Applied Physiology, Nutrition, and Metabolism
Physiologie appliquée, nutrition et métabolisme

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Journal:	<i>Applied Physiology, Nutrition, and Metabolism</i>
Manuscript ID	apnm-2017-0758.R2
Manuscript Type:	Brief communication
Date Submitted by the Author:	27-Feb-2018
Complete List of Authors:	Willoughby, Taura; University of Lethbridge, Kinesiology and Physical Education Doan, Jon; University of Lethbridge Currie, Cheryl; University of Lethbridge Copeland, Jennifer L.; University of Lethbridge,
Keyword:	sedentary, inflammation, physical activity < exercise, saliva, C reactive protein
Is the invited manuscript for consideration in a Special Issue? :	N/A

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Short-term changes in daily movement behaviour influence salivary C-reactive protein in healthy women.

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ABSTRACT

This study assessed the effect of changing daily movement behaviour on C-reactive protein (CRP) measured in saliva. Two groups of women either reduced daily movement or increased physical activity for 10 days. Salivary CRP increased by 31% in the sedentary group (0.378 ± 0.596 to $0.487 \pm 0.793 \mu\text{g} \cdot \text{L}^{-1}$) and decreased by 22% in the active group (0.414 ± 0.640 to $0.259 \pm 0.284 \mu\text{g} \cdot \text{L}^{-1}$). These results suggest short-term changes in daily movement behaviour can affect salivary CRP, a marker of systemic inflammation.

Keywords: sedentary, physical activity, inflammation, C reactive protein, saliva,

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36 INTRODUCTION

37 Despite growing awareness of the potential health consequences of excessive sedentary
38 time, the underlying physiological mechanisms remain unclear. Several nationally representative
39 studies have shown a cross-sectional association between sedentary time and inflammatory
40 markers such as C-reactive protein (CRP) (Healy et al. 2011; Carson et al. 2014).

41 CRP is a plasma protein and a component of the innate immune system (Du Clos 2000).
42 It is used in several areas of clinical practice, such as identifying acute infection and monitoring
43 the progression of some autoimmune diseases, such as rheumatoid arthritis (Plant et al. 2000).
44 Importantly, several epidemiological studies have shown that even when within a normal range,
45 higher serum CRP concentration is predictive of future cardiovascular events (Ridker et al.
46 2000).

47 Physical activity is inversely associated with CRP (Beavers et al. 2010), however,
48 experimental studies of sedentary time and CRP are limited. One study reported an increase in
49 serum CRP following 14 days of reduced activity in older adults (Breen et al. 2013). More
50 longitudinal and intervention studies of CRP and movement behaviours are needed to confirm
51 cross-sectional evidence.

52 Currently, CRP is most commonly measured via blood sampling. Salivary diagnostics
53 represent a promising new technique that is cost effective, easy to use, and less invasive than
54 blood sampling (Malathi et al. 2014). Evidence supports an association between serum and
55 salivary CRP concentrations (Ouellet-Morin et al. 2011), and correlations have been established
56 between salivary CRP and subclinical atherosclerosis (Labat et al. 2013) and chronic obstructive
57 pulmonary disease (Patel et al. 2015). Salivary CRP has also been shown to increase acutely in
58 response to minimally invasive surgery (Foley et al. 2012). Together these data suggest salivary

CRP is a valid measure of low grade inflammation. Thus, the purpose of this study was to explore the effect of changes in daily movement behaviour on salivary CRP among healthy women. We hypothesized that CRP would increase with reduced daily movement and decrease with increased daily physical activity.

MATERIALS AND METHODS

Participants. Healthy females aged 40-60 years were recruited for this study. Inclusion criteria were that participants be somewhat active or active, quantified by an average of 7,000 to 13,000 steps per day (Tudor-Locke et al. 2013), but not engaged in purposeful exercise. Exclusion criteria included recent injury, acute illness or infection, obesity ($\text{BMI} > 30 \text{ kg} \cdot \text{m}^{-2}$), or any known chronic conditions. Participants provided written informed consent, and the study was approved by the Institutional Human Subject Research Committee.

