through salivary polyamine concentration increase (Venza et al., 2000; Venza et al., submitted). Therefore, polyamine determination in saliva permits a correct diagnosis and the management of sub-clinical and adverse periodontal effects that, if not diagnosed early, may induce serious and, in some instances, irreversible damages to soft and hard tissues adjacent to the archwires (Pre, 1998).

Although we demonstrated that salivary polyamine content in healthy conditions is age- and sex-independent (Venza et al., 2001), nothing is known about the influence of fertility hormones on salivary polyamine content in subjects under orthodontic treatment with Ni-Ti alloy.

Since several data show that during treatment with fixed orthodontic appliances, levels of gingival inflammation were significantly greater for adolescents than for adults (Aydemir et al., 1999; Boyd and Baumrind, 1992) and prevention is a primary target for young people, we attempted to determine a possible role of fertility hormones in gingival homeostasis during the long-term orthodontic treatment in adolescents by measuring salivary polyamine content.

Materials and methods

Patient population

Sixty young people, whose age ranged between 6 and 17 years, under orthodontic treatment with Ni-Ti archwires for 12 months, were enrolled for this study. The archwires

employed were guided by metal slots, which were attached to the tooth surface by resin-based bonding agents.

None of the patients suffered from systemic or salivary gland disease that could affect saliva secretion, nor had they been administered drugs. Subjects were also requested to refrain from eating two hours prior to saliva withdrawal.

Patients were divided into three groups: the pre-pubertal (6–10 years, n = 20), the mid-pubertal (11–13 years, n = 20), the late-pubertal (14–17 years, n = 20).

Three control groups, composed by healthy people of the same age of the test groups, were processed in parallel.

Clinical examination

All the patients were scored for plaque deposits and gingival health through plaque (PI) and gingival (GI) indexes, respectively, expressed by mean values and standard deviations for each group (Löe and Silness, 1963).

Polyamine analysis by HPLC

a) Sample collection and storage

The collected saliva, drawn from the sublingual region using a polyethylene transfer pipette, was centrifuged at 3,000 rpm for 30 min to remove cellular elements. The clear supernatant fluid was mixed with 0.2 M perchloric acid (1:1) and centrifuged at 1,500 rpm for 10 min. The acidic extract was decanted and stored at −20°C until analysis.

b) Apparatus and chromatographic conditions

The HPLC system consisted of two pumps model LC-10 Ai (Shimadzu) coupled to a high pressure mixer, a Rheodyne injection valve fitted with a 20-μL loop. Separation was achieved on a Nucleosil ODS column (250 × 4.6 mm I.D., 5 μm). The elution procedure was performed with two mobile phases, A (water) and B (methanol), at a flow-rate of 0.8 ml/min. It first consisted of an isocratic elution with 80% solvent B for 2 min, then of a linear gradient elution raised to 85% B within 15 min. Detection was accomplished using a spectrofluorimeter (RF-10Axl, Shimadzu) at an excitation wavelength of 360 nm and an emission wavelength of 510 nm.

c) Reagents

SP free base, SPD free base and PUT dihydrochloride, purchased from Sigma-Aldrich (Milano, Italy), were dissolved in water (10 μg/mL). The o-phthaldialdehyde (OPA) reagent solution (Sigma-Aldrich, Milano, Italy), containing OPA (1 mg/mL), Brij 35, methanol, potassium hydroxide, boric acid and 2-mercaptoethanol as reducing agent, was diluted in methanol (1:10). The organic solvents used (LC grade) were purchased from Sigma-Aldrich (Milano, Italy).

d) o-Phtaldyaldeide derivatization

To 30 μL of saliva extract or standard mixture 50 μL of borate buffer (0.01 M, pH = 9) and 30 μL of the diluted OPA reagent solution were added. The derivatization mixture was shaken for 4 min and 50 μL were injected into the HPLC system.

Fertility hormone assay

Plasmatic levels of LH, FSH, estradiol, prolactin and testosterone were measured by electrochemiluminescence assay (ECLIA) with an automatic analyzer, Elecsys 2010 (Roche Diagnostic S.P.A., Monza, Italy).

Highly reactive species on an electrode surface were generated by stable precursors, such as ruthenium-chelate and 3-propylamine. The former was employed as a marker and the latter as a washing solution and electron donor.

