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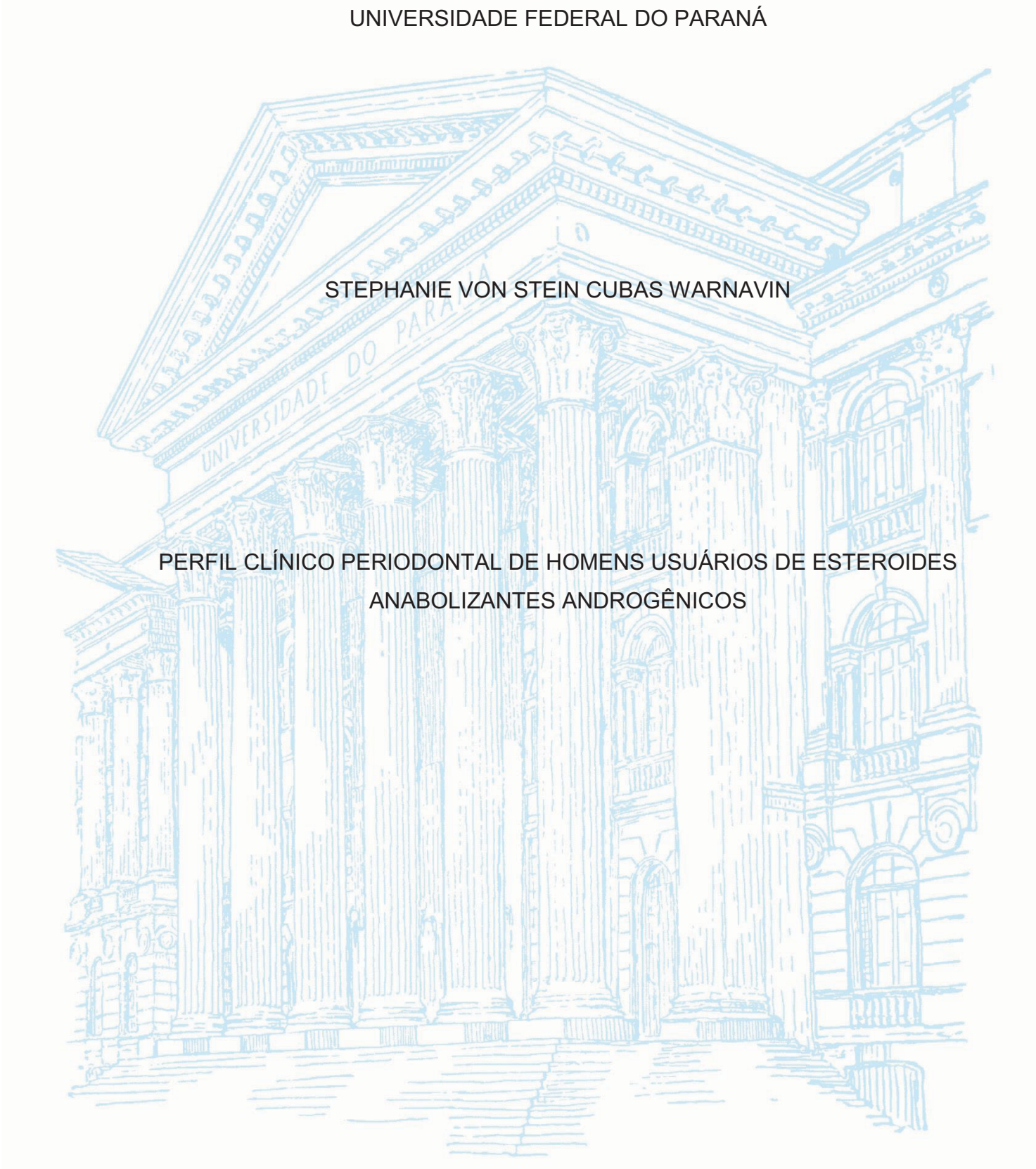

STEPHANIE VON STEIN CUBAS WARNAVIN

PERFIL CLÍNICO PERIODONTAL DE HOMENS USUÁRIOS DE ESTEROIDES ANABOLIZANTES ANDROGÊNICOS

CURITIBA
2018

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Dissertação apresentada ao Programa de Pós-Graduação em Odontologia, Setor de Ciências da Saúde, Universidade Federal do Paraná, como parte das exigências para obtenção do título de Mestre em Odontologia.

Orientador: Prof. Dr. João Paulo Steffens
Coorientadora: Profª. Dra. Geisla Mary Silva Soares

CURITIBA
2018

Warnavin, Stephanie Von Stein Cubas
Perfil clínico periodontal de homens usuários de esteroides anabolizantes androgênicos / Stephanie Von Stein Cubas Warnavin / – Curitiba, 2018.
63 f. : il. ; 30 cm

Orientador: Professor Dr. João Paulo Steffens
Coorientadora: Professora Dra. Geisla Mary Silva Soares
Dissertação (mestrado) – Programa de Pós-Graduação em Odontologia, Setor de Ciências da Saúde. Universidade Federal do Paraná.

Inclui referência

1. Testosterona. 2. Anabolizantes. 3. Periodonto. 4. Inflamação. I. Steffens, João Paulo. II. Soares, Geisla Mary Silva. III. Universidade Federal do Paraná. IV. Título.

CDD 617.632

Maria da Conceição Kury da Silva – CRB – 9/1275

TERMO DE APROVAÇÃO

STEPHANIE VON STEIN CUBAS WARNAVIN

TÍTULO DA DISSERTAÇÃO

PERFIL CLÍNICO PERIODONTAL DE HOMENS USUÁRIOS DE ESTEREÓIDES ANABOLIZANTES ANDROGÊNICOS

Dissertação aprovada como requisito parcial à obtenção do grau de mestre no Programa de Pós-Graduação em Odontologia, Setor de Ciências da Saúde, Universidade Federal do paraná, pela seguinte Banca Examinadora:

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Curitiba, 24 julho de 2018.

MINISTÉRIO DA EDUCAÇÃO
SETOR CIÊNCIAS DA SAÚDE
UNIVERSIDADE FEDERAL DO PARANÁ
PRÓ-REITORIA DE PESQUISA E PÓS-GRADUAÇÃO
PROGRAMA DE PÓS-GRADUAÇÃO ODONTOLOGIA

TERMO DE APROVAÇÃO

Os membros da Banca Examinadora designada pelo Colegiado do Programa de Pós-Graduação em ODONTOLOGIA da Universidade Federal do Paraná foram convocados para realizar a arguição da dissertação de Mestrado de **STEPHANIE VON STEIN CUBAS WARNAVIN** intitulada: **PERFIL CLÍNICO PERIODONTAL DE HOMENS USUÁRIOS DE ESTEREÓIDES ANABOLIZANTES ANDROGÊNICOS**, após terem inquirido a aluna e realizado a avaliação do trabalho, são de parecer pela sua APROVAÇÃO no rito de defesa.

A outorga do título de mestre está sujeita à homologação pelo colegiado, ao atendimento de todas as indicações e correções solicitadas pela banca e ao pleno atendimento das demandas regimentais do Programa de Pós-Graduação.

Curitiba, 24 de Julho de 2018.

JOÃO PAULO STEFFENS
Presidente da Banca Examinadora (UFPR)

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FÁBIO ANDRÉ DOS SANTOS
Avaliador Externo (UEPG)

Dedico esta dissertação a meus pais Anatolio Warnavin e Simone von Stein Cubas Warnavin, que sempre têm sido inspiração da coragem, força e determinação; meus irmãos, primos e amigos que souberam estar ao meu lado e serem minha fortaleza em todas as dificuldades.

AGRADECIMENTOS

A Deus, por cuidar dos meus caminhos e ser o maior alicerce para enfrentar todos os desafios e dificuldades e por possibilitar a realização deste sonho, que me permitirá multiplicar em favor de outros. À minha família, por ser o melhor presente na minha vida, por me apoiar sempre e acreditar, junto comigo, nos meus sonhos. A meu pai, por me ensinar a lutar todos os dias sem desaminar, a acreditar em mim mesma e nunca me deixar desistir, obrigada por sempre estar ao meu lado. À minha mãe, pelo incentivo constante e apoio em todos os momentos de estudos. Aos meus professores João Paulo Steffens, Cassius Carvalho Torres Pereira, Geisla Mary Silva Soares e Humberto Osvaldo Schwartz Filho, por serem um exemplo de compromisso, dedicação, excelência na prática da docência. Ao meu orientador João Paulo Steffens, por cada ensinamento, fazendo eu sempre me desafiar e buscar o melhor de mim, e por sempre acreditar e sonhar junto comigo nessa trajetória, muito obrigada por todo conhecimento transmitido e compartilhado, por ter me ajudado a crescer em todos os sentidos. Sempre serei muito grata a cada um de vocês por me deixar crescer junto com vocês. Aos meus colegas de turmas, por me acolherem e compartilharem conhecimentos, em especial à Bruna Luiza Maximo Ramos, pelo seu carinho e apoio, principalmente nos momentos mais difíceis, dentro e fora das salas de aula e que, com certeza, levarei sempre no meu coração. A Pricila Teixira, Gabriela Moraes, Eduardo Oliveira e Natalie Temporão por me acompanharem nessa trajetória, pelo acolhimento, desabafos e momentos de alegria compartilhados. Cada um de vocês tem um grande significado para mim. À Taize Figueiredo e Thainá Buides, alunas de Iniciação Científica, as quais contribuíram enormemente ao desenvolvimento do trabalho. Ao João Daniel Paganella Chaves, por sua total dedicação com a pesquisa, ao trabalho árduo e executado com perfeição e por ser esse exemplo de pessoa, dentro e fora do mundo acadêmico. Ao Henrique Valenga, por sua contribuição na pesquisa e apoio nos momentos difíceis da vida acadêmica. À Universidade Federal do Paraná e seu programa de Pós-graduação, por ser uma excelente instituição formadora de profissionais. À Ana, secretária de pós-graduação, pela sua recepção e disposição a sempre ajudar em todos os momentos. À Diana Elias Francisco, que esteve ao meu lado desde o primeiro momento, sempre me incentivou, nunca me deixou desistir e foi essencial para o andamento da pesquisa; obrigada por estar sempre presente em

todos os momentos nesse caminho. Aos meus primos Evandro e Larissa, por serem exemplos de profissionais e me darem o auxílio necessário para continuar minha trajetória em busca do meu sonho.

