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Impact on hemostatic parameters of interrupting sitting with intermittent activity

Short title: Interrupting sitting and hemostasis

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ABBREVIATIONS

aPTT – activated partial thromboplastin time

Hb – hemoglobin

Hct – hematocrit

MCV – mean cell volume

MPV – mean platelet volume

PC – platelet count

PT – prothrombin time

RBC – red-cell count

vWF – von Willebrand factor

WBC – white-cell count

SUMMARY

Excessive sitting has been associated with an elevated risk of vascular conditions, particularly venous thrombosis. Interrupting sitting time with intermittent physical activity can reduce venous stasis; however, impacts on other aspects of thrombogenesis are less understood. **Purpose:** To examine the effects of interrupting sitting time on blood coagulation and blood volume parameters in sedentary, middle-aged, overweight/obese adults (11 men/8 women; 53.8 ± 4.9yrs, BMI: 31.2 ± 4.1 $kg.m^{-2}$). **Methods:** The randomized three-period, three-treatment acute cross-over trial consisted of: uninterrupted sitting; sitting interrupted by 2 min bouts of either light- or moderate-intensity treadmill walking every 20min. In each trial-condition, blood samples were collected at baseline prior to the consumption of a standardized meal (-2hrs) and post-intervention (5hrs). **Results:** Plasma fibrinogen increased from baseline with uninterrupted sitting (0.24$g.l^{-1}$, [0.13—0.34], $P<0.001$). Light-intensity but not moderate-intensity activity breaks attenuated the increase by 0.17$g.l^{-1}$ ([0.01—0.32], $P<0.05$). There were no between-condition differences in prothrombin time, activated partial thromboplastin time, von Willebrand factor, D-dimer or platelet count. Uninterrupted sitting reduced plasma volume and increased hematocrit, hemoglobin and red-cell count; effects attenuated by both light- and moderate-intensity breaks ($P<0.05$). White-cell count increased with uninterrupted sitting and further increased with moderate-intensity breaks. Mean platelet volume increased with moderate, but not light-intensity breaks or uninterrupted sitting. **Conclusion:** Uninterrupted sitting increased fibrinogen and reduced plasma volume, with associated increases in hemoglobin and hematocrit. Activity breaks attenuated these responses, indicative of an ameliorating influence on the pro-coagulant effects of uninterrupted sitting.

Keywords: fibrinogen, blood volume, sedentary behavior, physical activity, venous thrombosis.

Introduction

Paragraph Number 1 Sedentary behaviors – characterized by posture (sitting or reclining) and low levels of metabolic energy expenditure – occupy the major portion of adults' waking hours, primarily through sitting at work, television (TV) viewing and other screen-based entertainment, and time spent sitting in cars (4). Several recent studies have reported detrimental associations of sedentary behaviors (most commonly TV viewing) with all-cause and cardiovascular mortality, even when accounting for leisure-time physical activity (32). Furthermore, recent epidemiological evidence has suggested that adults whose sedentary time is mostly uninterrupted (prolonged unbroken sitting) have a poorer cardio-metabolic health profile compared to those characterized as having more frequent interruptions or breaks in their sedentary time (9, 10). This has been corroborated by experimental findings from our laboratory indicating that the inclusion of regular light-intensity or moderate-intensity ambulatory breaks reduced postprandial glucose and insulin in sedentary overweight/obese adults to a similar extent, relative to uninterrupted sitting (5).

Paragraph Number 2 From the perspective of thrombotic risk, advocating interruptions in sitting time is not an entirely novel approach. In the 1950's, frequent breaks (getting up and moving around) in sitting time were recommended to reduce the risk of venous thrombosis (VT) (13, 22). These recommendations were subsequently supported by evidence indicating that frequent ambulatory breaks (2min every 30min) throughout a work day are sufficient to avoid the loss of plasma volume induced by uninterrupted sitting (36), yet the optimal intensity of the ambulation is unknown. It is possible that light-intensity activity would be sufficient to reduce the risk of VT, since active foot movements have been reported to reduce sitting induced venous stasis (11, 20). However, these findings relate to just one component (hemodynamic imbalance) of Virchow's (35) triad that characterizes the pathogenesis of VT. Evidence is also required on the effects of breaking up sitting time with activity of varied intensity on the other two components– hyper-coagulation and endothelial dysfunction, and their possible interactions (15).