Results

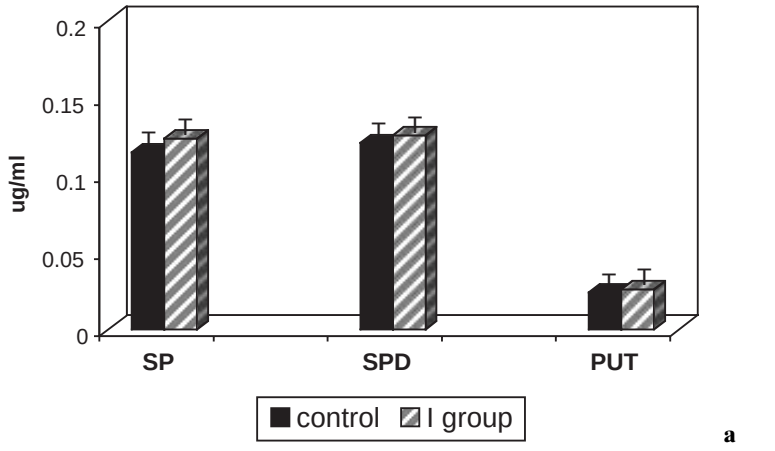
Figure 1 reports the salivary levels of SP, SPD and PUT in adolescents wearing Ni-Ti archwires for 12 months, divided in three groups, the pre-pubertal (6–10 years, n = 20), the mid-pubertal (11–13 years, n = 20) and the late-pubertal (14–17 years, n = 20) groups. The group of subjects whose age ranged from 14 to 17 years exhibited significant increases of SP and SPD salivary content. On the contrary, in the other two groups no modification of polyamine concentrations was observed in relation to the same age control group. PUT resulted unmodified in all the three groups considered.

Figure 2 reports the mean values of LH, FSH, estradiol, prolactin and testosterone in groups I, II and III. The concentrations of the fertility hormones significantly and progressively increase with the age, reaching the greatest value at the late-pubertal period (peak period). Prolactin showed a biphasic trend, since its concentration lowers during the mid-pubertal period and peaks to maximum values in late-pubertal age.

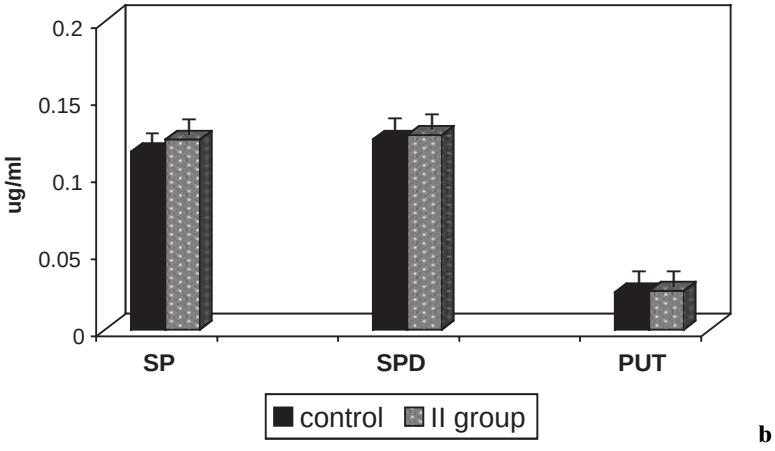
Figure 3 shows P.I. and G.I. mean values in the three test groups. P.I. does not show any significant variation as compared to the corresponding controls in all the three groups studied. On the contrary, G.I. is very significantly increased in late-pubertal adolescents in respect to the control and the other tested groups.

Pearson correlation coefficients comparing polyamine salivary levels with fertility hormones in the three adolescent groups are reported in Table 1. In group I, exhibiting low fertility hormone concentrations (Fig. 2) and low salivary polyamine levels (Fig. 1), a high correlation between these two parameters occurs ($p < 0.001$). In group II, whose sexual hormones increase and salivary polyamine content is still low, no correlation can be found ($p = 1$). A significant correlation for SP and SPD again occurs in group III, whose sexual hormones peak at the maximal levels ($p < 0.001$).

