

# 1 QUALITY OF LIFE, SALIVARY CORTISOL AND 2 ATOPIC DISEASES IN YOUNG CHILDREN

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21

## 22 ABSTRACT

### 23 *Background*

24 Children with atopic disease may have reduced health-related quality of life (QoL) and morning  
25 cortisol. The link between QoL, cortisol and atopic disease is unclear.

26 We aimed to determine if QoL was associated with morning salivary cortisol at two years of age, and if  
27 asthma, atopic dermatitis and/or allergic sensitisation influenced this association. Secondly, we aimed  
28 to determine if QoL at one year of age was associated with salivary cortisol one year later.

29

### 30 *Methods and findings*

31 From the Bronchiolitis All SE-Norway study, enrolling infants during hospitalisation for acute  
32 bronchiolitis in infancy (bronchiolitis group) and population based control infants (controls), we  
33 included all 358 subjects with available Infant Toddler Quality of Life Questionnaire™ (ITQOL)  
34 consisting of 13 domains, and morning salivary cortisol measurements at two years of age. Additionally,  
35 QoL nine months after enrolment was available for 289 of these children at one year of age. Recurrent  
36 bronchial obstruction was used as an asthma proxy. Atopic dermatitis was defined by Hanifin and Rajka  
37 criteria and allergic sensitisation by a positive skin prick test. Associations between QoL and cortisol  
38 were analysed by multivariate analyses, stratified by bronchiolitis and control groups due to interaction.  
39 At two years of age, QoL was significantly associated with 8/13 QoL domains in the bronchiolitis  
40 group, but only with General health in the controls. The associations in the bronchiolitis group showed  
41 0.06-0.19 percentage points changes per nmol/L cortisol for each of the eight domains (p-values 0.0001-  
42 0.034). The associations for all domains remained significant, but were diminished by independently  
43 including recurrent bronchial obstruction and atopic dermatitis, but remained unchanged by allergic  
44 sensitisation.

45 In the bronchiolitis group only, 8/13 age and gender adjusted QoL domains in one-year old children  
46 were significantly associated with cortisol levels at two years (p= 0.0005-0.04).

47

48 *Conclusions:*

49 At two years, most QoL domains were associated with salivary cortisol in children who had been  
50 hospitalised for acute bronchiolitis in infancy, but for one domain only in controls. The associations  
51 were weakened, but remained significant by taking into account asthma and atopic dermatitis. The QoL  
52 in one-year old children was associated with salivary cortisol 10 months later.

53

54 **Abbreviations:**

55 QoL – Health related quality of life

56 ITQOL – The Infant Toddler Quality of Life Questionnaire

57 AD – Atopic dermatitis

58

59 **Key words:** Quality of life, morning salivary cortisol, allergic disease, children, acute bronchiolitis

60 **CONTRIBUTIONS BY EACH AUTHOR**

61 **Leif Bjarte Rolfjord, M.D.**, main author, has given substantial contributions to the conception and  
62 design of the work, acquisition of the data, analysis and interpretation of the data, drafted the work,  
63 finally approved the version to be published and agreed to be accountable for all aspects of the work in  
64 ensuring that questions related to the accuracy or integrity of any part of the work are appropriately  
65 investigated and resolved.

66 **Håvard Ove Skjerven, M.D., Ph.D.** is PI of the Bronchiolitis study, has given substantial  
67 contributions to the conception and design of the work and contributed to acquisition of the data for the  
68 work. He has revised it critically for important intellectual content. He has finally approved the version  
69 to be published and agreed to be accountable for all aspects of the work in ensuring that questions  
70 related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

71 **Egil Bakkeheim, M.D., Ph.D.** has given substantial contributions to the conception and design of the  
72 work, especially morning salivary cortisol data and contributed to analysis of the data for the work. He  
73 has revised it critically for important intellectual content. He has finally approved the version to be  
74 published and agreed to be accountable for all aspects of the work in ensuring that questions related to  
75 the accuracy or integrity of any part of the work are appropriately investigated and resolved.

