

1 **Type of submission:** RESEARCH

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3 **TITTLE:** Intraocular pressure fluctuations assessment in professional wind instrument players.

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19 **Running title:** Intraocular pressure variation in musicians

20 **Key Words:** Glaucoma risk factors, Intraocular pressure fluctuation, Ocular risk factors, Wind  
21 instrument musicians

22 **Clinical relevance:** Due to the long-time that wind musicians spend playing their instruments, it  
23 is important to investigate if intraocular pressure could be affected by this activity.

24 **Background:** To assess the intraocular pressure fluctuations and fluctuations affecting factors  
25 in professional wind musicians while playing different tones.

26 **Methods:** 30 professional wind musicians ( $23.0 \pm 3.20$  years) were recruited from the  
27 Professional Music College of A Coruña. A questionnaire about environmental/demographic  
28 factors was given to participants. Intraocular pressure was measured four times by ICare IC100  
29 tonometer: before, during low and high-pitched tones, and immediately after stopping playing  
30 the wind instrument.

31 **Results:** Pairwise comparison revealed statistical differences between measurement points  
32 (Sidak, all  $p \leq 0.019$ ), except between before playing and while playing low-pitched tones  
33 (Sidak,  $p = 1.000$ ). Intraocular pressure increases during high pitch playing and decreases after  
34 stopping playing. No significant differences in intraocular pressure fluctuation were reported  
35 between physically active ( $> 2$  days/week) and non-physically active participants (Unpaired t-  
36 test,  $p = 0.680$ ). Regarding correlations, all intraocular pressure values were positively  
37 correlated (Pearson's correlation, all  $r \geq 0.505$ ,  $p \leq 0.004$ ). Intraocular pressure fluctuations  
38 were negatively correlated with musical playing years (Pearson's correlation,  $r = -0.396$ ,  $p =$   
39  $0.030$ ). There were no significant correlations among intraocular pressure fluctuation and  
40 gender, age, weight, height, or daily time playing (Pearson's correlation, all  $p \geq 0.058$ ).

41 **Conclusion:** Professional wind musicians suffer intraocular pressure peaks while playing high-  
42 pitched tones, therefore, ocular fundus evaluation and visual campimetry should be performed  
43 as routine tests in the visual exam of this population.

44 The intraocular pressure (IOP) value is an indicator of the balance between the production and  
45 drainage of aqueous humor.<sup>1</sup> The homeostasis of this process is essential to maintain good eye  
46 function, since its increase or decrease is related to different eye pathologies, such as glaucoma  
47 (increase) or retinal detachment (decrease).<sup>1,2</sup> In addition to the absolute value, the fluctuation of  
48 IOP has also been determined as a risk factor.<sup>3</sup> Due to circadian rhythm, IOP physiologically  
49 changes throughout the day, being the difference between the mean diurnal and nocturnal IOP  
50 ranges from 3 to 5 mmHg,<sup>4</sup> or even more in pathological populations.<sup>5</sup> Normally, the minimum  
51 value is obtained at the end of the afternoon, meanwhile, the maximum value appears between  
52 the middle and the end of the night period. It means there is an IOP peak in the early morning  
53 that decreases during the day (bathypase) until reaching the lowest IOP value in the late  
54 afternoon when IOP starts to rise again (acrophase).<sup>4</sup>

55 In addition to the physiological functioning, other exogenous factors have been established as a  
56 cause of IOP fluctuations, such as the body posture (greater IOP value in a supine position than  
57 in a sitting position),<sup>6</sup> the respiration (there is an IOP increase during Valsalva manoeuvre),<sup>6</sup>  
58 physical efforts (decreasing IOP while aerobic exercise<sup>7</sup> and increasing with isometric efforts in  
59 high-load condition,<sup>8</sup> perhaps related to a Valsalva manoeuvre), cognitive efforts (showing  
60 higher IOP values during an academic exam than in the control session, and higher before than  
61 after the exam, which could point to IOP as an indicator of stress),<sup>9</sup> or even the use of a  
62 smartphone (especially in low light conditions, probably due to mechanism of accommodation  
63 and convergence, the external ocular muscles contraction, the psychophysiological stress, or the  
64 neck-flexion posture).<sup>10</sup>