In Table 2 correlation coefficients between salivary polyamine concentrations and dental parameters are reported. A close correlation between SP, SPD and PUT low concentrations and the two low dental parameters was observed in groups I and II ($p < 0.001$). In group III no correlation between the increased levels of SP and SPD and the low values of P.I. ($p = 1$) was found, despite the significant positive correlation with the high values of G.I. ($p < 0.001$). In the same group, the low levels of PUT well correlated with low P.I. ($p < 0.001$), but not with high G.I. ($p = 1$) values.



a



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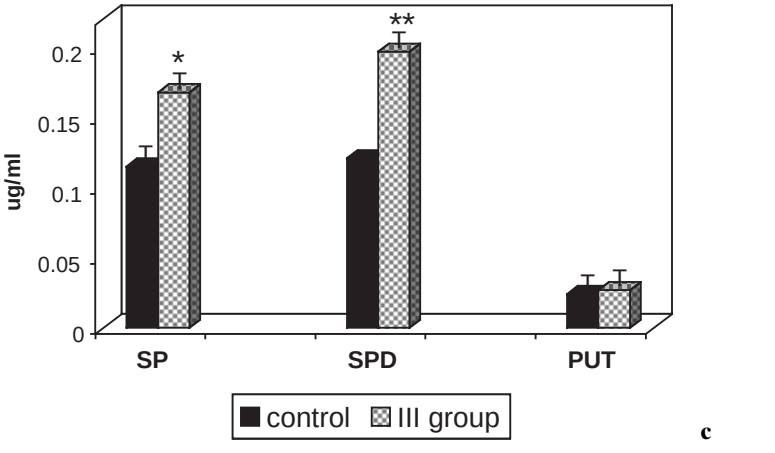

c

Fig. 1. Salivary levels of SP, SPD and PUT in adolescents under orthodontic treatment with Ni-Ti archwires for 12 months whose age ranged (**a**) from 6 to 10 years old (group I), (**b**) from 11 to 13 years old (group II) and (**c**) from 14 to 17 years old (group III). Significant *$P < 0.05$ and **$P < 0.01$ by Student's test as compared with controls. Column and bars represent mean ± SD

