



UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL  
FACULDADE DE ODONTOLOGIA  
PROGRAMA DE PÓS-GRADUAÇÃO EM ODONTOLOGIA  
DOUTORADO ÁREA DE CONCENTRAÇÃO  
CLÍNICA ODONTOLÓGICA/ CARIOLOGIA-DENTÍSTICA

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CÁRIE DENTÁRIA E PERDA DENTÁRIA EM ADOLESCENTES DO SUL  
DO BRASIL: UM ESTUDO DE COORTE

Porto Alegre

2022

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Tese apresentada ao Programa de Pós-Graduação em Odontologia como requisito parcial à obtenção do título de Doutora em Clínica Odontológica/ Cariologia-Dentística pela Universidade Federal do Rio Grande do Sul.

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Porto Alegre

2022

*“O cientista não é o homem que fornece as verdadeiras respostas; é quem faz as verdadeiras perguntas”.* Claude Lévi-Strauss

## DEDICATÓRIA

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Dedico este trabalho:

Ao *Senhor*, por ser a razão do meu viver.

Ao *Gabriel* e à *Sarah*, meus filhos e ao *André*, meu marido, meus tesouros.

## AGRADECIMENTOS

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Agradeço ao *meu marido André* por todo amor, suporte e apoio em todo o doutorado; à *minha tia Beti* pelas ajudas em diversos momentos durante esse período. Aos *meus pais Carlos e Andréa e a todos meus irmãos (Cláudia, Miriam, Raquel, João Pedro, Ana Maria, Samuel e André Carlos)* pelas palavras e ações sempre positivas a respeito dessa conquista.

À professora *Marisa Maltz*, orientadora deste trabalho, por todo carinho, atenção e ensinamento precioso.

À professora *Luana Severo Alves* por sua ajuda impecável. Pela sua gentileza. Por ter sido sempre tão doce e acolhedora. Uma verdadeira amiga, que vai além do seu profissionalismo. Quem diria que iríamos trabalhar juntas novamente no meu doutorado? Que grata surpresa! Serei sempre muito agradecida a ti, Lu.

Aos meus colegas de pós-graduação e alunos de graduação. Um agradecimento especial aos mais próximos com quem dividi tantos momentos: *Lais Ev, Gustavo Eidt, Rafael Azambuja, Ariel Rup, Manuella Goulart Buchmann e Luiza Vanzella*.

Às professoras *Clarissa Fatturi Parolo e Sandra Henz*, por toda a atenção e ajuda durante o doutorado e pela grande amizade.

Aos professores *Lina Naomi Hashizume, Juliana Jobim e Rodrigo Alex Arthur* pela amizade, carinho e incentivo.

À *Luisa Weber Mercado* e ao *Douglas*, laboratoristas do LABIM, pela amizade e pelas ajudas.

Ao *Programa de Pós-graduação em Odontologia da UFRGS*. Agradeço à coordenação, à equipe da secretaria e aos professores. Obrigada por todo empenho e dedicação que permitem sentir-me grata por adquirir mais este título nesta casa.

À *CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior)*, pelo apoio financeiro que permitiu o desenvolvimento desta pesquisa e a manutenção de meus custos durante minha formação.

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## APRESENTAÇÃO

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A presente tese baseia-se nos seguintes artigos, referidos no texto pelos seus números romanos:

**Artigo I:** Brusius CD, Alves LS, Maltz M. Is patient's caries activity associated with caries increment among adolescents, regardless of caries experience?

**Artigo II:** Brusius CD, Alves LS, Maltz M. Dental caries and tooth loss among adolescents and their association with tooth brushing frequency: a cohort study in southern Brazil.



## RESUMO

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**Objetivos:** O objetivo geral desta tese foi estudar a cárie dentária e a perda dentária em adolescentes de Porto Alegre, RS, após um período de 2,5 anos. Seus objetivos específicos foram: (1) Investigar se a atividade de cárie do paciente está independentemente associada ao incremento de cárie entre adolescentes, mesmo após o ajuste para experiência prévia de doença; (2) Avaliar a associação entre frequência de escovação e incremento de cárie e de perda dentária em adolescentes de Porto Alegre, RS. **Metodologia:** Na linha de base, entre 2009 e 2010, um estudo observacional transversal analítico foi desenvolvido em uma amostra representativa da população de escolares de 12 anos de Porto Alegre (n=1.528). A coleta de dados incluiu a aplicação de questionários e exame clínico odontológico. Entre 2012 e 2013, após um período de tempo médio de 2,5 anos ( $\pm 0,35$ ), 801 indivíduos foram reexaminados, representando 52,42% da amostra inicialmente examinada. As variáveis preditoras principais do Artigo I foram atividade de cárie e experiência de cárie. Quanto à atividade de cárie, foi considerado cárie-ativo o indivíduo que apresentou pelo menos uma lesão cariosa ativa no exame clínico inicial, não cavitada ou cavitada. Com relação à experiência de cárie, os adolescentes foram classificados como livres de cárie (índice CPO-D=0 no exame inicial) ou com experiência prévia de doença (índice CPO-D $\geq$ 1 no exame inicial), o que foi determinado tanto no nível de cavidade quanto no nível de lesões não cavitadas. No Artigo II, a variável preditora principal foi a frequência de escovação, classificada em  $\geq 3$  vezes/dia, 2 vezes/dia ou  $\leq 1$  vez/dia. Os desfechos do estudo foram incremento de cárie no nível de cavidade (Artigos I e II) e incremento de perda dentária (Artigo II). Modelos de regressão de Poisson (não ajustados e ajustados) foram usados para estimar o risco de incremento de cárie e incremento de perda dentária ao longo do período de estudo. Foram estimadas as razões de risco de incidência (RRI) e seus respectivos intervalos de confiança (IC) de 95%. **Resultados:** No Artigo I, observou-se que, mesmo após o ajuste para variáveis sociodemográficas (sexo, nível socioeconômico e tipo de escola) e experiência prévia de cárie, adolescentes classificados como cárie-ativos no início do estudo apresentaram um risco cerca de 2 vezes maior de incremento de cárie do que aqueles classificados como cárie-inativos (experiência de cárie avaliada no nível de cavidade, IRR ajustada = 2,03, IC 95% = 1,52-2,72; experiência de cárie avaliada no nível de lesões não cavitadas, IRR ajustada = 2,16, IC 95% = 1,63-2,86). No Artigo II, observou-se que escovar os dentes duas vezes/dia proporcionou risco 40% maior (IRR =

1,40; IC 95% = 1,02-1,92) enquanto escovar os dentes  $\leq 1$  vez/dia proporcionou risco cerca de 2 vezes maior (IRR=1,96; IC 95%=1,38-2,77) de incremento cárie do que a frequência  $\geq 3$  vezes/dia, mesmo após o ajuste para variáveis sociodemográficas, comportamentais e clínicas. Em relação ao incremento de perda dentária, os adolescentes que relataram escovar os dentes duas vezes ao dia tiveram um risco ao redor de 4 vezes maior (IRR ajustada = 3,92; IC 95% = 1,23-12,49) enquanto a menor frequência de escovação dentária ( $\leq 1$  vez/dia) resultou em um risco 7 vezes maior (IRR ajustada = 7,09; IC 95% = 2,24-22,49) do que aqueles que relataram uma frequência  $\geq 3$  vezes/dia. Além disso, o Artigo II demonstrou que sexo, tipo de escola e gengivite modificaram o efeito da frequência de escovação sobre o incremento de perda dentária, sendo que os indivíduos mais suscetíveis (sexo feminino, estudantes de escolas públicas e aqueles apresentando  $\geq 50\%$  dos sítios gengivais sangrantes) foram mais prováveis de se beneficiar de uma terceira escovação dentária por dia. **Conclusões:** (1) Atividade de cárie do paciente foi significativamente associada ao incremento de cárie entre adolescentes, mesmo após o ajuste para experiência prévia de doença; (2) Adolescentes que escovavam os dentes duas vezes/dia ou menos apresentaram maior incremento de cárie e perda dentária comparados com os que escovavam os dentes 3 vezes/dia; (3) Adolescentes mais suscetíveis são os mais beneficiados por uma terceira escovação dentária ao dia.

### **Palavras-chave**

Cárie dentária, fatores de risco, adolescentes, dentição permanente, estudo de coorte, escovação dentária, estudos longitudinais, perda de dentes.

## ABSTRACT

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**Aims:** The aim of this thesis was to study dental caries and tooth loss in adolescents from Porto Alegre, RS, after a period of 2.5 years. Its specific objectives were: (1) To investigate whether patient caries activity is independently associated with caries increment among adolescents, even after adjusting for previous disease experience; (2) To evaluate the association between brushing frequency and increased caries and tooth loss in adolescents from Porto Alegre, RS. **Methodology:** At baseline, between 2009 and 2010, an analytical cross-sectional observational study was carried out in a representative sample of the population of 12-year-old schoolchildren in Porto Alegre (n=1,528). Data collection included the application of questionnaires and clinical dental examination. Between August 2012 and May 2013, after an average time of 2.5 years ( $\pm 0.35$ ), 801 individuals were re-examined, representing 52.42% of the sample initially examined. The clinical examination followed the same procedure as the previous examination. The main predictor variables for Article I were caries activity and caries experience. As for caries activity, the individual who had at least one active carious lesion in the initial clinical examination, non-cavitated or cavitated, was considered caries active. Regarding caries experience, adolescents were classified as caries-free (DMF-D index=0 in the initial exam) or with previous experience of disease (DMF-D $\geq$ 1 in the initial exam), which was determined both at the cavity level and at the level of non-cavitated lesions. In Article II, the main predictor variable was brushing frequency, classified as  $\geq 3$  times/day, 2 times/day, or  $\leq 1$  time/day. Study outcomes were caries increment at cavity-level (Articles I and II) and tooth loss increment (Article II). Poisson regression models (unadjusted and adjusted) were used to estimate the risk of increasing caries and increasing tooth loss over the study period. Incidence hazard ratios (IRI) and their respective 95% confidence intervals (CI) were estimated. **Results:** In Article I, it was observed that even after adjusting for socio-demographic variables (gender, socioeconomic level and type of school) and previous caries experience, adolescents classified as caries-active at the beginning of the study presented a risk of about 2 times greater caries increment than those classified as caries-inactive (caries experience assessed at cavity level, adjusted IRR = 2.03, 95% CI = 1.52-2.72; caries experience assessed at non-cavitated level of, adjusted IRR = 2.16, 95% CI = 1.63-2.86). . In Article II, it was observed that brushing teeth twice/day provided a 40% higher risk (IRR = 1.40; 95% CI = 1.02-1.92) while brushing teeth  $\leq 1$  time/day provided a risk about 2 times greater (IRR=1.96; 95% CI=1.38-2.77)

of caries increment than frequency  $\geq 3$  times/day, even after adjusting for sociodemographic, behavioral and clinical variables. Regarding the increase in tooth loss, adolescents who reported brushing their teeth twice/day had a risk around 4 times greater (adjusted IRR = 3.92; 95% CI = 1.23-12.49) while lower frequency of toothbrushing ( $\leq 1$  time/day) resulted in a 7-fold higher risk (adjusted IRR = 7.09; 95% CI = 2.24-22.49) than those who reported a frequency  $\geq 3$  times/day. In addition, Article II showed that sex, type of school and gingivitis modified the effect of brushing frequency on the increase in tooth loss, with the most susceptible individuals (female, public school students and those presenting  $\geq 50\%$  of bleeding gingival sites) were more likely to benefit from a third tooth brushing per day. **Conclusions:** (1) Patient caries activity was significantly associated with caries increment among adolescents, even after adjusting for previous disease experience; (2) Adolescents who brushed their teeth twice a day or less had a greater increase in caries and tooth loss compared to those who brushed their teeth 3 times a day; (3) More susceptible adolescents benefit most from having a third tooth brushing per day.

