Impact of aerobic exercise on haemostatic indices in paediatric patients with haemophilia

Results from a prospective cohort study

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Summary
This study investigated the impact of aerobic exercise on laboratory assessments of haemostatic activity in boys (5–18 years of age) with haemophilia A (HA) or B (HB), examining the hypothesis that laboratory coagulation parameters temporarily improve with exercise. Thirty subjects meeting eligibility criteria (19 HA; 11 HB; mean age: 12.8 years) were invited to participate. They underwent a replacement factor washout period and were advised against strenuous activity for three days prior to the planned intervention. At study visit, baseline blood samples were drawn prior to exercise on a stationary cycle ergometer, aiming to attain 3 minutes (min) of cycling at 85% of predicted maximum heart rate. Blood work was repeated 5 min (t5) and 60 min (t60) post exercise completion. Samples were assessed for platelet count (PC), factor VIII activity (FVIII:C), von Willebrand antigen (VWF:Ag), ristocetin cofactor activity (VWF:RCo) and platelet function analysis (PFA-100); maximum rate of thrombus generation (MRTG) in blood was measured via thromboelastography and plasma peak thrombin generation (PTG) via calibrated automated thrombography. Mean duration of exercise was 13.9 (±2.6) min. On average, t5 samples showed significant elevation, relative to baseline in PC, FVIII:C, VWF:Ag, VWF:RCo and PTG, while FVIII:C, VWF:Ag, VWF:RCo and MRTG were significantly elevated in t60 samples. Within the cohort, participants with severe HA showed no change in FVIII:C levels with exercise. The greatest improvement in haemostatic indices was observed in post-adolescent males with mild-moderate HA, who thus represent the group most likely to benefit from a reduction of bleeding risk in the setting of exercise.

Keywords
Haemophilia, exercise, factor VIII

Introduction
Haemophilia, an X-linked recessive bleeding disorder, results from deficiency of coagulation factor VIII (HA) or factor IX (HB). The incidence of HA is estimated to be 1 in 5,000 males, whereas that of HB is estimated to be 1 in 30,000 males (1, 2). Based on coagulation factor activity, haemophilia may be subdivided into severe (factor activity <0.01 IU/ml), moderate (factor activity 0.01–0.05 IU/ml and mild (factor activity >0.05–0.40 IU/ml). Patients with severe haemophilia typically develop spontaneous and recurrent bleeds into joints (haemarthrosis) and muscles, while those with mild-moderate haemophilia tend to experience bleeding related to trauma and surgery (3). Prophylactic replacement with appropriate factor concentrate forms the mainstay of therapy for haemophilia in the developed world and effectively reduces bleeding frequency and its consequences (4, 5).

Historically, people with haemophilia (PWH) were discouraged from participating in sports, given the perceived risk of sports-associated trauma and subsequent haemorrhage and morbidity (6). Recent studies have since documented physical, medical and psychosocial benefits of exercise and appropriate sports activities (non-collision, non-contact sports) in PWH (7). Dynamic, isokinetic and isometric exercises are associated with improved muscle strength, proprioception and joint health, in addition to increasing social inclusion and adaptation (8–10). Additionally, studies in
PWH have suggested a possible improvement in coagulation parameters including FVIII:C and von Willebrand factor (VWF) with exercise, suggesting that appropriate exercise, to a certain extent, might diminish the risk of bleeding (11–13). These studies, however, typically investigated small cohorts of subjects performing sub-maximal exercise; additionally, they did not use global haemostatic assays to assess coagulation.

Despite the multiple benefits of exercise, there is a lack of awareness among PWH about the need to participate in appropriate sports and exercise. In a recent telephone survey conducted in the United States, 60% of the young haemophilia respondents believed that their condition could be managed by avoiding physical activity (14). Other studies from Europe have documented lower physical activity and fitness scores in PWH, compared to age-matched controls (15, 16). As expected, this has resulted in an increase in the prevalence of obesity and consequent decrease in quality-of-life in PWH (17–20). Large, well designed, prospective studies are needed to generate further evidence on the effects of exercise and appropriate sports activities on PWH.

In this hypothesis-generating, pilot study we investigated the impact of aerobic-exercise on multiple haemostatic indices in boys with HA and HB. Our hypothesis was that boys with non-severe HA and HB would exhibit incremental changes in haemostatic indices (VWF, FVIII:C) with exercise. We further hypothesised that this improvement could be quantified using global haemostatic assays, namely thromboelastography (TEG) and calibrated automated thromboelastography (CAT).