Paragraph Number 3 Prolonged sitting is widely believed to induce a pro-coagulatory state, yet the relevant evidence remains inconsistent. Most studies have focused on the elevated risk of VT during air travel (25-27), and thereby have typically evaluated the combination of hypoxia (12, 34, 37) and not specifically the influence of reduced skeletal muscle contractile activity induced through sitting. Two cross-over studies included everyday-activity conditions (27, 31), however, in both studies direct comparisons between active and sitting conditions could not be made, since the frequency, volume and intensity of activity during each condition was not rigorously monitored. Furthermore, the studies that have permitted a specified volume of activity or standing during their interventions did not report on the total sitting time or frequency and volume of interruptions (25, 34).

Paragraph Number 4 In order to better understand the mechanisms that may lead to the increased thrombotic risk of excessive sitting it is necessary to control the relevant activity parameters: frequency, volume, type and intensity of activity breaks. It is also pertinent to study these mechanisms in an at risk population. The majority of previous studies that have investigated the effects of excessive sitting on haemostatic parameters have been limited to younger (< 40yrs) healthy, non-obese populations (12, 30, 34, 37). Examining responses among those at a greater risk of thrombotic events – specifically older and overweight obese populations – may provide greater insights into the mechanisms underlying the adverse hemostatic consequences of too much sitting.

In a randomized, cross-over trial involving sedentary overweight older adults (45-65yrs), we examined the acute effects of a single period of uninterrupted sitting on hemostatic and hematological parameters, compared to sitting interrupted by intermittent bouts of either light-intensity or moderate-intensity walking. We hypothesized that uninterrupted sitting would increase blood coagulation parameters and reduce blood volume and, that brief intermittent bouts of either light- or moderate-intensity walking would attenuate such effects.

Methods

Paragraph Number 5 Descriptions of the participant characteristics and the screening and testing procedures for this study have been described in detail previously (5). Briefly, 19 overweight or obese (BMI > 25 kg.m^{-2}) adults (11 men and 8 women) were recruited from the general community between April 2009 and August 2010 into a randomized, three-period, three treatment crossover trial involving a single bout of sitting with and without intermittent bouts of light- or moderate-intensity activity. Exclusion criteria consisted of: employment in a non-sedentary occupation, low daily TV viewing (<2hrs/day); regularly engaged in the recommended amount of physical activity (≥150min/week); BMI > 45 kg.m^{-2}, current smoker, pregnant, non-English speaking; on anticoagulant, carbohydrate or lipid-lowering medication; clinically diagnosed acute/chronic illness or known physical activity contraindications. The study was approved by the Alfred Hospital and Deakin University Human Ethics committees, and was carried out in accordance with the Declaration of Helsinki. Participants provided signed, written informed consent. The study was registered as a clinical trial with the Australian New Zealand Clinical Trials Registry (ACTRN12609000656235).

Study Protocol

Paragraph Number 6 Participants were provided with written and verbal instructions to refrain from moderate-to-vigorous intensity exercise, alcohol and caffeine in the 48 hours prior to each condition. During this time, physical activity and sedentary time was objectively measured using an Actigraph GT1M accelerometer (Actigraph, Pensacola, FL) in combination with activity and food diary records. A minimum wash-out period of 6 days was employed between each condition to eliminate potential carry-over effects.

Paragraph Number 7 Participants reported to the laboratory setting for each condition between 0700 and 0800 hours after an overnight fast (≥ 8hrs), to control for circadian variance. Body mass was assessed prior to the commencement of each trial day. In each of the three conditions, sodium citrate and EDTA blood samples were collected via an indwelling venous catheter from the antecubital vein of the forearm at baseline (pre = -2hrs) and on completion (post = 5hrs), 18 minutes after the last activity bout. Following initial blood collection, participants remained seated for two hours prior to the consumption of a standardized test drink (Time = 0hrs), consisting of 75g carbohydrate (100% corn maltodextrin powder; Natural Health, Australia) and 50g fat (Calogen; Nutricia, Australia) to assess responses in a postprandial state (5). Throughout each condition participants were permitted to consume water, with consumption kept constant across the trial. Under supervision, participants complied with the respective trial-condition protocols for the remaining five hours.