### **Key words**

Dental caries, risk factors, adolescents, permanent dentition, cohort study, toothbrushing frequency, longitudinal studies, tooth loss.

## INTRODUÇÃO

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### Cárie dentária em adolescentes

Uma redução significativa nas estimativas de cárie dentária tem sido observada em todo o mundo nas últimas décadas (KASSEBAUM; SMITH; BERNABÉ; FLEMING *et al.*, 2017). Apesar desses avanços, a prevalência de cárie permanece elevada em vários países em desenvolvimento e entre pessoas carentes em países desenvolvidos. Ainda, estima-se que 35% da população global seja afetada pela cárie dentária não tratada (KASSEBAUM; BERNABÉ; DAHIYA; BHANDARI *et al.*, 2015), permanecendo um grande desafio para a saúde pública (PERES; MACPHERSON; WEYANT; DALY *et al.*, 2019) devido ao seu impacto na qualidade de vida dos indivíduos afetados (NORA; DA SILVA RODRIGUES; DE OLIVEIRA ROCHA; SOARES *et al.*, 2018), bem como os elevados custos do tratamento (LISTL; GALLOWAY; MOSSEY; MARCENES, 2015).

Dados muito relevantes sobre a epidemiologia global da cárie dentária entre crianças de 12 anos foi gerada nos últimos anos, pois esta é considerada uma idade-índice para o diagnóstico e monitoramento das tendências dessa doença em todo o mundo. No Brasil, o índice CPO-D (número de dentes permanentes cariados, perdidos ou restaurados) médio de 7,3 em 1980 diminuiu para 2,78 em 2003 (NARVAI; FRAZÃO; RONCALLI; ANTUNES, 2006) e para 2,07 em 2010 (MINISTÉRIO DA SAÚDE, 2011), evidenciando a importante melhora na saúde bucal observada nesta faixa etária.

Dados epidemiológicos relacionados à saúde bucal em adolescentes têm revelado melhorias mais discretas quando comparados aos dados coletados durante o período da infância (SAWYER; AFIFI; BEARINGER; BLAKEMORE *et al.*, 2012). De acordo com o Projeto SB Brasil 2003, um índice CPO-D médio de 2,78 aos 12 anos aumentou para 6,17 aos 15-19 anos (MINISTÉRIO DA SAÚDE, 2004). Já no Projeto SB Brasil 2010, um índice CPO-D médio de 2,07 na faixa etária dos 12 anos aumentou para 4,25 na faixa etária dos 15-19 anos (MINISTÉRIO DA SAÚDE, 2011) Em ambos os estudos, é possível observar o importante incremento de cárie que ocorre durante a adolescência. Sua repercussão vai além do comprometimento do bem-estar físico, estendendo-se aos contextos emocional, mental, econômico e social (GLICK; WILLIAMS; KLEINMAN; VUJICIC *et al.*, 2016).

A idade de transição dos 12 aos 15 anos foi usada no presente estudo pelo aumento significativo dos índices de cárie, devido à importância de instituir medidas preventivas adequadas (MEJÀRE; AXELSSON; DAHLÉN; ESPELID *et al.*, 2014).

Os comportamentos relacionados à saúde bucal variam entre a infância e a adolescência, sendo esta última fase um período altamente formativo para a saúde futura, durante o qual padrões de comportamento e de estilo de vida estão sendo formados (VINGILIS; WADE; SEELEY, 2002). Durante esta fase, os adolescentes passam a se tornar responsáveis por seu cuidado em saúde e sujeitos a suas próprias escolhas (SAWYER; AFIFI; BEARINGER; BLAKEMORE *et al.*, 2012). Há um aumento da autonomia em relação às suas atitudes, com a diminuição da influência familiar (HARRIS, 2010). A vulnerabilidade do adolescente ao envolvimento em comportamentos de risco altamente prejudiciais à saúde é potencializada (DALLA NORA; KNORST; COMIM; RACKI *et al.*, 2022). E este cenário representa um desafio às estratégias de saúde pública. No entanto, poucos estudos avaliaram a incidência de cárie em adolescentes. Por isso, se faz necessário mais estudos que avaliem a incidência, a progressão, os fatores associados e que identifiquem os indivíduos mais suscetíveis nesta faixa etária.

### **Perda dentária em adolescentes**

A redução da experiência de cárie observada no Brasil tem sido acompanhada pela redução proporcional do componente P do índice CPO. Em 1980, o componente P era responsável por 17,24% do CPO-D aos 12 anos (PINTO, 1983), reduzindo para 6,4% em 2003 (MINISTÉRIO DA SAÚDE, 2006) e 5,8% em 2010 (MINISTÉRIO DA SAÚDE, 2011). Apesar desta redução gradual, uma parcela da população de crianças e adolescentes ainda apresenta dentes permanentes extraídos por cárie. Taxas de prevalência de perda dentária aos 14-19 anos entre 20,5% e 40,9% têm sido encontradas em diferentes populações (ATIEH, 2008; CASANOVA-ROSADO; MEDINA-SOLÍS; VALLEJOS-SÁNCHEZ, 2005; BARBATO; PERES, 2009).

De uma maneira geral, os estudos avaliando os fatores associados à perda dentária na adolescência demonstraram que fatores demográficos (sexo, idade, raça), socioeconômicos (renda, nível socioeconômico, educação), comportamentais (fumo, estilo de vida, consumo de açúcar, uso de medicamentos, frequência de escovação, visita ao dentista) e clínicos (gengivite, má oclusão, número de dentes cariados), quando

desfavoráveis, aumentam a probabilidade de apresentar perda de dentes permanentes na adolescência (ATIEH, 2008; CASANOVA-ROSADO; MEDINA-SOLÍS; VALLEJOS-SÁNCHEZ, 2005; BARBATO; PERES, 2009). Apesar destes estudos prévios, não há na literatura estudos longitudinais avaliando fatores de risco associados à incidência e ao incremento de perda dentária na faixa etária da adolescência.

Perdas dentárias pontuais e isoladas, majoritariamente acometendo primeiros molares, podem não ser percebidas negativamente pelos indivíduos, não representando uma barreira frente a novas extrações. Já foi demonstrado na literatura que a ocorrência de perda dentária em idades precoces tende a tornar os indivíduos mais receptivos a novas extrações ao longo da vida (EKLUND; BURT, 1994), o que reforça a necessidade de se estudar este desfecho em populações jovens e desenvolver estratégias que visem impedir a sua ocorrência em idades tão precoces.

### **Atividade e experiência de cárie**

Um planejamento de tratamento correto é baseado em um diagnóstico adequado. O diagnóstico da doença cárie é definido pela detecção do processo de desmineralização dos tecidos dentários, ou seja, pela presença de perda mineral em curso que pode ser avaliada clinicamente (FEJERSKOV, 2008). A destruição localizada dos tecidos duros, a lesão de cárie, é o sinal clinicamente detectável do processo de doença cárie. A presença de lesão de cárie ativa indica que os eventos de perda mineral superaram os eventos de ganho mineral (CURY; TENUTA, 2009) e que é necessário um tratamento para restabelecer esse desequilíbrio. O controle da doença interrompe a progressão da lesão, convertendo assim uma lesão ativa em uma lesão inativa. Portanto, tanto o diagnóstico quanto a classificação da atividade de cárie do paciente são importantes para direcionar o tratamento.

Um estudo prévio (MALTZ; LEAL; WAGNER; ZENKNER *et al.*, 2020) demonstrou a possibilidade de definir o perfil de atividade de cárie de um paciente com base nas características da lesão a nível de superfície e mostrou que o risco de progressão de cárie nas superfícies hígidas foi maior entre adolescentes classificados como cárie-ativos na linha de base em comparação com aqueles classificados como cárie-inativos ou livres de cárie, os quais se comportaram de forma semelhante. Porém esse estudo não fez análise a nível do paciente, ou seja, não considerou seu incremento geral de cárie.

O Quadro 1 apresenta um resumo de estudos que usaram modelos/combinções ou fatores de risco isolados para prever a ocorrência de cárie em crianças e adolescentes. Como pode ser observado, a experiência passada de cárie tem sido descrita como o melhor preditor de cárie no futuro.

Os índices de cárie mais utilizados medem a experiência acumulada ao longo da vida e, embora seja importante para entender a história natural da doença, não fornecem informações sobre os níveis da doença atual, o que é sem dúvida mais importante para a avaliação da carga da doença e planejamento de serviços de atendimento odontológico (KASSEBAUM; BERNABÉ; DAHIYA; BHANDARI *et al.*, 2015).



Quadro 1. Estudos com resumo de preditores de cárie em crianças e adolescentes.

<b>Autores, ano</b>	<b>Local</b>	<b>N</b>	<b>Idade</b>	<b>Preditores</b>	<b>Desfecho</b>
RUSSELL <i>et al.</i> , 1991	Escócia	372	12.6- 14.6	CPOS, placa, espécies bacterianas, testes salivares	Incremento de cárie correlacionado com a experiência anterior de cárie, capacidade tampão salivar e contagem de microrganismos.
KASSAWARA <i>et al.</i> , 2010	Piracicaba-SP-BR	765	7-10	Presença de experiência de cárie em dentes permanentes e a presença de lesão inicial	A associação entre o incremento do CPOD e a presença de lesões ativas iniciais foi significativa apenas para crianças de 9 a 10 anos.
PERES <i>et al.</i> , 2009	Pelotas-RS-BR	359	6 e 12	Aspectos sociodemográficos, comportamento, prevalência de cárie	Associação entre fatores sociais, biológicos e comportamentais e cárie dentária aos 12 anos. Entretanto os achados evidenciam falta de precisão dos preditores de cárie dentária.
VANOBERGEN <i>et al.</i> , 2001	Flanders (Bélgica)	3303	7	Fatores sociodemográficos, placa bacteriana, dieta, higiene oral e CPOS	CPOS basal e índices de placa foram significativamente associados a um alto incremento de cárie.
VALLEJOS-SÁNCHEZ <i>et al.</i> , 2006	México	452	6-9	Experiência anterior de cárie.	Cárie dentária é indicador importante do desenvolvimento de cárie subsequente neste grupo de crianças em um país de renda média.
SÁNCHEZ-PÉREZ <i>et al.</i> , 2009	México	110	6	Morfologia da fissura, experiência de cárie, taxa de fluxo salivar, resultados do teste de Snyder e contagens de bactérias.	Experiência de cárie, teste Snyder e morfologia da fissura tiveram associação mais forte com o incremento de cárie.

Mejare e colaboradores (2014) realizaram uma revisão sistemática com o objetivo de avaliar a capacidade de modelos multivariados e fatores únicos predizerem o desenvolvimento futuro de cárie em crianças e adolescentes. A revisão incluiu 90 estudos, com somente um estudo na faixa etária de 12-13 anos e nenhum com faixa etária superior. Dos preditores únicos, a experiência de cárie inicial teve acurácia moderada/boa em pré-escolares e acurácia limitada em escolares/adolescentes. Considerando o limitado número de estudos em adolescentes, o objetivo do Artigo I da presente tese foi comparar esses dois preditores em adolescentes: experiência acumulada de doença e atividade de cárie.