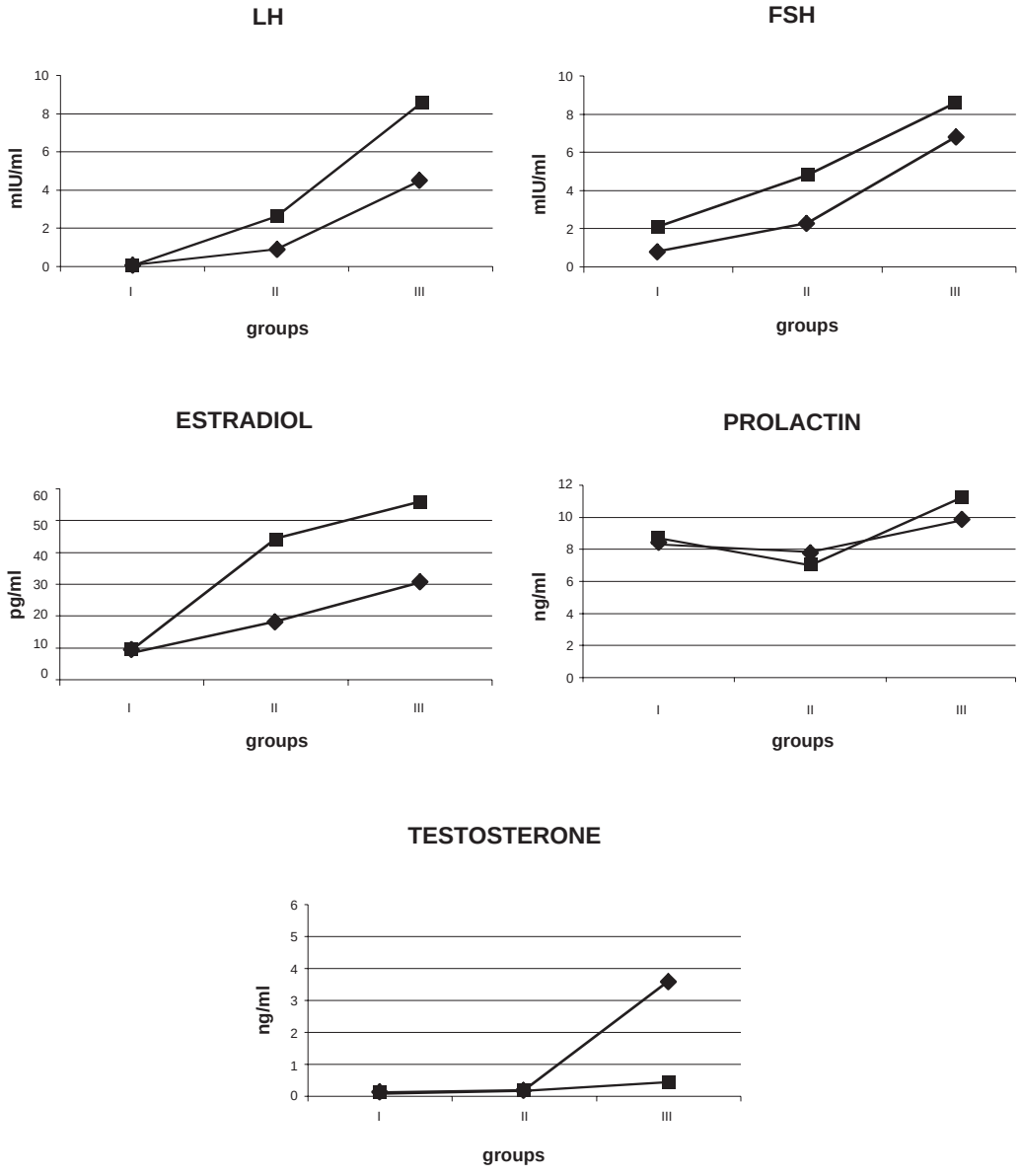

Fig. 2. Plasmatic concentrations of LH, FSH, estradiol, prolactina and testosterone in female and male adolescents under orthodontic treatment with Ni-Ti archwires for 12 months. Groups are designed as in Fig. 1

Discussion

Data reported here demonstrated that salivary SP and SPD but not PUT levels increase during the late-pubertal period in subjects wearing Ni-Ti archwires for 12 months. The increase of the two polyamines was well correlated to the maximum levels of fertility hormones occurring in 15–17 year old adolescents (peak period).

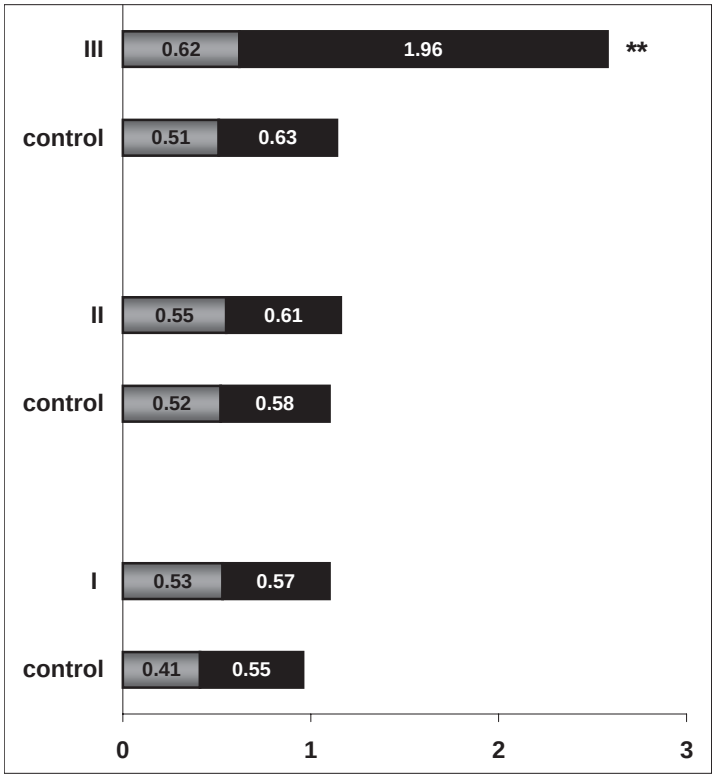

Fig. 3. Plaque and gingival values in adolescents under orthodontic treatment with Ni-Ti archwires for 12 months. Significant **$P < 0.01$ by Student's test as compared with controls. Groups are designed as in Fig. 1. ▩ plaque index; ■ gingival index

Results obtained by us are in agreement with literature data reporting that there is a higher tendency to develop gingivitis during the course of pubertal maturation processes (Gusberti et al., 1990) and that the elevation in serum levels of testosterone in boys and estradiol and progesterone in girls is positively correlated with the degree of gingival inflammation (Nakagawa et al., 1994; Matsson and Goldberg, 1985). Moreover, it has been demonstrated that during and after orthodontic treatment with fixed appliances, both maxillary and mandibular banded molars showed greater gingival inflammation and plaque accumulation than did bonded molars. When banded and bonded teeth were grouped by patient age, mean values for plaque accumulation and gingival inflammation in the maxillary molar regions were significantly greater for adolescents than for adults (Boyd and Baumrind, 1992). Interestingly, in the adolescent period ranging from 12 to 14 years greatest growth changes occur in the maxilla, mandible and maxillary-mandibular relationship of subjects who do not undergo orthodontic treatment (Aydemir et al., 1999).

