Journal of Clinical Periodontology
Official Journal of The European Federation of Periodontology
Founded by the British, Dutch, French, German, Scandinavian, and Swiss Societies of Periodontology

J Clin Periodontol 2009; 36: 388–395 doi: 10.1111/j.1600-051X.2009.01394.x

Journal of Clinical Periodontology

Physical activity, inflammatory biomarkers in gingival crevicular fluid and periodontitis

Anne E. Sanders[1], Gary D. Slade[1], Tracy R. Fitzsimmons[2] and Peter Mark Bartold[2]

[1]Australian Research Centre for Population Oral Health, School of Dentistry; [2]Colgate Australian Clinical Dental Research Centre, The University of Adelaide, Adelaide, Australia

Sanders AE, Slade GD, Fitzsimmons TR, Bartold PM. Physical activity, inflammatory biomarkers in gingival crevicular fluid and periodontitis. J Clin Periodontol 2009; 36: 388–395. doi: 10.1111/j.1600-051X.2009.01394.x.

Abstract

Aims: To examine the associations of physical activity with interleukin 1-β (IL-1β), C-reactive protein (CRP) and periodontitis and to investigate whether any relationship between physical activity and inflammatory mediators differs between periodontitis cases and non-cases.

Material and Methods: In this population-based case control study of Australians aged 18+ years, dentists conducted oral epidemiologic examinations identifying cases with moderate or severe periodontitis and periodontally healthy controls. Gingival crevicular fluid samples collected during examinations were analysed for inflammatory biomarkers. Subject-completed questionnaires assessed leisure-time physical activity. Exposure odds ratios (ORs) were estimated in multivariable logistic regression models adjusting for periodontitis risk indicators.

Results: Of 751 subjects (359 cases, 392 controls), those meeting a prescribed threshold for leisure-time physical activity had lower adjusted odds of elevated IL-1β: OR = 0.69, (95% CI = 0.50–0.94) and detectable CRP: OR = 0.70 (0.50–0.98) than less active adults. Physical activity was not associated with periodontitis: OR = 1.14 (0.80–1.62). Periodontitis modified the association between levels of physical activity and detectable CRP. Increasing quartiles of physically activity were associated with decreasing probability of detectable CRP, but the effect was limited to periodontitis cases and was not apparent among non-cases.

Conclusion: Leisure-time physical activity may protect against an excessive inflammatory response in periodontitis.

Key words: epidemiology; exercise; inflammatory biomarkers; periodontal disease; protective effect

Accepted for publication 09 February 2009

Conflict of interest and sources of funding statements

The authors declare that they have no conflict of interests.

The National Survey of Adult Oral Health was supported by National Health and Medical Research Council (NHMRC) Project Grants No 299060 and 349514; Australian Government Department of Health and Ageing, Population Health Division; Australian Institute of Health and Welfare; Colgate Oral Care; Australian Dental Association; and US Centers for Disease Control and Prevention's Research Participation Program. Dr. Anne Sanders is supported by an NHMRC Sidney Sax (Public Health) Fellowship.

The inflammatory response to infection, while intended to restore homeostasis, can become harmful if excessive or dysregulated (Medzhitov 2008). Under these conditions, inflammation plays an essential role in the pathogenesis of many chronic diseases including periodontitis. In the acute response to bacterial pathogens, toll-like receptors activate macrophages in local tissues to produce pro-inflammatory cytokines. One of these cytokines, interleukin-1β (IL-1β), is associated with the loss of connective periodontal tissue attachment and resorption of alveolar bone (Masada et al. 1990, Stashenko et al. 1991, Bulut et al. 2001). When the acute inflammatory response is insufficient, these cytokines stimulate hepatocytes to secrete acute-phase proteins such as C-reactive protein (CRP) in a chronic non-specific systemic inflammatory response (Medzhitov 2007). Surveys of the general population find elevated levels of both IL-1β (Zhong et al. 2007, Fitzsimmons et al. in press) and CRP (Fitzsimmons et al. in press) in the gingival crevicular fluid (GCF) of individuals with periodontitis. Large prospective studies demonstrate that CRP is a risk predictor of coronary heart disease (Pradhan et al. 2002, Pai et al. 2004), diabetes mellitus (Pradhan et al. 2001) and cerebrovascular events (Makita et al. 2008).

Because these conditions are major causes of morbidity and mortality in populations, it is of considerable public health interest to limit the pro-inflammatory component of pathogenesis through strategies that are not reliant on a pharmaceutical or a clinical intervention. Regular physical exercise may offer a behavioural strategy to limit inflammation. There is increasing evidence that, in addition to its other benefits, physical activity has an anti-inflammatory effect. The US National Health and Nutrition Examination Survey (NHANES III) found that a higher frequency of physical activity was associated with lower odds of elevated CRP and white blood cell count (Abramson & Vaccarino 2002, Ford 2002). Elsewhere, an inverse association between physical activity and several inflammatory biomarkers, including CRP, was found even at a low intensity of exercise (Elosua et al. 2005). In fact, walking 5 days a week for 30 min. daily has been associated with lower levels of IL-6, tumor necrosis factor-α and CRP (Yates et al. 2008). Strengthening this cross-sectional body of evidence is a small number of prospective studies showing a reduction in CRP following the initiation of physical activity (Kasapis & Thompson 2005).