### **Frequência de escovação dentária e cárie**

O biofilme cariogênico dos dentes é um pré-requisito para o estabelecimento da cárie dentária (FEJERSKOV; MANJI, 1990). Uma boa higiene oral por meio de uma escovação eficaz desempenha um papel fundamental na manutenção da saúde bucal. A escovação uma vez ao dia deve ser suficiente para o controle da cárie, pois o biofilme dental deve ter 2 dias de idade para produzir níveis de ácido suficientes para causar a desmineralização do esmalte (IMFELD; LUTZ, 1980). No entanto, a eficácia da limpeza com uma escova de dentes não é alta e a cárie dentária é uma doença multifatorial, com inúmeros determinantes que influenciam a doença, como consumo de açúcar, taxa de fluxo salivar, capacidade tampão da saliva, entre outros. Deste modo, não existe um padrão estabelecido de higiene oral considerado eficaz para controlar o desenvolvimento e progressão da lesão de cárie. Além disso, o flúor tende a diminuir o processo de desmineralização. A higiene bucal realizada com dentifício fluoretado (DF) combina limpeza mecânica e aplicação tópica de flúor, melhorando o efeito protetor da escovação dentária (DIJKMAN; HUIZINGA; RUBEN; ARENDS, 1990).

Duas revisões sistemáticas avaliando frequência de escovação mostraram que escovar os dentes  $\geq 2$  vezes ao dia é mais eficaz para o controle da cárie dentária do que  $< 2$  vezes ao dia (KUMAR; TADAKAMADLA; JOHNSON, 2016; MARINHO; HIGGINS; SHEIHAM; LOGAN, 2003). Porém, a maioria dos estudos não compara a frequência de escovação 2 vezes ao dia com 3 vezes ao dia ou mais, a frequência mais comumente realizada no Brasil e recomendada pelo Ministério da Saúde (Brasil Sorridente). Em um estudo *in situ*, Souza *et al.* (SOUZA; HASHIZUME; EIDELWEIN; MALTZ, 2010) demonstraram que o uso de dentifício com flúor 3 vezes/dia aumentou

a proteção contra desmineralização quando comparado a 2 vezes/dia, evidenciando que há um benefício adicional de uma terceira aplicação diária de flúor para o controle da cárie dentária. No entanto, poucos estudos investigaram o possível benefício decorrente de uma terceira escovação diária.

Tendo em vista observações de estudos laboratoriais e epidemiológicos acerca desse possível benefício adicional do aumento da frequência de escovação no controle da cárie dentária, o Artigo II que compõe a presente tese se propôs a avaliar a associação entre essa frequência de escovação e incremento de cárie e de perda dentária em adolescentes.

## OBJETIVOS

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### **Objetivo geral**

Estudar o incremento de cárie dentária e o incremento de perda dentária em adolescentes de 14 a 15 anos de Porto Alegre, RS após 2,5 anos de acompanhamento.

### **Objetivos específicos**

- Investigar se a atividade de cárie do paciente está independentemente associada ao incremento de cárie entre adolescentes, mesmo após o ajuste para experiência prévia de doença (Artigo I);
- Avaliar a associação entre frequência de escovação e incremento de cárie e de perda dentária em adolescentes de Porto Alegre, RS (Artigo II).

**Is patient's caries activity associated with caries increment among adolescents, regardless of caries experience?**

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**Short title:** Caries experience vs. caries activity as predictor of future caries

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**Number of tables: 2**

**Number of figures: 0**

**Word count: 2,562**

**Keywords:** Dental caries. Adolescent. Permanent dentition. Cohort study. Risk assessment.

## **Abstract**

This 2.5-year cohort study investigated whether patient's caries activity is independently associated with caries increment among adolescents, regardless of previous caries experience, in a sample of 801 adolescents from South Brazil. Caries examination was performed at baseline (12y) and at follow-up (14–15y). Caries activity was significantly associated with caries increment even after adjustment for sex, socioeconomic status, type of school, and previous caries experience at both cavity and non-cavitated levels. Caries-active adolescents had approximately 2-fold higher risk of caries increment than those without caries activity (cavity level, IRR=1.90, 95%CI=1.45-2.49,  $p<0.001$ ; non-cavitated level, IRR=2.16, 95%CI=1.63-2.86,  $p<0.001$ ).

## **Introduction**

Dental caries is a dynamic process involving acid production by the dental biofilm overlaying tooth surfaces. When the demineralization process is underway (the disease is established) and the accumulated sum of mineral loss reaches certain threshold levels, a clinically detectable lesion is originated. This lesion acquires specific clinical characteristics of an active lesion. When the balance is re-established, the progression of the lesion is controlled, and an active lesion is converted into an inactive lesion, which also presents specific clinical characteristics [Holmen et al., 1985; Nyvad and Fejerskov, 1986; Artun and Thylstrup, 1989; Nyvad et al., 1999; Fejerskov et al., 2015]. Disease activity is almost impossible to measure accurately unless the site-specific acidogenicity of biofilm and demineralization can be quantified in a real-time manner. Due to this difficulty, clinical assessment of lesion features has been used to evaluate at a given timepoint the state, not the process, of demineralization. Surface color, texture/consistency, and light reflection has been advocated as indicators of the activity status of a lesion, being widely used in the literature [Nyvad et al., 2003; Ferreira Zandoná et al., 2012; Maltz et al., 2020; Cabral et al., 2022]. Notwithstanding, although active and inactive lesions have different clinical features, the use of lesion features as an indicator of caries disease has two main limitations. First, there is a possibility that the patient is undergoing a process of mineral loss that has not achieved a clinically detectable threshold, being restricted to ultrastructural or microscopic levels. Second, the disease is under control, but the clinical features of the lesion have not yet changed.

It is known that information on lesion activity derived from a single clinical examination can only reveal the result of mineral loss, and not caries/disease activity. However, a previous study by our research group demonstrated the possibility of defining a patient's caries activity profile (disease activity) based on the clinical characteristics of the lesions after a single examination [Maltz et al., 2020]. At baseline, 801 12-year-old South Brazilian adolescents were classified as caries-free, caries-inactive (with no active lesion), or caries-active (with at least one active lesion). After 2.5 years of follow-up, we observed that the risk of caries development on sound tooth surfaces was higher in caries-active adolescents, whereas it was similar between caries-inactive and caries-free individuals. Nevertheless, the previous study only included the surfaces classified as sound at baseline in the risk analysis, because that was the only type of surface present in the three types of patients. Additionally, the study performed a surface-level analysis with a binary outcome (caries development: “yes” or “no”), not considering the overall

patient's caries increment ( $\Delta$  Decayed, Missing, and Filled Teeth/Surfaces [DMF-T/S]) over time.

Considering that (1) no sufficient evidence is available to confirm that different caries risk assessment models including sociodemographic, behavioral, clinical, salivary, and microbiological variables are effective in caries prediction among adolescents [Cagetti et al., 2018], and (2) past caries experience has been considered the best predictor of future caries [Mejàre et al., 2014], this study aimed to investigate whether patient's caries activity is independently associated with caries increment among adolescents, regardless of previous caries experience. We hypothesized that caries activity is associated with caries increment, even after the adjustment for caries experience, as it refers to the current disease status of a patient instead of the accumulated history of the disease. To the best of our knowledge, no study has investigated this issue until this date.

## **Methods**

This study was based on the data from a 2.5-year prospective cohort study conducted in South Brazil to assess several oral health outcomes. At baseline (2009–2010), a representative sample of the population of 12-year-old schoolchildren from Porto Alegre, South Brazil was drawn using a multistage probability sampling strategy. To estimate a caries prevalence of 60% [Maltz et al., 2001] with a precision level of  $\pm 3\%$  for a 95% confidence interval (CI) and a design effect of 1.3, a minimum sample size of 1,331 schoolchildren was required for this study. A non-response error of 40% was added, and the final sample size estimated was 1,837. In total, 1,528 schoolchildren attending 42 schools (33 public and 9 private schools) were examined, yielding a response rate of 83.2%. This sample size far exceeded that needed for an association study investigating caries experience and caries activity (statistical power calculated a posteriori achieved 100% for both predictors). Further details on the sampling strategy and non-response analysis can be found elsewhere [Alves et al., 2014].

Data collection included questionnaire administration and clinical examinations. At baseline, a structured questionnaire on sociodemographic information was sent to the parents/legal guardians of the selected students.

The clinical examination was conducted at the schools with the students in a supine position and under artificial light, using a flat mirror, periodontal probe, and portable equipment (air compressor and suction). Professional tooth cleaning and drying were performed. Following this, the examiner recorded the presence of caries lesions



according to the following criteria: 1) active non-cavitated (ANC) lesion, opaque enamel with a dull-whitish surface; 2) inactive non-cavitated (INC) lesion, shiny appearance of the surface area with white or different degrees of brownish discoloration; 3) active cavitated (AC) lesion, localized surface destruction with active characteristics (dull-whitish enamel and soft dentin); and 4) inactive cavitated (IC) lesion, localized surface destruction with arrested characteristics (shiny enamel and hard dentin) [Maltz et al., 2003]. The presence of underlying dentin shadows and missing or filled teeth were also recorded. Clinical examinations were performed at baseline and follow-up for all erupted permanent teeth according to the same protocol.

At follow-up, 801 schoolchildren were re-examined (2012–2013) after a mean period of 2.5 years (standard deviation=0.3), representing 52.4% of the sample examined initially.

### *Reliability*

At baseline, caries examination was performed by a single calibrated examiner (LSA). Training with clinical photographs and calibration by the double examination of patients were performed under the supervision of a benchmark examiner before the study began. During the survey, calibration was monitored by repeated examinations conducted on 5% of the sample. The overall unweighted Cohen kappa value was 0.84.

During the follow-up, the clinical examination was conducted by another examiner (CDB), who was trained and calibrated by the first examiner (LSA). The inter-examiner unweighted Cohen kappa value was 0.78. During the survey, this calibration was also monitored by repeated examinations of 5% of the sample. The lowest intra-examiner unweighted Cohen kappa value was 0.81 (CDB).

### *Data analysis*

The primary outcome of this study was caries increment, defined as the difference between follow-up and baseline DMFS scores. DMFS was calculated as the sum of the decayed, missing, or filled surfaces according to the World Health Organization criterion (cavity level). The main predictor variables were caries activity (no [adolescents with sound, INC or IC lesion, or filled/missing surfaces, but no active caries lesion] or yes [at least one active lesion, either non-cavitated or cavitated]) and caries experience (defined at the cavity level [ $D_5MFS=0$  or  $D_5MFS \geq 1$ ] and at the non-cavitated level [ $D_1MFS=0$  or  $D_1MFS \geq 1$ ]). Other predictor variables included in the study as the adjusting variables

were sex (female or male), socioeconomic status (SES) classified according to the cut-offs proposed by the standard Brazilian economic classification (high, mid-high, mid-low, or low) [ABEP, 2015], and type of school (private or public).

Data analysis was performed using STATA (Stata 14.2; Stata Corp., College Station, TX, USA). Baseline characteristics of the individuals followed up and those lost to follow-up were compared using the chi-square test and Wald test. A weight variable considering the inverse probability of participation at follow-up according to sex and SES was used in the statistical analysis. Poisson regression models (unadjusted and adjusted) were used to estimate the risk of caries increment over the study period. Two different adjusted models were proposed, one including caries experience at the cavity level (model 1) and another including caries experience at the non-cavitated level (model 2). The incidence risk ratios (IRR) and their respective 95% confidence intervals (CI) were estimated. Sex, SES, and type of school were included and maintained in the adjusted models, irrespective of their p-values.

## **Results**

The comparison of baseline characteristics showed a significantly higher proportion of public school attendees and a lower caries experience in the followed-up participants than in those lost to follow-up ( $p < 0.05$ ). No significant differences were observed in the other sociodemographic and behavioral characteristics analyzed, as shown previously [Lock et al., 2019].

As shown in Table 1, this study population had an overall average caries increment of 0.97 surfaces. A significant gradient was found among the different caries activity categories, with a mean increase of 1.58 new caries-affected surfaces in caries-active adolescents, 0.71 in caries-inactive adolescents, and 0.39 in caries-free adolescents. Those with caries experience at baseline had a significantly higher caries increment than those without (1.39 and 0.49 at the cavity level, and 1.11 and 0.39 at the non-cavitated level, respectively).