**Methods**

**Participant characteristics**

The study was conducted at the physiological research unit (PRU) at the Hospital for Sick Children (SickKids). Paediatric subjects with haemophilia, aged 5 years (and weighing ≥ 21 kg) to 18 years were solicited for participation and written informed consent/assent was obtained. For patients with mild haemophilia, only those with FVIII:C or factor IX activity levels (FIX:C) of 0.05–0.1 IU/ml were included, and a mean of the three most-recent clotting factor activity measurements was used to determine eligibility. The limitation of inclusion to patients with factor activity of 0.05–0.1 IU/ml was done to make the results of this study relevant to those mild haemophilia patients most likely to experience sports related bleeds.

Exclusion Criteria for participation in the study included (i) a current circulating or history of an inhibitor (≥ 0.5 Bethesda units (BU)) within the past five years; (ii) weight less than 21 kg; (iii) co-existence of additional bleeding diatheses (e.g. von Willebrand disease, platelet function abnormality); (iv) being enrolled in studies investigating extended half-life factor concentrates; (v) having severe arthropathy interfering with the ability to exercise; (vi) being on beta-blockers, anti-platelet agents or non-steroidal anti-inflammatory medications; (vii) having an active infectious or inflammatory condition (HIV, active hepatitis B or C); (viii) being active cigarette smokers or having reactive airway disease; (ix) a history of a recent bleed (preceding 2 weeks) in any location or a joint/muscle bleed in the lower limbs (in preceding 4 weeks). Baseline demographic data for all participants was abstracted from a review of their medical records. The study was approved by the institutional research ethics board. An institutional data safety monitoring board was established to monitor safety of the planned intervention.

**Exercise protocol**

Upon arrival to the PRU, all subjects were evaluated by one of two investigators (RK or MC) with a complete medical history and physical examination. Baseline participant exercise conditioning was determined using a standardised questionnaire (21, 22). Body mass index (BMI) was determined, vitals were collected and the exercise protocol was explained to the participants and their guardians. Participants had been previously instructed not to participate in any strenuous physical activity (e.g. participation in sports) for 72 hours (h) prior to the study date, and adherence to this instruction was assessed on the day of participation. Participants with HA were instructed not to infuse any prophylactic factor concentrate for 72 h before the date of participation while participants with HB were instructed not to infuse for 96 h before the date of participation.

A peripheral intravenous-line (PIV) was placed in the forearm/hand of all participants to facilitate blood sampling. To minimise pre-analytical variables, all PIVs were placed by the same haemophilia nurse-practitioner (VB). After baseline blood work was drawn, participants were instructed to exercise on an electrically braked cycle-ergometer (Upright Corival, Lode, The Netherlands) using the previously-validated, progressively-incremental Godfrey protocol (23–25). Per the Godfrey protocol, the participant started cycling on the calibrated cycle-ergometer with an initial exercise load dependent on their height. The workload was increased every minute in standard increments also based on the participant’s height (see Suppl. Appendix I for details, available online at www.thrombosis-online.com) (23–25). All participants were instructed to exercise until they completed 3-minutes (min) of cycling at 85% of their predicted maximum heart rate (MPHR). Heart rate (Polar HR monitor, Lachine, QC, Canada), blood pressure (Dash Monitor, GE Health care, Camarillo, CA, USA) and oxygen saturation (forehead reflectance probe, Massimo Radical, Irvine, CA, USA) were measured throughout the exercise protocol. Upon completion of planned exercise, work load was decreased to zero watts and participants were instructed to continue cycling at this cool-down rate for an additional 3 min. Second and third sets of laboratory specimens were drawn from the pre-existing PIV at 5 min (t5) and 60 min (t60) after completion of exercise. Prior to dismissal from the PRU, participants previously on-prophylaxis were given their routine prophylactic dose of factor concentrate. The entire intervention was administered by a certified exercise physiologist (JS) familiar with the protocol, in the presence of a haematologist (RK or MC).
Laboratory variables