RESUMO

A testosterona é o principal hormônio sexual masculino e promove, entre suas várias funções, o crescimento muscular. Desta forma, derivados sintéticos deste hormônio (Esteroides Anabolizantes Androgênicos – EAAs) são amplamente usados por atletas e fisiculturistas. Entretanto, o uso inadequado dos EAAs pode gerar efeitos colaterais, entre eles toxicidade hepática, acne, alterações comportamentais e podem alterar os tecidos periodontais. O objetivo deste estudo foi avaliar a condição periodontal de homens usuários de EAAs. Foram recrutados 15 homens, com idades igual ou superior a 18 anos, em academias de Curitiba, PR, Brasil, que reportassem uso de EAAs no momento da entrevista (grupo "caso"). O grupo controle foi composto por outros 15 homens, pareados por idade e hábito (frequentadores de academia) e que nunca fizeram uso de EAAs. Todos os pacientes foram avaliados clinicamente quanto a Índice de Placa (IP), Sangramento Marginal (SM), Profundidade de Sondagem (PS), Nível Clínico de Inserção (NIC) e Sangramento à Sondagem (SS). Considerou-se "periodontite" a presença de pelo menos dois sítios com PS maior que 3mm, com perda de inserção e SS no mesmo sítio. A idade dos pacientes variou entre 23 a 40 anos (grupo caso: 30,07±4,4 anos; grupo controle:28,1±4,6 anos), sendo a maioria de etnia branca (grupo caso: 92,5%; grupo controle: 100%) e com conclusão do ensino médio (grupo caso: 60%; grupo controle: 73,3%). O grupo caso apresentou significativamente mais sítios com doença periodontal, com PS≥4mm e PS≥4mm com SS e mais sítios com SS ($p<0,05$). O grupo controle apresentou maior porcentagem de sítios sem doença periodontal ($p<0,05$). Não houve diferença estatisticamente significante para IP, SM e nas médias de NIC de todos os sítios ou dos sítios com perda de Inserção ($p>0,05$). O uso de EAAs pode afetar a saúde periodontal, principalmente quanto aos parâmetros inflamatórios como PS e SM.

Palavras-chave: Testosterona, anabolizante, periodonto, inflamação.

ABSTRACT

Testosterone is the main male sex hormone and promotes muscle growth among its many functions. In this way, synthetic derivatives of this hormone (Androgenetic Anabolic Steroids - AAS) are widely used by athletes and bodybuilders. However, inadequate use of AAS can generate side effects, including liver toxicity, acne, behavioral changes and may alter periodontal tissues. The objective of this study was to evaluate the periodontal status of the men using AAS. Fifteen men, aged 18 years and over, were recruited from gyms in Curitiba, PR, Brazil, who reported use of AAS at the time of the interview (case group). The control group consisted of 15 other men, matched by age and habit (gymgoers) who had never used AAS. All patients were clinically evaluated for the Plaque Index (PI), Marginal Bleeding (MB), Probing Pocket Depth (PD), Clinical Attachment Loss (CAL) and Bleeding on Probing (BoP). The presence of at least two sites with PD greater than 3mm, with attachment loss and BoP at the same site, was considered "periodontitis". The patients' ages ranged from 23 to 40 years (case group: 30.07 ± 4.4 years, control group: 28.1 ± 4.6 years), most of them white (case group: 92.5%, control group: 100%) and with high school education (case group: 60%, control group: 73.3%). The case group presented significantly more sites with periodontal disease, PD ≥4 mm with our without BoP ($p < 0.05$). The control group had a higher percentage of sites without periodontitis ($p < 0.05$). There was no statistically significant difference for PI, MB and in the means of CAL of all sites or sites with attachment loss ($p > 0.05$). The use of AAS can affect periodontal health, especially in relation to inflammatory paraments such as PD and MB.

Key words: Testosterone, anabolic agents, periodontium, inflammation.

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1 INTRODUÇÃO

Os hormônios sexuais esteroidais desempenham grande papel no desenvolvimento, crescimento e regulação de tecidos-alvo, como órgãos sexuais e glândulas prostáticas e mamárias (HILAKIVI-CLARKE et al., 1997; NIKOLOVA et al., 1998). São derivados do colesterol e destacam-se por apresentarem efeito direto no metabolismo mineral ósseo e estarem envolvidos na sustentação da integridade esquelética, inclusive do osso alveolar (KATZ e EPSTEIN, 1993). Desenvolvem suas funções por receptores específicos que regulam a transcrição de genes alvo (citocinas e proteínas reguladoras do ciclo celular, enzimas proteolíticas e macromoléculas da matriz extracelular) (ARANEO et al., 1991; PRALL et al., 1998; JENSTER, 2000).

A testosterona é o principal hormônio sexual masculino e suas funções básicas englobam a sexualidade, desenvolvimento das características sexuais adultas primárias e secundárias (massa muscular, pelos faciais, libido e produção de espermatozoides) e, em menores proporções, dos equilíbrios hídrico e eletrolítico. Este hormônio também está envolvido nos processos de aumento da perfusão cerebral, influenciando sintomas cognitivos; e na saúde óssea através da inibição da ação de osteoclastos estimulando o crescimento ósseo (BAIN, 2010; RAHNEMA et al., 2015).

Em virtude dos benefícios da testosterona no organismo foram desenvolvidos os Esteroides Anabolizantes Androgênicos (EAAs), pertencentes a um grupo hormonal sintético derivado da testosterona. Esta categoria apresentou aumento significativo nos últimos 75 anos, com mais derivados desenvolvidos. Estes EAAs apresentam propriedades anabólicas que ocasionam efeitos diretos na estrutura corporal, promovendo crescimento muscular e diminuição da gordura. O uso desta substância também acentua os efeitos relacionados às características masculinas previamente citadas, como crescimento de barba, alteração da voz e características sexuais secundárias (KANAYAMA et al., 2018).

Homens que apresentam hipogonadismo, puberdade tardia ou algum tipo de impotência possuem indicação para uso de EAAs como forma de suplementar os níveis reduzidos da testosterona (BROWER et al., 1990). Todavia, estes esteroides sintéticos são amplamente utilizados tanto por atletas profissionais de alto rendimento quanto por atletas que fazem o uso recreativos, visando o aumento do

desempenho e rendimento, aumento de massa muscular magra e diminuição do percentual de gordura corporal (BASARIA, 2010). O uso indevido de EAAs por atletas e fisiculturistas acarreta em efeitos colaterais (hormonais e tóxicos), principalmente, quando há uso de grandes doses e um longo período de tempo (FANTON et al., 2009). Estes efeitos relacionam-se ao fato de todos tecidos possuírem receptores suscetíveis à ação anabólica e androgênica destes esteroides (WU, 1997; VAN AMSTERDAM et al., 2010). Os efeitos adversos implicam em toxicidade hepática, acne, alterações comportamentais e alterações nos níveis plasmáticos de lipoproteínas de baixa densidade (GOLDBERG et al., 1996; BHASIN et al., 2001)

Os hormônios esteroidais podem, também, influenciar na fisiologia dos tecidos orais e periodontais, devido à homeostase das funções anabólicas e catabólicas na matriz do tecido conjuntivo e ósseo (SOORY, 2000).

Na fisiologia gengival, o primeiro sinal clínico de inflamação dá-se com a transdução do fluido gengival, lentamente convertido em um líquido composto por soro e leucócitos. A coloração avermelhada, típica da inflamação, ocorre pela diminuição da queratina e aumento dos vasos sanguíneos no tecido conjuntivo subepitelial. Decorrente desse processo há perda na textura da gengiva livre, refletindo a perda de tecido conjuntivo fibroso (LANG et al., 2009). A inflamação gengival é precursora das doenças periodontais, podendo evoluir à periodontite. A periodontite é uma doença inflamatória crônica multifatorial associada a biofilme disbiótico e caracterizada por destruição progressiva do aparato de suporte dental (PAPAPANOU et al., 2018).

Hormônios estereoidais e seus derivados podem desempenhar um papel fundamental na progressão da doença periodontal, incluindo alteração na composição da microbiota e no processo de cicatrização (KASASA e SOORY, 1996; COLETTA et al., 2002; SOORY e TILAKARATNE, 2003; FAMILI et al., 2007; ENGELAND et al., 2009; BRUSCA et al., 2014).