In the unadjusted models, caries-active adolescents and those with previous caries experience (at both cavity and non-cavitated levels) had around 2-to-3-fold increased risks of caries increment ( $p < 0.001$ ) than individuals without caries activity or experience (Table 2). Adjusted models showed that caries activity was significantly associated with caries increment even after adjustment for sex, SES, type of school, and previous caries experience at both cavity (adjusted model 1) and non-cavitated (adjusted model 2) levels.

Caries-active adolescents had approximately 2-fold higher risk of caries increment than those without caries activity (cavity level, IRR=1.90, 95%CI=1.45-2.49,  $p<0.001$ ; non-cavitated level, IRR=2.16, 95%CI=1.63-2.86,  $p<0.001$ ). Previous caries experience at the cavity level remained significantly associated with caries increment after the inclusion of caries activity in model 1 ( $D_5MFS \geq 0$ , IRR=2.03, 95%CI=1.52-2.72,  $p<0.001$ ), but the same was not true for caries experience at the non-cavitated level in model 2 ( $D_1MFS \geq 0$ , IRR=1.66, 95%CI=0.94-2.92, 0.08).

## Discussion

This study was conducted to assess the usefulness of ongoing caries activity in addition to past caries experience as a risk factor for caries increment over a period of 2.5 years in a population-based sample of adolescents. Our hypothesis that caries activity would be independently associated with caries increment irrespective of caries experience was accepted, and this finding was consistently found at both cavity and non-cavitated levels of caries experience ( $D_5MFS \geq 0$  or  $D_1MFS \geq 0$ ). To the best of our knowledge, this is the first study to address this issue.

Baseline caries experience has been widely used as a predictor of future caries based on the premise that those who already have caries are more likely to develop new lesions. A systematic review by Mej re *et al.* found that baseline caries experience was the most accurate predictor of future caries, with moderate/good accuracy in preschool-aged children but limited accuracy in school-aged children and adolescents [Mej re *et al.*, 2014]. In this study, after adjustment for important sociodemographic variables and caries activity, adolescents with  $D_5MFS \geq 1$  at baseline had a 2-fold increased risk of caries increment than those with  $D_5MFS = 0$ . Our findings corroborated the findings of Kassawara *et al.*, who found that 9–10-year-old children from Brazil with baseline caries experience in the permanent dentition ( $DMFT \geq 1$ ) presented a 3-fold higher risk than those without ( $DMFT = 0$ ) [Kassawara, 2010]. Further corroborating these findings, Stenlund *et al.* found that the relative risk of having at least one new proximal caries lesion increased linearly as the number of approximal lesions at baseline increased among 11–13-year-old Swedish children [Stenlund, 2002]. Notwithstanding, this approach does not differentiate individuals with ongoing disease from those with previous disease episodes, as detecting caries lesions is different from diagnosing the disease process. It is important to point out that caries experience at the non-cavitated level lost its significant association with caries increment after the adjustment for caries activity. This is a

conceivable finding since the inclusion of those adolescents who have only non-cavitated inactive and active lesions in the group  $D_1MDS \geq 1$  may inflate this category with mildly affected individuals, thus reducing the discriminatory power of the variable.

Despite several studies on past caries experience, the reliability of using the patient's caries activity to predict future lesions has scarcely been investigated. In this study, the clinical features of the lesions were considered indicators of the presence of an ongoing demineralization process, as widely performed in previous studies [Thylstrup et al., 1994; Nyvad et al., 1999; Nyvad et al., 2003; Maltz et al., 2003; Carvalho et al., 2016]. Despite attempts to propose objective methods to classify caries lesion activity including biofilm (plaque stagnation area) as an indicator of activity [Ekstrand et al., 2007], this criterion tends to overestimate caries activity, as previously shown in the literature [Oliveira et al., 2015; Braga et al., 2010]. After addressing the same sample of South Brazilian adolescents at the surface-level, we had previously shown that compared to caries-inactive and caries-free adolescents, caries-active individuals were more than two times likely to develop caries lesions over 2.5 years in surfaces classified as sound at baseline [Maltz et al., 2020]. The present study performed a patient-level analysis, evaluating the person as a whole and using the DMFS increment as the outcome and corroborated our previous findings. In the adjusted models, it was shown that caries-active individuals had around 2-fold higher risk of caries increment than individuals without caries activity, even after the adjustment for past caries experience and sociodemographic variables. This finding indicates that once a patient has caries activity during the baseline examination, it is likely that the presence of disease will result in the continuity of the demineralization process, with the consequent progression of lesions detected previously (from non-cavitated to cavitated lesions) and/or development of new lesions in the future. Considering that both caries activity and caries experience at the cavity level remained significantly associated with DMFS increment in the adjusted model 1, it is possible to infer that both variables exert an independent influence on the study outcome.

Achieving a high follow-up rate is a major challenge in cohort studies, especially in this age group, because the period from 12 years of age to 14–15 years includes the transition from primary school to high school in Brazil. Of the original sample of 1,528 adolescents, 801 (52.4%) were reexamined after 2.5 years, and the main reason for loss to follow-up was moving to another school. To mitigate the impact of non-response, a weight variable considering the probability of participation was used to adjust the

estimates. It could be argued that important confounders such as dietary and oral hygiene habits were not included in the adjusted models and that this might have compromised the study findings. Notwithstanding, we understand that these behavioral and clinical variables may influence or be influenced by a patient's caries activity and should not be included in the adjusted models. Even so, we tested the adjusted models including tooth brushing frequency, soft drinks consumption, and gingivitis and no major effect on the study findings was observed. Furthermore, the possibility that some adolescents may have received dental treatment between the two clinical examinations cannot be ruled out, and this could be a possible limitation of the study. The strengths of this study include its longitudinal design, the follow-up period of 2.5 years, and the fact that only one examiner performed the clinical examinations at each time point, one calibrated by the other, which may have improved the internal validity of the study.

In conclusion, the results of this population-based cohort study demonstrated that caries activity was significantly associated with DMFS increment over a 2.5-year period in a population of South Brazilian adolescents, regardless of previous caries experience.

## **Statements**

### *Acknowledgements*

We thank the Colgate-Palmolive Company for toothbrushes and toothpastes donating.

### *Statement of ethics*

The study protocol was approved by the Federal University of Rio Grande do Sul Research Ethics Committee (299/08) and by the Municipal Health Department of Porto Alegre Research Ethics Committee (process number 001.049155.08.3/register number 288 and process n° 001.028618.12.2/register n° 807). The research was conducted ethically, in accordance with the World Medical Association Declaration of Helsinki. All participants and their parents/legal guardians provided written informed consent.

### *Conflict of interest statement*

The authors have no conflicts of interest to declare.

### *Funding sources*

This study was partially supported by National Coordination of Post-graduate Education (CAPES), Ministry of Education, Brazil (funding code 001), and by Federal University of Rio Grande do Sul.

### *Author contributions*

Marisa Maltz and Luana Severo Alves conceived the ideas; Luana Severo Alves and Carolina Doege Brusius collected the data; Marisa Maltz, Luana Severo Alves and Carolina Doege Brusius analyzed the data and led the writing.

### *Data availability statement*

All data generated or analyzed during this study are included in this article. Further enquiries can be directed to the corresponding author.

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Table 1. Sample distribution and caries increment over 2.5 years among adolescents.

	n (%)	Mean (95% CI)
<b>Sex</b>		
Female	387 (48.3)	1.05 (0.85-1.25) <sup>a</sup>
Male	414 (51.7)	0.90 (0.73-1.07) <sup>a</sup>
<b>Socioeconomic status</b>		
High	63 (7.9)	0.74 (0.40-1.09) <sup>ab</sup>
Mid-high	195 (24.3)	0.70 (0.46-0.93) <sup>a</sup>
Mid-low	461 (57.5)	1.06 (0.88-1.24) <sup>bc</sup>
Low	82 (10.2)	1.36 (0.88-1.84) <sup>c</sup>
<b>Type of school</b>		
Private	117 (14.6)	0.51 (0.31-0.71) <sup>a</sup>
Public	684 (85.4)	1.05 (0.90-1.20) <sup>b</sup>
<b>Caries activity</b>		
Caries-free	155 (19.3)	0.39 (0.18-0.60) <sup>a</sup>
Caries-inactive	346 (43.2)	0.71 (0.55-0.86) <sup>b</sup>
Caries-active	300 (37.5)	1.58 (1.31-1.85) <sup>c</sup>
<b>Caries experience (cavity level)</b>		
D <sub>5</sub> MFS=0	370 (46.2)	0.49 (0.37-0.61) <sup>a</sup>
D <sub>5</sub> MFS≥1	431 (53.8)	1.39 (1.18-1.60) <sup>b</sup>
<b>Caries experience (non-cavitated level)</b>		
D <sub>1</sub> MFS=0	155 (19.4)	0.39 (0.18-0.60) <sup>a</sup>
D <sub>1</sub> MFS≥1	646 (80.6)	1.11 (0.96-1.27) <sup>b</sup>
<b>TOTAL</b>	<b>801 (100)</b>	<b>0.97 (0.84-1.11)</b>

Different letters indicate statistically significant differences between categories (p<0.05, adjusted Wald test)

Table 2. Association between patient's caries activity and caries experience at baseline with caries increment over 2.5 years among adolescents.

	Unadjusted		Adjusted*	
	IRR (95% CI)	p	IRR (95% CI)	p
<b>Model 1</b>				
Caries activity				
No	1.00		1.00	
Yes	2.59 (1.98-3.38)	<0.001	1.90 (1.45-2.49)	<0.001
Caries experience (cavity level)				
D <sub>5</sub> MFS=0	1.00		1.00	
D <sub>5</sub> MFS≥1	2.83 (2.11-3.79)	<0.001	2.03 (1.52-2.72)	<0.001
<b>Model 2</b>				
Caries activity				
No	1.00		1.00	
Yes	2.59 (1.98-3.38)	<0.001	2.16 (1.63-2.86)	<0.001
Caries experience (non-cavitated level)				
D <sub>1</sub> MFS=0	1.00		1.00	
D <sub>1</sub> MFS≥1	2.84 (1.65-4.90)	<0.001	1.66 (0.94-2.92)	0.08

\*Estimates are adjusted for sex, socioeconomic status, and type of school.

## ARTIGO II

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### **Association between tooth brushing frequency and the increment of dental caries and tooth loss in adolescents.**

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#### **Acknowledgements**

We would like to thank the Colgate-Palmolive Company for toothbrushes and toothpastes donating. We also thank all volunteers, their parents/legal guardians, and the schools for their contribution.

**TITLE:**

Association between tooth brushing frequency and the increment of dental caries and tooth loss in adolescents after 2.5 years of follow-up.

**SHORT TITLE:**

Tooth brushing frequency in adolescents

## SUMMARY

This cohort study assessed the association between tooth brushing frequency and the increment of dental caries and tooth loss in a population-based sample of South Brazilian adolescents in order to investigate whether there is an additional benefit in a third daily brushing. At baseline, 1,528 12-year-old schoolchildren attending 42 schools were examined for gingivitis and dental caries and answered a questionnaire. After a mean period of 2.5 years, 801 schoolchildren were re-examined. The primary outcomes were caries and tooth loss increment. The main predictor variable was tooth brushing frequency ( $\geq 3$  times/day vs. 2 times/day or  $\leq 1$  time/day). Poisson regression models were used to estimate the risk for caries and tooth loss increment. Incidence risk ratios (IRR) and 95% confidence intervals (CI) were estimated. After adjustment for sociodemographic, behavioural and clinical variables, brushing teeth twice/day afforded 40% greater risk (IRR=1.40; 95%CI=1.02-1.92) for caries increment than  $\geq 3$  times/day. Regarding tooth loss increment, adolescents who brushed their teeth twice/day had a 4-fold greater risk (IRR=3.92; 95%CI=1.23-12.49) than those who brushed  $\geq 3$  times/day. Sex, school type, and gingivitis was found to act as effect modifiers, with a third daily brushing being advantageous in terms of tooth loss for girls, for public school attendees, and for those with  $\geq 50\%$  of bleeding sites. This study suggests that adolescents benefit from a third daily tooth brushing. Increasing brushing frequency to 3 times/day may be a suitable strategy to control dental caries and tooth loss among high-risk adolescents.