Paragraph Number 8 As described previously (5), each participant performed the three trial conditions in a randomized order, determined via balanced blocks prepared for male and female participants.1) Uninterrupted sitting: participants remained seated. 2) Sitting interrupted by light-intensity activity breaks: participants rose from the seated position every 20 minutes throughout the intervention period (Time: 0-5hrs), and completed a 2 minute bout of light-intensity treadmill walking at 3.2 $km.hr^{-1}$. Upon completion of each bout they returned to the seated position. 3) Sitting interrupted by moderate-intensity activity breaks: identical procedure to condition 2, however the activity bouts were of a moderate-intensity corresponding with a treadmill speed between 5.8 and 6.4$km.hr^{-1}$ that yielded a Borg scale rating of perceived exertion (RPE) of 12 to 14. In all three conditions participants were seated in a lounge chair and instructed to minimize excessive movement, only rising to void, with the exception of activity breaks. The pathology technicians and team statistician were blinded to the trial condition.

Plasma preparation and analytical methods

Paragraph Number 9 Sodium citrate blood samples were analyzed by Alfred Pathology on the day of testing using an automated coagulation analyser (DiagnosticaStago STA (R)) for activated partial thromboplastin time (aPTT), prothrombin time (PT) via international normalized ratio (INR) and fibrinogen via the Clauss (2) method with their respective reagents: aPTT s (Trinity Biotech TriniCLOT), neoplastine C1 plus and fibrinogen (5) reagent (DiagnosticaStago STA (R)). Hematocrit (Hct), hemoglobin (Hb), red-cell count (RBC), white-cell count (WBC), mean platelet volume (MPV) and platelet count (PC) were measured with a Beckman Coulter LH 785 analyzer using standard methods. The intra-assay coefficient of variation for these analyses ranged from 2% to 4.4%. Plasma volume was calculated using Dill & Costill’s (3) method.

Paragraph Number 10 EDTA blood samples were kept on ice and centrifuged for 15 minutes at 13,000rpm at 4°C, the plasma removed and stored at -80°C. Stored samples were analyzed in duplicate on site (Baker IDI) via ELISA for von Willebrand factor (vWF) antigen (Imubind; American Diagnostica, Stamford, CT, USA) and D-dimer (Technozym; Technolone, Vienna, Austria), with intra-assay coefficient of variations ≤10%. Plasma fibrinogen, vWF, D-dimer and WBC were corrected for plasma volume.

Statistical analyses

Paragraph Number 11 Generalized estimating equations (GEE models) with exchangeable working correlation matrix to account for the dependency in the data (repeated measures) were used to evaluate the differential effects of the trial conditions on the outcomes (21, 28). The GEE models enabled the examination of between-condition differences in all variables and adjustment for potentially important covariates (age, gender, body mass), baseline (-2hrs) outcome values and order effects (28). Where >5 missing samples were present, GEE models were replaced with multilevel generalized latent and mixed models (GLAMM), to ensure missing samples were not treated as missing completely at random (29). Carry-over effects were not formally tested, given the minimum six day wash-out period between conditions and the fact that interventions were performed in a randomized order. A probability level of 0.05 was adopted. All statistical analyses were performed using Stata 12 for Windows (StataCorp LP). Data are reported as mean [95% CI] in the text and table, and as estimated marginal means ± SEM in the figures.

Paragraph Number 12 All hemostatic parameters were available for the 19 participants. Data for Hct, Hb, RBC, WBC, PC and MPV were missing for one participant in the moderate condition, with another participant missing data for PC in the uninterrupted sitting condition. For MPV there was a total of nine missing data, with data unavailable in all conditions for two participants, and an additional two missing from the moderate condition and one from the light-intensity condition. Data were missing either due to unsuitable (sample clotting or outside of measurable range) or incomplete analysis. Where plasma volume correction was unavailable the unadjusted values for all conditions and parameters were used.

Results

Paragraph Number 13 The unadjusted means at baseline and at the completion of each trial-condition along with the adjusted (age, sex, body mass, baseline outcome values and order effects) net differences (post minus baseline) for each variable and condition are presented in the Table. There were no differences at baseline between conditions for any of the parameters with the exception of WBC which was $0.43^{10^{\wedge}9}.l^{-1}$ ([0.02, 0.85], P=0.042) lower at baseline in the moderate-intensity activity condition compared with uninterrupted sitting, but did not differ between the remaining conditions. As previously reported, there were no differences between trials for dietary and accelerometer-derived physical activity data prior to each of the respective conditions (5).