In vitro, demonstrou-se que altas doses de testosterona aumentaram a osteoclasteogênese diretamente, a partir de células RAW264.7 (STEFENS, et al.,2015), além de aumentarem a razão RANKL/OPG em cultura primária de osteoblastos murinos, sugerindo uma sobrerregulação indireta na osteclastogênese (STEFFENS, et al., 2014). Em animais, observou-se que o tratamento com doses

suprafisiológicas de testosterona levou a um aumento da perda óssea induzida por ligadura em ratos machos orquiectomizados (STEFFENS, et al., 2012).

Em 2009, os autores ORWOLL et al., realizaram um estudo com o objetivo de determinar se os esteroides sexuais endógenos estão associados a periodontite e perda dentária. Para isso, utilizaram 1210 homens idosos, com a faixa de idade entre 66 a 95 anos. Foram avaliados os níveis de testosterona e estradiol, Nível de Clínico de Inserção (NIC), Profundidade de Sondagem (PS), Sangramento de Sondagem (SS) e número de dentes. Foi considerado periodontite um NIC proximal maior que 5mm em pelo menos 30% dos dentes examinados, sendo que foram avaliados 6 sítios por dente. A progressão da periodontite se classificou por pelo menos 2 dentes com perda próximal de 3 mm. Observou que 38% dos participantes apresentavam periodontite severa no início do estudo, a progressão da doença ocorreu em 32% e que 22% destes pacientes tinham perda dentária. Este estudo concluiu que não houve evidências de que concentrações séricas de esteroides sexuais estão relacionadas com a periodontite, progressão da doença e a perdas de dentes.

Ao comparar os níveis médios de testosterona endógena e densidade mineral óssea (DMO) em homens com e sem perda dentária, SINGH et al., (2011) analisaram 203 homens com idade entre 30 a 65 anos, que tivessem diagnóstico de periodontite crônica generalizada moderada (30% ou mais dos sítios examinados com NIC de 3-4mm), com ou sem perda dentária e que não apresentassem história ou tratamento de doenças endócrinas, metabólicas ou esqueléticas, tabagismo ou ingestão de álcool, ou qualquer tratamento medicamentoso que pudesse afetar o periodonto. Foram avaliadas a DMO, níveis de testosterona, NIC, PS, mobilidade e perda dentária associada a DP. Foram analisados 4 sítios por dente. Observou-se que o nível de testosterona e DMO em indivíduos com perda dentária foram significativamente menores do quem em indivíduos sem perda dentária. Conclui-se que este hormônio é um bom preditor de perda dentária, entretanto, sua eficiência diminiu com a perda dentária.

Outro estudo, que utilizou dados da Terceira Pesquisa Nacional de Saúde e Nutrição (NHANES), teve o objetivo de explorar a associação entre os níveis de hormônios sexuais e periodontite em homens, onde foram analisados os dados de 755 homens, com idade igual ou superior 30 anos juntamente com seus níveis séricos hormonais. A periodontite foi classificada em leve: 2 ou mais sítios

interproximais com NIC entre 3 a 4 mm e 2 ou mais sítios interproximais com PS≥4 mm (não no mesmo dente) ou 1 local com PS≥5 mm), moderada: 2 ou mais sítios interproximais com NIC entre 4 e 6 mm (não no mesmo dente) ou 2 ou mais sítios interproximais com PS≥ 5 mm, também não no mesmo dente, e severa: presença de 2 ou mais sítios interproximais com NIC≥ 6 mm (não no mesmo dente) e 1 ou mais locais interproximais com PS≥ 5 mm. A correlação entre a presença e severidade da periodontite e níveis de hormônios sexuais foram determinadas e apresentadas como odds ratios. O resultado encontrado foi de que homens com altos níveis de testosterona apresentam maior prevalência e severidade da doença periodontal (STEFFENS et al., 2015).

Uma pesquisa desenvolvida por SAMIETZ et al., (2016) teve como objetivo determinar a associação entre as concentrações de esteroides sexuais com a progressão da doença periodontal e a incidência da perda dentária em homens e mulheres. Para isso, avaliou-se 1465 mulheres e 1939 homens, com a faixa etária entre 20 a 81 anos). Avaliou-se NIC, número de dentes e níveis hormonais (Testosterona total e livre, SHBG e índice de andrógenos livres). Não foram encontradas associações consistentes de esteróides sexuais com progressão periodontal ou perda dentária.

Os derivados sintéticos da testosterona podem atuar sobre os tecidos bucais, como demonstrado por OZCELIK et al (2006) que tiveram o objetivo de avaliar os efeitos do abuso de EAA sobre os tecidos periodontais. Foram avaliados 24 atletas, fisioculturistas e levantadores de peso, com idades entre 17 a 29 anos, usuários de EAA por mais de 1 ano, sem doenças sistêmicas, que não tivessem realizado tratamento periodontal por pelo menos 6 meses ou antibióticos ou antiinflamatórios por 3 meses. Outros 20 participantes, com o mesmo perfil, mas não usuários de EAA, foram incluídos e pareados. Avaliou-se IP, índice gengival e aumento gengival. Foram avaliados 6 sítios por dente, nos quais se analisou o grau de esperssura gengival. A média destes exames clínicos foi realizada através de um score. Nos resultados, não foi observada diferença estatística entre os grupos para IP e índice gengival, entretanto, os usuários de EAA apresentaram scores estatisticamente maiores de aumento gengival, espessura gengival e extensão da invasão gengival.

Em 2014, BRUSCA et al., avaliaram as diferenças no periodonto entre homens usários de EAA e não usuários. Foram analisados 92 homens fisioculutristas e praticantes de musculação, com idades entre 19 a 40 anos, sem

doenças sistêmicas e não tabagistas. Entre estes, 42 usavam EAA. Os parâmetros avaliados foram IP, PS, radiografias para analisar a perda óssea interproximal e níveis de inflamação gengival. Foram avaliados 6 sítios por dente. O diagnóstico de gengivite se dava por sítios com PS entre 2 a 4 mm sem perda óssea radiográfica. A periodontite crônica foi classificada de acordo com sua severidade: leve (NIC de 1 a 2 mm com perda óssea radiográfica), moderada (NIC de 3 a 4mm com perda óssea radiográfica) e seveva (NIC≥5mm com perda óssea radiográfica). Constatou-se que há evidências de que níveis altos de testosterona estão relacionados a formas severas de periodontite, tal como a alta prevalência de microorganismos periodontopatogênicos e *Candida spp*. em homens submetidos ao uso de EAA.

Apesar das limitações encontradas nos estudos epidemiológicos, abuso de EAA e suplementos nutricionais já é considerado um fenômeno de ordem mundial (KERSEY et al., 2012). A prática deste consumo, geralmente tem início em idades jovens e predominantemente no sexo masculino (ROGOL, 2010; POPE et al., 2014). Em alguns países como os Estados Unidos o uso abusivo de EAA é considerado uma epidemia silenciosa (EVANS, 2004).

Um estudo desenvolvido nos Estados Unidos, envolveu 3 mil homens do 12° ano (ensino médio) e revelou que 6,6% dos rapazes já aviam usado EAA, onde 67% iniciaram o uso por volta dos 16 anos de idade e 40% já tinham realizado outros ciclos de EAA. Aproximadamente 21% dos casos tinha sido um proficional de saúde a orientalos (BUCKLEY, et al., 1988).

Na Arábia Saudita, uma pesquisa avaliou o conhecimento, comportamento e a prática de atividade física de homens que frequentavam academias, através de um questionário, com informações sobre dados demográficos, uso de EAA e hábitos de vida. Participaram um total de 4860 homens com média de idade 28,6±6,2 anos. A maioria era solteiro, com nível superior, entre os participantes 9,8% utilizaram EAA, onde 76,7% relatou melhor rendimento. Entre as fontes de informações sobre os EAA, amigos ou conhecidos foi a mais relatada, mas apenas 38% de usuários destas drogas procuraram acompanhamento médico. Concluiu também estes usuários de EAA eram mais propensos a estar envolvido em hábitos de risco, como tabagismo e abuso de hormônio do crescimento. Eles estavam menos conscientes dos riscos e complicações oriundas do uso EAA sem acompanhamento médico, e os instrutores das academias sendo a fonte predominante de substâncias EAA (ALTHOBITI, et al., 2018).

Decorrente do aumento e da prevalência do uso, sem prescrição médica, de EAAs os cirurgiões-dentistas e periodontistas devem familiarizar-se com os efeitos adversos dos derivados sintéticos da testosterona no organismo e nos tecidos gengivais (OZCELIK et al., 2006).

2 OBJETIVOS

O objetivo deste trabalho foi avaliar a condição periodontal de pacientes homens usuários de esteroides anabolizantes androgênicos (EAAs).

2.1 Objetivos específicos:

- Comparar a Profundidade de Sondagem em pacientes usuários e não usuários de EAAs;
- Comparar o Nível Clínico de Inserção em pacientes usuários e não usuários de EAAs;
- Comparar Índice de Placa em pacientes usuários e não usuários de EAAs;
- Comparar Sangramento Marginal em pacientes usuários e não usuários de EAAs;
- Comparar Sangramento à Sondagem em pacientes usuários e não usuários de EAAs;
- Comparar a prevalência de doenças periodontais em pacientes usuários e não usuários de EAAs.

3 DESENVOLVIMENTO

3.1 Artigo 1

PERIODONTAL CLINICAL PROFILE OF MEN UNDER ANDROGENIC ANABOLIC STEROIDS ABUSE

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Word count: 1998

Number of figures: 4

Number of tables:1

Number of references:28

Short running title: Periodontium of men using androgenic anabolic steroids.