## KEYWORDS

Tooth brushing; dental caries; tooth loss; adolescent; cohort studies; risk assessment.

## INTRODUCTION

Brushing teeth twice a day has been considered a social norm in many countries [Kumar, Tadakamadla et al. 2016]. The American Dental Association recommends “For a healthy mouth and smile the ADA recommends you brush your teeth twice/day with a soft-bristled brush”. The Centers for Disease Control and Prevention (CDC) recommends brushing twice a day specifically for preventing dental caries. International comparison studies on oral hygiene practices in 22 European countries showed that most children (73%-83%) brushed twice/day in Sweden, Denmark, Germany, Austria, and Norway. More-than-once-a-day tooth brushing was especially uncommon (26-33%) among boys in Finland, Lithuania, Russia, Estonia, and Latvia [Kuusela, Honkala et al. 1997].

Most studies in children/adolescents consider the frequency of brushing twice/day as the cut-off and find a higher caries risk in individuals who brush their teeth less than twice/day [Tanner, Kämpfi et al. 2014; Marinho, Higgins et al. 2003]. In a systematic review with meta-analysis, Kumar et al. [2016] studied the effect of tooth brushing frequency on dental caries incidence and increment. Infrequent brushers demonstrated higher incidence and increment of caries lesions than frequent brushers. Brushing  $<2$  times/day caused a significantly higher increment of caries lesions compared with  $\geq 2$  times/day (standardized mean difference = 0.34; 95%CI=0.18 to 0.49). Only two studies evaluated a brushing frequency  $>2$  times/day and no differences between  $>2$  times/day and  $\leq 2$  times/day brushers was found regarding caries incidence [Tagliaferro, Ambrosano et al. 2008] and increment [Dummer, Oliver et al. 1990].

Teeth cleaning can be a highly effective method for controlling the development and progression of caries, especially when using a fluoride toothpaste. Consensus between researchers and local public health authorities consider tooth brushing with fluoride toothpaste the method of choice to prevent dental caries. The effect of tooth brushing frequency is related not only to the frequent cleaning effect but also to the fact that fluoride effectiveness is increased by maintaining high fluoride levels throughout the day through frequent applications of small amounts of fluoride. In a systematic review investigating the effect of fluoride toothpaste in the prevention of dental caries, Marinho et al. [2003] included 74 studies, almost all reporting a daily frequency of once or twice. They reported an increase in the prevented fraction of 14% (CI 6% to 22%) moving from once to 2 times/day. No comparison between 2 and 3 times/day was performed.

A tooth brushing frequency of 3 times/day is the most prevalent in Brazil [Freddo, Aerts et al. 2008], where there is a culture of associating brushing teeth with the three main meals of the day. A brushing frequency equal to or greater than 3 times/day is recommended by the Brazilian Ministry of Health (Smiling Brazil), which advises “brushing your teeth after each meal and before bedtime”. Furthermore, an *in situ* study showed that the use of fluoride toothpaste 3 times/day enhanced demineralization protection when compared to 2 times/day [Souza, Hashizume et al. 2010]. In this context, it is important to assess whether there is an additional benefit in a third daily brushing. Therefore, the aim of this cohort study was to assess the association between tooth brushing frequency and the increment of dental caries and tooth loss in a population-based sample of South Brazilian adolescents over a 2.5-year period. We hypothesized that brushing teeth 3 times/day afforded higher protection against caries and tooth loss increment.

## METHODS

This is a cohort study conducted in southern Brazil. At baseline (2009-2010), a representative sample of the population of 12-year-old schoolchildren from Porto Alegre was drawn using a multistage probability sampling strategy. A total of 1,528 schoolchildren attending 42 schools (33 public and 9 private) were examined (response rate of 83.2%), far more than the minimum sample size of 1,331 required for the study. The sampling strategy can be found elsewhere [Lock, Susin et al. 2019].

At baseline, a structured questionnaire containing questions on sociodemographic information and behavioural aspects was sent to the parents/legal guardians of the selected students. The clinical examination was conducted at the schools, with the students in a supine position, under artificial light, using a flat mirror, a periodontal probe, and portable equipment (air compressor and suction). First, a trained examiner recorded the gingival bleeding index [Ainamo and Bay, 1975] in four sites per tooth (buccal, lingual, mesial, and distal). After professional tooth cleaning and drying, the examiner recorded the presence of caries lesions (non-cavitated and cavitated, active and inactive), missing, and filled surfaces. Clinical examinations comprised all erupted permanent teeth.

At follow-up, a total of 801 schoolchildren were re-examined (2012-2013) after a mean period of 2.5 years (standard deviation=0.3), representing 52.4% of the sample initially examined. Clinical examination was performed according to the same protocol.



The flowchart of the study including the reasons for non-participation and the comparison between followed individual and those lost to follow-up can be found elsewhere [Lock, Susin et al. 2019].

### *Reliability*

At baseline, caries examination was performed by a single calibrated examiner (LSA). Training and calibration were performed before the study began. During the survey, calibration was monitored by repeated examinations conducted on 5% of the sample. The overall unweighted Cohen's kappa value was 0.84.

At follow-up, the clinical examination was conducted by another examiner (CDB), who was trained and calibrated by the first examiner (LSA). The inter-examiner unweighted Cohen's kappa value was 0.78. During the survey, calibration was also monitored by repeated examinations of 5% of the sample (10 double examinations at every 200 schoolchildren included in the sample). The lowest intra-examiner unweighted Cohen's kappa value was 0.81.

### *Data analysis*

The primary outcomes of this study were caries increment and tooth loss increment, modelled as count outcomes. Caries increment was defined as the difference between follow-up DMFS and baseline DMFS. DMFS was calculated as the sum of decayed, missing, or filled surfaces according to the WHO criteria (cavity level). Tooth loss increment was defined as the difference between the number of missing teeth at follow-up and at baseline and included extracted teeth as well as those indicated for extraction (residual roots).

The main predictor variable was tooth brushing frequency ( $\geq 3$  times/day vs. 2 times/day or  $\leq 1$  time/day). Other predictor variables included in the study as adjusting variables were sociodemographic, behavioural and clinical variables. Sociodemographic variables were sex (girls vs. boys), socioeconomic status (classified according to the cut-offs proposed by the standard Brazilian economic classification [ABEP 2015] as high, mid-high, mid-low or low), and school type (private vs. public). In addition to tooth brushing frequency, the other behavioural variable assessed was soft drinks consumption

(daily vs. non-daily) and the clinical variable was gingivitis, defined based on the proportion of bleeding sites (<50% of bleeding sites vs.  $\geq 50\%$  to < 75% or  $\geq 75\%$ ).

Data analysis was performed using STATA (Stata 14.2; Stata Corp., College Station, TX, USA). A weight variable considering the inverse probability of participation at follow-up according to sex and socioeconomic status was used in the statistical analysis. Poisson regression models (unadjusted and adjusted) were used to estimate the risk for caries increment and tooth loss increment over the study period. Incidence risk ratios (IRR) and their respective 95% confidence intervals (CI) were estimated. Two different adjusted models were presented. The first one adjusted for sociodemographic and behavioural variables (sex, socioeconomic status, type of school and soft drinks consumption) and the second one including the clinical variable (gingivitis). These variables were included and maintained in the adjusted models irrespective of their p-values.

The mean increment of dental caries and tooth loss and the risk assessment analysis comparing tooth brushing frequencies of  $\geq 3$  times/day versus 2 times/day were calculated after stratifying the sample by predictor variables to investigate whether any of them acted as an effect modifier of the association between tooth brushing frequency and the study outcomes.

### *Ethical aspects*

The study protocol was approved by the Federal University of Rio Grande do Sul Research Ethics Committee (299/08) and by the Municipal Health Department of Porto Alegre Research Ethics Committee (process number 001.049155.08.3/register number 288 and process n° 001.028618.12.2/register n° 807). The research was conducted ethically, in accordance with the World Medical Association Declaration of Helsinki. All participants and their parents/legal guardians provided written informed consent.

## RESULTS

The comparison between followed individuals and those lost to follow-up showed a significantly higher proportion of public school attendees and a lower caries experience among participants than among those lost to follow-up ( $p < 0.05$ ) [Lock, Susin et al. 2019]. Among the 801 followed adolescents, 370 were classified as caries-free (DMFT=0) at

baseline, of whom 91 developed caries. It yielded a caries incidence of 24.5% (95%CI=20.1-29.0). Regarding tooth loss, an incidence of 3% was detected (95%CI=1.8-4.2), representing 23 adolescents out of 761 who had no missing teeth at baseline.

The sample distribution and increment of dental caries and tooth loss over 2.5 years by predictor variables are described in Table 1. This population had a mean DMFS of 2.06 (95%CI=1.83-2.29) and a mean DMFT of 1.35 (95%CI=1.23-1.47) at baseline, and an overall caries increment of 0.97 (95%CI=0.84-1.11) surfaces, on average, was observed after a 2.5-year period. Adolescents who brushed their teeth once/day or less had a mean caries increment of 1.45 (95%CI=1.08-1.82) surfaces, which was significantly higher than among those who reported a brushing frequency of twice/day (0.95, 95%CI=0.76-1.15) or  $\geq 3$  times/day (0.70, 95%CI=0.53-0.88). The comparison between these two last categories showed a borderline p-value of 0.054. Regarding tooth loss, an overall increment of 0.05 (95%CI=0.03-0.07) tooth was found on average. Tooth loss increment was lower among adolescents who brushed their teeth  $\geq 3$  times/day compared with the other two categories.

Table 2 describes the association between predictor variables and dental caries increment. Brushing teeth twice/day afforded 40% greater risk for caries increment than a frequency of  $\geq 3$  times/day while brushing teeth  $\leq 1$  time/day afforded about 2-fold greater risk for caries increment than  $\geq 3$  times/day in both adjusted models. The inclusion of gingivitis in the second adjusted model exerted no effect on this association.

As shown in Table 3, adolescents who reported brushing their teeth twice/day had a 4-fold greater risk for tooth loss increment than those who reported a frequency of  $\geq 3$  times/day. The lowest tooth brushing of  $\leq 1$  time/day resulted in a 7-fold greater risk for tooth loss increment compared with the highest frequency of  $\geq 3$  times/day, even after the inclusion of gingivitis in the second adjusted model.

Sex, school type, and gingivitis were found to modify the effect of tooth brushing frequency on tooth loss estimates (Table 4). A significantly greater risk for tooth loss increment was found among girls and among adolescents with  $\geq 50\%$  of bleeding sites who brushed their teeth twice/day than among those who brushed their teeth  $\geq 3$  times/day while no association was found among boys or those with  $< 50\%$  of bleeding sites. Regarding school type, a significantly higher risk for tooth loss increment was found among public school attendees who brushed their teeth twice/day than among those with  $\geq 3$  times/day. No case of tooth loss was found among private school attendees who

reported a tooth brushing frequency of  $\geq$ twice/day, which enabled this comparison. No effect modification was detected regarding caries increment.