65 There are also a few studies that have found an increase in IOP while playing a wind  
66 instrument, showing a different level of IOP variation concerning the tonal frequency or  
67 intensity during playing<sup>11,12</sup>; some authors have suggested that the IOP rising could be related  
68 to an involuntary Valsalva maneuver.<sup>13</sup> As a consequence of the amount of time that musicians  
69 spend playing or practicing with their instruments, in most cases since childhood, this activity  
70 could be a risk factor for ocular hypertension development or even pathologies such as  
71 glaucoma. Previous reports have found a greater incidence of visual field defects in wind  
72 musicians, correlated with cumulative practice time.<sup>14</sup> However, several of these studies  
73 included a small or inadequately defined sample population, encompassing a wide age range,  
74 both professional and amateur musicians, and individuals with and without glaucoma;  
75 moreover, in some studies, measurements of IOP were conducted in non-representative  
76 positions during instrument play.<sup>15-17</sup> Based on the hypothesis that playing wind instruments  
77 could cause fluctuations in IOP, the current study aimed to evaluate such fluctuations during the  
78 playing different tones and to investigate other potential factors influencing these changes,

79 while exclusively examining young, non-glaucomatous, professionally trained musicians in a  
80 natural posture for instrument playing.

81

## 82 **METHODS**

### 83 *Participants*

84 For the sample size calculation, PS Power and Sample Size Calculations Software Version 3.1.2  
85 (Copyright © by William D. Dupont and Walton D. Plummer) was used. Data from previous  
86 studies with a similar design to the present study indicate that the mean standard deviation (SD)  
87 of repeated IOP measures is normally distributed with a value of 2 mmHg; in order to have a  
88 90% power for a significance level of  $\alpha = 0.05$  (Type I error associated) with a confidence level  
89 of 95% to detect an anticipated mean difference of 1.5 mmHg, the minimum numbers of  
90 participants required was 21.<sup>18</sup> Since all the final participants recruited were volunteers from  
91 various courses at the Professional Music College of A Coruña (CSM A Coruña, Galicia,  
92 Spain), the sample size was expanded beyond the initially calculated value to enhance  
93 representativeness and minimize potential biases in the study. This adjustment aimed to ensure  
94 that the study encompassed a broader spectrum of musicians.

95 The initial recruitment process was conducted by the management team of the Professional  
96 Music College of A Coruña, who received the information about the study and disseminated it  
97 to all students, regardless of the year of enrolment at the institution. Once the initial sample was  
98 recruited, the researchers conducted a rigorous evaluation of the inclusion and exclusion criteria  
99 for the voluntary participants. Only those who met these criteria were subsequently enrolled to  
100 participate in the study. Enrolment was done one by one, following a sequential enrolment list,  
101 until the estimated sample size of 30 participants was reached. All other volunteers, both those  
102 who have not meet the inclusion and exclusion criteria and those who exceed the pre-  
103 determined recruitment number, were excluded. This approach ensured that there was no  
104 preselection or bias based on research predisposition or human factors.

105 Participants were included if they were wind instrument players enrolled at the Professional  
106 Music College of A Coruña (Table 1 shows instruments included in the study), had a refractive  
107 error between - 8.00 and + 4.00 dioptres, an absence of previous diagnosis of any retinal  
108 pathology, have not been diagnosed with ocular infection at the time of the study, not being  
109 under treatment with corticosteroids, or have not undergone corneal surgery.<sup>11,19</sup> All participants  
110 were informed of the procedures and gave their written informed consent to be included in the  
111 study. The study protocol complies with the Declaration of Helsinki and was approved by both  
112 the Institutional and Regional Research Ethics Committees (CEIC2013/360).

113

114 ***Outcome measurements***

115 ***Part 1: Questionnaire***

116 A researcher-administered questionnaire about factors that could affect IOP, personal musical  
117 history and habits was self-reported by each participant. The first part of the questionnaire  
118 included open-ended questions about the date of birth, height, weight, biological sex, and an  
119 anamnesis asking about the presence of systemic and ocular health and drug intake. In addition,  
120 dichotomous (yes/no) questions about family history of glaucoma and practice of physical  
121 activity more than 2 days per week were asked. The second part of the questionnaire deals with  
122 personal musical history: instrument played, years of musical training, daily time playing the  
123 instrument and academic year attended at the Professional Music College of A Coruña. Table 2  
124 summarizes the questions and type of the questionnaire.