One-sentence summary: The use of AAS negatively affects the periodontal profile, especially regarding inflammatory parameters such as Probing Pocket Depth and Marginal Bleeding.

ABSTRACT

Background: Testosterone is the main male sex hormone and promotes muscle growth among its many functions. The synthetic derivatives of this hormone (Androgenic Anabolic Steroids - AAS) are widely used by athletes and bodybuilders. However, inadequate use of AAS can lead to side effects such as liver toxicity, behavioral changes, and periodontal tissues changes. The objective of this study was to evaluate the periodontal clinical profile of men using AAS.

Methods: Fifteen men, 18 years of age or older who reported the use of AAS at the time of interview, were recruited from gyms. The control group consisted of 15 other men, matched by age and habit (gymgoers) who had never used AAS. All patients were clinically evaluated for plaque index (PI), marginal bleeding (MB), probing pocket depth (PD), clinical attachment loss (CAL) and bleeding on probing (BoP). The presence of at least two sites with PD greater than 3mm, with attachment loss and BoP at the same site, was considered "periodontitis".

Results: The patients' ages ranged from 23 to 40 years. The case group presented significantly more sites with periodontitis, PD≥4mm with our without BoP ($p<0.05$). There was no statistically significant difference between groups for plaque index, marginal bleeding and means of CAL of all sites or sites with attachement loss ($p>0.05$).

Conclusion: The use of AAS can affect the periodontal health of users, especially in relation to inflammatory parameters such as PD and MB.

Key words: Testosterone, anabolic agents, periodontal, inflammation

INTRODUCTION

Testosterone is the main male sex hormone and its basic functions encompass sexuality, development of primary and secondary adult sexual characteristics (muscle mass, facial hair, libido and sperm production) and, to a lesser extent, water and electrolyte balance.[1,2]

Because of the the benefits of this hormone, the Androgenic Anabolic Steroids (AAS), belonging to a synthetic hormone group derived from testosterone, have been developed.[3] Their use is indicated for men who present hypogonadism, late puberty and some types of impotence.[4] However, these synthetic steroids are widely used by professional athletes or recreational athletes, aiming to increase performance, increase lean muscle mass and decrease body fat percentage.[5] The use of AAS by athletes and bodybuilders leads to side effects (hormonal and toxic).[6] The adverse effects of inappropriate and abusive use of AASs vary according to the individual's age and sex, such as dose, duration, and type of steroid. In addition, the effects of hepatic enzymes on hepatic toxicity, acne, behavioral changes and changes in plasma levels of low-density lipoprotein.[7,8]

It is know that steroid hormones can influence the physiology of oral and periodontal tissues due to the homeostasis of anabolic and catabolic functions in the connective tissue and bone matrix.[9] These hormones and their derivatives may play a key role in the progression of periodontal disease, including alterations in the composition of the microbiota and the healing process.[10-15]

In vitro, it was demonstrated that high doses of testosterone increased osteoclasteogenesis directly from RAW264.7 cells,[16] in addition to increasing RANKL/OPG ratio in murine osteoblast primary culture, suggesting an overregulation in osteoclastogenesis.[17] In animals, treatment with supraphysiological doses of testosterone has been shown to increase bone loss induced by ligature in orchiectomized male rats.[18]

There is evidence that high levels of testosterone are related to severe forms of periodontitis, such as the high prevalence of periodontopathogenic microorganisms and *Candida spp.* in men who underwent the use of AAS.[15] Another study showed that men with high levels of testosterone present higher prevalence and severity of periodontal disease.[19] The use of AAS was intimately associated with significant levels of gingival increase.[20]

Hence, the objective of this study was to evaluate the periodontal status of men under AAS abuse.

MATERIALS AND METHODS

This study was approved by the Brazilian National Commission for Research Ethics – CONEP (1.906.729 - 2.443.657). It is a case-control study, carried out from March 2017 to June 2018, at the Federal University of Paraná-UFPR, Curitiba, PR, Brazil.

Recruitment of patients

The convenient sample included adult males 18 years of age or older, who reported being AAS users at the time of the interview, physical activity practitioners, nonsmokers, without systemic diseases that exhibit inflammatory, immunological, or hormonal changes. For inclusion in the study, participants required at least 20 teeth in the mouth, excluding third molars.

Adult men with similar characteristics, but not ASA users, were also invited. The groups were matched according to age, with a difference of up to 3 years. Recruitment was done through leaflets, interviews on radios and local television network and also visits to gyms and bodybuilding events.

All interested in participating in the research were elucidated on the steps of the methodology, as well as the risks and benefits of the study. All individuals who agreed to participate signed an Informed Consent Term.

Clinical examination

Dental clinical examination was performed to evaluate: Plaque index (O'Leary, 1972),[21] marginal bleeding (MB) - 4 sites per tooth, probing depth (PD), bleeding on probing (BoP) and Clinical Attachment Loss (CAL) - 6 sites per tooth.

The gingival health categorization of the participants was based on the criteria adopted by the classification of periodontal diseases of the American Academy of Periodontology and European Federation of Periodontology (2018),[22] where up to 10% of sites with BoP represent gingival health, otherwise, gingivitis is diagnosis. The diagnosis of periodontitis occurred when the patient presented CAL in 2 or more interproximal sites, non-adjacent or CAL≥ 3 mm, buccal or lingual, in at least 2 teeth without association of other factors.[23] The evaluation procedures were

performed by a previously trained and calibrated examiner (SVSCW, Kappa ± 1mm = 0.97).

Statistical analysis

Statistical analysis was performed with GraphPad Prism Software (version 7.0®, La Jolla, CA, US). In order to observe the correct pairing (± 3 years), the groups were compared in relation to age using Student's t-test. All quantitative variables were compared using paired t-test or Wilcoxon test, depending on the homogeneity of variances and normal distribution of samples. Fisher's exact test was used for frequency comparisons. Data were presented as means and standard deviations. Statistical significance was set at p <5%.

The calculation of the power of the test was performed for the primary outcome probing pocket depth found in each group. At a significance level of 5%, 15 patients per group represented 96% power.

RESULTS

In this study, 30 men were included in two groups containing 15 participants: AAS users (case group) and non-AAS users (control group). The mean age of participants was 29.1±4.55 years, ranging from 23 to 40 years. In relation to ethnicity, the majority was classified as white, representing 93.3% of the total sample. The average income was $1083.64, with no statistical difference between groups. The educational level of the individuals was categorized into high school and College/University. The highest level of education was high school education, in both groups, with 60% (case group) and 73.3% (control group) (Table 1).

As to the periodontal diagnosis of the patients, the case group, the prevalence of periodontitis was 73.34%; Gingivitis was diagnosed in 26.66% of the patients. The control group presented 40.01% of the participants with periodontitis, Gingivitis in 53.33% and one patient diagnosed as healthy (Table 1).

PI and BoP were higher in the case group, but the difference was statistically significant only for MB (p<0.005) (Figure 1).

The mean attachment loss in the control group was higher, but there was no statistically significant difference (Figure 2).

Shallow site (PD between 1-3 mm) were more prevalent in the control group (p<0,004). The indicative parameters of periodontitis, that is, PD ≥4 mm associated with attachment loss and BoP were present in significantly higher percentage in the case group (p=0.0007)(Figure 3).

The case group presented a significantly higher percentage of sites with attachment loss (p = 0.003) (Figure 4).

DISCUSSION

This study aimed to evaluate the periodontal status of patients users of AAS. Our results demonstrated a greater number of sites with PD≥4 mm and a higher percentage of sites with marginal bleeding in individuals who used AAS.

The results of this research suggest that the use of AAS influences the periodontal profile of these patients. Individuals who consumed AAS had greater clinical changes in gingival inflammation. There were significant differences between the groups regarding PD and MB parameters, whereas the case group presented greater inflammation. These data are in agreement with other studies,[15,19] which relate the use of these synthetic derivatives of testosterone to periodontal inflammatory changes.

Differently from some studies[15,19] and animal research reports,[18] our results do not demonstrate differences between users of AAS and non-AAS regarding attachment loss of sites with periodontitis. Although the control group had a higher average of attachment loss among the sites that presented loss, the case group had a significantly higher percentage of sites with attachement loss per patient.

It was observed that, although there is a tendency for the users of AAS to present higher PI, there was no statistically significant difference between the two groups. These results are similar to those found on other study,[20] but diferente from another one,[15] where plaque index was significantly higher in users of AAS. It is noteworthy that different evaluations of this parameter were used between our work (O'Leary, 1972)[21] and the work of other authors (Silness and Löe, 1964).[24]

Scientific background shows that men with high levels of testosterone have a higher prevalence and severity of periodontal disease.[15,19] Our results indicate that, even if the case group presented a higher percentage of individuals diagnosed with

periodontitis, there were no significant differences between the groups regarding the prevalence of the disease.

In this study, it was verified the use of 9 types of drugs, where 8 were AAS. Anastrozole is not considered AAS, but belongs to a class of medicines called aromatase inhibitors, a substance that affects the level of some female sex hormones, such as estrogens. The use of this drug by this group of patients, aims to minimize the conversion of testosterone to estradiol, in order to obtain higher levels of testosterone. It was observed that these drugs were not used in isolated or single forms, most of the time, they were used concomitantly. In addition, it was possible to evaluate that athletes who participated actively in competitions and bodybuilding events, used larger doses and/or longer duration. Regardless of the administration of these drugs, it was found that the men who used AAS who participated in this study had no medical prescription.