INSERT TABLE ABOUT HERE

Hemostatic parameters

Paragraph Number 14 Plasma fibrinogen increased from baseline after consumption of the test drink with uninterrupted sitting and sitting interrupted with moderate-intensity activity; but not in the sitting interrupted with light-intensity activity condition (Table). Following adjustment for potential confounders, the change in plasma fibrinogen was $0.17g.l^{-1}$ ([0.01, 0.32], $P<0.05$) less after sitting interrupted by light-intensity activity compared to uninterrupted sitting (Fig. 1). There were no significant differences between the remaining conditions for plasma fibrinogen.

INSERT FIGURE 1 ABOUT HERE

Paragraph Number 15 PT decreased significantly in all three conditions from baseline, whilst aPTT decreased significantly in the sitting interrupted with moderate-intensity activity condition only (Table). vWF increased significantly in the sitting interrupted with moderate-intensity activity condition only. No significant changes were observed for D-dimer in any of the conditions. There were no significant between-condition net differences for PT, aPTT, vWF or D-dimer.

Hematological parameters

Paragraph Number 16 Plasma volume decreased significantly across all three conditions (Table), to a lesser extent with the interrupted sitting conditions compared to uninterrupted sitting (Fig. 2). Hct, Hb, RBC, WBC and PC increased significantly from baseline in all three conditions (Table). Compared with uninterrupted sitting the net difference in Hct, Hb and RBC was less in the two activity conditions, whereas WBC increased more in the sitting interrupted with moderate-intensity activity condition only (Fig. 2). MPV did not change with uninterrupted sitting or sitting with light-intensity activity, but increased significantly after the sitting with moderate-intensity activity condition (Table), and was significantly higher in this condition relative to uninterrupted sitting (Fig. 2). There were no between-condition differences in PC.

INSERT FIGURE 2 ABOUT HERE

Discussion

Paragraph Number 17 These are the first findings on the hemostatic effects of interrupting sitting time with intermittent short walking breaks. In support of our hypothesis, activity breaks attenuated increases seen in plasma fibrinogen (light-intensity only), Hct, Hb and RBC and the reduction in plasma volume with uninterrupted sitting in sedentary obese/overweight adults. In the context of VT and broader aspects of cardio-metabolic health, this may be a potential beneficial outcome, since these parameters – particularly fibrinogen – have been shown to be highly correlated with atherogenesis, thrombosis and ischemia (16). In contrast, WBC and MPV were higher in the moderate-intensity condition compared to uninterrupted sitting, indicating a possible acute inflammatory effect of the higher intensity activity breaks.

Paragraph Number 18 Uninterrupted sitting is thought to increase thrombotic risk through multiple mechanisms, but predominantly via venous stasis (11). In the current study and earlier studies (12, 36), this is characterized by increases in key blood viscosity parameters that influence blood flow including plasma fibrinogen, Hct, Hb, RBC and reduced plasma volume (6). Our observation of an elevation in plasma fibrinogen (irrespective of correction for plasma volume changes) also supports the hypothesis for hyper-coagulation, since fibrinogen is integral to both primary and secondary clot formation (17). However, given there were no between-condition differences in the remaining hemostatic parameters it is difficult to determine the relative role of venous stasis versus direct effects on coagulation signaling cascades. Based on previous research (25, 30, 31, 34, 37) it is probable that in the acute context, changes in blood volume that increase blood viscosity and decrease blood flow are more likely to underlie the increased risk of thrombosis associated with uninterrupted or excessive sitting.