Preliminary Assessment. A 7-day preliminary assessment of movement behaviour was completed; participants were blind to the step-count inclusion criteria and were told to maintain their typical lifestyle. A Piezo Rx pedometer (Deep River, ON) was used to measure step count, and an ActiGraph GT3X accelerometer (ActiGraph LLC, Pensacola, FL) was used to assess sedentary time and physical activity. Baseline measures were then collected; eligible participants were matched based on age and BMI then randomly assigned to one of two intervention groups, sedentary or active.

Intervention Protocol. The intervention was 10 days. The active group increased their individual daily step count by 3,000 steps, which is approximately equivalent to adding 30 min/day of moderate intensity activity (Tudor-Locke et al. 2008). The sedentary group reduced their daily step count to < 5000 steps per day to mimic an inactive and sedentary lifestyle (Tudor-Locke et al. 2008). Participants were directed to maintain their normal diet, report any illness or

injury, and refrain from using any anti-inflammatory medications. (For study timeline see Supplemental Figure 1).

Measurement of Movement Behaviour. During the intervention, participants wore a pedometer to self-monitor their daily step count. Participants also wore an ActiGraph GT3X accelerometer (ActiGraph, Pensacola, FL) on the hip. The ActiGraph recorded in 10-s periods, and non-wear time was identified by 90 consecutive minutes of zero counts with a 2-min spike tolerance (Choi et al. 2011). Sedentary time was defined as <100 counts/min, and moderate to vigorous physical activity (MVPA) was defined as >1951 counts/min (Freedson et al. 1998). Accelerometer data were reduced using ActiLife version 6.1 software (ActiGraph, LLC, Pensacola, Fla., USA).

Measurement of C-Reactive Protein. Saliva samples were collected at the end of the preliminary assessment and at day 0 and Day 10 of the intervention. Serum CRP concentrations >10 mg/L are often due to acute conditions and individuals with values above that should be excluded (Pearson et al. 2003). Based on the conversion formula suggested by Ouellet-Morin et al. (2011), we chose a salivary CRP cut-off, a priori, of 7 µg/L.

All saliva samples were collected using the SalivaBio Oral Swab (Salimetrics, LLC., State College, PA) according to manufacturer's instructions. Participants were asked to avoid eating, smoking, or drinking for 1 hour prior to sample collection, consuming alcohol for 12 hours prior, and to refrain from strenuous activity the day of sampling. CRP concentrations were assessed using a high sensitivity enzyme-linked immunosorbent assay (ELISA) (Salimetrics, LLC., State College, PA). Average intra- and inter- assay variability was 3.4% and 9.1%, respectively.

Statistical Analysis. Statistical analyses were performed using IBM SPSS Statistics (version 22.0; IBM Corp., Armonk, N.Y., USA). Salivary CRP concentrations did not meet the

criteria of a normal distribution so logarithmic transformations were applied prior to analyses; actual concentrations are presented. Preliminary comparisons between groups were performed using independent sample t-tests (two-tailed). Difference in CRP from the preliminary assessment to day 0 of the intervention were examined using a paired samples t-test. Difference in CRP between groups and across time during the intervention were examined using a two-way repeated measures ANOVA (group x time). Statistical significance was set at $p < 0.05$. All values are reported as mean $\pm$ SD.

RESULTS

Twenty women began the study; one participant in the sedentary group was injured during the intervention and was omitted from analyses. In addition, preliminary CRP samples from 2 participants were not collected due to non-compliance with sampling guidelines. Table 1 shows the baseline data.

Change in Movement Behaviour. During the intervention, all 19 participants wore the accelerometer for 10 days, with an average wear time of 15.2 ± 1.2 hours per day. The sedentary group significantly decreased their daily step count from $9,392 \pm 702$ to $3,854 \pm 147$ ($p < 0.001$) and the active group increased from $8,772 \pm 427$ to $12,815 \pm 454$ steps per day ($p < 0.001$). The sedentary group increased their sedentary time by 69.8 minutes per day and significantly decreased both their LPA and MVPA ($p < 0.001$). The active group added 19 minutes of MVPA per day ($p < 0.001$) and sedentary time did not change significantly. During the intervention, the sedentary group spent a greater proportion of waking time sedentary than the active group (85% versus 72%, respectively) ($p < 0.001$). Detailed activity profiles for each group can be seen in Supplemental Table 2.