Complete blood count (CBC), activated partial thromboplastin time (aPTT), FVIII:C, FIX:C, VWF:Ag, VWF:RCo, PFA-100 closure time, CAT and TEG were measured at all three time points (baseline, t5 and t60). For the first 10 participants, D-dimer and fibrinogen activity were determined at all three time points, but given the lack of any significant change in these parameters with exercise, they were subsequently collected only at baseline for the remaining 20 participants. CBC was performed using the Abbott CELL-DYN analyzer (Abbott Park, IL, USA). aPTT, D-dimer and fibrinogen activity were determined using the Stago STA-R evolution analyzer (Diagnostica Stago, Theale, England). FVIII:C and FIX:C were both measured using a one-stage assay, VWF:Ag was estimated using an immune-turbidimetric assay and VWF:RCo using agglutination of formalin-fixed platelets also though the Stago STA-R evolution analyzer. Closure times were run on the platelet function analyzer, PFA-100 (Siemens, Munich, Germany).

TEG 5000 thromboelastography analyzers were used for TEG analysis (Haemonetics Corporation, Braintree, ME, USA). Whole blood samples were collected in tubes containing 20 µg/ml corn trypsin inhibitor (CTI) and 3.2% trisodium-citrate as anticoagulant. This concentration of CTI has been shown to be sufficient to inhibit contact activation (26). At the beginning of each TEG assay blood samples were re-calcified with 12.5 mM calcium chloride to inhibit contact activation (26). At the beginning of each TEG assay blood samples were re-calcified with 12.5 mM calcium chloride and low concentration (0.5 pM) tissue factor (TF) was used to initiate coagulation. TEG assay results were expressed as a cumulative plot of clot amplitude, or cloting profile, which shows the time to clot initiation and maximum clot amplitude. Clot formation kinetics were depicted via a plot of the first derivative of clot amplitude, or clotting velocity curve, from which was obtained the maximum rate of thrombus generation.

Thrombin generation was assessed in thawed samples of frozen platelet-poor plasma (PPP) using the CAT assay in a Thrombinscope system based on the methods developed by Hemker et al., which are optimal for studying thrombin generation in normal individuals and those with hemophilia (27). The results of the CAT assays are presented as a plot of thrombin concentration (in nM) over time, or thrombogram, where the key parameters are the peak thrombin level, time to reach the peak and the endogenous thrombin potential (area under the curve).

Statistical analysis

Standard statistical methods were used to summarize the parameters: frequency and percent for categorical parameters and mean (± SD), median, and range for ordinal or continuous scaled parameters. Baseline characteristics of sub-cohorts of interest (severe HA, mild-moderate HA and HB) were compared using one-way-ANOVA. To compare pre- and post-exercise levels of the laboratory assays, we performed paired t-tests when the paired differences were normally distributed; non-parametric sign-tests were performed in cases when the paired differences were not normally distributed. For participants with mild-moderate haemophilia, changes in FVIII:C with exercise were compared to changes in FVIII:C with previously performed desmopressin challenge also using the paired t-test. Given that the analyses were performed as part of a preliminary, hypothesis generating pilot project, we did not perform adjustments for multiple comparisons. Analyses were performed using the SAS version 9.2 software package (SAS Institute, Inc., Cary, NC, USA). All calculated p-values were two-sided and p-values less than 0.05 were considered statistically significant.

Results

Patients and exercise

Thirty subjects were recruited for the study – 19 with HA (6: severe; 6: moderate; 7: mild) and 11 with HB (3: severe; 6: moderate; 2: mild).

Table 1: Baseline characteristics of the sub-cohorts investigated in this report.

<table>
<thead>
<tr>
<th>Demographic</th>
<th>Severe HA (n=6)</th>
<th>Moderate-mild HA (n=13)</th>
<th>HB (all severities) (n=11)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (± SD)</td>
<td>14.2 (± 4) yrs</td>
<td>12.8 (± 3.7) yrs</td>
<td>11.6 (± 4.1) yrs</td>
<td>0.43</td>
</tr>
<tr>
<td>BMI (± SD)</td>
<td>22.5 (± 5)</td>
<td>21.8 (± 4.8)</td>
<td>17.6 (± 3.1)</td>
<td>0.03</td>
</tr>
<tr>
<td>Type of therapy</td>
<td>6: prophylaxis</td>
<td>1: prophylaxis 12: on-demand</td>
<td>4: prophylaxis (all with severe haemophilia) 7: on-demand</td>
<td>nd</td>
</tr>
<tr>
<td>History of arthropathy</td>
<td>1: arthropathy</td>
<td>none</td>
<td>3: arthropathy</td>
<td>nd</td>
</tr>
<tr>
<td>Mean time to achieve 85 % of MPHR (± SD)</td>
<td>10.8 (± 3.4) min</td>
<td>10.3 (± 3.2) min</td>
<td>9.7 (± 1.7) min</td>
<td>0.7</td>
</tr>
<tr>
<td>Mean duration of exercise (± SD)</td>
<td>13.5 (± 2.2) min</td>
<td>13.9 (± 2.6) min</td>
<td>13.8 (± 2.6) min</td>
<td>0.9</td>
</tr>
</tbody>
</table>