## DISCUSSION

This study was carried out to investigate whether a third daily tooth brushing would provide benefits in terms of dental caries and tooth loss in a population-based sample of adolescents from southern Brazil over a period of 2.5 years. Our hypothesis was accepted, as adolescents who brushed their teeth at least 3 times/day were less likely to present increment of dental caries and tooth loss than those who brushed their teeth twice/day. To the best of our knowledge, no cohort study has investigated the effect of brushing teeth 3 times/day on tooth loss increment among adolescents.

The main finding of the present study was that brushing teeth twice/day afforded greater risk for caries and tooth loss increment than  $\geq 3$  times/day. This is in disagreement with the systematic review on tooth brushing frequency and dental caries by Kumar et al. [2016] that found no difference between caries increment estimates of  $>2$  times/day and  $\leq 2$  times/day brushers. The authors stressed, however, that this estimate came from only one study and should be considered with caution. This previous study included a similar sample in terms of size and age group (798 adolescents followed from 11-12 to 15-16 years); however, the tooth brushing frequency was categorized on a weekly basis, being the category of  $>14$  times/week considered a frequency  $>2$  times/day. It can explain, at least in part, the lack of difference between groups (standardize mean difference =  $-0.12$ ; 95%CI =  $-0.38$  to  $0.15$ ;  $p = 0.39$ ).<sup>6</sup> In the present study, the brushing frequency was categorized as  $\geq 3$  times/day, which would result in a weekly frequency of  $>21$  times, clearly indicating a greater daily frequency of fluoride application than the previous study by Dummer [1990]. The other study addressing a brushing frequency  $>2$  times/day was conducted by Tagliaferro et al. [2008]. Although tooth brushing frequency was significantly associated with a high caries level at baseline, no association was found with a 'high caries increment' over a 7-year follow-up period, defined as DMFS increment  $\geq 4$ . The variables that remained in the final model were only baseline dmfs and mother's educational level. The definition of a binary outcome instead of a counting one and the cut off used ( $\geq 4$  surfaces) as well as the reduced sample size ( $n=206$ ) may have compromised the statistical power of the study, thus explaining the disagreement between their findings and ours.

Reducing the tooth brushing frequency from three to two times/day increased the risk of caries and tooth loss increment in this sample of Brazilian adolescents by 40% and 4-fold, respectively. Notwithstanding, an interesting finding of our study was that the benefit provided by a third daily brushing was not observed in the whole sample. Sex, school type, and gingivitis modified the effect of tooth brushing on tooth loss increment. A greater risk of tooth loss increments among adolescents who brushed their teeth twice/day was observed only in girls, in public school attendees, and in those with  $\geq 50\%$  of bleeding sites. All of these are conceivable findings. The relationship between sex and caries/tooth loss during childhood and adolescence may be explained by the different time of tooth eruption, with girls having an earlier tooth eruption than boys, which results in a longer timer at risk for caries development and progression [Lock, Susin et al. 2019; Alves, Susin et al. 2014; Fabruccini, Alves et al. 2016]

.Type of school has been consistently used as a proxy for socioeconomic status [al-Mohammadi, Rugg-Gunn et al. 1997; González, Cabrera et al. 1993], with public school attendees being more likely to have caries than their counterparts attending private schools. Maltz & Silva previously showed that parents of public school attendees tend to have a lower socioeconomic level and lower education than parents of the private school attendees, being more affected by caries and gingivitis. The authors discussed that, probably, the composition of the variables parental education level and per capita family income, within the public and private Brazilian education networks, associated with other variables, such as lifestyle, may turn this variable more sensitive to catch the effect of socioeconomic condition on oral health outcomes than the variable “socioeconomic status” itself [Maltz and Barbachan e Silva, 2001]. The feasibility of using type of school as an alternative indicator for socioeconomic status was also shown by Piovesan et al. [2011]. Finally, gingivitis was used as an indicator of oral hygiene pattern targeting biofilm control. The association between greater proportion of bleeding sites and greater risk of caries and tooth loss has been previously shown in the literature [Alves, Susin et al. 2014; Begum S, 2016]. In general, these results may support a recommendation of a higher tooth brushing frequency for patients considered at “high caries risk”.

The benefit of the third daily brushing observed in the present study does not seem to be related to mechanical cleaning or the presence of biofilm, since the adjustment for gingivitis exerted no effect in the models. This is aligned with the literature review by Bellini et al., conducted prior to the widespread use of fluoride toothpaste, making it possible to study the effect of brushing frequency without fluoride interference. The

authors did not find a relationship between brushing frequency and dental caries [Bellini, Arneberg et al. 1981]. There is no evidence that an increase in brushing frequency is associated with an improvement in the quality of biofilm control. In fact, brushing once a day should be sufficient for caries control, as dental biofilm must be 2 days old to produce acid levels sufficient to cause enamel demineralization [Imfeld and Lutz 1980]. However, the effectiveness of cleaning with a toothbrush is not high and dental caries is a multifactorial disease, with numerous determinants influencing the disease, as sugar consumption, salivary flow rate, saliva buffer capacity, and others. Oral hygiene performed with fluoride toothpaste (FT) combines mechanical cleaning and topical fluoride application. Twice-daily brushing frequency has been suggested to increase the frequency of fluoride availability in the oral environment. In an *in situ* study, Dijkman et al. [1990] evaluated two oral hygiene protocols - twice-daily brushing with FT (1250 ppm F) and twice-daily brushing with non-FT, compared to a control group with no brushing. The authors demonstrated that brushing twice/day with non-FT resulted in a 50% reduction in mineral loss compared to the non-brushing control group (cleaning effect only), with this protection increasing to 90% when a FT was used (combination of two factors, a cleaning effect and a fluoride effect). In another *in situ* study, Souza et al. [2010] observed that the use of FT 3 times/day enhanced demineralization protection when compared to 2 times/day. This result showed the added benefit of a third daily fluoride application. The decrease in caries increment and tooth loss observed in our study with a brushing frequency of  $\geq 3$  times/day compared to 2 times/day is likely due to an even higher frequency of topical fluoride application.

Among the strengths of this study, we can highlight its longitudinal design with a 2.5-year follow-up period and the high intra- and inter-examiner reproducibility. In addition, its pioneering aspect has also to be acknowledged, as this is the first cohort study assessing tooth loss among adolescents and the first to investigate the tooth brushing frequency of 3 times/day. Of the original sample of 1,528 adolescents, 801 (52.4%) were re-examined after 2.5 years and the main reason for the losses was moving to another school, which is a common finding in this age group that are moving from elementary to high school. To mitigate the impact of non-response, a weighting variable was used considering the possibility of participation to adjust the characteristics.

In conclusion, the results of this population-based cohort suggest that Brazilian adolescents benefit from a third daily tooth brushing in terms of dental caries and tooth loss. Adolescents considered at “high caries risk” (girls, public school attendees, and

those with  $\geq 50\%$  of bleeding sites) were more likely to benefit from a third daily brushing in terms of tooth loss.

## RESUMO

Este estudo de coorte avaliou a associação entre a frequência de escovação dentária e o incremento de cárie e perda dentária em uma amostra de base populacional de adolescentes sul-brasileiros, a fim de investigar se há um benefício adicional em uma terceira escovação diária. No início do estudo, 1.528 escolares de 12 anos de idade de 42 escolas foram examinados para gengivite e cárie dentária e responderam a um questionário. Após um período médio de 2,5 anos, 801 escolares foram reexaminados. Os desfechos primários foram cárie e incremento de perda dentária. A principal variável preditora foi a frequência de escovação dentária ( $\geq 3$  vezes/dia vs. 2 vezes/dia ou  $\leq 1$  vez/dia). Modelos de regressão de Poisson foram usados para estimar o risco de cárie e incremento de perda dentária. Razões de risco de incidência (IRR) e intervalos de confiança de 95% (IC) foram estimados. Após ajuste para variáveis sociodemográficas, comportamentais e clínicas, escovar os dentes duas vezes/dia conferiu um risco 40% maior (IRR=1,40; IC95%=1,02-1,92) para apresentar incremento de cárie do que  $\geq 3$  vezes/dia. Em relação ao incremento de perda dentária, adolescentes que escovavam os dentes duas vezes/dia apresentaram risco 4 vezes maior (IRR=3,92; IC95%=1,23-12,49) do que aqueles que escovavam  $\geq 3$  vezes/dia. Sexo, tipo de escola e gengivite atuaram como modificadores de efeito, com uma terceira escovação diária sendo vantajosa em termos de perda de dentes para meninas, para frequentadores de escolas públicas e para aqueles com  $\geq 50\%$  de sítios sangrantes. Este estudo sugere que adolescentes se beneficiam de uma terceira escovação diária. Aumentar a frequência de escovação para 3 vezes/dia pode ser uma estratégia adequada para controlar a cárie dentária e a perda dentária em adolescentes.



## ACKNOWLEDGEMENTS

We acknowledge the support of the National Coordination of Post-graduate Education (CAPES), Ministry of Education, Brazil (funding code 001), and of the Federal University of Rio Grande do Sul. We also thank Colgate-Palmolive Company for toothbrushes and toothpastes donating. We also thank all volunteers, their parents/legal guardians, and the schools for their contribution.

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TABLES

Table 1. Sample distribution, increment of dental caries and tooth loss over 2.5 years among South Brazilian adolescents. Mean (95% confidence interval).

	n (%)	Dental caries	Tooth loss
<i>Sociodemographic</i>			
Sex			
Girls	387 (48.3)	1.05 (0.85-1.25) <sup>a</sup>	0.06 (0.03-0.09) <sup>a</sup>
Boys	414 (51.7)	0.90 (0.73-1.07) <sup>a</sup>	0.04 (0.02-0.07) <sup>a</sup>
Socioeconomic status			
High/mid-high	258 (32.2)	0.71 (0.51-0.90) <sup>a</sup>	0.03 (0.01-0.05) <sup>a</sup>
Mid-low	461 (57.6)	1.06 (0.88-1.24) <sup>b</sup>	0.06 (0.03-0.08) <sup>a</sup>
Low	82 (10.2)	1.36 (0.88-1.84) <sup>b</sup>	0.10 (0.02-0.18) <sup>a</sup>
School type			
Private	117 (14.6)	0.51 (0.31-0.71) <sup>a</sup>	0.01 (-0.01-0.02) <sup>a</sup>
Public	684 (85.4)	1.05 (0.90-1.20) <sup>b</sup>	0.06 (0.04-0.08) <sup>b</sup>
<i>Behavioral</i>			
Tooth brushing frequency			
≥ 3 times/day	275 (34.3)	0.70 (0.53-0.88) <sup>a</sup>	0.01 (0.01-0.03) <sup>a</sup>
2 times/day	349 (43.6)	0.95 (0.76-1.15) <sup>a</sup>	0.05 (0.02-0.08) <sup>b</sup>
≤ 1 time/day	177 (22.1)	1.45 (1.08-1.82) <sup>b</sup>	0.11 (0.05-0.16) <sup>b</sup>
Soft drinks consumption			
Daily	227 (28.3)	0.94 (0.71-1.17) <sup>a</sup>	0.03 (0.01-0.06) <sup>a</sup>
Non-daily	574 (71.7)	0.99 (0.83-1.15) <sup>a</sup>	0.06 (0.03-0.08) <sup>a</sup>
<i>Clinical</i>			
Gingivitis <sup>†</sup>			
<50%	341 (42.7)	0.80 (0.63-0.97) <sup>a</sup>	0.03 (0.01-0.05) <sup>a</sup>
≥ 50% and < 75%	388 (48.6)	1.05 (0.85-1.25) <sup>ab</sup>	0.06 (0.03-0.09) <sup>a</sup>
≥ 75%	69 (8.7)	1.47 (0.90-2.04) <sup>b</sup>	0.09 (0.01-0.16) <sup>a</sup>
TOTAL	801 (100)	0.97 (0.84-1.11)	0.05 (0.03-0.07)

<sup>†</sup> Figures do not totalize 801 due to missing data.