Paragraph Number 19 Further to the previously-reported benefits of breaks in sitting time for glucose (5) we observed attenuations in blood volume parameters and (for the first time) plasma fibrinogen following sitting interrupted with light-intensity activity. Although the average difference in plasma fibrinogen for the light-intensity activity condition compared to uninterrupted sitting was small (0.17g.l^{-1} or 6.5%), considerable variations were observed at the individual level with a 1.0g.l^{-1} (39%) difference observed for one participant and ≥0.40 g.l^{-1} (17-38%) for a further seven participants. The magnitude of such changes observed with light-intensity activity breaks in these individuals, exceeds the 10-15% reduction in fibrinogen reported following longer-term pharmaceutical intervention with certain fibrate drugs (18) and may therefore have potential relevance for disease states, particularly if future investigations can demonstrate that these findings persist over accumulated bouts across several days. It is possible that with a larger sample size, a significant difference in fibrinogen for moderate-intensity may have also been observed. However, it is also possible that the higher intensity of the moderate-activity condition may have induced an acute inflammatory response including fibrinogen release, as part of the acute-phase reaction (19). The higher

WBC and MPV in the moderate condition relative to uninterrupted sitting are consistent with this possibility (1).

Paragraph Number 20 Findings from acute exercise studies may be of relevance to interpreting the current findings. For instance, five- to 20-fold increases in WBC and fibrinogen (23) and greater MPV (33) have been reported following high-intensity exercise and are consistent with an acute inflammatory response (1). However, chronic exercise training can lead to a reduction in these parameters (7). Therefore, despite moderate-intensity activity interruptions in sitting time not appearing to be as beneficial as light-intensity activity interruptions in the acute context, if accumulated across consecutive days, it is possible that a reduction may be observed. Irrespective of the findings for moderate-intensity activity, from a practical perspective, the changes observed in the light-intensity activity condition may provide a feasible and acceptable approach to reducing and interrupting overall sitting time.

Paragraph Number 21 A major strength of the current study is the strict monitoring of the activity parameters within a rigorously controlled laboratory setting, allowing for the comparison of the effects of uninterrupted sitting with interrupted sitting. In addition, we were able to assess the integrated effects of uninterrupted sitting and the test meal, an important consideration since the postprandial state predominates during waking hours. In previous studies that have assessed postprandial hemostatic responses to various macronutrient composition meals, fibrinogen has not been altered (8, 14, 24, 38). However, most if not all studies that have evaluated post-prandial states have not controlled for physical activity. As such, it is not possible to reach conclusions about whether the meal provided in the current study amplified the response to uninterrupted sitting or had no effect.

Paragraph Number 22 There are some limitations to our study. First, we examined only an acute exposure to uninterrupted sitting in a single day with interruptions of a fixed frequency and duration, with two different activity-break intensities. To better understand implications for long term cardiovascular risk, it will be important to understand the changes induced in hemostatic risk factors after repeated long-term exposure to sitting, as well as the dose-response relationships associated with different frequencies, durations and/or intensities of activity-break conditions. Additionally, interpretation of the current findings is limited to sedentary middle aged overweight/obese adults who are at greater hemostatic risk than younger, active, non-obese individuals. It will be important to study larger and more heterogeneous populations so that the influence of factors such as age, sex, fitness and body composition can also be examined. In addition, investigating the responses in individuals with clinically impaired vascular or hemostatic functioning (e.g., diabetics or patients with peripheral or coronary artery disease) may provide further insights.

Conclusions

Paragraph Number 23 In summary, compared to uninterrupted sitting, we observed less increase in plasma fibrinogen and blood volume parameters (Hct, Hb and RBC) that influence blood viscosity and less reduction in plasma volume with the introduction of intermittent activity (walking) bouts during sitting. These findings suggest that in the acute setting, changes in both fibrinogen and blood viscosity may contribute to the well-documented increased risk of thrombosis associated with excessive sitting. However, future research using larger sample sizes and a more extended exposure should be directed at elucidating potential mechanisms, in addition to determining the optimal timing, intensity and type of activity undertaken to interrupt sitting time. Assuming that these findings can be replicated, they further support the case that reducing and frequently breaking up sedentary time may have important health benefits. In addition to the widely-promoted guidelines on physical activity participation, public health recommendations should also be directed at efforts to interrupt sitting time.

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

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FIGURE LEGENDS

Figure 1: The effect of the 3 trial conditions on Δ plasma fibrinogen corrected for plasma volume. Data represents marginal means ± SEM (adjusted for age, sex, body mass, baseline outcome values and order effects). *significantly different from uninterrupted sitting condition, $P<0.05$.