BMI: body mass index; SD: standard deviation; nd: not done; MPHR: maximum predicted heart rate; yrs: years; min, minute.
Table 2: Impact of exercise on haemostasis for the entire cohort (n=30).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Baseline</th>
<th>t5</th>
<th>t60</th>
<th>P-value (t5 vs baseline)</th>
<th>P-value (t60 vs baseline)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean (± SD)</td>
<td>Mean (± SD)</td>
<td>Mean (± SD)</td>
<td>Mean (± SD)</td>
<td>Mean (± SD)</td>
</tr>
<tr>
<td>APTT (sec)</td>
<td>55.7 (± 9.7)</td>
<td>57.4 (± 10)</td>
<td>1.03</td>
<td>0.02</td>
<td>54.5 (± 9.9)</td>
</tr>
<tr>
<td>VWF:Ag (IU/ml)</td>
<td>0.98 (± 0.3)</td>
<td>1.32 (± 0.5)</td>
<td>1.3</td>
<td>&lt;0.0001</td>
<td>1.14 (± 0.4)</td>
</tr>
<tr>
<td>VWF:RCo (IU/ml)</td>
<td>1.0 (± 0.4)</td>
<td>1.19 (± 0.5)</td>
<td>1.2</td>
<td>0.003</td>
<td>1.17 (± 0.5)</td>
</tr>
<tr>
<td>Platelet count</td>
<td>253.6 (± 48.5)</td>
<td>309.3 (± 54.9)</td>
<td>1.2</td>
<td>&lt;0.0001</td>
<td>246.5 (± 52.7)</td>
</tr>
<tr>
<td>(x 10^9/l)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PFA-100</td>
<td>127.3 (± 20.9)</td>
<td>116.8 (± 26)</td>
<td>0.9</td>
<td>0.02</td>
<td>124.1 (± 36.1)</td>
</tr>
<tr>
<td>Col/Epi (sec)</td>
<td>96.7 (± 28.4)</td>
<td>84.8 (± 23.3)</td>
<td>0.9</td>
<td>0.12</td>
<td>88.9 (± 21.2)</td>
</tr>
<tr>
<td>PFA-100</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Col/ADP (sec)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>


Vitals were stable throughout. Another participant, a 16-year-old male with severe HA who was fasting at the time of the study, complained of feeling light-headed during the exercise protocol. He turned on a later date to redo the exercise protocol successfully. His symptoms resolved without interrupting the study. Further details for this sub-cohort are provided in supplementary appendix III.

Severe haemophilia A

Six patients with severe HA participated in the study. A 1.2 fold increase in platelet count was appreciated at t5 (p=0.01). No change was appreciated in the FVIII:C. A 1.3 and 1.2 fold increase was appreciated at t5 in VWF:Ag and VWF:RCo, respectively, though this did not reach statistical significance (p=0.07 and p=0.1, respectively). Details for this sub-cohort are provided in supplementary appendix III.

Mild to moderate haemophilia A

Thirteen patients with mild-moderate HA participated in the study. Platelet counts increased significantly by t5 (p<0.001), though this increase was lost by t60 (p=0.52). FVIII:C in this sub-cohort increased by 1.9 fold at t5 (p=0.01), and remained significantly elevated by 1.5 fold at t60 (p=0.04). VWF:Ag was 1.4 fold higher at t5 as compared to baseline (p=0.002) (Table 3).

Haemophilia B

Eleven patients with haemophilia B participated in the study. Platelet count (1.2 fold increase), FVIII:C (1.4 fold increase) and VWF:Ag (1.4 fold increase) were all significantly increased at t5 (p values of <0.001; 0.01 and 0.005, respectively), although only VWF:Ag remained significantly elevated at t60 (1.3 fold increase;
p=0.03). Exercise had no impact on FIX:C. Details for this sub-cohort are provided in Suppl. Appendix IV (available online at www.thrombosis-online.com).