76 **Teresa Løvold Berents, M.D., Ph.D.** has given substantial contributions to the conception and design  
77 of the work and contributed to acquisition of the data for the work, especially of atopic dermatitis data.  
78 She has revised it critically for important intellectual content. She has finally approved the version to be  
79 published and agreed to be accountable for all aspects of the work in ensuring that questions related to  
80 the accuracy or integrity of any part of the work are appropriately investigated and resolved.

81 **Kai-Håkon Carlsen, M.D., Ph.D.** has given substantial contributions to the analysis and interpretation  
82 of the data for the work. He has revised it critically for important intellectual content. He has finally

83 approved the version to be published and agreed to be accountable for all aspects of the work in  
84 ensuring that questions related to the accuracy or integrity of any part of the work are appropriately  
85 investigated and resolved.

86 **Karin C. Lødrup Carlsen, M.D., Ph.D.** has given substantial contributions to the conception, design,  
87 analysis and interpretation of the data of the work. She has given substantial contributions to drafting  
88 the work and has revised it critically for important intellectual content. She has finally approved the  
89 version to be published and agreed to be accountable for all aspects of the work in ensuring that  
90 questions related to the accuracy or integrity of any part of the work are appropriately investigated and  
91 resolved.

92 **Conflicts of interests:** Each named author has reported no conflicts of interest.

93

## 94 INTRODUCTION

95

96 Reduced health related quality of life (QoL) has been reported in children with asthma (1, 2) and atopic  
97 dermatitis (AD) (3) as well as in infants admitted to hospital for acute bronchiolitis (4-8), a disease  
98 known to increase the risk of later asthma (9, 10). Also, the generic Infant Toddler Quality of Life  
99 Questionnaire™ (ITQOL) has shown reduced QoL in young children with obstructive airways disease  
100 (11), AD (7) and other diseases (5), while reduced QoL also may be associated with psychological and  
101 physical stress (12).

102 Acute bronchiolitis may represent acute stress to the infant, as we recently showed that infants with  
103 moderate to severe acute bronchiolitis had higher morning salivary cortisol than healthy infants (13), in  
104 line with the higher morning salivary cortisol observed in children and adults during acute stress (14).  
105 Furthermore, the severity of acute bronchiolitis has been associated with plasma cortisol suppressing the  
106 T-helper cell type 1 (Th1) immune response (15), possibly leading to a shift to a Th2 response in acute  
107 bronchiolitis through inhibition of the interferon gamma response acting directly on T cells or indirectly  
108 through IL-12. Glucocorticoids may stimulate the secretion of IL-4 and IL-10, enhancing the Th-2  
109 response, and stimulate Th2-cells directly (16), as well as possibly suppressing Th2-inflammation (17).

110 The reduced basal morning cortisol levels observed in children with asthma, also without concurrent use  
111 of inhaled corticosteroids (ICS) (18), may on the other hand indicate chronic immunological stress. The  
112 subsequent blunted cortisol responses to acute stress in subjects with asthma related to a disturbance of  
113 the hypothalamus-pituitary-adrenal (HPA) axis differs from the chronic stress in non-atopic children  
114 that can lead to a higher cortisol response (19, 20). Similarly, a blunted cortisol response to acute stress  
115 has been suggested in children with AD (21). On the other hand, a higher morning salivary cortisol was  
116 observed in two year old children with AD and allergic sensitisation, while a non-significant tendency to  
117 lower cortisol was found in children with at least three wheeze episodes (22).

118 In adults with poorly controlled asthma, both reduced morning salivary cortisol as well as reduced QoL  
119 have been found (23), while possible links between QoL, cortisol and atopic disease in children are not  
120 known.

121 Morning salivary cortisol, reflecting the non-protein bound fraction of serum cortisol (24, 25) was  
122 recently shown to be similar in two year old children with and without acute bronchiolitis in infancy  
123 (13) with values ranging from 2.5 to 189.0 nmol/L, and with higher levels in girls than in boys.

124 Based upon our previous findings of high morning cortisol levels during acute bronchiolitis but with  
125 levels at two years of age similar to those of controls (13), we hypothesised that *low* cortisol levels in  
126 periods without acute illness may contribute to development of asthma. We further hypothesised that  
127 reduced QoL some months after severe acute illness in early life may be a marker of chronic stress, with  
128 subsequent lower future salivary cortisol levels.