Studies have shown that there is a strong association between the level of physical activity and periodontitis.[25-28] It was observed that adequate levels of physical activity improve periodontal health, regardless of sociodemographic characteristics and risk factors, [25,26] as well as can protect against an excessive inflammatory response in periodontitis.[27] Another study showed that high performance athletes present greater problems related to oral health. [28]

Some limitations of this study should be observed: The convenience sample was finalized with 15 patients per group due to the secretive nature of anabolic substance abuse. However, the power calculation of a posteriori test indicated power of 96% for the primary endpoint Probing Depth. This outcome was selected due to the hypothesis of the study, that users of AAS would present altered parameters of gingival inflammation and, due to their age, not necessarily attachment loss. In addition, operator blinding became infeasible due to differences in body structures between the two groups.

CONCLUSIONS

AAS abuse can affect the clinical periodontal profile of users, especially regarding the inflammatory parameters such as PD and MB. Therefore, these individuals should be accompanied by their dentists at the time of abuse of these substances.

ACKNOWLEDGMENTS

This study was supported by a grant from the Coordination for the Improvement of Higher Education Personnel (CAPES, Brasília, DF, Brazil) awarded to SVSCW and JDPC was a beneficiary of a scholarship from the Araucária Foundation for Scientific and Technological Development Support of the State of Paraná (Curitiba, PR, Brazil). We also thank the staff from the Department of Stomatology of the Federal University of Paraná. All authors state that they have no conflict of interest.

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FIGURE LEGENDS

Table 1: Demographic, socioeconomic, periodontal clinical data of patients belonging to the case group (AAS) and control group (non-AAS).

Variable	Case	Control	p value	Test
Age (Years)	30.07±4.4	28.1±4.6	0.3	t test
Ethnic Group (%, n)				Fisher
White	92.5 (n=14)	100 (n=15)	0.48	
Non-White	7.5 (n=1)	0 (n=0)		
Income (Dolars)	1070.97±923.18	1087.46±333.65	0.18	Mann Whitney
Educational Level (%)				Fisher
High School	60% (n=9)	73.3% (n=11)	0.7	
College/University	40% (n=6)	26.6% (n=4)		
-Nandrolone (Deca Durabolin®), Schering-Plough, Brazil	40% (n=6)	-	N/A	-
-Testosterone Propianate (Durateston®), Schering-Plough, Brazil	40% (n=6)	-		
-Testosterone Cypionate (Deposteron®), EMS, Brazil	26.6% (n=4)	-		
-Methandrostenolone (Dianabol®), CIBA, Switzerland	20% (n=3)	-		
-Testosterone enanthate (Testovir on Depot®, Bayer Pharma, Germay)	13.3% (n=2)	-		
-Drostanolone Propionate (Masteron®), Syntex, Argentina	6.6% (n=1)	-		
-Boldenone Undecylenate (Boldenone®), USP Labs, USA	6.6% (n=1)	-		
-Trenbolone Acetate (Trembolona Acetato®), Landerlan, Paraguay	6.6% (n=1)	-		
-Anastrozole* (Anastrozol®), Eurofarma, Brazil	6.6% (n=1)	-		
Diagnosis (%)				
Healthy	0	6.66		Fisher
Gingivitis	26.66	53.33	0.17	
Periodontitis	73.34	40.01		
Probing Depth (mm)	2.6±0.3	2.3±0.1	0.004	t test
PD≥4 (% sites; n)	11.55 ±10.48 (n=14)	1.99 ±1.82 (n=11)	0.003	Wilcoxon
PD≥ 5 (% sites; n)	0.60 ± 1.15 (n=6)	0.15 ± 0.27 (n=4)	0.06	Wilcoxon
PD≥6 (% sites; n)	0.08 ±0.22 (n=2)	0.04 ±0.15 (n=1)	0.5	Wilcoxon
CAL (mm)	0.1±0.08	0.06±0.06	0.26	Wilcoxon

PD: Probing Depth; CAL: Clinical Attachment Loss; *Not AAS

Figure 1: (A) Percentage of sites accordingts by the Plaque Index; (B) Marginal Bleeding; (C) Bleeding on Probing.

* p <0.05 (paired t-test)

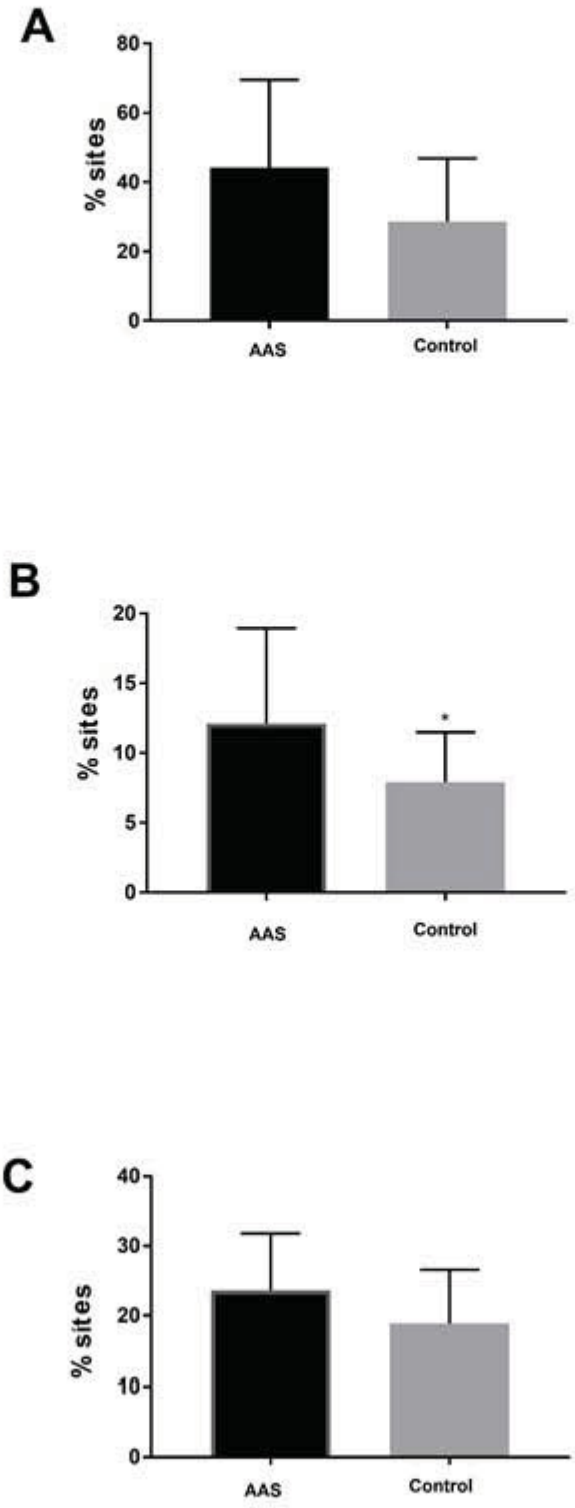


Figure 2: Mean Attachement Loss on the sites that presented attachment loss

** p<0.01 (paired t-test)

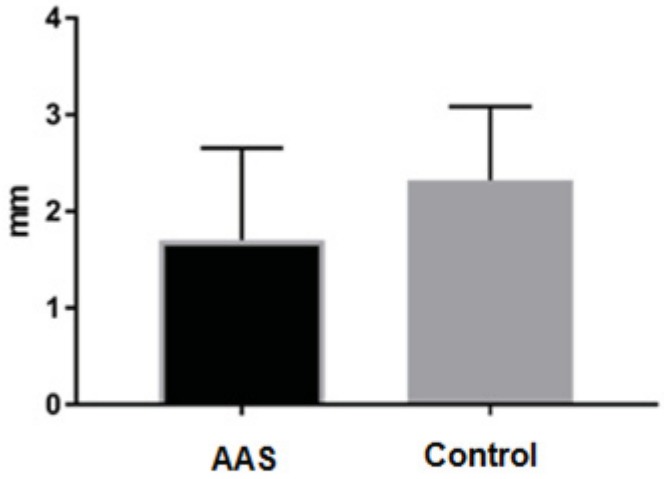


Figure 3: (A) Percentage of Sites with Probing Depth between 1 and 3mm (B) Percentage of Sites with Probing Depth ≥4 mm with attachment Loss and Bleeding on Probing.