**Impact of age on exercise-induced changes in haemostasis**

During the course of the study, it became apparent that age had a significant impact on haemostatic changes associated with exercise. Post-hoc, we arbitrarily divided our patients into pre-adolescent (age <13 years) and post-adolescent (age ≥13 years). For the entire cohort, post-adolescent participants had significantly greater fold-increase in VWF:Ag and VWF:RCo at t5 (p < 0.001 and 0.005, respectively) as compared to pre-adolescent males. This observation remained significant at t60 (p = 0.01 and 0.009, respectively) (Table 4). In the participants with mild-moderate HA, the mean fold-increase in FIX:C (t5 compared to baseline) in pre-adolescent males was only 1.11; whereas the mean fold-increase in post-adolescent males was 2.34 (p = 0.02). Again this observation remained significant at t60 (p = 0.03) (Figure 1). VWF:Ag and VWF:RCo also showed a significantly greater fold-

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Baseline</th>
<th>t5</th>
<th>fold increase (t5 vs baseline)</th>
<th>P-value (t5 vs baseline)</th>
<th>t60</th>
<th>fold increase (t60 vs baseline)</th>
<th>P-value (t60 vs baseline)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FVIII:C (IU/ml)</td>
<td>0.08 (± 0.07)</td>
<td>0.15 (± 0.1)</td>
<td>1.9</td>
<td>0.01</td>
<td>0.12 (± 0.1)</td>
<td>1.5</td>
<td>0.04</td>
</tr>
<tr>
<td>VWF:Ag (IU/ml)</td>
<td>0.99 (± 0.3)</td>
<td>1.34 (± 0.5)</td>
<td>1.4</td>
<td>0.002</td>
<td>1.11 (± 0.4)</td>
<td>1.1</td>
<td>0.06</td>
</tr>
<tr>
<td>VWF:RCo (IU/ml)</td>
<td>1.03 (± 0.5)</td>
<td>1.24 (± 0.5)</td>
<td>1.3</td>
<td>0.08</td>
<td>1.13 (± 0.5)</td>
<td>1.1</td>
<td>0.16</td>
</tr>
<tr>
<td>Platelet count (x 10^9/l)</td>
<td>241.2 (± 49.4)</td>
<td>297.3 (± 52.2)</td>
<td>1.3</td>
<td>&lt;0.0001</td>
<td>237.5 (± 51.6)</td>
<td>0.9</td>
<td>0.52</td>
</tr>
<tr>
<td>PFA-100 Col/Epi (sec)</td>
<td>137.5 (± 19.4)</td>
<td>117 (± 20.8)</td>
<td>0.9</td>
<td>0.02</td>
<td>129.8 (± 35.6)</td>
<td>1</td>
<td>0.45</td>
</tr>
<tr>
<td>PFA-100 Col/ADP (sec)</td>
<td>102.2 (± 40.2)</td>
<td>92.4 (± 27.9)</td>
<td>1</td>
<td>0.77</td>
<td>93.5 (± 21.6)</td>
<td>1</td>
<td>0.39</td>
</tr>
</tbody>
</table>