But, until now the influence of the onset of puberty and the peak of sexual hormones on the homeostasis of gingival tissue has not been studied in regard to the periodontal response to a long-term Ni-Ti appliance use in the three pubertal ages, pre-, mid- and late-pubertal.

Table 1. Pearson correlation coefficients comparing salivary polyamine levels with plasmatic concentrations of fertility hormones

	Correlation coefficient for								
	I Group[a]			II Group[a]			III Group[a]		
	[SP]	[SPD]	[PUT]	[SP]	[SPD]	[PUT]	[SP]	[SPD]	[PUT]
LH	$r = 0.9793$ $p < 0.001$	$r = 0.9798$ $p < 0.001$	$r = 0.9791$ $p < 0.001$	$r = 0.2869$ $p = 1$	$r = 0.2836$ $p = 1$	$r = 0.2995$ $p = 1$	$r = 0.9989$ $p < 0.001$	$r = 0.9935$ $p < 0.001$	$r = 0.1962$ $p = 1$
FSH	$r = 0.9781$ $p < 0.001$	$r = 0.9751$ $p < 0.001$	$r = 0.9665$ $p < 0.001$	$r = 0.2754$ $p = 1$	$r = 0.2626$ $p = 1$	$r = 0.2680$ $p = 1$	$r = 0.9865$ $p < 0.001$	$r = 0.9752$ $p < 0.001$	$r = 0.1856$ $p = 1$
Estradiol	$r = 0.9689$ $p < 0.001$	$r = 0.9658$ $p < 0.001$	$r = 0.9662$ $p < 0.001$	$r = 0.2779$ $p = 1$	$r = 0.2881$ $p = 1$	$r = 0.2995$ $p = 1$	$r = 0.9995$ $p < 0.001$	$r = 0.9968$ $p < 0.001$	$r = 0.2123$ $p = 1$
Prolactin	$r = 0.9702$ $p < 0.001$	$r = 0.9748$ $p < 0.001$	$r = 0.9788$ $p < 0.001$	$r = 0.2789$ $p = 1$	$r = 0.2854$ $p = 1$	$r = 0.2762$ $p = 1$	$r = 0.9823$ $p < 0.001$	$r = 0.9792$ $p < 0.001$	$r = 0.2369$ $p = 1$
Testosterone	$r = 0.9733$ $p < 0.001$	$r = 0.9654$ $p < 0.001$	$r = 0.9689$ $p < 0.001$	$r = 0.6621$ $p = 1$	$r = 0.6812$ $p = 1$	$r = 0.5959$ $p = 1$	$r = 0.9751$ $p < 0.001$	$r = 0.9763$ $p < 0.001$	$r = 0.2042$ $p = 1$

[a] Groups are designed as in Fig. 1.

Table 2. Pearson correlation coefficients comparing salivary polyamine levels with dental parameter mean values

Dental parameters	Correlation coefficient for								
	I Group[a]			II Group[a]			III Group[a]		
	[SP]	[SPD]	[PUT]	[SP]	[SPD]	[PUT]	[SP]	[SPD]	[PUT]
P.I.[b]	$r = 0.9878$ $p < 0.001$	$r = 0.9786$ $p < 0.001$	$r = 0.9832$ $p < 0.001$	$r = 0.9869$ $p < 0.001$	$r = 0.9671$ $p < 0.001$	$r = 0.9733$ $p < 0.001$	$r = 0.2312$ $p = 1$	$r = 0.1853$ $p = 1$	$r = 0.9752$ $p < 0.001$
G.I.[c]	$r = 0.9781$ $p < 0.001$	$r = 0.9779$ $p < 0.001$	$r = 0.9863$ $p < 0.001$	$r = 0.9765$ $p < 0.001$	$r = 0.9754$ $p < 0.001$	$r = 0.9780$ $p < 0.001$	$r = 0.9965$ $p < 0.001$	$r = 0.9852$ $p < 0.001$	$r = 0.1926$ $p = 1$

[a] Groups are designed as in Fig. 1.
[b] *P.I.* plaque index.
[c] *G.I.* gingival index.