C-reactive protein. From the end of the preliminary assessment period to the start of the intervention (range of 9-21 days, N=17), CRP did not change significantly (0.320 ± 0.571 to $0.297 \pm 0.435 \mu\text{g} \cdot \text{L}^{-1}$, $p > 0.05$). During the intervention (N=19) there was a significant crossover interaction ($p < 0.001$) showing that the two groups responded differently over time. In the sedentary group CRP increased in seven of nine individuals with an average 31% increase (0.378 ± 0.596 to $0.487 \pm 0.793 \mu\text{g} \cdot \text{L}^{-1}$) and in the active group CRP decreased in all 10 participants with an average 22% decrease (0.414 ± 0.640 to $0.259 \pm 0.284 \mu\text{g} \cdot \text{L}^{-1}$). The individual relative change and individual absolute concentration change can be seen in Supplemental Figure 2.

DISCUSSION

The present study adds experimental evidence to the increasing number of cross-sectional studies that have shown an association between movement behaviour and CRP (Carson et al. 2014; Healy et al. 2011). Limited experimental studies have been conducted, most using serum CRP and clinical populations. Breen et al. (2013) showed a 12% increase in serum CRP after 2 weeks of reduced activity in elderly individuals. The only other study to use salivary CRP to examine activity effects reported a 54% decrease in CRP after 8 weeks of exercise in male cardiac patients (Jamshidpour et al. 2013). This is the first study to show that salivary CRP is sensitive to changes in daily movement behaviour in healthy women.

Quantifying CRP, as well as other biochemicals, via saliva is a useful advancement in bioanalysis techniques for researchers who are often constrained by budget, personnel, and willingness of volunteer participants. Like blood, saliva is a dynamic and complex fluid that contains an array of enzymes, hormones, antibodies, and cytokines, that can reflect the physiologic state of the body (Aps and Martens 2005). CRP likely enters the oral cavity via the

gingival crevicular fluid, which can be either a transudate or inflammatory exudate of serum, that mixes to become a constituent of whole saliva (Lamster and Ahlo 2007).

The mechanisms by which movement influences CRP are unclear. (Hamilton et al. 2004). Previous studies have suggested that physical activity reduces inflammation by decreasing body fat (Oberbach et al. 2006), increasing insulin sensitivity (Oberbach et al. 2006), improving endothelial function (Taddei et al. 2000), or by the acute release of anti-inflammatory cytokines with each bout of exercise (Gleeson et al. 2011). The increase in CRP in the sedentary group could also be due to a number of factors, such as inhibition of anti-inflammatory myokines due to reduced muscular contractions (Henson et al. 2013). The sedentary group significantly increased sedentary time, but also decreased their MVPA. Therefore, it is possible that the removal of daily MVPA, rather than the increased sedentary time, caused the increase in CRP; it is also plausible that both changes contributed.

This exploratory study does have limitations. The sample size is small, and we did not control for menstrual status. Also, dietary records were not used and it is presumed that participants adhered to instructions about diet and medication use during the intervention. Due to matching and randomization procedures, the time between the end of preliminary assessment and start of the intervention varied across participants (9-21 days) and this limits the interpretation of the CRP concentrations between these two samples.

In conclusion, 10 days of altered movement behaviour resulted in significant changes in salivary CRP, consistent with cross-sectional evidence of associations between sedentary time and serum CRP. Inflammation may be a potential link between movement behaviour and health outcomes in healthy middle-aged women. The use of salivary measures of CRP and other

inflammatory markers should be considered for future investigations as this may allow
researchers to explore and develop this field more quickly.

Conflict of interest statement

The authors have no conflicts of interest to declare.