**Impact of age on haemostatic changes associated with exercise.**

<table>
<thead>
<tr>
<th>Cohort investigated</th>
<th>Lab value</th>
<th>Pre-adolescent mean fold increase (t5 vs baseline)</th>
<th>Post-adolescent mean fold increase (t5 vs baseline)</th>
<th>P-value</th>
<th>Pre-adolescent mean fold increase (t60 vs baseline)</th>
<th>Post-adolescent mean fold increase (t60 vs baseline)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Entire cohort (n=30)</td>
<td>Plt ct</td>
<td>1.19</td>
<td>1.26**</td>
<td>0.25</td>
<td>0.98</td>
<td>0.97**</td>
<td>0.77</td>
</tr>
<tr>
<td>pre-adolescent: 11 post-adolescent: 19</td>
<td>FVIII:C</td>
<td>1.14*</td>
<td>1.77</td>
<td>0.04</td>
<td>1.05</td>
<td>1.45</td>
<td>0.08</td>
</tr>
<tr>
<td>VWF:Ag</td>
<td>1.07</td>
<td>1.54</td>
<td>&lt;0.0001</td>
<td>1.02</td>
<td>1.27</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td>VWF:RCo</td>
<td>0.96</td>
<td>1.41</td>
<td>0.005</td>
<td>0.96</td>
<td>1.39</td>
<td>0.009</td>
<td></td>
</tr>
<tr>
<td>Mild-mod. HA (n=13)</td>
<td>Plt ct</td>
<td>1.19</td>
<td>1.28</td>
<td>0.22</td>
<td>0.95</td>
<td>1.01</td>
<td>0.35</td>
</tr>
<tr>
<td>pre-adolescent: 5 post-adolescent: 8</td>
<td>FVIII:C</td>
<td>1.11</td>
<td>2.34</td>
<td>0.02</td>
<td>1.06</td>
<td>1.81</td>
<td>0.03</td>
</tr>
<tr>
<td>VWF:Ag</td>
<td>1.07</td>
<td>1.56</td>
<td>0.01</td>
<td>0.98</td>
<td>1.22</td>
<td>0.09</td>
<td></td>
</tr>
<tr>
<td>VWF:RCo</td>
<td>0.92</td>
<td>1.48</td>
<td>0.03</td>
<td>0.93</td>
<td>1.26</td>
<td>0.02</td>
<td></td>
</tr>
<tr>
<td>HB (n=11)</td>
<td>Plt ct</td>
<td>1.19</td>
<td>1.27</td>
<td>0.53</td>
<td>1.03</td>
<td>0.95</td>
<td>0.24</td>
</tr>
<tr>
<td>pre-adolescent: 5 post-adolescent: 6</td>
<td>FVIII:C</td>
<td>1.18</td>
<td>1.66</td>
<td>0.12</td>
<td>1.06</td>
<td>1.34</td>
<td>0.79</td>
</tr>
<tr>
<td>VWF:Ag</td>
<td>1.09</td>
<td>1.61</td>
<td>0.01</td>
<td>1.06</td>
<td>1.46</td>
<td>0.08</td>
<td></td>
</tr>
<tr>
<td>VWF:RCo</td>
<td>1.01</td>
<td>1.41</td>
<td>0.79</td>
<td>0.99</td>
<td>1.70</td>
<td>0.43</td>
<td></td>
</tr>
</tbody>
</table>

Plt ct: platelet count; mod: moderate; * n=10, secondary to missing data; ** n=18, secondary to missing data.
increase at t5 in post-adolescent males with mild-moderate HA (p = 0.01 and 0.03, respectively), though only fold-increase in VWF:RCo remained significant at t60 (p=0.02). For patients with mild-moderate HA, baseline FVIII:C did not impact the fold-increase associated with exercise. Pre-adolescent males exercised for a longer duration of time as compared to the older participants (15.1 min vs 13.2 min; p=0.04), though they achieved similar mean increment in their MPHR (88% of MPHR for pre-adolescent males vs 92% of MPHR for post-adolescent males).

**Global haemostatic assays**

Although blood was taken from all patients for global haemostatic assays there was significant data attrition secondary to blood sample haemolysis. Complete data were available for 19 participants (5: severe HA; 8: mild-moderate HA; 6: HB).

**TEG**

For the entire cohort, mean maximum rate of thrombus generation (MRTG) increased at t5, though not significantly (from 5.09 ± 2.2 to 5.48 ± 1.8 mm/min; p=0.16); and continued to increase till t60 (to 5.75 ± 2.3 mm/min; p=0.04 vs baseline). Similar findings were appreciated for patients with mild-moderate HA. MRTG increased from 4.8 ± (2.1) mm/min at baseline to 5.5 ± (2.2) mm/min at t5 (p=0.11); and eventually to 6 ± (2.6) mm/min at t60 (p=0.002).

**CAT**

For the entire group of patients Peak thrombin generation (PTG) using CAT was significantly higher at t5 (93.33 ± 35.3 nmol/l) in comparison to baseline (87 ± 33.8 nmol/l; p<0.0001), but returned to baseline by t60 (86.1± 33.6 nmol/l).
Similarly, in patients with mild-moderate HA, PTG improved significantly by 15 (from 95.4 [± 22.9] nmol/l to 112.6 [± 19.8 nmol/l]); p<0.0001), though it returned to baseline by 160 (95.72 [± 24.9] nmol/l).