It is plausible that physical activity protects the periodontium by attenuating an excessive host inflammatory response. There is some supporting cross-sectional evidence (Al-Zahrani et al. 2005a, b) and one prospective study (Merchant et al. 2003) finding that physically active adults have a lower risk of periodontitis. To date, no study has jointly explored the relationships of physical activity with inflammatory biomarkers and with periodontitis. Hence, the first aim of this study was to examine the relation of physical activity to levels of IL-1β and CRP and periodontitis case status. The second aim was to determine whether the association between physical activity and inflammation is the same for periodontitis cases and non-cases. The rationale for the second hypothesis was based on the view that periodontitis is, in part, one manifestation of a ''hyperinflammatory'' trait that elicits a tissue-destructive response to periodontal pathogens (Beck et al. 1998). Accordingly, the hypothesis for this second aim was that any protective effect of physical exercise on inflammatory mediators would be more apparent in periodontitis cases than in non-cases.

Material and Methods

Study design and subjects

This case–control study was nested in the National Survey of Adult Oral Health, a cross-sectional survey of a nationally representative sample of adults in Australia conducted between 2004 and 2006. Full details of sampling and design are available online (Slade et al. 2007). The case–control component included epidemiologic examination data from dentate adults aged 18 years and older. Subjects firstly provided sociodemographic information in a computer-assisted telephone interview, secondly participated in a standardized oral epidemiologic examination and thirdly responded to a self-administered questionnaire that included questions about leisure-time physical activity and height and weight.

Data collection

Telephone interview

Subjects provided demographic information about their sex, age in years and country of birth (Australia or other). They described their tobacco smoking status (current smoker, former smoker or never smoked), and they indicated whether they had ever received a medical diagnosis of diabetes (yes or no).

Periodontal examination

Using a modified NHANES periodontal examination protocol, periodontal measurements were made on all teeth except third molars. Probing pocket depth (PPD) and recession were measured at three sites: mesio-buccal, mid-buccal and disto-buccal, to enable calculation of clinical periodontal attachment level. Examination data were recorded during examination onto a notebook computer.

Periodontitis case status

Periodontitis case status met the diagnostic criteria for population-based studies of periodontitis developed by a consensus panel convened by the US Centers for Disease Control and Prevention and the American Academy of Periodontology (Page & Eke 2007). Cases had moderate periodontitis or more severe levels of disease and non-cases had mild levels or no signs of the disease. Moderate periodontitis is defined as, ''two or more interproximal sites with clinical attachment level $\geqslant$4 mm, not on the same tooth, or two or more interproximal sites with probing depth $\geqslant$5 mm, not on the same tooth'' (Page & Eke 2007 p1397).

Sampling and collection of GCF

An algorithm in the examination program selected four periodontal sites at random for collection of GCF samples from among all periodontally measured sites within each person's mouth, yielding up to two sites with PPDs of at least 4 mm and the remaining sites with shallower PPDs. GCF was collected at four sites where recession and PPD were recorded. Where all PPD measurements were $<$4 mm a simple random sampling method was used to select all four sites. Otherwise, a stratified random sampling method was used to collect GCF from up to two sites with PPD$\geqslant$4 mm, and the remaining samples from sites with PPD$<$4 mm. From each site, two samples were collected on Periopaper™ strips (Oraflow Inc, Plainview, NY, USA). The area was isolated from saliva with cotton rolls and air dried before placing the strip into the gingival crevice for 10 s. Following its removal, a second strip was immediately inserted into the same site also for 10 s. On removal, both strips were air dried, wrapped in an aluminium foil and sealed in an identified tube before shipment to the Adelaide laboratory, where they were stored frozen at -20°C until processed.

Self-administered questionnaire

Leisure-time physical activity. Eight core questions from the Active Australia Survey (Australian Institute of Health and Welfare 2003) measured leisure-time physical activity for the week preceding the survey. The reliability and validity of these questions is established (Booth et al. 1996a, b, Brown et al. 2004, Timperio et al. 2004). Subjects offered literal responses to recall the number of sessions (of $\geqslant$10 min.' duration) engaged in four activities: continuous walking for at least 10 min.; vigorous gardening or heavy work around the yard; other vigorous activity (e.g., jogging, cycling or aerobics); and other more moderate physical activities (e.g., gentle swimming or golf). The amount of time spent in these activities was also recorded. Overall physical activity was calculated by summing the time spent in walking and moderate

activity and twice the time spent in vigorous activity (not including gardening and yard work). Guidelines from the Australian Institute of Health and Welfare recommend at least five sessions of physical activity per week culminating in at least 2.5 h of activity – the threshold at which people derive a health benefit. This corresponds to at least 30 min. of exercise over 5 days. In this analysis, physical activity was dichotomized at this threshold value to yield a sufficiently active group ($\geqslant$150 min. over $\geqslant$5 sessions) *versus* an insufficiently active group ($<$150 min. over $<$5 sessions). In addition, quartiles of total time in minutes engaged in physical activity were computed to test for a potential dose–response effect of exercise on inflammation.

Body mass index (BMI)

Questions about height and weight were used to calculate BMI [BMI = weight (kg)/height $(m)^2$]. Based on World Health Organization classification of BMI for adults (WHO 2000), a BMI of $<$25 was coded underweight or normal, and a BMI of $\geqslant$25 classified as overweight or obese.