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References

- Aps, J.K.M., and Martens, L.C. 2005. Review: The physiology of saliva and transfer of drugs into saliva. *Forensic Sci. Int.* **150**(2-3): 119-131. doi:10.1016/j.forsciint.2004.10.026
- Beavers, K.M., Brinkley, T.E., and Nicklas, B.J. 2010. Effect of exercise training on chronic inflammation. *Clin. Chim. Acta.* **411**(11-12): 785-793. doi:10.1016/j.cca.2010.02.069
- Breen, L., Stokes, K.A., Churchward-Venne, T.A., Moore, D.R., Baker, S.K., Smith, K. et al. 2013. Two weeks of reduced activity decreases leg lean mass and induces "anabolic resistance" of myofibrillar protein synthesis in healthy elderly. *J. Clin. Endocrinol. Metab.* **98**(6): 2604-2612. doi:10.1210/jc.2013-1502
- Carson, V., Wong, S.L., Winkler, E., Healy, G.N., Colley, R.C., and Tremblay, M.S. 2014. Patterns of sedentary time and cardiometabolic risk among canadian adults. *Prev. Med.* **65**: 23-27. doi:10.1016/j.ypmed.2014.04.005
- Choi, L., Liu, Z., Matthews, C.E., and Buchowski, M.S. 2011. Validation of accelerometer wear and nonwear time classification algorithm. *Med. Sci. Sports Exerc.* **43**(2): 357-364. doi:10.1249/MSS.0b013e3181ed61a3
- Du Clos, T.W. 2000. Function of c-reactive protein. *Ann. Med.* **32**(4): 274-278. doi:10.3109/07853890009011772
- Foley, J.D., Sneed, J.D., Steinhubl, S.R., Kolasa, J., Ebersole, J.L., Lin, Y.S. et al. 2012. Oral fluids that detect cardiovascular disease biomarkers. *Oral Surgery Oral Medicine Oral Pathology Oral Radiology.* **114**(2): 207-214. doi:10.1016/j.oooo.2012.03.003

- 199 Freedson, P.S., Melanson, E., and Sirard, J. 1998. Calibration of the computer science and
200 applications, inc. Accelerometer. *Med. Sci. Sports Exerc.* **30**(5): 777-781.
201 doi:10.1097/00005768-199805000-00021
- 202 Gleeson, M., Bishop, N.C., Stensel, D.J., Lindley, M.R., Mastana, S.S., and Nimmo, M.A. 2011.
203 The anti-inflammatory effects of exercise: Mechanisms and implications for the
204 prevention and treatment of disease. *Nat. Rev. Immunol.* **11**(9): 607-615.
205 doi:doi:10.1038/nri3041
- 206 Hamilton, M.T., Hamilton, D.G., and Zderic, T.W. 2004. Exercise physiology versus inactivity
207 physiology: An essential concept for understanding lipoprotein lipase regulation. *Exerc.*
208 *Sport Sci. Rev.* **32**(4): 161-166.
- 209 Healy, G.N., Matthews, C.E., Dunstan, D.W., Winkler, E.A., and Owen, N. 2011. Sedentary time
210 and cardio-metabolic biomarkers in us adults: Nhanes 2003-06. *Eur. Heart J.* **32**(5): 590-
211 597. doi:10.1093/eurheartj/ehq451
- 212 Henson, J., Yates, T., Edwardson, C.L., Khunti, K., Talbot, D., Gray, L.J. et al. 2013. Sedentary
213 time and markers of chronic low-grade inflammation in a high risk population. *PLoS*
214 *One.* **8**(10): e78350. doi:10.1371/journal.pone.0078350
- 215 Jamshidpour, B., Moghadam, B.A., Vasaghi-Gharamaleki, B., Mirzaii-Dizgah, I., and Nejatian,
216 M. 2013. The effects of phase iii cardiac rehabilitation in serum and salivary hs-crp and
217 anthropometric measurements in patients with coronary artery disease. *J. Contemp. Dent.*
218 *Pract.* **14**(5): 819-824.