Discussion

In this pilot study, we have documented that aerobic-exercise in children with haemophilia is associated with an immediate increase in platelet count, FVIII:C, VWF:Ag and VWF:RCo. Of these laboratory variables, FVIII:C, VWF:Ag and VWF:RCo remain significantly elevated 1 h post exercise completion. Participants with severe HA had no change in FVIII:C with exercise. Participants with mild-to-moderate HA had an impressive increase in FVIII:C immediately after exercise (1.9 fold increase) and this remained significantly elevated 1 h post-exercise (1.5 fold increase in relation to baseline). FIX:C levels did not change in any patient (HA or HB) with exercise.

We observed a significant impact of age on the haemostatic changes associated with exercise – this improvement was most dramatic in the eight post-adolescent males with mild-moderate HA where we documented an overall 2.3 fold increase in FVIII:C, immediately with exercise (including a greater than 3-fold increase in FVIII:C in two, 17-year-old participants [Figure 1]). For the oldest boys (age ≥ 15 years; n=4) with mild-moderate HA, the 1 h rise in FVIII:C post exercise was similar to their historical response to DDAVP (2.6 [± 0.9] fold rise with exercise vs 2.4 [± 1.6] fold rise with DDAVP). It should be noted, however, that they had undergone their DDAVP challenge at much younger ages (mean age at time of DDAVP challenge 5.8 [± 4.3] years). Previous work from our group has shown that most boys with MHA experience a two- to four-fold rise in their FVIII:C after administration of DDAVP, and this increase is partly dependent on patient age (older boys respond better than very young boys) (28, 29). Exercise mediated increase in FVIII:C and VWF levels likely has a similar mechanism of action (30, 31), and a head-to-head comparison of exercise versus DDAVP remains an interesting topic for future studies.

The impact of exercise on both primary and secondary haemostasis in healthy (non-haemophilic) adults is well documented (32). More than 30 years ago, Dimitriadou et al assessed platelet count and aggregation in nine Finnish amateur runners participating in a non-competitive marathon race (33). Post-exercise, both the platelet count and aggregation to ADP and collagen were found to be significantly increased. Multiple studies have since confirmed these original findings by Dimitriadou et al. (34–36). In 1997, Rock et al. noted a nearly 300 % increase in FVIII:C, VWF:Ag and VWF:RCo in 14 well-trained individuals after a cycle-ergometer based exercise protocol (43). The fold-increase documented by Koch et al. is similar to what we noticed in our pre-adolescent cohort (mean age: 8.8 [± 2] years; n=5) with mild-moderate HA (1.11 fold). In 2006, Roya et al. performed a study in 10 mild-moderate HA patients (mean age: 24.5 years), again using a cycle ergometer based protocol. The authors noted a non-significant, 1.3 fold increase in the FVIII:C immediately post-exercise (13). This study was unique in that the participants performed submaximal exercise over a prolonged period of time (mean duration of exercise: 37.4 min), as opposed to a short burst of intense exercise. More recently, Beltrame et al. published their experience on the impact of low intensity, aquatic exercise on coagulation parameters in men with moderate to severe haemophilia A and B (mean age: 22 years) (11). Participants of this study performed 20 min of aquatic exercise aiming for 70 % of their MPHR. This intervention showed a non-significant 1.3 fold improvement in FVIII:C. In summary, previous work in both children and adults with haemophilia, have not noted a significant improvement in coagulation parameters with sub-optimal exercise. This is particularly relevant to children, who rarely perform prolonged duration of low-intensity exercise. Physical activity in children, particularly non-organized sports as occurs in school playgrounds and in gym class, is characterised by short bursts of high-intensity exercise interrupted by periods of rest (24). Our exercise protocol was designed to mimic the typical duration of physical activity that children might perform routinely. Hence we aimed only for about 15 minutes of exercise and generally not to exhaustion, but instead to 85 % of MPHR. Additionally, given the shorter attention span and sometimes lower motivation in younger children, we were concerned about compliance with long monotonous exercise protocols (24).

The findings from our study closely match those of Groen et al. (12). In that study 15 adults (mean age: 26.5 years) with non-severe HA were exercised using a progressively incremental cycle ergometer protocol, until exhaustion. Blood work was drawn before and 8 min after exercise. The authors noted a 2.5 fold increase in FVIII:C in addition to significant increase in both VWF:Ag and VWF:RCo. The improvement in FVIII:C was similar to the 2.34 fold increase noted in post-adolescent males with mild-moderate HA in our cohort.