Laboratory procedures

GCF was eluted from each pair of paper strips separately, in a two-step centrifugation procedure described in detail previously (Fitzsimmons et al. in press). Eluted samples were pooled before analysis by enzyme linked immunosorbent assays (ELISA). Levels of CRP and the amount of IL-1β collected over 20 s (10 s/strip) were assayed in duplicate using commercial ELISA kits (IL-1β, R&D Systems, Minneapolis, MN, USA; CRP, Bender MedSystems, Vienna, Austria) according to the manufacturer's instructions. Concentrations were determined from standard curves generated with KC4 software using linear regression analysis (CRP) or a four-parameter logistic curve-fit (IL-1β).

Quantifying inflammatory response

The eluted IL-1β was multiplied by 0.48 to yield the amount in picograms at each periodontal site. Because of the skewed nature of its distribution, the natural log of the average amount collected within each mouth was computed to create a person-level variable. In this study, this continuous variable was dichotomized at the median value for all subjects to produce a variable coded zero and one where one denotes levels above the median labelled ''elevated IL-1β''. The lower limit of detection of the CRP ELISA was 3 pg/ml. The eluted concentration of CRP fell below the ELISA's level of detection for 53.7% of sites, and so the data were dichotomized to indicate whether or not an amount of three or more picograms per millilitre was detected. A person-level variable was computed to indicate whether or not the study subject had detectable CRP at this threshold.

Dependent variables

Physical activity was examined for its relations with three binary dependent variables. These were (1) elevated IL-1β, defined as levels above the median value for the study population, (2) detectable CRP, defined as 3 pg/ml or higher, and (3) periodontitis case status.

Covariates

Covariates were sex, age in years, country of birth, diabetes status, smoking status and body mass index. Age was included as a risk indicator for periodontitis because prevalence is greater at each successive age group. In adjusting for age, the authors recognize that periodontitis is more a consequence of the cumulative effects of tissue destruction over time rather than an age-related intrinsic deficiency (Genco 1996, Nunn 2003), and they acknowledge that the rate of periodontal destruction throughout adulthood is constant rather than accelerating (Abdellatif & Burt 1987). Age, like country of birth, are not directly causally associated with periodontitis but is associated with other factors, which may in turn be related to physical activity.

Statistical analysis

To test aim one, unconditional logistic regression was used to examine the association of physical activity with the two inflammatory biomarkers and with periodontitis. The insufficiently active group was set as the reference category to evaluate the potential protective effect of physical activity. Results were expressed as the odds ratio (OR) with its 95% confidence interval (CI).

To screen for potential confounding, we conducted stratified analyses, computing exercise-periodontitis ORs separately for each stratum of a potential confounder. When stratum-specific ORs were homogenous, as signified by a non-significant Breslow–Day test, we then computed a pooled, Mantel–Haenszel OR that adjusted the physical activity–inflammatory biomarker/periodontitis odds for the confounding variable. Where the crude log-OR differed from the adjusted log-OR by $\geqslant$20%, we considered this to be evidence of confounding. In the subsequent multivariate analyses our criteria for including a variable in the model were whether it: (1) was statistically significant in the model (p<0.05); (2) was a confounder, i.e. whether it resulted in a change of $\geqslant$20% in the regression coefficient of physical activity; and (3) was the exposure of interest (physical activity) or a variable of special interest, such as an important predictor in other studies, such as age or smoking status.

To test for a possible inverse dose–response trend in the relationship between physical activity and inflammation, the ordinal variable of quartiles of total time was treated as a grouped linear variable in logistic regression. Results were interpreted as the average change in odds of inflammation (IL-1β or CRP) for every one quartile increase in physical activity. To test the second aim of a potential effect modification of periodontitis case status on the relation between physical activity and inflammation, an interaction term was computed as the product of the physical activity quartiles and CRP. An interaction of physical activity quartiles and IL-1β was also tested. Models were examined with and without these interaction terms. Findings from the final model were depicted as predicted probabilities of elevated biomarkers. Probabilities and their 95% CIs were computed for each quartile of physical activity, both for cases and for non-cases. All statistical analyses were performed using STATA release 10.1.

Ethical considerations

The project was reviewed and approved by the Human Research Ethics Committee of the University of Adelaide. All examined subjects provided signed informed consent before the examination.

Results

Of the 1139 adults for whom biomarkers were quantified, 751 adults (359 cases, 392 controls) completed the questionnaire, representing a response rate of 65.9%. Among periodontitis cases, 38.3% had elevated levels of both inflammatory biomarkers, compared with 20.9% of non-cases. Fourteen percent of adults reported having no physical activity in the previous week.

Sixty percent of adults engaged in at least five sessions of physical activity in the previous week, accumulating at least 2.5 h of physical activity – a threshold considered sufficient for a health gain (Table 1). Fifty percent of adults were former or current tobacco smokers and 54 percent were overweight or obese. Six percent were diagnosed diabetics and nearly half (46%) had detectable levels of CRP in GCF.

No statistically significant differences were found between the proportion of people who had sufficient physical activity and those less physically active according to socio-demographic characteristics and periodontitis risk indicators, although diabetics ($p = 0.07$) and overweight/obese adults ($p = 0.06$) were likely to be less active than non-diabetics and adults of low or normal weight (Table 2). When examined separately on the basis of periodontitis case status, associations remained non-significant (findings not tabulated).

Physically active adults had lower unadjusted odds of elevated IL-1β (Table 3). Unadjusted odds of elevated IL-1β were higher among males, people born outside of Australia, overweight/obese adults, those with detectable CRP and periodontitis cases, compared with referenced peers. Physically active adults also had lower unadjusted odds of detectable CRP (Table 3). Higher odds of detectable CRP were found among older adults, diagnosed diabetics, the overweight/obese, those with elevated IL-1β and periodontitis cases. However, there was no significant association between physical activity and periodontitis case status.