129 We therefore primarily aimed to determine if QoL was associated with morning salivary cortisol at two  
130 years of age, and if asthma, atopic dermatitis and/or allergic sensitisation modified this association.  
131 Secondly, we aimed to determine if QoL at one year of age was associated with salivary cortisol at  
132 two years.

133

## 134 MATERIALS AND METHODS

### 135 Study design

136 From the source population of 644 children included in the Bronchiolitis ALL SE-Norway study  
137 enrolling infants who were hospitalised for acute bronchiolitis and controls recruited from a general  
138 population (7), we included all 358 children with available salivary cortisol and QoL at 24 months of  
139 age. The bronchiolitis group consisted of 203 infants with moderate to severe acute bronchiolitis at  
140 inclusion, and 155 were controls. For details, see figure 1 and Supporting Information.

### 141 Legend Figure 1:

142 The figure outlines the number of infants enrolled in the Bronchiolitis All SE-Norway study (top,  
143 n=644) who were subsequently included in the present study (n=358) for analyses based upon available

144 Quality of life (QoL) and/or salivary morning cortisol at 24 months of age. The QoL questionnaires  
145 were completed nine months after enrolment at approximately 14 months of age (QoL<sub>1</sub>) as well as at the  
146 time of the clinical examination at 24 months of age (QoL<sub>2</sub>).

147

148 Investigations at enrolment and at two years of age included clinical assessment, structured parental  
149 interviews and morning salivary sampling for cortisol, whereas skin prick test (SPT) for common  
150 inhalant and food allergens was performed at two years only. Quality of life questionnaires were  
151 completed nine months after enrolment (QoL<sub>1</sub>) (7, 8) and at two years of age (QoL<sub>2</sub>).

152 Caregivers of all children signed the informed written consents prior to study enrolment. The study was  
153 approved by the Regional Committee for Medical and Health Research Ethics and The Norwegian Data  
154 Protection Authority and registered in the Norwegian bio bank registry. The randomised clinical trial  
155 part of the study was registered in Clinical Trials.gov, no. NCT00817466 (26).

## 156 **Study subjects**

157 The mean (range) age of the 358 children in the present study was 5.2 (0.2-13.4) months at enrolment  
158 and 24.2 (17.6-34.7) months at the two-year investigation. The children in the bronchiolitis group  
159 compared to controls were shorter, more often exposed to second-hand smoke at inclusion and their  
160 parents had lower income, lower educational attainment and less often allergic rhinitis or AD (Table 1).

161

162 **Table 1**

163 Characteristics and asthma risk factors of the children at two years of age. All data are given as n and %,  
 164 unless otherwise stated. The control group is the group from the reference population.

	Bronchiolitis group N=203	Control group N=155
Boys n (%)	117 (57.6%)	89 (57.4%)
Age months, mean (SD)	24.2 (3.2)	24.3 (3.7)
Weight kg, mean (SD)	13.2 (1.6)	12.9 (1.5)
Length cm, mean (SD)	87.0 *** (4.1)	88.7(4.2)
Breastfeeding at enrolment n (%)	149 (73.4%)	122 (78.7%)
Second-hand smoke exposure in infancy n (%)	25 (14.4%)**	5 (3.3%)
Second-hand smoke exposure at 2 years	5 (2.5%)	1 (0.7%)
Atopic manifestations defined at 2 years		
At least one n (%)	103 (50.7%)	37 (23.9%)
Two or more n (%)	19 (9.4%)	25 (7.3%)
rBO (at least 3 wheeze episodes) n (%)	98 (48.3%***)	22 (14.2%)
Atopic dermatitis at 2 years n (%)	30 (14.8%)	25 (16.1%)
Allergic sensitisation <sup>1</sup> n (%)	17 (8.4%)	11 (7.4%)
At least one parent asthma n (%)	35 (22.2%)	46 (29.7%)
At least one parent AD n (%)	33 (18.2%)*	46 (29.7%)
At least one parent allergic rhinoconjunctivitis n (%)	62 (34.4%***)	84 (54.2%)
Higher education mothers <sup>2</sup> n (%)	122 (70.1%***)	142 (91.6%)
Higher education fathers <sup>2</sup> n (%)	100 (57.8%***)	129 (83.8%)
Income mothers <sup>3</sup> , mean (SD)	1.92 **	2.13
Income fathers <sup>3</sup> , mean (SD)	2.32 ***	2.59
Caucasian mother n (%)	189 (93.6%)	147 (94.8%)
Caucasian father n (%)	191 (95.0%)	143 (92.3%)