Different letters indicate statistically significant differences between categories (p<0.05, adjusted Wald test).

Table 2. Association between predictor variables and dental caries increment over 2.5 years among South Brazilian adolescents.

	Unadjusted		Adjusted <sup>a</sup>		Adjusted <sup>b</sup>	
	IRR (95% CI)	p	IRR (95% CI)	p	IRR (95% CI)	p
<i>Sociodemographic</i>						
Sex						
Girls	1.00		1.00		1.00	
Boys	0.86 (0.66-1.13)	0.27	0.78 (0.60-1.01)	0.06	0.76 (0.61-1.04)	0.09
Socioeconomic status						
High/mid-high	1.00		1.00		1.00	
Mid-low	1.49 (1.08-2.06)	0.01	1.19 (0.84-1.67)	0.32	1.16 (0.82-1.64)	0.39
Low	1.92 (1.22-3.01)	0.005	1.45 (0.92-2.28)	0.11	1.42 (0.90-2.24)	0.13
School type						
Private	1.00		1.00		1.00	
Public	2.06 (1.36-3.12)	0.001	1.65 (1.04-2.60)	0.03	1.59 (1.01-2.53)	0.049
<i>Behavioral</i>						
Tooth brushing frequency						
≥ 3 times/day	1.00		1.00		1.00	
2 times/day	1.36 (0.99-1.87)	0.06	1.41 (1.03-1.93)	0.03	1.40 (1.02-1.92)	0.04
≤ 1 time/day	2.07 (1.45-2.94)	<0.001	2.01 (1.41-2.86)	<0.001	1.96 (1.38-2.77)	<0.001
Soft drinks consumption						
Daily	1.00		1.00		1.00	
Non-daily	1.05 (0.78-1.40)	0.74	1.05 (0.79-1.39)	0.75	1.05 (0.79-1.38)	0.76
<i>Clinical</i>						
Gingivitis <sup>†</sup>						
<50%	1.00		-	-	1.00	
≥ 50% and < 75%	1.31 (0.98-1.74)	0.07	-	-	1.12 (0.84-1.49)	0.43
≥ 75%	1.83 (1.18-2.85)	0.007	-	-	1.49 (0.96-2.30)	0.08

IRR = Incidence risk ratio; CI = Confidence interval.

<sup>a</sup> Adjusted for sociodemographic and behavioral variables.

<sup>b</sup> Adjusted for sociodemographic, behavioral and clinical variables.

Table 3. Association between predictor variables and tooth loss increment over 2.5 years among South Brazilian adolescents.

	Unadjusted		Adjusted <sup>a</sup>		Adjusted <sup>b</sup>	
	IRR (95% CI)	p	IRR (95% CI)	p	IRR (95% CI)	p
<i>Sociodemographic</i>						
Sex						
Girls	1.00		1.00		1.00	
Boys	0.74 (0.35-1.55)	0.43	0.58 (0.28-1.19)	0.14	0.60 (0.29-1.25)	0.17
Socioeconomic status						
High/mid-high	1.00		1.00		1.00	
Mid-low	2.06 (0.85-4.99)	0.11	1.15 (0.48-2.78)	0.76	1.12 (0.45-2.80)	0.80
Low	3.52 (1.17-10.60)	0.03	1.74 (0.60-5.02)	0.30	1.69 (0.58-4.94)	0.34
School type						
Private	1.00		1.00		1.00	
Public	7.11 (0.97-52.02)	0.06	4.71 (0.62-35.72)	0.13	4.44 (0.58-34.24)	0.15
<i>Behavioral</i>						
Tooth brushing frequency						
≥ 3 times/day	1.00		1.00		1.00	
2 times/day	3.64 (1.17-11.38)	0.03	3.96 (1.24-12.67)	0.02	3.92 (1.23-12.49)	0.02
≤ 1 time/day	7.53 (2.48-11.89)	<0.001	7.53 (2.40-23.66)	0.001	7.09 (2.24-22.49)	0.001
Soft drinks consumption						
Daily	1.00		1.00		1.00	
Non-daily	1.61 (0.72-3.62)	0.25	1.63 (0.75-3.54)	0.22	1.61 (0.73-3.52)	0.23
<i>Clinical</i>						
Gingivitis <sup>†</sup>						
<50%	1.00		-	-	1.00	
≥ 50% and < 75%	1.91 (0.82-4.47)	0.13	-	-	1.36 (0.54-3.41)	0.51
≥ 75%	2.72 (0.88-8.37)	0.08	-	-	1.79 (0.58-5.56)	0.31

IRR = Incidence risk ratio; CI = Confidence interval.

<sup>a</sup> Adjusted for sociodemographic and behavioral variables.

<sup>b</sup> Adjusted for sociodemographic, behavioral and clinical variables.

Table 4. Tooth loss increment and its association with tooth brushing frequency stratified by sex, school type, and gingivitis.

	Increment	Unadjusted		Adjusted*		Adjusted**	
	Mean (95% CI)	IRR (95% CI)	p	IRR (95% CI)	p	IRR (95% CI)	p
<i>Sex</i>							
<i>Female</i>							
≥ 3 times/day	0.02 (-0.01-0.04) <sup>a</sup>	1.00		1.00		1.00	
2 times/day	0.07 (0.02-0.13) <sup>a</sup>	4.23 (1.08-16.6)	0.04	4.34 (1.09-17.29)	0.04	4.38 (1.10-17.3)	0.04
<i>Male</i>							
≥ 3 times/day	0.01 (-0.01-0.03) <sup>a</sup>	1.00		1.00		1.00	
2 times/day	0.03 (0.01-0.06) <sup>a</sup>	3.51 (0.43-28.89)	0.24	3.52 (0.43-28.66)	0.24	3.26 (0.41-26.04)	0.27
<i>School type</i>							
<i>Private</i>							
≥ 3 times/day	0	-	-	-	-	-	-
2 times/day	0	-	-	-	-	-	-
<i>Public</i>							
≥ 3 times/day	0.02 (0.01-0.03) <sup>a</sup>	1.00		1.00		1.00	
2 times/day	0.06 (0.03-0.10) <sup>b</sup>	3.65 (1.17-11.37)	0.03	3.99 (1.24-12.78)	0.02	3.97 (1.24-12.67)	0.02
<i>Gingivitis</i>							
<i>&lt;50% bleeding sites</i>							
≥ 3 times/day	0.01 (-0.01-0.3) <sup>a</sup>	1.00		1.00		-	-
2 times/day	0.02 (-0.01-0.04) <sup>a</sup>	1.38 (0.23-8.15)	0.72	1.53 (0.26-8.99)	0.64	-	-
<i>≥ 50% bleeding sites</i>							
≥ 3 times/day	0.01 (-0.01-0.03) <sup>a</sup>	1.00		1.00		-	-
2 times/day	0.08 (0.02-0.13) <sup>b</sup>	5.46 (1.18-25.25)	0.03	6.00 (1.22-29.48)	0.03	-	-

IRR = Incidence risk ratio; CI = Confidence interval.

Different letters indicate statistically significant differences between categories (p<0.05, adjusted Wald test).

<sup>a</sup> Adjusted for sociodemographic and behavioral variables.

<sup>b</sup> Adjusted for sociodemographic, behavioral and clinical variables.



## CONSIDERAÇÕES FINAIS

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Após a realização da presente tese, pode-se concluir que:

- Ambas as variáveis, atividade de cárie do paciente e experiência prévia de doença, desempenham um efeito independente no incremento de cárie de adolescentes do Sul do Brasil (Artigo I);

- A frequência de escovação mais prevalente no Brasil,  $\geq 3$  vezes/dia, justifica-se em virtude do menor incremento de cárie e perda dentária observada comparativamente à frequência de 2 vezes/dia, principalmente entre os adolescentes mais suscetíveis (Artigo II).

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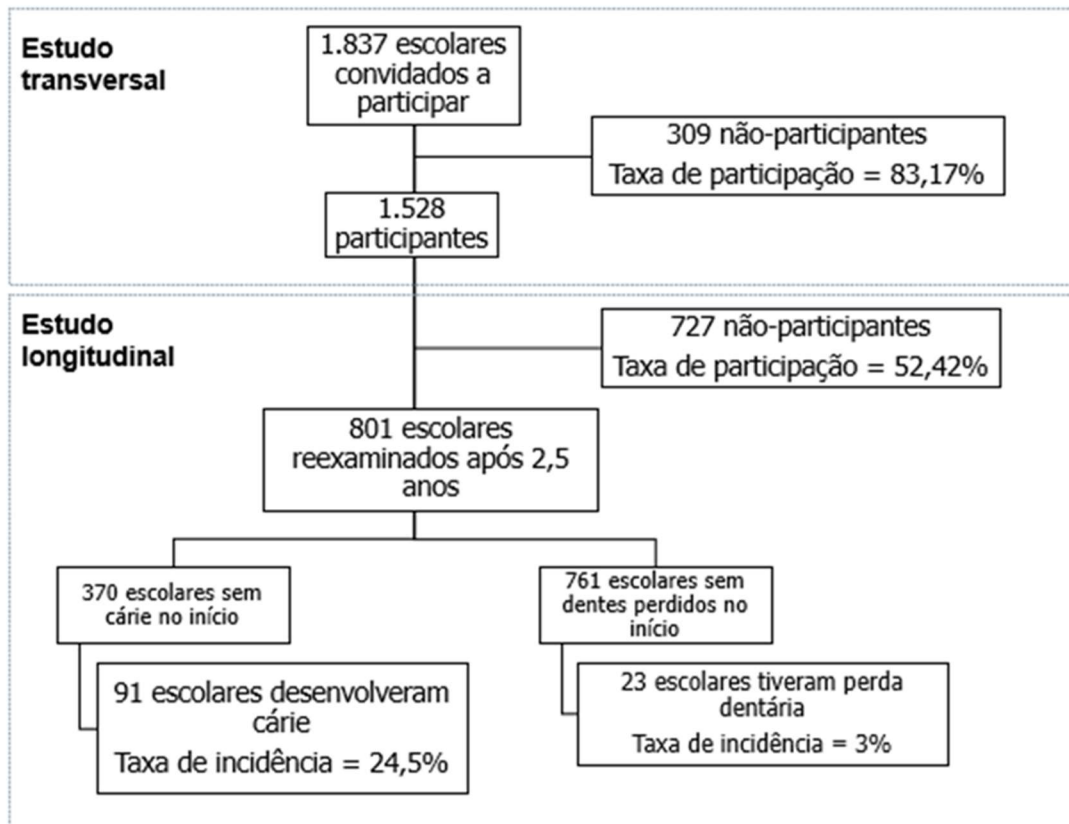
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## APÊNDICES

Flowchart do estudo



Universidade Federal do Rio Grande do Sul  
Faculdade de Odontologia

TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO

Estudo da prevalência de cárie dentária, gengivite e fluorose dentária  
em escolares de Porto Alegre, RS: em 1998 e 2009

- 1. Objetivo do estudo:** Estudar a ocorrência de gengivite (inflamação na gengiva), cárie, fluorose (manchas nos dentes ocasionadas pela ingestão de flúor em excesso durante o seu período de formação), erosão (desgastes nos dentes) e traumatismos dentários (fratura) em estudantes de 12 anos de idade residentes em Porto Alegre-RS;
- 2. Seleção dos indivíduos:** As escolas e os estudantes foram sorteados aleatoriamente;
- 3. Duração:** A participação na pesquisa consiste no preenchimento de um questionário pelos pais ou responsáveis e um pelo estudante e exame odontológico da criança, a ser realizado em um único dia, com duração prevista de aproximadamente dez minutos.
- 4. Procedimentos:** Os indivíduos terão seus dentes limpos com escova, fio e pasta dental, fornecidos pela equipe da pesquisa e examinados pela cirurgiã-dentista Luana Severo Alves (CRO-RS 16588) (estudante de doutorado da UFRGS).
- 5. Importância do estudo:** Este levantamento epidemiológico será muito importante para avaliação do estado atual de saúde bucal das crianças de nossa cidade.
- 6. Danos:** Não existem danos previstos. Todo o instrumental (espelho, pinça e sonda) utilizado estará devidamente esterilizado. Somente participarão dos exames os estudantes que assim concordarem e assinarem este termo, juntamente com a assinatura dos pais ou responsáveis.
- 7. Benefícios:** Os pais ou responsáveis conhecerão as condições de saúde bucal de seu filho e receberão, posteriormente, um relatório do exame realizado, assim como a indicação de lugares que ofereçam atendimento odontológico gratuito. O estudante receberá uma escova dental.
- 8. Confidencialidade:** As informações contidas nos questionários e a identidade dos estudantes ficarão sob o poder restrito dos pesquisadores e não serão divulgadas nos trabalhos resultantes desta pesquisa.