Stratified analyses revealed no evidence of confounding bias. Consequently, initial multivariate models were examined with inclusion all potential predictor variables. Diabetes status and body mass index failed to reach statistical significance ($p < 0.05$) in modelling odds for IL-1β and periodontitis and were excluded from the final model (Table 4). Similarly, country of birth and diabetes status were omitted in modelling odds for CRP.

Physical activity remained significantly associated with lower levels of IL-1β and CRP after adjustment for periodontitis risk indicators significant in unadjusted analysis (Table 4). Physical activity also remained non-significantly associated with periodontitis (adjusted OR = 1.14, 95% CI = 0.80–1.62). Significantly associated with higher odds of periodontitis were male sex, older age, non-Australian birth, tobacco smoking and inflammatory biomarkers.

An inverse linear effect was found between quartiles of length of time spent in physical activity and odds of detectable CRP (test for trend $p = 0.002$). For every quartile increase in time spent in physical activity, odds of detectable CRP decreased by 22% (adjusted OR = 0.82, 95% CI = 0.72–

Table 1. Characteristics of the study subjects, Australia 2004–2006

	Frequency	Percent		Frequency	Percent
Physical activity			Smoking status		
Insufficient	299	39.8	Never	375	49.9
Sufficient	452	60.2	Former	257	34.2
			Current smoker	119	15.9
Sex					
Female	441	58.7	Body mass index		
Male	310	41.3	Underweight/normal	298	39.7
			Overweight/obese	407	54.2
Age group			Missing	46	6.1
18–44 years	234	31.2			
45–64 years	336	44.7	Interleukin-1β		
65+ years	181	24.1	$<$Median	360	47.9
			$\geqslant$Median	360	47.9
Country of birth			Missing	31	4.1
Australia	554	73.8			
Other	196	26.1	C-reactive protein		
Missing	1	0.1	<3 pg/ml	403	53.7
			$> = 3$ pg/ml	348	46.3
Diabetes diagnosis					
Not diagnosed	703	93.6	Periodontitis		
Diagnosed	48	6.4	Non-case	392	52.2
			Case	359	47.8

Table 2. Number (%) of adults with levels of leisure-time physical activity classified as sufficient for a health benefit, Australia 2004–06*

	Sufficient physical activity for a health benefit			Sufficient physical activity for a health benefit	
Sex			Smoking status		
Female	256	(58.1)	Never smoked	228	(60.8)
Male	196	(63.2)	Former smoker	155	(60.3)
p-value	0.154		Current smoker	69	(58.0)
			p-value	0.860	
Age group					
18–44 years	143	(61.1)	Body mass index		
45–64 years	201	(59.8)	Underweight/normal	192	(64.4)
65+ years	108	(59.7)	Overweight/obese	234	(57.5)
p-value	0.941		*p*-value	0.063	
Country of birth			Periodontitis		
Australia	336	(60.7)	Non case	239	61.0
Other	116	(59.2)	Case	213	59.3
p-value	0.718		*p*-value	0.647	
Diabetes diagnosis					
Not diagnosed	429	(61.0)			
Diagnosed	23	(47.9)			
p-value	0.073				

*When stratified by periodontitis case status, differences for all characteristics between cases and non-cases remained non-significant.

Table 3. Percentage of subjects and unadjusted odds ratios (95% CI) for the association of physical activity and covariates with elevated interleukin-1β (median and above), detectable C-reactive protein ($\geqslant$3 pg/ml) and periodontitis case status, Australia 2004–06

	Elevated interleukin-1β			Detectable CRP			Periodontitis case		
	percent	OR	95% CI	percent	OR	95% CI	percent	OR	95% CI
Physical activity									
Insufficient	56.6	Reference		53.9	Reference		48.8	Reference	
Sufficient	45.6	0.64	(0.48–0.87)	41.4	0.60	(0.45–0.81)	47.1	0.93	(0.70–1.25)
Sex									
Female	45.4	Reference		46.7	Reference		40.6	Reference	
Male	56.5	1.57	(1.16–2.11)	45.8	0.96	(0.72–1.29)	58.1	2.03	(1.51–2.72)
Age group									
18–44 years	45.5	Reference		40.6	Reference		23.9	Reference	
45–64 years	50.9	1.24	(0.88–1.75)	46.7	1.28	(0.92–1.80)	51.8	3.41	(2.36–4.94)
65+ years	54.0	1.41	(0.94–2.09)	53.0	1.65	(1.12–2.44)	71.3	7.89	(5.08–12.25)
Country of birth									
Australia	46.0	Reference		45.3	Reference		41.7	Reference	
Other	60.9	1.82	(1.30–2.56)	49.0	1.16	(0.84–1.61)	65.3	2.63	(1.87–3.69)
Diabetes diagnosis									
Not diagnosed	49.2	Reference		45.1	Reference		46.9	Reference	
Diagnosed	62.2	1.70	(0.91–3.17)	64.6	2.22	(1.21–4.09)	60.4	1.73	(0.95–3.13)
Smoking status									
Never smoked	50.8	Reference		44.0	Reference		40.0	Reference	
Former smoker	51.6	1.03	(0.75–1.43)	47.9	1.17	(0.85–1.61)	53.7	1.74	(1.26–2.40)
Current smoker	43.9	0.76	(0.49–1.15)	50.4	1.29	(0.86–1.96)	59.7	2.22	(1.46–3.38)
Body mass index									
Underweight/normal	44.5	Reference		34.2	Reference		47.0	Reference	
Overweight/obese	53.8	1.45	(1.07–1.97)	54.3	2.28	(1.68–3.11)	47.2	1.01	(0.75–1.36)
Interleukin-1β									
$<$Median	NA			34.4	Reference		40.3	Reference	
$\geqslant$Median	NA			58.3	2.66	(1.97–3.60)	54.7	1.79	(1.33–2.41)
C-reactive protein									
$<$3 pg/ml	38.9	Reference		NA			38.2	Reference	
$\geqslant$3 pg/ml	62.9	2.66	(1.97–3.60)	NA			58.9	2.32	(1.73–3.11)
Periodontitis status									
Non-case	43.12	Reference		36.48	Reference		NA		
Case	57.60	1.79	(1.33–2.41)	57.10	2.32	(1.73–3.11)	NA		