Figure 2: The effect of the 3 trial conditions on Δ haematological variables: (A) haematocrit, (B) haemoglobin, (C) red-cell count (D) plasma volume, (E) white-cell count corrected for plasma volume and (F) mean platelet volume. Data represents marginal means ± SEM (adjusted for age, sex, body mass, baseline outcome values and order effects). *significantly different from uninterrupted sitting condition, $P<0.05$; †significantly different from uninterrupted sitting condition, $P<0.01$; and ‡significantly different from uninterrupted sitting condition, $P<0.001$.

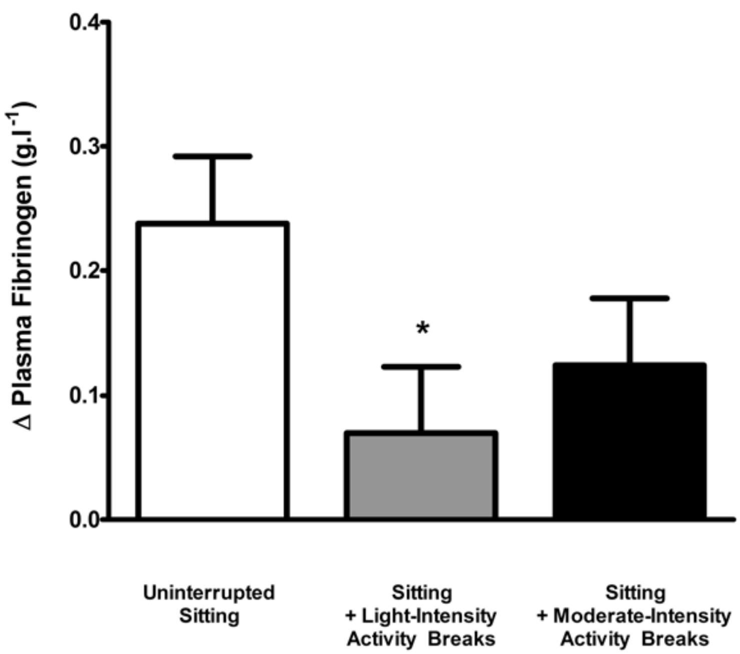
Table - Within-condition responses in haemostatic and haematological parameters