A participação na pesquisa é totalmente voluntária e o indivíduo tem a liberdade de se recusar a participar ou retirar seu consentimento em qualquer momento do estudo sem nenhum tipo de penalidade.

No caso de dúvidas ou acontecimentos associados à pesquisa, o participante poderá entrar em contato com a pesquisadora Luana Severo Alves, através do telefone 3308 5193 ou com a orientadora deste projeto, profª. Drª. Marisa Maltz(3308 5247), e terá a garantia de resposta a qualquer pergunta ou informação extra.

**Confirmo que entendi a natureza da pesquisa e autorizo a participação do estudante**

Assinatura dos pais ou responsável: \_\_\_\_\_

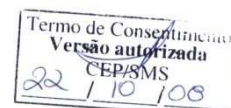
**Confirmo que entendi a natureza da pesquisa e me disponho a participar voluntariamente.**

Assinatura do estudante: \_\_\_\_\_

Pesquisadora Luana Severo Alves: \_\_\_\_\_

Porto Alegre, \_\_\_\_ de \_\_\_\_\_ de 20 \_\_\_\_

Comitê de Ética em Pesquisa da Faculdade de Odontologia da UFRGS: 3308 5187  
Comitê de Ética em Pesquisa da Secretaria Municipal de Saúde de Porto Alegre-RS: 3212 4623



## COMITÊ DE ÉTICA EM PESQUISA

### RESOLUÇÃO

O Comitê de Ética em Pesquisa e a Comissão de Pesquisas da Faculdade de Odontologia da Universidade Federal do Rio Grande do Sul analisaram o Projeto:

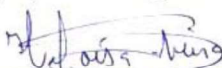
**Número: 299/08**

**Título: ESTUDO DA PREVALÊNCIA DE CÁRIE DENTÁRIA, GENGIVITE E FLUOROSE DENTÁRIA EM ESCOLARES BRASILEIROS: EM 1998 E 2008.**

**Investigador(es) principal(ais): Professores Berenice Barbachan e Silva, Marisa Maltz, Cristiano Susin e CD. Luana Severo Alves.**

O Projeto foi aprovado na reunião do dia 14/08/2008, Ata nº 08/08 do Comitê de Ética em Pesquisa e da Comissão de Pesquisas, da UFRGS, por estar adequado ética e metodologicamente de acordo com a Resolução 196/96 do Conselho Nacional de Saúde.

Porto Alegre, 15 de agosto de 2008.

  
Prof<sup>ª</sup>. Heloísa Emília Dias da Silveira  
Coordenadora do Comitê de Ética em Pesquisas

  
Prof<sup>ª</sup>. Deise Ponzoni  
Coordenadora da Comissão de Pesquisas



**Prefeitura Municipal de Porto Alegre**  
**Secretaria Municipal de Saúde**  
**Comitê de Ética em Pesquisa**

**PARECER CONSUBSTANCIADO**

Pesquisador (a) Responsável: Mariza Maltz

Equipe executora:

Registro do CEP: 288 Processo Nº. 001.049155.08.3

Instituição onde será desenvolvido: Escolas municipais

Utilização: TCLE


Situação: APROVADO

O Comitê de Ética em Pesquisa da Secretaria Municipal de Saúde de Porto Alegre analisou o processo Nº.001.049155.08.3, referente ao projeto de pesquisa: "Estudo da prevalência de cárie dentária, gengivite e fluorose dentária em escolares de Porto Alegre, RS: em 1998 e 2009", tendo como pesquisador responsável Mariza Maltz cujo objetivo é "Geral: Estudar a prevalência de cárie dentária, gengivite e fluorose dentária em escolares de 12 anos do município de Porto Alegre, RS, 2008 e comparar os dados obtidos com dados coletados em 1998. Objetivos específicos: Verificar as condições atuais de saúde bucal de escolares de 12 anos regularmente matriculados em escolas públicas e particulares do município de Porto Alegre, RS; • Avaliar a presença de modificações na prevalência de cárie dentária, gengivite e fluorose dentária na população estudada nos últimos dez anos; • Avaliar a existência de associação entre variáveis demográficas, socioeconômicas e comportamentais e as doenças em estudo".

Assim, o projeto preenche os requisitos fundamentais das resoluções. O Comitê de Ética em Pesquisa segue os preceitos das resoluções CNS 196/96, 251/97 e 292/99, sobre as Diretrizes e Normas Regulamentadoras de Pesquisa Envolvendo Seres Humanos, do Conselho Nacional de Saúde / Conselho Nacional de Ética em Pesquisa / Agência nacional de Vigilância Sanitária. Em conformidade com os requisitos éticos, classificamos o presente protocolo como **APROVADO**.

O Comitê de Ética em Pesquisa, solicita que :

1. Enviar primeiro relatório parcial em seis meses a contar desta data;
2. Informar imediatamente relatório sobre qualquer evento adverso ocorrido;
3. Comunicar qualquer alteração no projeto e no TCLE;
4. Entregar junto com o relatório, todos os TCLE assinados pelos sujeitos de pesquisas e a apresentação do trabalho.
5. Após o término desta pesquisa, o pesquisador responsável deverá apresentar os resultados junto à equipe da unidade a qual fez a coleta de dados e/ou entrevista, inclusive para o Conselho Local da Unidade de Saúde.

Porto Alegre, 22/10/08  
  
Elen Maria Borba  
Coordenadora do CEP



## TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO

**Título da pesquisa:** Condições de saúde bucal de adolescentes de Porto Alegre e fatores associados: estudo de coorte

**Pesquisadora responsável:** Marisa Maltz

**Pesquisadora:** Carolina Doege Brusius

**Instituição/Departamento:** Universidade Federal do Rio Grande do Sul/Faculdade de Odontologia

- 1. Objetivo do estudo:** Estudar a incidência e a progressão da cárie, gengivite, fluorose e erosão (desgaste nos dentes) em estudantes residentes em Porto Alegre- RS.
- 2. Seleção dos indivíduos:** Os estudantes foram examinados anteriormente, em 2009/2010, sendo escolhidos por sorteio aleatório.
- 3. Duração:** A participação na pesquisa consiste em exame odontológico do estudante, a ser realizado em um único dia, na própria escola, com duração prevista de aproximadamente dez minutos.
- 4. Procedimentos:** Os indivíduos terão seus dentes limpos com escova, fio e pasta dental, fornecidos pela equipe da pesquisa e examinados pelas cirurgiãs-dentistas Carolina Doege (CRO-RS 17.892) e Luana Severo Alves (CRO-RS 16.588) (estudantes de Pós-Graduação da UFRGS).
- 5. Importância do estudo:** Este levantamento epidemiológico será muito importante para avaliação da incidência e da progressão de problemas bucais dos estudantes de nossa cidade.
- 6. Danos:** Não existem danos previstos. Todo o instrumental (espelho, pinça e sonda) utilizado estará devidamente esterilizado. Somente participarão dos exames os estudantes que assim concordarem e assinarem este termo, juntamente com a assinatura dos pais ou responsáveis.
- 7. Benefícios:** Os adolescentes e os pais conhecerão as condições de saúde bucal do estudante e receberão, posteriormente, um relatório do exame realizado, assim como a indicação de lugares que ofereçam atendimento odontológico gratuito. O estudante receberá uma escova dental.
- 8. Confidencialidade:** As informações contidas nos questionários e a identidade dos estudantes ficarão sob o poder restrito dos pesquisadores e não serão divulgadas nos trabalhos resultantes desta pesquisa.

A participação na pesquisa é totalmente voluntária e o indivíduo tem a liberdade de se recusar a participar ou retirar seu consentimento em qualquer momento do estudo sem nenhum tipo de penalidade.

Caso tenha alguma dúvida sobre este estudo, você pode entrar em contato com Carolina Doege no telefone (51) 3308.5193, Marisa Maltz (51) 3308.5247 e com o Comitê de Ética em Pesquisa da Secretaria Municipal de Saúde (51) pelo número 3289.5517 – Rua Capitão Montanha, 27, 7. Andar – Porto Alegre.

O Termo de Consentimento Livre e Esclarecido será assinado em duas vias, ficando uma com você e a outra com a pesquisadora.

Porto Alegre, \_\_\_\_ de \_\_\_\_\_ de 2012.

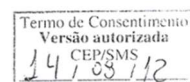
**Confirmo que entendi a natureza da pesquisa e autorizo a participação do estudante chamado:** \_\_\_\_\_

**Assinatura dos pais ou responsável:** \_\_\_\_\_

**Confirmo que entendi a natureza da pesquisa e me disponho a participar voluntariamente.**

**Assinatura do estudante:** \_\_\_\_\_

**Pesquisadora Carolina Doege:** \_\_\_\_\_



Reg. Cop. 307



**Prefeitura Municipal de Porto Alegre  
Secretaria Municipal de Saúde  
Comitê de Ética em Pesquisa**

**PARECER CONSUBSTANCIADO**

**Pesquisador (a) Responsável:** Marisa Maltz

**Registro no CEP:** 807 **Processo N.º:** 001.028618.12.2

**Instituição onde será desenvolvido:** 42 escolas do município de Porto Alegre (09 particulares e 33 públicas)

**Utilização:** TCLE

**Situação:** APROVADO

O Comitê de Ética em Pesquisa da Secretaria Municipal de Saúde de Porto Alegre analisou o processo N.º.001.028618.12.2, referente ao projeto de pesquisa: **"Condições de saúde bucal e de adolescentes de Porto Alegre e fatores associados: estudo de coorte"**.

De acordo com os procedimentos internos estabelecidos nesta instituição, bem como as exigências das Resoluções do Conselho Nacional de Saúde n.ºs 196/96, 251/97 e 292/99, este Comitê de Ética em Pesquisa considera **APROVADO** o referido projeto, em sua Reunião Ordinária realizada em 14 de agosto de 2012.

O Comitê de Ética em Pesquisa solicita o atendimento aos itens abaixo:

1. Enviar primeiro relatório parcial em seis meses a contar desta data;
2. Informar imediatamente qualquer evento adverso ocorrido;
3. Comunicar qualquer alteração no projeto e no TCLE;
4. Entregar com o relatório final todos os TCLEs assinados pelos sujeitos de pesquisas, juntamente com o formulário disponível no site e CD com trabalho concluído;
5. Após o término desta pesquisa, o pesquisador responsável deverá apresentar os resultados junto à equipe da unidade a qual fez a coleta de dados e/ou entrevista, inclusive para o Conselho Local da Unidade de Saúde.

Porto Alegre, 14/08/2012.

  
Maria Mercedes Bendati  
Coordenadora do CEP