Salivary polyamine SP and SPD increase has been tied to an early sign of gingivitis hyperplastica occurring in a sufficient percentage of subjects under orthodontic treatment with Ni-Ti archwires (Venza et al., submitted). So, salivary SP and SPD increase observed by us in late-pubertal adolescents who had been wearing for a long time Ni-Ti archwires and having a good correlation with the maximal levels of sexual hormones accounts for a greater periodontal susceptibility of this age to the cytotoxic effect of the alloy. These data may be of pathophysiological relevance considering that in healthy subjects who did not undergo orthodontic treatment, low concentrations of salivary polyamines have been detected and were age- and sex-independent (Venza et al., 2001).

Ni-Ti alloy has been demonstrated to have an antimicrobial effect (Grimsdottir and Hensten-Pettersen, 1993) and the low levels of P.I. in all the subjects enrolled in this study confirm that the modifications of G.I. and of salivary polyamine content may be due to a cytotoxic effect of the alloy rather than a microbic adhesion. Moreover, our data support the hypothesis that orthodontic devices may have an inhibitory effect on dental plaque viability (Grimsdottir and Hensten-Pettersen, 1993) and that they can cause a chemical-induced gingival hyperplasia coinciding with peaked increases of testosterone as well as LH, FSH, estradiol and prolactin hormones. Recently, Petti et al. (1997) have studied the microbiological and clinical changes occurring during the first six months of orthodontic therapy with fixed and removable appliances to evaluate the consequent risk for gingivitis and periodontal disease. They demonstrated in both the groups a decrease of Gram positive cocci and that gingivitis and periodontitis do not occur during the first six months of treatment.

On the other hand, it's well known that a cytotoxic effect of the devices per se might contribute to a localized gingivitis and that local tissue irritation caused by corrosion products cannot clinically be distinguished from gingivitis of bacterial origin (Grimsdottir et al., 1992). The simultaneous checking of low P.I. and elevated levels of polyamines that we observed accounts for a cytotoxic effect exerted by Ni-Ti archwires worn for a long period of time in adolescents whose fertility hormones reached the maximum levels in late-puberty. This observation may be of particular interest given that single-component devices, like the archwires employed by us, have been demonstrated to be, in adult subjects, less cytotoxic than the multi-component devices joined with silver- and copper-based brazing alloys (Grimsdottir et al., 1992).

Although *in vitro* researches have revealed the absence of any toxic effect for Ni-Ti alloys and no decrease in cell proliferation (Ryhanen et al., 1997; Wever et al., 1997) and several *in vivo* studies have claimed the biocompatibility of porous nickel-titanium, indicating a good tissue tolerance (Rhalmi et al., 1999; Ryhanen et al., 1998; Ryhanen et al., 1999), all the data obtained are related to very brief periods of contact time between Ni-Ti alloy and cells (Wataha et al., 2000).

More recent data report that corrosive products of nitinol wires following constant electrochemical breakdown voltage were toxic to the primary cul-

tured aortic smooth muscle cells. Corrosion products altered cell morphology, induced cell necrosis and decreased cell number by inhibiting replication at the G0–G1 to S transition phase. These effects are discussed in that corrosion products of current nitinol might affect the postimplantation neointimal hyperplasia (Shin et al., 2000). A similar reactive hyperplastic response observed in vascular smooth muscle cells may occur in periodontal tissue subjected to the cytotoxic effect of Ni-Ti alloy and to endocrine maximal stimulation. The enhancing effect of endocrine factors may lower the concentrations and the time necessary for Ni-Ti to exert its cytotoxic action.

Since others reported that after 24–72 h exposure the nickel-based alloys altered a number of fibroblast functions, such as DNA, RNA and protein synthesis, intracellular ATP levels and glucose-6-phosphate dehydrogenase activity (Messer and Lucas, 2000), we postulate that these early modifications may occur in gingival tissue and may induce a long-term adaptive response of periodontium, resulting in an increase of the proliferative rate favoured by steroid hormone peak-induced gene activation.

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