** p <0.004 (Wilcoxon)

*** p = 0.0007 (Wilcoxon)

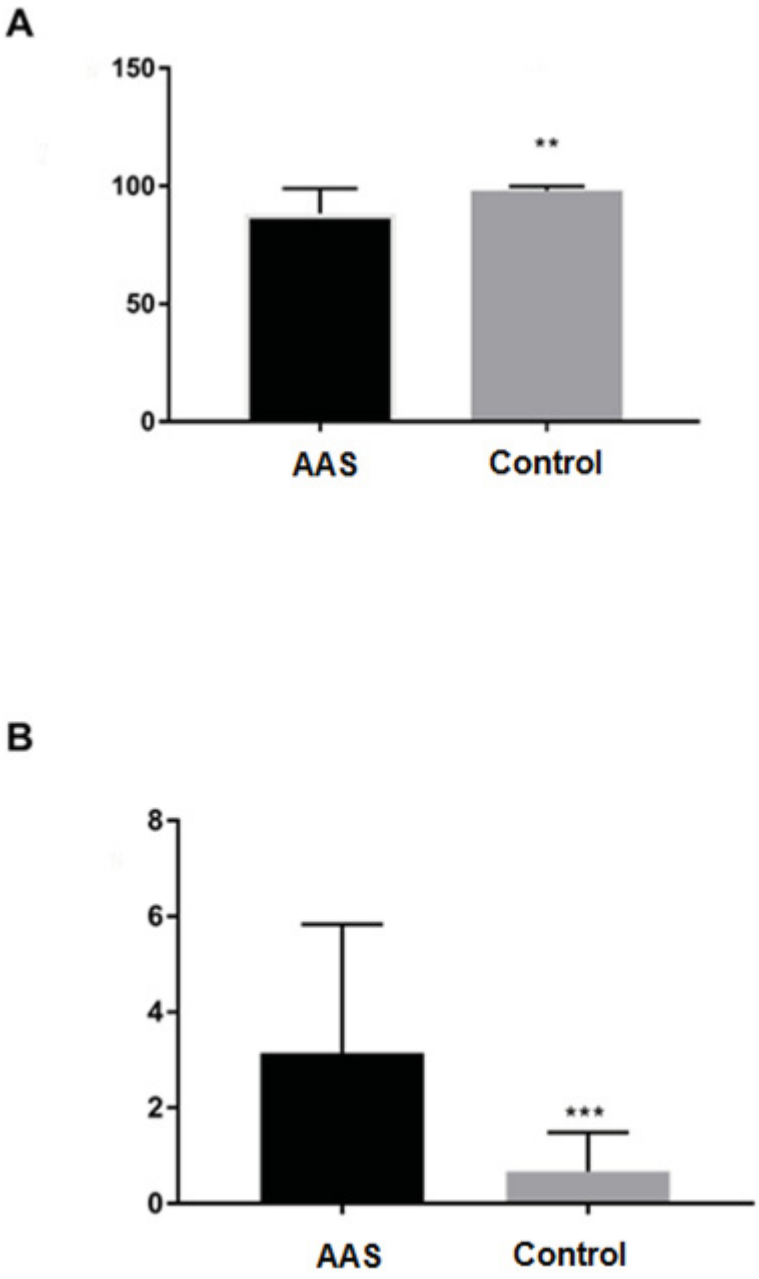
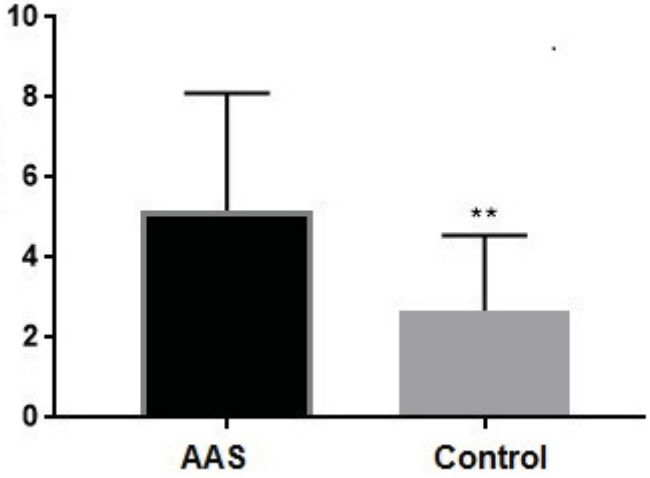


Figure 4: Percentage of sites with attachement loss (CAL>0)

** p = 0.003 (paired t-test).



4 CONCLUSÃO

Concluímos que o uso indevido de EAA pode afetar a saúde periodontal de seus usuários, especialmente em relação aos fatores inflamatórios como Profundidade de Sondagem e Sangramento Marginal. Portanto, esses indivíduos devem ser acompanhados por seus dentistas no momento do abuso dessas substâncias.

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APÊNDICE 1 – METODOLOGIA DETALHADA

Após primeira análise, realizada em novembro de 2016, pelo Comitê de Ética em Pesquisa- Setor de Ciências da Saúde (CEP/SD), da Universidade Federal do Paraná (UFPR), este trabalho não foi aceito e aprovado. Entretanto, depois do envio ao Comissão Nacional de Ética em Pesquisa –CONEP (1.906.729 - 2.443.657), foi aprovado. Trata-se de um estudo caso-controle, realizado no período de março de 2017 a junho de 2018, nas dependências da Universidade Federal do Paraná-UFPR.

RECRUTAMENTO DE PACIENTES

Foram convidados a participar do estudo: homens adultos com idade igual ou superior a 18 anos, usuários auto reportados de anabolizantes andrógenos, praticantes de atividades física, não tabagistas, sem doenças sistêmicas que evidenciem alterações inflamatórias, imunes ou hormonais. Os participantes, também, necessitavam de pelo menos 20 dentes em boca, excluindo-se terceiros molares e não terem o recebido tratamento periodontal ou medicação nos últimos 6 meses, para inclusão no estudo. Os participantes foram recrutados em academias de ginástica e musculação, eventos de fisioculturismo, com a utilização de cartazes explicativos da pesquisa.

A primeira tentativa de recrutamento de pacientes foi realizada através da elaboração de flyers que foram entregues em academias, nos cursos de educação física das universidades locais e no Serviço de Endocrinologia e Metabologia do Hospital de Clínicas da Universidade Federal do Paraná. A segunda tentativa foi por meios de comunicação e mídias locais, com entrevistas em rádio e jornal transmitido em rede aberta local. Junto a isso, frequentou-se um evento de competicação de fisioculturismo e academias de treinamentos específicos para esse tipo de competição, onde foi possível o acesso aos competidores usuários de EAA.

Foram convidados também, homens adultos com características semelhantes, entretanto, não usuários de qualquer tipo de fármaco que apresentassem esse hormônio. Os grupos foram pareados de acordo com idade. Ambos os grupos continham o mesmo número de integrantes, para correto pareamento.

Aos que demonstraram interesse na participação, foram explicados todos os passos da metodologia, bem como os riscos e benefícios inerentes do estudo. Todos os indivíduos que concordaram com a participação, assinaram o Termo de Consentimento Livre e Esclarecido (TCLE).

EXAME CLÍNICO

Após a formação de ambos os grupos, foi realizado o exame clínico odontológico para avaliação dos índices relacionados a saúde periodontal dos participantes. Os índices utilizados foram: índice de placa (*O'Leary*), sangramento marginal (SM), profundidade de sondagem (PS), sangramento à sondagem (SS) e nível de inserção clínica (NIC) - em 6 sítios por dente. O paciente com ao menos dois sítios com profundidade de sondagem superior a 3 mm, com perda de inserção e sangramento à sondagem no mesmo sítio foi diagnosticado com periodontite. Para o exame clínico utilizou-se espelho clínico n°5, pinça clínica e sonda periodontal milimetrada Hu-Friedy®.

A categorização da saúde gengival dos participantes baseou-se nos critérios adotados pela classificação de doenças periodontais de 2018 da Academia Americana de Periodontia e Federação Europeia de Periodontia. O diagnóstico de periodontite crônica deu-se na presença de pelo menos dois sítios com profundidade de sondagem maior que 3mm, com perda de inserção e sangramento à sondagem no mesmo sítio. Os procedimentos de avaliação foram realizados por examinador único previamente treinado e calibrado.

Todos os participantes receberam orientações de higiene bucal e cuidados para a manutenção da saúde periodontal. Após o exame clínico inicial para os padrões periodontais, os participantes foram submetidos a profilaxia e avaliação de outras condições odontológicas. Sendo constatada a necessidade de outros tratamentos dentários, os participantes foram encaminhados ao Serviço de Atendimento Odontológico da Universidade Federal do Paraná para atendimento adequado, restabelecendo a saúde bucal. Os nomes dos pacientes foram substituídos por códigos, resguardando a identidade dos mesmos e as fichas clínicas foram arquivadas de acordo com a legislação em vigor.

ANÁLISE ESTATÍSTICA

A análise estatística foi executada com o software GraphPadPrism 7®. Percentagens, médias e desvios padrão, foram obtidos para descrição das características socioeconômicas, demográficas e clínicas dos participantes. Os procedimentos de avaliação foram realizados por um examinador previamente treinado e calibrado (SVSCW, Kappa ± 1mm = 0,97).