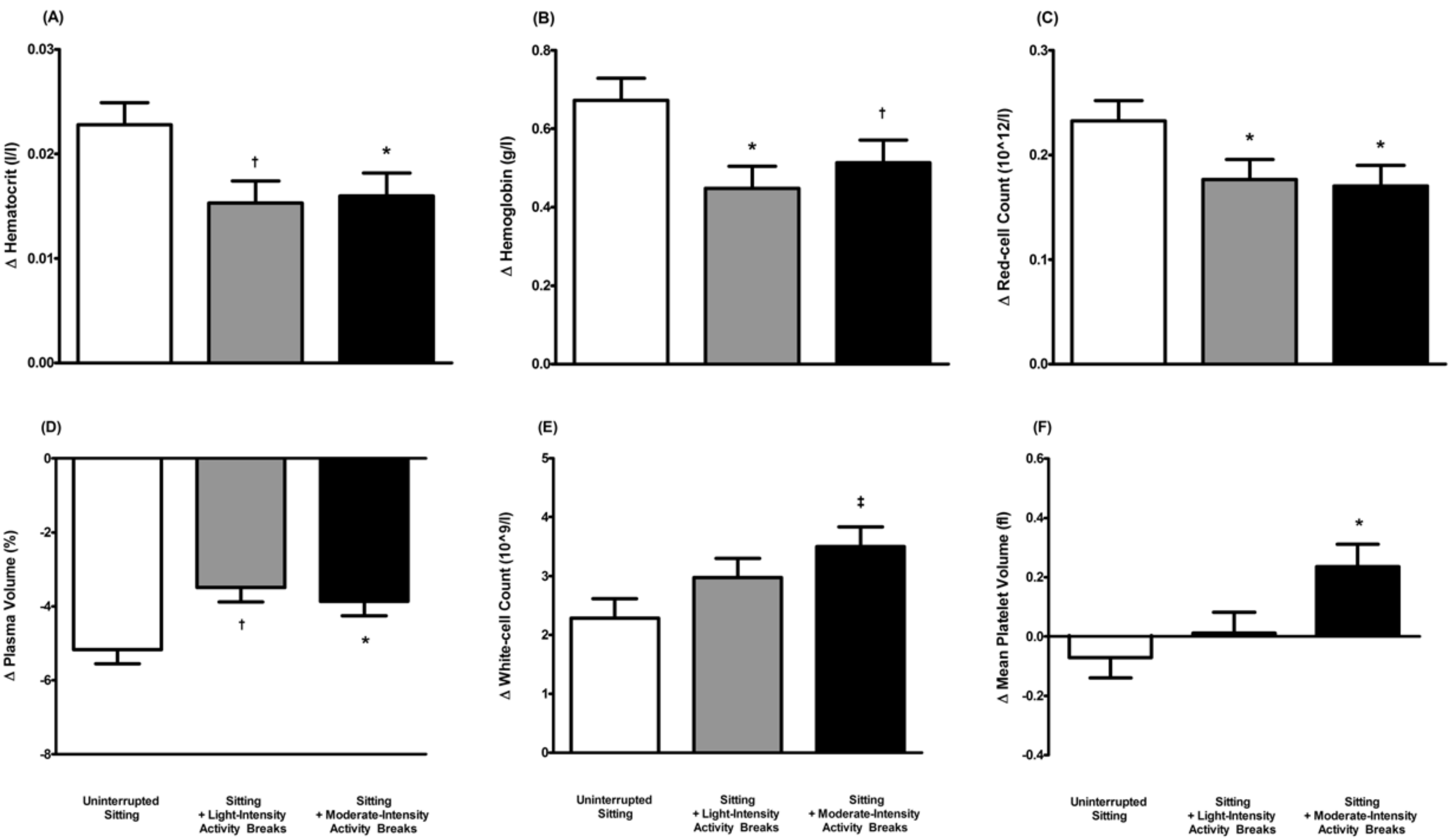
	Time	Uninterrupted Sitting		Sitting Interrupted by Light-Intensity Activity		Sitting Interrupted by Moderate-Intensity Activity	
Fibrinogen ($g.L^{-1}$)	Pre	3.11	(2.84, 3.37)	3.24	(3.00, 3.49)	3.20	(2.93, 3.47)
	Post §	3.37	(3.15, 3.61)	3.29	(3.00, 3.58)	3.31	(3.02, 3.61)
	Net Difference	0.24	(0.13, 0.34)‡	0.07	(-0.04, 0.18)	0.12	(-0.02, 0.23)*
vWF ($m\mu.mL^{-1}$)	Pre	360	(227, 492)	341	(221, 461)	354	(252, 457)
	Post §	400	(280, 518)	425	(298, 551)	488	(337, 640)
	Net Difference	11	(-114, 136)	15	(-108, 139)	135	(11, 259)*
D-dimer ($ng.mL^{-1}$)	Pre	17.6	(3.1, 32.1)	30.5	(4.2, 56.8)	27.8	(7.0, 48.7
	Post §	29.5	(15.8, 43.1)	42.1	(5.3, 78.9)	40.6	(19.2, 61.9)
	Net Difference	11.4	(-3.4, 26.2)	12.57	(-2.0, 27.2)	12.29	(-2.3, 26.9)
PT (s)	Pre	13.5	(13.2, 13.8)	13.6	(13.2, 13.9)	13.5	(13.3, 13.8)
	Post	13.4	(13.1, 13.6)	13.4	(13.1, 13.7)	13.3	(13.1, 13.6)
	Net Difference	-0.15	(-0.26, -0.03)*	-0.14	(-0.25, 0.02)*	-0.21	(-0.33, -0.09)‡