The impact of age on the differential haemostatic response to exercise has been previously reported in non-haemophilia subjects – both young children and older adults tend to have inferior response as compared to adolescents and young adults (40, 44). Younger children in our cohort, with both mild-moderate HA, and
HB documented minimal changes in tested haemostatic parameters, despite having exercised for a longer duration of time. Given concerns about motivation and shorter attention span, we encouraged parents of younger children to exercise side-by-side with their children on a sham cycle-ergometer. The mechanism responsible for post-exercise FVIII:C and VWF increase is thought to involve beta adrenergic receptor activation and release of stored VWF from endothelial Weibel-Palade bodies (45, 46), and it therefore remains plausible that aging associated conditioning of the endothelial function is responsible for this difference in response to exercise between pre-pubertal patients and post pubertal patients (40). Another explanation could be a lack of well-developed leg extensor muscles in younger children, which may have resulted in leg-fatigue prior to core (cardio-respiratory) fatigue, particularly on a cycle-ergometer based exercise regimen (24, 25). To overcome this perceived concern, we had initially considered using a treadmill-based exercise protocol (21, 22). During the feasibility phase of our study, two healthy adult volunteers were made to exercise on a treadmill following the Bruce protocol; and it was felt that the steep rise in incline may be particularly difficult for smaller children and as well for all children with previous ankle arthropathy. A cycle ergometer was thought to be less intimidating for younger participants and to pose little risk of joint (particularly ankle) damage.

CAT detected an immediate effect with exercise after 5 min but not after 1 h whereas TEG demonstrated a significant effect only after 1 h. This suggests that there is significant thrombin generation after 5 min which is not sustained (measured in plasma alone), whereby the cumulative effect of plasma in the presence of platelets and other blood cells demonstrated significantly increased MRTG after 1 h using TEG.

Another interesting observation in our study was the relative prolongation of APTT (t5) despite the increase in FVIII:C. While not statistically significant, this observation was appreciated in the mild-moderate HA cohort (54.9 [±6.2] sec at t5 vs 53.3 [± 6.2] sec at baseline), and in the post-adolescent mild-moderate HA cohort (51.3 [± 6.6] sec at t5 vs 51.3 [± 7.4] sec at baseline) despite the more robust increase in FVIII:C observed in these sub-cohorts. Changes in pH or other coagulation factors associated with exercise, resulting in a shortening in APTT despite increase in FVIII:C are attractive hypothesis for testing in future studies. Lastly, the increase in platelet count by t5 followed by return to baseline by t60 warrants further discussion. This change in platelet count may be secondary to haemoconcentration, given that the mean haematocrit for the entire cohort changed from 44 (± 4)% at baseline to 46 (± 5)% at t5 and 42 (± 4)% at t60. Another explanation for the acute rise in platelet count with subsequent return to baseline could be splenic contraction from exercise induced adrenergic stimulation resulting in a release of platelets into peripheral circulation. This has been previously demonstrated in non-haemophilic individuals (47, 48).

Some of the limitations of our study include a relatively small cohort size (although larger than most studies previously conducted on patients with haemophilia), inability to study fibrinolysis and significant data attrition while investigating global haemostatic assays. Given that this was a hypothesis generating study, we did not adjust for multiple comparisons – this may have resulted in spurious associations. Additionally, it should be recognised that we had our patients abstain from exercise in the previous 72 h prior to study. As such it is possible that in such patients, their VWF stores in their Weibel-palade bodies were at maximal amounts. Therefore, it remains plausible that in subjects who exercise regularly, the changes in haemostatic indices (particularly VWF and FVIII:C) with exercise may be less substantial. This, of course, needs to be tested prospectively. Lastly, while investigating the impact of age, we arbitrarily divided our patients in pre- and post-adolescent based on age. For future studies, we recommend using a more objective definition of age, such as Tanner stage.

In summary, our study investigates the impact of exercise on coagulation parameters in children and adolescents with haemophilia. Our patients followed a well-defined exercise protocol and we noted that exercise was associated with significant increase in multiple laboratory markers of haemostasis, particularly in post-adolescent males with mild-moderate HA. Results from our study suggest that 15 min of exercise may result in an increase in FVIII:C, VWF and platelet counts that may then decrease the risk of sports associated haemorrhage. One can speculate from this study that encouraging children with haemophilia (particularly post-adolescent boys with mild-moderate haemophilia A) to warm-up prior to engaging in appropriate sports might lessen their risk of subsequent bleeding – though this would need to be demonstrated in a larger study.

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Conflicts of interest
None declared.

References