ANEXO 1 – PARECER DA APROVAÇÃO DO PROJETO DE PESQUISA PELA COMISSÃO NACIONAL DE ÉTICA EM PESQUISA – *CONEP*

COMISSÃO NACIONAL DE ÉTICA EM PESQUISA

PlataForma Brasil

PARECER CONSUBSTANCIADO DA CONEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: Perfil clínico, microbiológico e imunológico da condição periodontal de homens com hipogonadismo

Pesquisador: João Paulo Steffens

Área Temática:

Versão: 3

CAAE: 59617016.8.0000.0102

Instituição Proponente: Departamento de Estomatologia

Patrocinador Principal: Financiamento Próprio

DADOS DO PARECER

Número do Parecer: 1.906.729

Apresentação do Projeto:

INTRODUÇÃO

Os hormônios sexuais masculinos (andrógenos) incluem a androstenediona (ASD), androstenediol (ADIOL), testosterona (T) e a dihidrotestosterona (DHT). A T é o principal andrógeno e está envolvida no desenvolvimento e manutenção da massa muscular; no estímulo à eritropoiese; no aumento da perfusão cerebral, influenciando no humor e cognição; e na saúde óssea através da inibição da ação de osteoclastos e reabsorção óssea e estímulo à atividade osteoblástica e crescimento ósseo. 1 Noventa e cinco porcento da T circulante é produzida pelos testículos, enquanto os 5% restantes são sintetizados localmente nos tecidos-alvo a partir da ASD e ADIOL. Estas duas são sintetizadas a partir da dihidroepiandrosterona (DHEA) ou DHEA-sulfato (DHEAS), as quais são secretadas pelo córtex adrenal. 2 A ação da T no local onde exercerá seu efeito biológico pode se dar diretamente através da ligação a receptores de andrógenos, ou indiretamente através de sua conversão a DHT ou estradiol (E2). A DHT é formada a partir da enzima 5–redutase, é metabolicamente mais ativa que a T e é produzida no fígado, rins, pele e próstata. As enzimas hidroxiesteroide-desidrogenase podem converter a DHT reversivelmente aos metabólitos androstano 3,17-diol ou androsterona (ADT). Por outro lado, o E2 é produzido em macrófagos e fibroblastos no fígado, rins, cérebro e tecido adiposo, e acredita-se que é o maior

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COMISSÃO NACIONAL DE ÉTICA EM PESQUISA

Continuação do Parecer: 1.906.729

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Situação do Parecer:
Aprovado

BRASILIA, 07 de Março de 2017

Assinado por:
Jorge Alvec de Almeida Venancio
(Coordenador)

ANEXO 2 – PARECER DA APROVAÇÃO DA EMENDA DO PROJETO DE PESQUISA PELA COMISSÃO NACIONAL DE ÉTICA EM PESQUISA – *CONEP*

COMISSÃO NACIONAL DE ÉTICA EM PESQUISA

Plataforma Brasil

PARECER CONSUBSTANCIADO DA CONEP

DADOS DA EMENDA

Título da Pesquisa: Perfil clínico, microbiológico e imunológico da condição periodontal de homens com hipogonadismo

Pesquisador: João Paulo Steffens

Área Temática: A critério do CEP

Versão: 5

CAAE: 59617016.8.0000.0102

Instituição Proponente: Departamento de Estomatologia

Patrocinador Principal: Financiamento Próprio

DADOS DO PARECER

Número do Parecer: 2.443.657

Apresentação do Projeto:

INTRODUÇÃO

Os hormônios sexuais masculinos (andrógenos) incluem a androstenediona (ASD), androstenediol (ADIOL), testosterona (T) e a dihidrotestosterona (DHT). A T é o principal andrógeno e está envolvida no desenvolvimento e manutenção da massa muscular; no estímulo à eritropoiese; no aumento da perfusão cerebral, influenciando no humor e cognição; e na saúde óssea através da inibição da ação de osteoclastos e reabsorção óssea e estímulo à atividade osteoblástica e crescimento ósseo. 1 Noventa e cinco porcento da T circulante é produzida pelos testículos, enquanto os 5% restantes são sintetizados localmente nos tecidos-alvo a partir da ASD e ADIOL. Estas duas são sintetizadas a partir da dihidroepiandrosterona (DHEA) ou DHEA-sulfato (DHEAS), as quais são secretadas pelo córtex adrenal. 2 A ação da T no local onde exercerá seu efeito biológico pode se dar diretamente através da ligação a receptores de andrógenos, ou indiretamente através de sua conversão a DHT ou estradiol (E2). A DHT é formada a partir da enzima 5–redutase, é metabolicamente mais ativa que a T e é produzida no fígado, rins, pele e próstata. As enzimas hidroxiesteroide-desidrogenase podem converter a DHT reversivelmente aos metabólitos androstano 3,17-diol ou androsterona (ADT). Por outro lado, o E2 é produzido em macrófagos e fibroblastos no fígado, rins, cérebro e tecido adiposo, e acredita-se que é o maior regulador da homeostase óssea no homem. Pode atuar através de receptores de estrógeno (ER) e ou GPR-30, todos expressos em tecido ósseo. Apesar disto, apenas o ER - parece estar envolvido

COMISSÃO NACIONAL DE ÉTICA EM PESQUISA

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Situação do Parecer:
Aprovado

BRASILIA, 17 de Dezembro de 2017

Assinado por:
Jorge Alves de Almeida Venancio
(Coordenador)

ANEXO 3 – NORMAS DA REVISTA: JOURNAL OF PERIODONTOLOGY

The *Journal of Periodontology* publishes articles relevant to the science and practice of periodontics and related areas. Manuscripts are accepted for consideration with the understanding that text, figures, photographs, and tables have not appeared in any other publication, except as an abstract prepared and published in conjunction with a presentation by the author(s) at a scientific meeting, and that material has been submitted only to this journal.

The *Journal of Periodontology* accepts manuscript submissions online at ScholarOne Manuscripts. To start a new submission, enter the Author Center and click "Click here to submit a new manuscript." Details regarding each submission step are located at the top of the page in ScholarOne Manuscripts. Authors should prepare manuscripts in accordance with the instructions below. Failure to do so may result in delays or manuscript unsubmission.

MANUSCRIPT CATEGORIES AND SPECIFIC FORMATS

Submissions to the *Journal of Periodontology* should be limited to one of the categories defined below. Specific information regarding length and format is provided for each category. Please also refer to the instructions provided under General Format and Style. All manuscripts will be reviewed by the Editors for novelty, potential to extend knowledge, and relevance to clinicians and researchers in the field. Some manuscripts will be returned without review, based on the Editors' judgment of the appropriateness of the manuscript for the *Journal of Periodontology*.

ORIGINAL ARTICLES

These are papers that report significant clinical or basic research on the pathogenesis, diagnosis, and treatment of the different forms of periodontal disease. Papers dealing with design, testing, and other features of dental implants are also included.

Format

Original articles must be limited to 4,000 words (excluding the abstract, references, and figure legends). The reference list should not exceed 50 references, and the total combined number of figures and tables must be six or fewer. Multi-panel figures are acceptable.

Abstract

All original articles should be submitted with a structured abstract, consisting of no more than 250 words and the following four paragraphs:

- Background: Describes the problem being addressed.
- Methods: Describes how the study was performed.
- Results: Describes the primary results.
- Conclusion(s): Reports what authors have concluded from these results, and notes their clinical implications.

Introduction

The Introduction contains a concise review of the subject area and the rationale for the study. More detailed comparisons to previous work and conclusions of the study should appear in the Discussion section.

Materials and Methods

This section lists the methods used in the study in sufficient detail so that other investigators would be able to reproduce the research. When established methods are used, the author need only refer to previously published reports; however, the authors should provide brief descriptions of methods that are not well known or that have been modified. Identify all drugs and chemicals used, including both generic and, if necessary, proprietary names and doses. The populations for research involving humans should be clearly defined and enrollment dates provided.

Results

Results should be presented in a logical sequence with reference to tables, figures, and supplemental material as appropriate.

Discussion

New and possible important findings of the study should be emphasized, as well as

any conclusions that can be drawn. The Discussion should compare the present data to previous findings. Limitations of the experimental methods should be indicated, as should implications for future research. New hypotheses and clinical recommendations are appropriate and should be clearly identified. Recommendations, particularly clinical ones, may be included when appropriate.

Publication of Accepted Original Articles

Please note that accepted manuscripts which are classified by the Editors as "Discovery Science" will be placed on an accelerated schedule for **online-only publication**. See Online-Only Publication below.

REVIEW ARTICLES

The JOP is no longer accepting submissions of reviews. Authors may be invited to submit reviews for potential publication, but unsolicited reviews will no longer be accepted.

COMMENTARIES

The purpose of these papers is to provide a forum for discussion of controversies and other issues as they relate to the practice of periodontics and implant dentistry. Full and balanced discussion of controversies on important issues is encouraged. This may result in several authors each presenting a relevant viewpoint. Commentaries should be concise (2,000 to 3,000 words) with no more than 50 references; however, they should be complete and balanced, which may require that the issue or controversy addressed be highly focused.

Introduction

This section should clearly state the clinical question or issues to be discussed and document their importance and timeliness.

Body

The body should present the information supporting all aspects of the issues. This portion of the Commentary may be subdivided as appropriate with headings. Figures,

tables, and other illustrative materials may be incorporated. The total combined number of figures and tables should not exceed six.

Summary

The summary should place the issue in perspective and point a way for future directions in addressing the controversy.

Acknowledgment(s)

Since these papers allow authors to express their opinions on a subject, it is extremely important that authors disclose any and all affiliations, financial position, or any other information that constitutes a real or perceived conflict of interest.

CASE SERIES

The *Journal of Periodontology* no longer publishes Case Reports. Authors are encouraged to submit Case Reports to *Clinical Advances in Periodontics*. The *Journal of Periodontology* publishes selected Case Series that describe unusual case presentations, complex diagnoses, and novel approaches to treatment within the scope of practice of periodontology. These Case Series provide valuable information for clinicians and teachers in the field.

Case Series report a sufficient number of consecutive or randomized cases to make a persuasive argument for or against the procedure, technique, or concept under discussion. Cases should be relatively homogeneous so that a systematic evaluation of one type of disease, lesion, or condition is made for the procedure under consideration. Also, treatment and documentation should be consistent and standardized for all cases. It is recognized that definitive evidence for the safety and efficacy of any procedure, drug, or device comes primarily from well-designed, randomized, controlled trials. However, well-executed Case Series may lead to hypotheses about the usefulness of new and innovative procedures, drugs, or devices and may therefore be of value to the progress of clinical science.