CRP, C-reactive protein.

Table 4. Multivariate logistic regression models of adjusted odds ratios (OR) and 95% confidence intervals (CI) for the relation of physical activity with inflammatory biomarkers and periodontitis, Australia 2004–2006

	Elevated interleukin-1-β (above median)		C-reactive protein ($\geqslant$3 pg/ml)		Periodontitis case (moderate or severe)	
	OR	95% CI	OR	95% CI	OR	95% CI
Male (reference = female)	1.69	1.22–2.33	0.71	0.51–1.00	1.63	1.15–2.30
Age in years	1.00	0.99–1.01	1.01	1.00–1.02	1.06	1.05–1.07
Not born in Australia (reference = Australia)	1.77	1.23–2.53	NA		2.32	1.56–3.45
Diagnosed diabetes (reference = no diagnosis)	NA		NA		NA	
Former smoker (= reference = never smoked)	0.91	0.64–1.28	1.15	0.80–1.65	1.51	1.03–2.20
Current smoker (reference = never smoked)	0.65	0.41–1.02	1.70	1.06–2.73	3.78	2.28–6.27
Body mass index	NA		1.12	1.08–1.16	NA	
Elevated IL-1β (reference = $<$median value)	DV		2.65	1.91–3.69	1.53	1.08–2.18
Detectable CRP (reference = no detectable CRP)	2.68	1.96–3.66	DV		2.08	1.46–2.95
Sufficient physical activity (reference = insufficient)	0.69	0.50–0.94	0.70	0.50–0.98	1.14	0.80–1.62

NA denotes omission on the basis of statistical non-significance in the multivariate analysis.
DV denotes dependent variable.
CRP, C-reactive protein.

0.94) (results not tabulated). There was no significant corresponding trend between physical activity and odds of elevated IL-1β or between physical activity and odds of periodontitis.

Periodontitis case status modified the association between leisure-time physical activity and levels of CRP (Table 5). Plotting-predicted probabilities from the logistic regression model (Fig. 1) showed that the probability of detectable CRP in GCF among periodontitis cases approximately halved across increasing quartiles levels of physical activity from 0.67 (95% CI = 0.59–0.76) in the quar-

Table 5. Multivariate logistic regression models of odds ratios (OR) and 95% confidence intervals (CI) showing the effect modification of periodontitis case status in the relationship between physical activity and detectable CRP, Australia 2004–2006

	OR	95% CI
Male (reference = female)	0.65	0.46–0.92
Age in years	1.00	0.99–1.01
Former smoker (reference = never smoked)	1.12	0.77–1.62
Current smoker (reference = current smoker)	1.43	0.88–2.33
Body mass index	1.12	1.08–1.17
Elevated IL-1β (reference = <median value)	2.57	1.84–3.60
Duration of physical activity	1.00	0.81–1.23
Periodontitis case (reference = non-case)	4.80	2.09–11.01
Duration of physical activity × Periodontitis case	0.71	0.53–0.96

CRP, C-reactive protein.

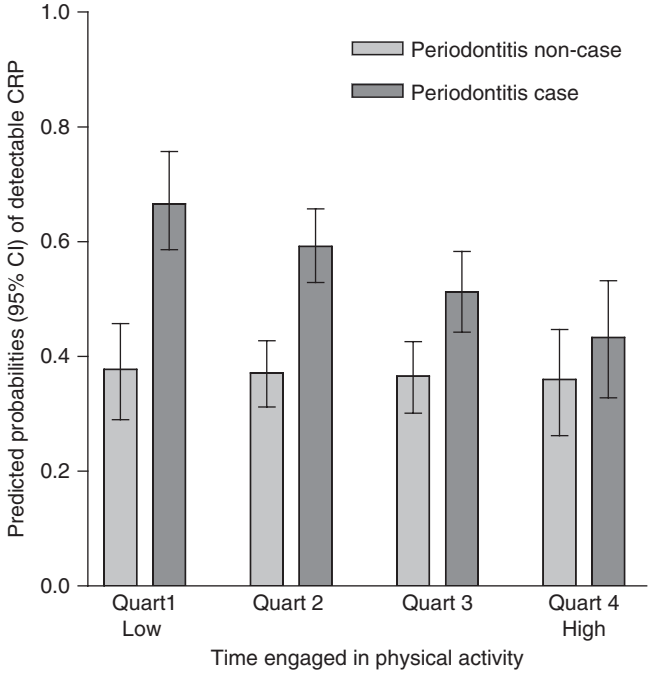

Fig. 1. Effect modification of case status on the physical activity and CRP relationship. Predicted probabilities of detectable C-reactive protein at four levels of leisure-time physical activity for periodontal cases and non-cases from a logistic regression model that included an interaction term for the duration of physical activity and periodontitis case status, adjusted for sex, age, smoking, body mass index and interleukin 1β. Note: the non-parallelism in the height of the bars and lack of overlap of errors bars is visual evidence of effect modification.

tile with the least physical activity to 0.43 (95% CI = 0.33–0.53) in the quartile with the most physical activity. In contrast, in adults without periodontitis, the probability of detectable CRP was not associated with time engaged in leisure-time physical activity.