aPTT (s)	Pre	30.9	(30.0, 31.7)	30.7	(29.7, 31.7)	30.8	(29.9, 31.8)
	Post	30.4	(29.40, 31.4)	30.4	(29.3, 31.5)	30.2	(29.2, 31.2)
	Net Difference	-0.46	(-1.01, 0.09)	-0.3	(-0.85, 0.25)	-0.65	(-1.2, -0.09)*
Hct ($L.L^{-1}$)	Pre	0.38	(0.37, 0.39)	0.38	(0.36, 0.39)	0.38	(0.36, 0.40)
	Post	0.40	(0.39, 0.42)	0.39	(0.38, 0.41)	0.40	(0.38, 0.41)
	Net Difference	0.02	(0.02, 0.03)‡	0.02	(0.01, 0.02)‡	0.02	(0.01, 0.02)‡
Hb ($g.L^{-1}$)	Pre	13.0	(12.5, 13.6)	13.0	(12.4, 13.5)	13.0	(12.4, 13.6)
	Post	13.7	(13.1, 14.3)	13.4	(12.8, 14.0)	13.5	(12.9, 14.1)
	Net Difference	0.67	(0.56, 0.78)‡	0.45	(0.34, 0.56)‡	0.51	(0.40, 0.63)‡
RBC($10^12.L^{-1}$)	Pre	4.28	(4.11, 4.45)	4.25	(4.07, 4.43)	4.29	(4.10, 4.48)
	Post	4.52	(4.31, 4.71)	4.43	(4.23, 4.63)	4.45	(4.25, 4.62)
	Net Difference	0.23	(0.19, 0.27)‡	0.18	(0.14, 0.21)‡	0.17	(0.13, 0.21)‡
Plasma Volume (%)	Pre	62.0	(60.6, 63.3)	62.2	(60.1, 63.7)	61.9	(60.2, 63.7)
	Post	56.7	(54.8, 58.5)	58.7	(56.8, 60.6)	58.2	(56.4, 60.0)
	Net Difference	-5.2	(-6.0, -4.4)‡	-3.5	(-4.3, -2.7)‡	-3.8	(-4.7, -3.1)‡
WBC ($10^9.L^{-1}$)	Pre	6.14	(5.30, 6.99)	6.03	(5.13, 6.93)	5.80	(5.13, 6.47)

	Post §	8.51	(7.16, 9.87)	8.98	(7.51, 10.45)	9.17	(8.10, 10.25)
	Net Difference	2.28	(1.64, 2.93)‡	2.98	(2.33, 3.62)‡	3.50	(2.84, 4.16)‡
MPV (fL)	Pre	8.26	(7.67, 8.86)	8.34	(7.76, 8.92)	8.57	(7.97, 9.16)
	Post	8.19	(7.59, 8.80)	8.34	(7.75, 8.93)	8.60	(8.09, 9.22)
	Net Difference	-0.07	(-0.21, 0.06)	0.01	(-0.13, 0.15)	0.24	(0.09, 0.38)†
PC $(10^9.L^{-1})$	Pre	233	(202, 264)	229	(205, 253)	228	(195, 260)
	Post	259	(224, 293)	249	(222, 277)	249	(220, 279)
	Net Difference	24	(17, 32)‡	20	(13, 28)‡	19	(11, 27)‡

Data represent means (95% CI). vWF = von Willebrand factor antigen, PT = prothrombin time, aPTT = activated partial thromboplastin time, Hct = haematocrit, Hb = haemoglobin, RBC = red-cell count, WBC = white-cell count, MPV = mean platelet volume and PC = platelet count. pre = time -2 hrs, post = time 5 hrs, net difference = post – pre, adjusted for age, sex, body mass, baseline outcome values and order effects. §adjusted for PV. *significant net difference from baseline, $P < 0.05$; †significant net difference from baseline, $P < 0.01$; and ‡significant net difference from baseline, $P < 0.001$.


Δ Plasma Fibrinogen (g.l-1)
0.4
0.3
0.2
0.1
0.0
*
Uninterrupted Sitting
Sitting + Light-Intensity Activity Breaks
Sitting + Moderate-Intensity Activity Breaks


(A)
Δ Hematocrit (l/l)
0.03
0.02
0.01
0.00
†
*
(B)
Δ Hemoglobin (g/l)
0.8
0.6
0.4
0.2
0.0
*
†
(C)
Δ Red-cell Count (10^12/l)
0.3
0.2
0.1
0.0
*
*
(D)
Δ Plasma Volume (%)
0
-2
-4
-6
-8
†
*
(E)
Δ White-cell Count (10^9/l)
5
4
3
2
1
0
‡
(F)
Δ Mean Platelet Volume (fl)
0.6
0.4
0.2
0.0
-0.2
-0.4
*
Uninterrupted Sitting
Sitting + Light-Intensity Activity Breaks
Sitting + Moderate-Intensity Activity Breaks