125

126 ***Part 2: IOP measurements***

127 IOP was measured with the ICare IC100 tonometer (Tiolat Oy, Helsinki, Finland) following the  
128 manufacturer's instructions.<sup>20</sup> The ICare IC100 is a rebound portable tonometer with high  
129 repeatability that does not require the use of topical anesthesia.<sup>20</sup> This device takes six  
130 consecutive measurements automatically, discards the highest and lowest readings, and averages  
131 the remaining four providing the mean IOP in mmHg, which was used as the final IOP value.  
132 While sitting in a normal playing position, participants were requested to fixate their sight on a  
133 straight target; a total of four IOP measurement points were established in the protocol. First,  
134 one measurement was taken before playing the instrument (Basal). Then, the second and third  
135 measurements were performed while the participants were playing the instrument for 8 seconds  
136 on a low (LP) and high pitch (HP) respectively with moderate loudness (see Table 1). The low  
137 pitch was the lowest A of the instrument's tessitura, and the high pitch was an A two octaves  
138 above the previous one. Previous to the measurement, the tone was given by a piano or a tuner  
139 phone app prior to playing to ensure that the pitch played was correct. Finally, the last  
140 measurement was recorded immediately after stopping playing (AP) the HP. IOP measurements  
141 were repeated six times on each pitch. Measurements were only performed in one eye of each  
142 participant; if the instrument allows access to both eyes, one eye was randomly selected,  
143 nevertheless, if the selected eye was difficult to be correctly measured because of the specific  
144 features of the respective instrument and the accessibility of the examiner, the other one was  
145 elected.<sup>11</sup>

146 All measurements were taken in the morning and afternoon (10.30 a.m. to 3.30  
147 p.m.)<sup>21</sup> by only one examiner to avoid the possible inter-observer variation.<sup>22</sup> To ensure the  
148 musicians' comfort and maintain their playing posture the examiner received specialized

149 training at the university optometry clinic previous to the study. Moreover, the examiner was  
150 instructed to check with the musicians if they experienced any discomfort during the procedure  
151 and to adjust as necessary.

152

### 153 ***Statistical analysis***

154 SPSS statistical software v. 25.0 for Windows (SPSS Inc., Chicago, United States) was used for  
155 data analyses. Significance was set at a  $p \leq 0.05$  for all the statistical analyses. Previous to the  
156 analysis, the normal distribution of the IOP results was checked using the Shapiro-Wilk test;  
157 parametric tests were applied in all the analyses.<sup>23</sup>

158 Differences between IOP results obtained on each measuring point were assessed using the  
159 Greenhouse-Geisser or Huynh-Feldt correction based on Mauchly's W test of Sphericity, while  
160 the Sidak test was used to detect significant pairwise differences.<sup>23</sup> IOP fluctuation was analysed  
161 with respect to weekly physical activity using a t-test for independent samples; participants were  
162 divided into two groups based on the frequency of physical activity reported in the  
163 questionnaire (see Table 2): those who engaged in physical activity more than twice a week and  
164 those who have not. For graphical purposes, Bland and Altman procedures were used; this  
165 method describes the agreement between two variables, representing averages versus  
166 differences.<sup>23,24</sup> The 95 % limits of agreement (95 % LoAs) were calculated (Mean difference  $\pm$   
167  $1.96 \times SD$ ), as well as the exact 95% Confidence Intervals (95% CI) for Upper and Lower LoAs  
168 considered as a pair (Mean difference  $\pm c_{0.025} \times SD$ ; Mean difference  $\pm c_{0.975} \times SD$ ).<sup>23,25</sup>

169 Relationships were assessed through Pearson's correlation for parametric data. The correlations  
170 between basal, LP, HP, and AP IOP values were calculated. Furthermore, the impact of the  
171 studied environmental and demographic factors on the increase in IOP during HP playing was  
172 also analysed (the increase in IOP was calculated as the difference between IOP HP and Basal  
173 IOP, expressed as  $\Delta IOP = IOP_{HP} - Basal\ IOP$ ). Correlation between variables was described as  
174 weak (0.20 – 0.40), moderate (0.41 – 0.60), good (0.61 – 0.80) or strong (0.81 – 1.00).<sup>26</sup>

175

## 176 **RESULTS**

177 The sample consisted of 30 eyes (56.67 % were right eyes) of 30 participants (22 men and 8  
178 women) with a mean  $\pm$  SD age of  $23.0 \pm 3.20$  years (range from 19 to 30 years), mean  $\pm$  SD  
179 height of  $176.7 \pm 9.33$  cm and mean  $\pm$  SD weight of  $77.1 \pm 17.13$  kg. Only one participant had a  
180 family history of glaucoma. Regarding their musical history, participants had been playing their  
181 wind instrument for a mean  $\pm$  SD of  $13.8 \pm 2.88$  years, with an average daily dedication of mean  
182  $\pm$  SD of  $3.3 \pm 1.32$  hours.