Discussion

Individuals who engaged in a near-daily physical activity of 30 min. or more had lower levels of the proinflammatory biomarkers IL-1β and CRP in GCF than those less active. This applied to all individuals irrespective of their periodontitis case status.

A major finding of this study was the effect modification of periodontitis case status on the relationship between physical activity and CRP. Among cases there was a dose–response relation of decreasing probability of detectable CRP at increasing levels of physical activity. This was in stark contrast to the invariant probability of detectable CRP among non-cases across levels of physical activity. This interaction of effects confirms our prior hypothesis and suggests that the putative ''hyperinflammatory trait'' (Beck et al. 1998) might be modifiable by physical exercise. Moreover, it implies that physical activity may reduce levels of inflammation, thus promoting periodontal health. This interpretation is made cautiously because despite the consensus that CRP levels are higher in periodontitis cases than non-cases (Paraskevas et al. 2008), the evidence remains sparse that CRP plays a causal role in the pathogenesis of the disease. In the sole cohort study to date, males recording elevated levels of CRP on two occasions a decade apart were at a higher risk of advanced periodontitis (Linden et al. 2008), strengthening the plausibility of a case for causal role of CRP. More commonly, the literature considers chronic infection and inflammatory mediators in the periodontium as a reservoir for spill-over with implications for systemic effects once entering the circulation (Li et al. 2000).

This is the first study to jointly assess the associations of physical activity on periodontitis and its inflammatory basis in the same analysis. A particular strength of this study is that cases and non-cases were drawn from a national survey of the Australian general adult population rather than from a dental practice-based population. A second strength is that periodontal health was assessed in epidemiologic examinations by dentists trained and calibrated to record valid and reliable measures of periodontal health at multiple sites per tooth. This builds on earlier research relating physical activity to self-reported periodontal disease (Merchant et al. 2003, Joshipura et al. 2004) and relating physical fitness with CPITN scores (Wakai et al. 1999). Findings also build on evidence of associations between periodontal disease and these inflammatory biomarkers in GCF (Fitzsimmons et al. in press). The estimated proportion of subjects reaching recommended levels of physical activity (60%) is similar to a national estimate of 57% obtained by the Australian Institute of Health and Welfare using the same core questions (Armstrong et al. 2000). This suggests that findings may be generalizable to adult populations beyond this study. Previous research shows that these physical activity questions exhibit acceptable validity and good to excellent reliability (Brown et al. 2004). Moreover, since the measures of physical activity adopted for this analysis are blunt instruments, they

do not require a high precision and any misclassification would tend to bias a true effect toward the null. Also biasing towards the null is the fact that physical activity may be precipitated by an existing medical condition.

This study has several limitations. Since data are cross-sectional, it is not possible to establish whether physical inactivity is causally involved in limiting the pro-inflammatory component of pathogenesis, or whether the effects are mediated through other mechanisms. Nevertheless, the finding of a dose–response gradient of a lower CRP with a longer duration of physical activity strengthens the plausibility of a protective effect of exercise in limiting inflammation in the periodontal tissues. A second limitation is that physical activity was limited to self-report. Other studies have additionally calculated weekly energy expenditure and applied a threshold value of 800 kcal/week consistent with the recommendation of the US Surgeon General (United States Department of Health and Human Services 1996). However, these energy expenditure estimates are biased for lighter weight persons and require further adjustment for age (AIHW 2003). Finally, because the findings are indicative only of activity over a single week, this study provides no information on the length of time that people have been physically active.

Studies evaluating levels of CRP levels in plasma or serum far outnumber those measuring CRP in GCF samples. One study, comparing levels of high-sensitivity CRP collected in serum and in GCF, found that only 18% of the variance in one measure was accounted for by the other ($r = 0.425$, $p = 0.001$) (Tüter et al. 2007). The present study presents evidence that physical activity may limit the potential harmful effects of excessive or dysregulated inflammation. One mechanism may be by limiting the harmful effect of inflammation. An alternative mechanism may be by enhancing the host immunological response or by simply promoting immune function. The dose–response relation between physical activity and detectable CRP among periodontitis cases in the presence of the invariant relationship among non-cases implies a greater potential health gain for people who already have the disease. The corollary of this conclusion is that the therapeutic mechanism of physical activity may be to reduce the risk of an excessive or a dysregulated systemic inflammatory response in periodontitis patients. The absence of this anti-inflammatory benefit among physically inactive individuals may increase the risk of an excessive inflammatory response and its harmful sequelae. This interpretation is consistent with the concept of an allostatic load, whereby neuroendocrinal regulatory processes designed to maintain homeostasis can become dysregulated under a sustained challenge, increasing the risk of pathophysiology.