- 219 Labat, C., Temmar, M., Nagy, E., Bean, K., Brink, C., Benetos, A. et al. 2013. Inflammatory
220 mediators in saliva associated with arterial stiffness and subclinical atherosclerosis. *J.*
221 *Hypertens.* **31**(11): 2251-2258. doi:10.1097/HJH.0b013e328363dccc
- 222 Lamster, I.B., and Ahlo, J.K. 2007. Analysis of gingival crevicular fluid as applied to the
223 diagnosis of oral and systemic diseases. *Ann. N. Y. Acad. Sci.* **1098**: 216-229.
224 doi:10.1196/annals.1384.027
- 225 Malathi, N., Mythili, S., and Vasanthi, H.R. 2014. Salivary diagnostics: A brief review. *ISRN*
226 *Dentistry.* **2014**: 1-8.
- 227 Oberbach, A., Tonjes, A., Kloting, N., Fasshauer, M., Kratzsch, J., Busse, M.W. et al. 2006.
228 Effect of a 4 week physical training program on plasma concentrations of inflammatory
229 markers in patients with abnormal glucose tolerance. *Eur. J. Endocrinol.* **154**(4): 577-585.
230 doi:10.1530/eje.1.02127
- 231 Ouellet-Morin, I., Danese, A., Williams, B., and Arseneault, L. 2011. Validation of a high-
232 sensitivity assay for c-reactive protein in human saliva. *Brain Behav. Immun.* **25**(4): 640-
233 646. doi:10.1016/j.bbi.2010.12.020
- 234 Patel, N., Belcher, J., Thorpe, G., Forsyth, N.R., and Spiteri, M.A. 2015. Measurement of c-
235 reactive protein, procalcitonin and neutrophil elastase in saliva of copd patients and
236 healthy controls: Correlation to self-reported wellbeing parameters. *Respir. Res.* **16**.
237 doi:10.1186/s12931-015-0219-1
- 238 Pearson, T.A., Mensah, G.A., Alexander, R.W., Anderson, J.L., Cannon, R.O., Criqui, M. et al.
239 2003. Markers of inflammation and cardiovascular disease application to clinical and

- 240 public health practice: A statement for healthcare professionals from the centers for
241 disease control and prevention and the american heart association. *Circulation*. **107**(3):
242 499-511. doi:10.1161/01.cir.0000052939.59093.45
- 243 Plant, M.J., Williams, A.L., O'Sullivan, M.M., Lewis, P.A., Coles, E.C., and Jessop, J.D. 2000.
244 Relationship between time-integrated c-reactive protein levels and radiologic progression
245 in patients with rheumatoid arthritis. *Arthritis Rheumatol*. **43**(7): 1473-1477.
246 doi:10.1002/1529-0131(200007)43:7<1473::aid-anr9>3.0.co;2-n
- 247 Ridker, P.M., Hennekens, C.H., Buring, J.E., and Rifai, N. 2000. C-reactive protein and other
248 markers of inflammation in the prediction of cardiovascular disease in women. *N. Engl.*
249 *J. Med.* **342**(12): 836-843. doi:10.1056/NEJM200003233421202
- 250 Taddei, S., Galetta, F., Viridis, A., Ghiadoni, L., Salvetti, G., Franzoni, F. et al. 2000. Physical
251 activity prevents age-related impairment in nitric oxide availability in elderly athletes.
252 *Circulation*. **101**(25): 2896-2901.
- 253 Tudor-Locke, C., Craig, C.L., Thyfault, J.P., and Spence, J.C. 2013. A step-defined sedentary
254 lifestyle index: <5000 steps/day. *Appl. Physiol. Nutr. Metab.* **38**(2): 100-114.
255 doi:10.1139/apnm-2012-0235
- 256 Tudor-Locke, C., Hatano, Y., Pangrazi, R.P., and Kang, M. 2008. Revisiting "how many steps
257 are enough?". *Med. Sci. Sports. Exerc.* **40**(7 Suppl): S537-543.
258 doi:10.1249/MSS.0b013e31817c7133
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Table 1. Anthropometric, physiological, and behavioral characteristics of sedentary and active groups during the preliminary assessment.

	Sedentary (n=9)	Active (n=10)	P
Age (yr)	49.6 ±5.6	49.9 ±5.2	0.891
Height (m)	1.64 ±5.3	1.66 ±5.2	0.413
Weight (kg)	76.5 ±7.7	73.6 ±10.5	0.518
BMI (kg·m ⁻²)	28.4 ±3.5	26.6 ±3.7	0.296
WC (cm)	90.7 ±10.4	87.0 ±8.3	0.399
CRP (µg/L)	0.45 ±0.78	0.53 ±1.04	0.857
Steps (steps/day)	9392 ±2106	8772 ±1350	0.451

All values are presented as mean ±SD