183

184 ***Differences between IOP values***

185 A statistical difference in IOP values between each measurement point was found (Mauchly's  
186 W:  $p < 0.001$ ,  $\epsilon = 0.570$ ; Greenhouse-Geisser, all  $p < 0.001$ ). Pairwise comparisons are reported  
187 in detail in Table 3. Figure 1 provides a Bland-Altman plot of means against the differences  
188 between the data obtained at each measurement point. On the paired analysis, there were no  
189 significant differences between Basal IOP and LP (Sidak post-hoc,  $p = 1.000$ ) (Table 3, Fig.  
190 1A). Significant differences were observed between all other values obtained in each  
191 measurement point: Basal IOP vs. HP (Fig. 1B) or vs. AP (Fig. 1C), LP vs. HP (Fig. 1D) or vs.  
192 AP (Fig. 1E), and HP vs. AP (Sidak post-hoc, all  $p \leq 0.019$ ) (Fig. 1F).

193

194 ***Correlation between IOP values***

195 There was a good positive correlation between Basal IOP and all other IOP values (Pearson's  
196 correlation, all  $r \geq 0.711$ ,  $p \leq 0.001$ ) and between LP and AP (Pearson's correlation,  $r = 0.831$ ,  $p$   
197  $< 0.001$ ). There was also a positive but described such as a moderate correlation between LP  
198 and HP, and between HP and AP (Pearson's correlation, all  $r \geq 0.505$ ,  $p \leq 0.004$ ).

199

200 ***Analysis of the relationship between the increase in IOP and environmental or demographic***  
201 ***factors***

202 IOP fluctuation was weakly negatively correlated with the years of musical training (Pearson's  
203 correlation,  $r = -0.396$ ,  $p = 0.030$ ). There were found no statistically significant correlations  
204 between IOP fluctuation and gender, age, weight, height, or daily time playing the instrument  
205 (Pearson's correlation, all  $p \geq 0.058$ ). There was also found no significant difference in IOP  
206 fluctuation between the group that performed physical activity more than two days per week and  
207 the group that did not (Unpaired t-test,  $p = 0.680$ ).

208

209 **DISCUSSION**

210 In the present study, the fluctuation of IOP on professional wind musicians during playing  
211 different tones was studied, as well as the possible influence of other factors on these  
212 fluctuations. The increase in IOP and its fluctuations are frequently related to the development  
213 of some ocular pathologies, such as glaucoma, which causes an irreversible visual field  
214 reduction due to the mechanical stress and ischemic effects of IOP on the retinal nerve fibre  
215 layer.<sup>1</sup> Previously, Schuman et al.<sup>13</sup> reported a significant correlation between playing high-  
216 resistance wind instruments and abnormal visual fields. This finding was later confirmed by Lin  
217 et al.,<sup>14</sup> who also observed an association between mean visual field defect and practice time.

218 Specifically, they found that there was a 0.07 dB increase in the mean visual field defect for  
219 every 1,000 cumulative hours of practice and playing time.

220 Results obtained here showed a mean Basal IOP and LP in normal lower values ( $16.1 \pm 0.68$   
221 and  $16.3 \pm 0.64$  mmHg respectively) with no significant difference between them (Fig. 1A).  
222 Contrary, while HP, the IOP rises to a mean value of  $20.0 \pm 0.99$  mmHg, which was  
223 significantly different to the Basal or LP value (Fig. 1B and D); moreover, it was also found a  
224 significant difference in the IOP value at the end playing ( $14.7 \pm 0.67$  mmHg) (Fig. 1F). In  
225 addition, significant differences have also been observed between AP and Basal or LP values  
226 (Fig. 1C and E). All these results showed that IOP fluctuations during normal playing are  
227 related to the HP: there is an increase during HP, which decreases to regular or even lower  
228 values after stopping playing the high-pitched note. These findings are in agreement with  
229 previous research in which an IOP increase related to the tone frequency played (greater in  
230 high-pitched tones), especially when playing at high intensity (*fortissimo*) was also found.<sup>11,12,27</sup>  
231 As a reinforcement of this idea, there was found a positive correlation between IOP values: the  
232 higher the basal IOP, the higher the LP, HP, and AP IOP.