Quite low levels of physical activity appear to have a beneficial effect on inflammatory response. Because chronic inflammation is critical in the pathogenesis of major causes of morbidity in populations, it is of considerable public health interest to limit the pro-inflammatory component of pathogenesis through behavioural strategies that are not reliant on pharmaceutical or clinical intervention. Public health recommendations of regular physical activity may also translate into clinical important gains for periodontal health.

It would be instructive in future research to investigate whether any therapeutic gain among people with moderate or severe periodontitis exceeds a prophylactic effect in people with minimal or no clinical signs of the disease.

Acknowledgements

The NHMRC funded this study: Project Grant #299060. Dr. Sanders is supported by a NHMRC Sidney Sax Public Health Fellowship #399222.

References

Abdellatif, H. M. & Burt, B. A. (1987) An epidemiological investigation into the relative importance of age and oral hygiene status as determinants of periodontitis. *Journal of Dental Research* **66**, 13–18.

Abramson, J. L. & Vaccarino, V. (2002) Relationship between physical activity and inflammation among apparently healthy middle-aged and older US adults. *Archives of Internal Medicine* **162**, 1286–1292.

Al-Zahrani, M. S., Borawski, E. A. & Bissada, N. F. (2005a) Increased physical activity reduces prevalence of periodontitis. *Journal of Dentistry* **33**, 703–710.

Al-Zahrani, M. S., Borawski, E. A. & Bissada, N. F. (2005b) Periodontitis and three health-enhancing behaviors: maintaining normal weight, engaging in recommended level of exercise, and consuming a high-quality diet. *Journal of Periodontology* **76**, 1362–1366.

Armstrong, T., Bauman, A. & Davies, J. (2000) *Physical Activity Patterns of Australian Adults. Results of the 1999 National Physical Activity Survey*. Canberra: Australian Institute of Health and Welfare.

Australian Institute of Health and Welfare (AIHW) (2003) *The Active Australia Survey: A Guide and Manual for Implementation, Analysis and Reporting*. Canberra: AIHW.

Beck, J. D., Offenbacher, S., Williams, R., Gibbs, P. & Garcia, R. (1998) Periodontitis: a risk factor for coronary heart disease? *Annals of Periodontology* **3**, 127–141.

Booth, M., Owen, N., Bauman, A. & Gore, C. (1996a) Relationship between a 14-day recall measure of leisure time physical activity and a submaximal test of physical work capacity in a population sample of Australian adults. *Research Quarterly for Exercise and Sport* **67**, 221–227.

Booth, M., Owen, N., Bauman, A. & Gore, C. (1996b) Retest reliability of recall measures of leisure time physical activity in Australian adults. *International Journal of Epidemiology* **25**, 153–159.

Brown, W. J., Trost, S. G., Bauman, A., Mummery, K. & Owen, N. (2004) Test–retest reliability of four physical activity measures used in population surveys. *Journal of Science and Medicine in Sport* **7**, 205–215.

Bulut, U., Develioglu, H., Taner, I. L. & Berker, E. (2001) Interleukin-1 beta levels in gingival crevicular fluid in type 2 diabetes mellitus and adult periodontitis. *Journal of Oral Science* **43**, 171–177.

Elosua, R., Bartali, B., Ordovas, J. M., Corsi, A. M., Lauretani, F. & Ferrucci, L. (2005) InCHIANTI investigators. Association between physical activity, physical performance, and inflammatory biomarkers in an elderly population: the InCHIANTI study. *The Journals of Gerontology. Series A, Biological Sciences and Medical Sciences* **60**, 760–767.

Fitzsimmons, T. R., Sanders, A. E., Slade, G. D. & Bartold, P. M. (In press) Biomarkers of periodontal inflammation in the Australia adult population. *Australian Dental Journal*.

Ford, E. S. (2002) Does exercise reduce inflammation? Physical activity and C-reactive protein among US adults. *Epidemiology* **13**, 561–568.

Genco, R. J. (1996) Current view of risk factors for periodontal diseases. *Journal of Periodontology* **67**, 1041–1049.

Joshipura, K. J., Wand, H. C., Merchant, A. T. & Rimm, E. B. (2004) Periodontal disease and biomarkers related to cardiovascular disease. *Journal of Dental Research* **83**, 151–155.

Kasapis, C. & Thompson, P. D. (2005) The effects of physical activity on serum C-reactive protein and inflammatory markers: a systematic review. *Journal of the American College of Cardiology* **45**, 1563–1569.

Li, X., Kolltveit, K. M., Tronstad, L. & Olsen, I. (2000) Systemic diseases caused by oral

infection. *Clinical Microbiology Reviews* **13**, 547–558.

Linden, G. J., McClean, K., Young, I., Evans, A. & Kee, F. (2008) Persistently raised C-reactive protein levels are associated with advanced periodontal disease. *Journal of Periodontology* **35**, 741–747.

Makita, S., Nakamura, M., Satoh, K., Tanaka, F., Onoda, T., Kawamura, K., Ohsawa, M., Tanno, K., Itai, K., Sakata, K., Okayama, A., Terayama, Y., Yoshida, Y. & Ogawa, A. (2008) Serum C-reactive protein levels can be used to predict future ischemic stroke and mortality in Japanese men from the general population. *Atherosclerosis* August 12. [Epub ahead of print].

Masada, M. P., Persson, R., Kenney, J. S., Lee, S. W., Page, R. C. & Allison, A. C. (1990) Measurement of interleukin-1 alpha and -1 beta in gingival crevicular fluid: implications for the pathogenesis of periodontal disease. *Journal of Periodontal Research* **25**, 156–163.