The requirements for patient consent, privacy, and institutional approval are well defined for manuscripts describing research on human subjects. These basic requirements are described by the International Committee of Medical Journal Editors (ICMJE) in their Uniform Requirements for Manuscripts Submitted to

Biomedical Journals (available at: www.icmje.org) and are interpreted in the instructions to authors of all peer-reviewed biomedical journals, including the *Journal of Periodontology*.

Due to the changing ethical and legal environment around the use of patient information, the editorial team has received multiple questions about the need for subject consent from patients described in Case Series submitted for publication.

The following applies to most Case Series. It should be noted that the Editors will determine whether specific Case Series require additional approvals beyond what is described below.

Requirement for Ethics Board Approval

Most Case Series are a retrospective description of clinical findings in cases or an observed course of events that document a new aspect of patient management during the normal course of clinical treatment. Since there is no hypothesis testing, no systematic data collection beyond that which is part of routine clinical practice, no data analysis, and the work has already been done, Case Series do not usually qualify as "research" requiring approval from ethical boards designed to protect humans involved in clinical research.

(U.S. Fed. definition: "RESEARCH is any systematic investigation, including research development, testing and evaluation, designed to develop or contribute to generalizable knowledge.")

Example 1: Series of private practice implant cases in patients who have been taking bisphosphonates. Authors describe the findings in each case, which are collected and reported in a table format.

Example 2: Authors collect series of private practice implant cases in patients who have or have not been taking bisphosphonates. The sample size is sufficient for data analysis, and authors analyze and report the incidence of complications.

Example 1 does not qualify as "research," but example 2 does qualify and requires ethical approval.

Please see "Does My Case Series Need IRB Approval?" for more information.

Privacy in Case Series

No patient identifiers should be included in Case Series. If the authors choose to include any subject identifiers, the authors must include the patient's informed written consent to publish the information.

Our policy conforms to the Uniform Requirements, which states: "Patients have a right to privacy that should not be violated without informed consent. Identifying information, including names, initials, or hospital numbers, should not be published in written descriptions, photographs, or pedigrees unless the information is essential for scientific purposes and the patient (or parent or guardian) gives written informed consent for publication. Informed consent for this purpose requires that an identifiable patient be shown the manuscript to be published. Authors should disclose to these patients whether any potential identifiable material might be available via the Internet as well as in print after publication."

It should be noted that patients may have given a signed "consent to treat," but that does not constitute permission to publish their case with personal identifiers unless they have explicitly approved the manuscript. Likewise, patient consent under government privacy rules, such as the Health Insurance Portability and Accountability Act (HIPAA) in the United States, does not constitute permission to publish their case with personal identifiers unless they have explicitly approved the manuscript.

Format

Case Series must be limited to 2,000 to 3,000 words (excluding the abstract, references, and figure legends). The reference list should not exceed 50 references, and the total combined number of figures and tables must be six or fewer. Multi-panel figures are acceptable.

Abstract

Case Series should be submitted with a structured abstract, consisting of no more than 250 words and the following four paragraphs:

- Background: Describes the clinical situation being discussed.
- Methods: Describes the clinical procedures (surgical and non-surgical) performed.
- Results: Describes the clinical results.

- Conclusion(s): Reports what authors have concluded, specifically clinical implications in practice situations.

Introduction

This section should include a critical review of the pertinent literature.

Case Description and Results

This section describes the cases, including all relevant data. For ease of presentation, tables describing longitudinal data in a chronological form may be useful. Carefully selected, high-quality clinical photographs in full color, as well as radiographs, are encouraged.

Discussion

This should include findings, put into perspective with respect to the field and literature. Unique arguments and new information gained should be summarized. Consideration of the clinical significance of the cases should be emphasized in all sections.

GUEST EDITORIALS

Guest Editorials may be invited or may be submitted from authorities in certain areas as a means of offering their perspective on one or more articles published in the *Journal*, or on other items of interest to the readership.

LETTERS TO THE EDITOR

Letters may comment on articles published in the *Journal* and should offer constructive criticism. If a letter comments on a published article, the author(s) will be provided 30 days to respond to the observations.

Letters to the Editor may also address any aspect of the profession, including education and training, new modes of practice, and concepts of disease and its management.

Letters should be brief (<1,000 words), focused on one or a few specific points or concerns, and can be signed by no more than five individuals.

Citations should be handled as standard references.

GENERAL FORMAT

Manuscripts must be submitted in Microsoft Word. Margins should be at least 1" on both sides and top and bottom and all text should be double-spaced. Materials should appear in the following order:

- Title Page
- Abstract (or Introduction) and Key Words
- Text
- Footnotes
- Acknowledgment(s)
- References
- Figure Legends
- Tables

Figures should not be embedded in the manuscript. Please see the *Journal of Periodontology* Digital Art Guidelines for more information on submitting figures.

Authors should retain a copy of their manuscript for their own records.

TITLE PAGE

The title page should contain:

1. a concise but informative title;
2. first name, middle initial, and last name of each author, with the highest academic degree and the current institutional affiliation, including department, for each (please use footnote symbols in the sequence *, †, ‡, §, ‖, ¶, #, **, etc. to identify authors and their corresponding institutions);
3. disclaimers, if any;
4. the name and address (including fax number and e-mail) of the author responsible for correspondence (please indicate whether fax number and e-mail can be published);
5. word count and number of figures, tables, and references in the manuscript;
6. a short running title of no more than 60 characters, including spaces;
7. a one-sentence summary describing the key finding(s) from the study.

KEY WORDS

A maximum of six key words or short phrases, drawn from MeSH documentation, to facilitate indexing should be listed below the abstract.

ACKNOWLEDGMENT(S) AND CONFLICTS OF INTEREST

Acknowledgment(s)

Following the Discussion, acknowledgments may be made to individuals who contributed to the research or the manuscript preparation at a level that did not qualify for authorship. This may include technical help or participation in a clinical study. Authors are responsible for obtaining written permission from persons listed by name. Acknowledgments must also include a statement that includes the source of any funding for the study, and defines the commercial relationships of each author.

Conflicts of Interest

In the interest of transparency and to allow readers to form their own assessment of potential biases that may have influenced the results of research studies, the *Journal of Periodontology* requires that all authors declare potential competing interests relating to papers accepted for publication. Conflicts of interest are defined as those influences that may potentially undermine the objectivity or integrity of the research, or create a perceived conflict of interest.

Authors are required to submit:

1. A statement in the acknowledgments section of the manuscript that includes the source of any funding for the study, and defines the commercial relationships of each author. If an author has no commercial relationships to declare, a statement to that effect should be included. This statement should include financial relationships that may pose a conflict of interest or potential conflict of interest. These may include financial support for research (salaries, equipment, supplies, travel reimbursement); employment or anticipated

employment by any organization that may gain or lose financially through publication of the paper; and personal financial interests such as shares in or ownership of companies affected by publication of the research, patents or patent applications whose value may be affected by this publication, and consulting fees or royalties from organizations which may profit or lose as a result of publication. An example is shown below.

2. A conflict of interest and financial disclosure form for each author. A link to this electronic form will be e-mailed to each author after manuscript submission.

Conflict of interest information will not be used as a basis for suitability of the manuscript for publication.

Example of Conflict of Interest Statement

This study was supported by a grant from the Acme Implant Corporation, Seoul, Korea. Dr. Lee is on the scientific advisory board for Acme Implant Corporation and gives lectures sponsored by the company. Dr. Smith is a consultant and shareholder of the Brownstone Implant Corporation, Boston, Massachusetts. Dr. Wang is employed full-time as chief technical officer of the Acme Implant Corporation. Drs. Able, Kim, and Bruce report no conflicts of interest related to this study.

REFERENCES

References should be numbered consecutively in the order in which they appear in the text. A journal, magazine, or newspaper article should be given only one number; a book should be given a different number each time it is mentioned, if different page numbers are cited.

All references are identified, whether they appear in the text, tables, or legends, by Arabic numbers in superscript. Journal title abbreviations should be those used by the U.S. National Library of Medicine. If you are uncertain about the correct abbreviation for a journal title, please search for the journal at http://www.ncbi.nlm.nih.gov/nlmcatalog.

The use of abstracts as references is strongly discouraged. Manuscripts accepted for publication may be cited and should include the manuscript's DOI, if known. Material submitted, but not yet accepted, should be cited in text as "unpublished observations." Written and oral personal communications may be referred to in text,

but not cited as references. Please provide the date of the communication and indicate whether it was in a written or oral form. In addition, please identify the individual and his/her affiliation. Authors should obtain written permission and confirmation of accuracy from the source of a personal communication. Presented papers, unless they are subsequently published in a proceedings or peer-reviewed journal, may not be cited as references. In addition, Wikipedia.org may not be cited as a reference. For most manuscripts, authors should limit references to materials published in peer-reviewed professional journals. In addition, authors should verify all references against the original documents. References should be typed double-spaced. Examples of references are given below. Authors are encouraged to consult EndNote for the *Journal of Periodontology*'s preferred reference style.

Journals

1. Standard journal reference. Note: list all authors if six or fewer; when seven or more, list only first three and add et al. Kurita-Ochiai T, Seto S, Suzuki N, et al. Butyric acid induces apoptosis in inflamed fibroblasts. *J Dent Res* 2008;87:51-55.
2. Corporate author. Federation Dentaire Internationale. Technical report no. 28. Guidelines for antibiotic prophylaxis of infective endocarditis for dental patients with cardiovascular disease. *Int Dent J* 1987;37:235.
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