233 It has been hypothesized that the increase in IOP during playing could be generated by the  
234 Valsalva maneuver,<sup>12,13</sup> although this hypothesis is controversial. On the one hand, this  
235 hypothesis has been disputed because to obtain a good quality sound playing a wind instrument,  
236 it is necessary to use diaphragmatic breathing with open glottis, a condition that does not occur  
237 during a Valsalva maneuver.<sup>28</sup> It should be noted that in the present study only professionally  
238 trained musicians from the Professional Music College of A Coruña were recruited, who  
239 presumptively have good breath control; nevertheless, an IOP increase was also found. Besides,  
240 de Crom et al.<sup>16</sup> found a higher increase in the IOP of professional musicians versus amateurs,  
241 which is difficult to explain by the hypothesis of an involuntary Valsalva manoeuvre due to bad  
242 breathing techniques. In a separate study, Schuman et al.<sup>13</sup> measured IOP and uveal thickness  
243 before and during playing, revealing a positive correlation between uveal volume increase and  
244 the musician's IOP; this relationship can be explained by the rise in intrathoracic pressure and  
245 compression of the intrathoracic venous system, which is transmitted through veins to the  
246 choroid, leading to an increase in choroidal volume and an increase in IOP. However, a  
247 standardized Valsalva manoeuvre did not produce an increase in uveal volume.<sup>29</sup>

248 On the other hand, also according to Schuman et al.<sup>13</sup> the pattern of IOP increase during playing  
249 a high-resistance wind instrument is similar to that seen in a Valsalva manoeuvre. However,  
250 Markoff<sup>28</sup> suggested that the categorization of instruments as high or low resistance was  
251 imprecise, since the authors did not take into account certain characteristics of the instruments

252 that affect the amount of lung volume and air velocity required to play. A finding of the present  
253 study that could also support the theory of the Valsalva manoeuvre as responsible for the  
254 increase in IOP while playing was that IOP fluctuation was negatively correlated with years of  
255 musical training; the more years of playing the instrument, the lower the IOP increase. This  
256 could be related to the fact that the more professional the musician, the more controlled the  
257 breathing, although the relationship was weak and therefore of poor predictive quality. To  
258 enlighten this controversy, novel studies which control if the Valsalva manoeuvre occurs while  
259 playing are necessary. One way to test this phenomenon may be through otoscopic  
260 examinations during playing time: the Valsalva manoeuvre can be objectively detected by  
261 otoscopy, as the increased pressure in the middle ear through the Eustachian tube causes the  
262 movement of the tympanic membrane.<sup>30</sup> Therefore, the relationship between both IOP  
263 fluctuations and the Valsalva manoeuvre can be clarified by evaluating the IOP value and the  
264 tympanic movements while playing a wind instrument.

265 One of the main limitations of the present study was that musicians were asked to play at  
266 medium intensity (*mezzoforte*), but the volume was not measured objectively by a sound level  
267 meter. The literature found that the IOP increase was greater when playing louder,<sup>11-13</sup> therefore,  
268 it may be differences between the IOP fluctuation in the participants due to the intensity and not  
269 to the tonal frequency. In addition, only professionally trained musicians were measured; a  
270 “control” group with no professional musicians should be recruited during future studies to  
271 perform comparisons. Moreover, future studies should consider the child population, since  
272 people usually start to play an instrument between 3 and 10 years old.

273 To conclude, in normal conditions of playing, the impact of playing a wind instrument on the  
274 IOP of professional musicians depends on the note played, with HP being responsible for the  
275 IOP peaks. These peaks could be risk factors for the development of some ocular pathologies,  
276 therefore, regular ocular fundus evaluation and visual campimetry should be routine tests in the  
277 visual exam of wind musicians for a preventive early diagnosis of any possible pathology.

278 Further research to establish the mechanism of IOP increase while playing is needed.

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<b>Instrument (n)</b>	<b>LP</b>	<b>HP</b>
Saxhorn (1)	55 Hz	220 Hz
Tuba (1)	55 Hz	220 Hz
Tenor saxophone (1)	110 Hz	440 Hz
Trombone (5)	110 Hz	440 Hz
Horn (1)	110 Hz	440 Hz
Piccolo trumpet (3)	110 Hz	440 Hz
Clarinet (5)	220 Hz	880 Hz
Alto saxophone (1)	220 Hz	880 Hz
Trumpet (6)	220 Hz	880 Hz
Oboe (2)	440 Hz	1568 Hz
Transverse flute (4)	440 Hz	1760 Hz

349 **Table 1.** Frequencies played by each instrument. LP = Low Pitch; HP = High Pitch.