Medzhitov, R. (2007) Recognition of microorganisms and activation of the immune response. *Nature* **449**, 819–826.

Medzhitov, R. (2008) Origin and physiological roles of inflammation. *Nature* **24**, 428–435.

Merchant, A. T., Pitiphat, W., Rimm, E. B. & Joshipura, K. (2003) Increased physical activity decreases periodontitis risk in men. *European Journal of Epidemiology* **18**, 891–898.

Nunn, M. E. (2003) Understanding the etiology of periodontitis: an overview of periodontal risk factors. *Periodontology 2000* **32**, 11–23.

Page, R. C. & Eke, P. I. (2007) Case definitions for use in population-based surveillance of periodontitis. *Journal of Periodontology* **78**, 1387–1399.

Pai, J. K., Pischon, T., Ma, J., Manson, J. E., Hankinson, S. E., Joshipura, K., Curhan, G. C., Rifai, N., Cannuscio, C. C., Stampfer, M. J. & Rimm, E. B. (2004) Inflammatory markers and the risk of coronary heart disease in men and women. *New England Journal of Medicine* **351**, 2599–2610.

Paraskevas, S., Huizinga, J. D. & Loos, B. G. (2008) A systematic review and meta-analyses on C-reactive protein in relation to periodontitis. *Journal of Clinical Periodontology* **35**, 277–290.

Pradhan, A. D., Manson, J. E., Rifai, N., Buring, J. E. & Ridker, P. M. (2001) C-reactive protein, interleukin 6, and risk of developing type 2 diabetes mellitus. *Journal of the American Medical Association* **286**, 327–334.

Pradhan, A. D., Manson, J. E., Rossouw, J. E., Siscovick, D. S., Mouton, C. P., Rifai, N., Wallace, R. B., Jackson, R. D., Pettinger, M. B. & Ridker, P. M. (2002) Inflammatory biomarkers, hormone replacement therapy, and incident coronary heart disease: prospective analysis from the Women's Health Initiative observational study. *Journal of the American Medical Association* **288**, 980–987.

Slade, G. D., Spencer, A. J. & Roberts-Thomson, K. F. (eds). (2007) *Australia's Dental Generations: the National Survey of Adult Oral Health 2004–06 (Dental Statistics and Research Series No. 34)*. Canberra: Australian Institute of Health and Welfare, available at: http://www.arcpoh.adelaide.edu.au/project/distribution/nsaoh_pdf%20files/nsaoh_report.pdf (accessed 20 November 2008).

Stashenko, P., Fujiyoshi, P., Obernesser, M. S., Prostak, L., Haffajee, A. D. & Socransky, S. S. (1991) Levels of interleukin 1 beta in tissue from sites of active periodontal disease. *Journal of Clinical Periodontology* **18**, 548–554.

Timperio, A., Salmon, J., Rosenberg, M. & Bull, F. C. (2004) Do logbooks influence recall of physical activity in validation studies? *Medicine and Science in Sports and Exercise* **36**, 1181–1186.

Tüter, G., Kurtis, B. & Serdar, M. (2007) Evaluation of gingival crevicular fluid and serum levels of high-sensitivity C-reactive protein in chronic periodontitis patients with or without coronary artery disease. *Journal of Periodontology* **78**, 2319–2324.

United States Department of Health and Human Services. (1996) *Physical Activity and Health: A Report of the Surgeon General*, pp. 11–37. Atlanta, GA: US Department of Health and Human Services, Centers for Disease Control and Prevention, National Center for Chronic Disease Prevention and Health Promotion.

Wakai, K., Kawamura, T., Umemura, O., Hara, Y., Machida, J., Anno, T., Ichihara, Y., Mizuno, Y., Tamakoshi, A., Lin, Y., Nakayama, T. & Ohno, Y. (1999) Associations of medical status and physical fitness with periodontal disease. *Journal of Clinical Periodontology* **26**, 664–672.

World Health Organization (2000) *Obesity: Preventing and Managing the Global Epidemic. Report of a WHO Consultation. Technical Report Series 894*. Geneva: WHO.

Yates, T., Davies, M., Brady, E., Webb, D., Gorely, T., Bull, F., Talbot, D., Sattar, N. & Khunti, K. (2008) Walking and inflammatory markers in individuals screened for type 2 diabetes. *Preventive Medicine* July 3. [Epub ahead of print].

Zhong, Y., Slade, G. D., Beck, J. D. & Offenbacher, S. (2007) Gingival crevicular fluid interleukin-1beta, prostaglandin E2 and periodontal status in a community population. *Journal of Clinical Periodontology* **34**, 285–293.

Address:
Anne E. Sanders
Australian Research Centre for Population Oral Health
School of Dentistry
The University of Adelaide
Adelaide
Australia 5005
E-mail: anne.sanders@adelaide.edu.au

Clinical Relevance

Scientific rationale for the study: Despite strong evidence for an anti-inflammatory effect of leisure-time physical activity and some evidence that physical activity may protect periodontal health, no single study has examined the relationship of physical activity with inflammatory biomarkers and periodontitis case status in the general population.

Principal findings: Adults who met public health recommendations for physical activity had significantly lower levels of the proinflammatory biomarkers IL-1β and CRP in GCF. The probability of detectable CRP decreased with increasing levels of physical activity among periodontitis cases, but not among periodontally healthy controls.

Practical implication: Recommendations of regular physical activity may confer a reduction in inflammation, especially among patients with moderate to severe levels of periodontitis.