350

Question	Answer type
1. Birth date	Open question
2. Biological sex	Dichotomous (Men/Women)
3. Height	Open question (in cm)
4. Weight	Open question (in kg)
5. General health: Hypertension	Dichotomous (Yes/No)
6. General health: Diabetes	Dichotomous (Yes/No)
7. General health: Cardiac insufficiency	Dichotomous (Yes/No)
8. General health: Sleep apnoea	Dichotomous (Yes/No)
9. General health: Other pathology	Open question
10. Drugs intake	Open question
11. Ocular health: Glaucoma	Dichotomous (Yes/No) to each eye
12. Ocular health: Ocular surgery	Dichotomous (Yes/No) to each eye
13. Ocular health: Other pathology	Open question
14. Refractive error: Myopia	Open-ended question to each eye
15. Refractive error: Hyperopia	Open-ended question to each eye
16. Refractive error: Astigmatism	Open-ended question to each eye
17. Family history of glaucoma	Closed question: I don't know / No / Yes (what relative?)
18. Physical exercise more than 2 days per week	Dichotomous (Yes/No)
19. Instrument played	Open question
20. Years playing the instrument	Open question
21. Time per day playing the instrument	Open question
22. Academic year attended at the Professional Music College	Closed question: 1°, 2°, 3°, 4°

352 **Table 2.** Diagram of the questionnaire.

	Mean $\pm$ SD	Mean difference $\pm$ SD	p	95 % LoAs		95 % CI of LoAs	
				Lower	Upper	Lower	Upper
<b>Basal</b>	16.1 $\pm$ 3.72						
<b>LP</b>	16.3 $\pm$ 3.49	-0.17 $\pm$ 2.601	1.000	-5.26	4.93	-4.28 /	3.94 /
<b>Basal</b>	16.1 $\pm$ 3.72						
<b>HP</b>	20.0 $\pm$ 5.43	-3.93 $\pm$ 3.823	<0.001	-11.43	3.56	-9.98 /	2.11 /
<b>Basal</b>	16.1 $\pm$ 3.72						
<b>AP</b>	14.7 $\pm$ 3.68	1.37 $\pm$ 2.326	0.019	-3.19	5.93	-2.31 /	5.04 /
<b>LP</b>	16.3 $\pm$ 3.49						
<b>HP</b>	20.0 $\pm$ 5.43	-3.77 $\pm$ 4.470	<0.001	-12.53	4.99	-10.83 /	3.30 /
<b>LP</b>	16.3 $\pm$ 3.49						
<b>AP</b>	14.7 $\pm$ 3.68	1.53 $\pm$ 2.096	<0.001	-2.58	5.64	-1.78 /	4.85 /
<b>HP</b>	20.0 $\pm$ 5.43						
<b>AP</b>	14.7 $\pm$ 3.68	5.30 $\pm$ 4.779	<0.001	-4.07	14.67	-2.25 /	12.85 /
						-7.53	18.13

355 **Table 3.** Descriptive statistics and differences of IOP values between measurement points. All  
356 values in mmHg. n = 30. SD = Standard Deviation. IOP = intraocular pressure. 95 % LoAs = 95  
357 % Limits of Agreement. 95 % CI of LoAs = 95 % Confidence Interval of Limits of Agreement.  
358 LP = Low Pitch; HP = High Pitch; AP = After Playing.

359 **FIGURE LEGENDS**

360 Figure 1. Mean versus differences (Bland-Altman plot) between the values obtained in each  
361 measurement point in n = 30 participants. The thick solid horizontal line indicates the mean  
362 difference while the closely dashed horizontal lines the 95% LoAs (Mean difference  $\pm$   
363 1.96xSD). The widely dashed horizontal lines indicate the 95% Confidence Interval of the  
364 LoAs. A) Basal vs. Low pitch, B) Basal vs. High pitch, C) Basal vs. After playing, D) Low  
365 pitch vs. High pitch, E) Low pitch vs. After playing, F) High pitch vs. After playing. 95% LoAs  
366 = 95% Limits of Agreement. 95% CI = 95% Confidence Interval.