165 <sup>1</sup>Allergic sensitisation was define by at least one positive skin prick test to common inhalant and food  
 166 allergens

167 <sup>2</sup>Higher education at least three years after secondary school

168 <sup>3</sup>Annual income before tax. 1: <300.000 NOK. 2: 300.000-500.000 NOK. 3: >500.000 NOK.

169 \* p<0.05 \*\* p<0.01 \*\*\*<0.001

170

171

172 **Methods**

173 Atopic manifestations determined at two years of age, consisted of recurrent bronchial obstruction  
 174 (rBO) as a proxy for asthma, atopic dermatitis and allergic sensitisation.

175 *Recurrent bronchial obstruction* was defined as at least three parentally reported episodes of wheeze at  
 176 two years of age, in line with previous reports (27), with acute bronchiolitis included as one episode.



177 *Atopic dermatitis* was defined based upon the modified Hanifin and Rajka's criteria (yes or no) (28),  
178 and severity by the SCORing AD index (SCORAD) (see Supporting Information for details).

179 *Allergic sensitisation*, determined by SPT using 17 common inhalant and food allergens with Soluprick  
180 SQ allergen extracts, ALK, Hørsholm, Denmark, was defined as positive with at least one mean wheal  
181 diameter at least 3 mm greater than the negative control. Further details are given in the Supporting  
182 Information.

183 *Morning salivary cortisol* was sampled by the parents on the first morning after enrolment in the  
184 bronchiolitis group, otherwise at home and brought to the investigation centre. Two Sorbette®  
185 hydrocellulose microsponges were applied in the child's mouth as soon as possible after their child's  
186 first awakening after 6:00 a.m., before the first meal, and placed in appropriate prepared containers, as  
187 described elsewhere (13). The samples were stored at -86°C and later analysed at Karolinska Institutet,  
188 Stockholm, with radioimmunoassay with monoclonal rabbit antibodies Codolet, France).

189 *The Infant Toddler Quality of Life™ Questionnaire (ITQOL-97)* (11) completed by the parents included  
190 97 questions within 13 domains scored from 0 (worst) to 100 (best), with no overall score. Accordingly,  
191 a change in QoL score is equivalent to the percentage point score change. The Overall health domain  
192 consisted of only one item: Is your child's health excellent, very good, good, fair or poor? In line with  
193 others (29) and as previously reported (7, 8), with permission from the copyright holder, we recoded the  
194 domain Change in health from the original scores from 1-5 to 0-100 (zero meaning worst deterioration  
195 of health from one year ago, 50 meaning no change). Four domains (Change in health, General  
196 behaviour, Overall behaviour and Getting along) were recorded in children older than 12 months only  
197 (7).

## 198 **Study outcomes and explanatory variables**

199 The main outcome for our primary aim, QoL<sub>2</sub>, was reported by quantitative values per domain, and  
200 secondarily by the number of domains with significantly reduced QoL<sub>2</sub> scores.

201 The main explanatory variables for the primary aim were morning salivary cortisol, and the three atopic  
202 manifestations rBO, AD and allergic sensitisation at two years of age. Further analyses reported in  
203 Supporting Information substituted the respective atopic manifestations by quantitative measures, i.e.  
204 the total number of wheeze episodes, the AD severity score SCORAD and the sum of SPT wheal  
205 diameters for influence on the associations between morning salivary cortisol and QoL<sub>2</sub>.

206 The main outcome of the secondary aim was morning salivary cortisol, with QoL<sub>1</sub> as the explanatory  
207 factor.

### 208 **Statistical analysis**

209 The bronchiolitis and control groups were compared by Pearson's chi-square tests for categorical data  
210 and Student's T- test for normally distributed numerical data, and otherwise with Welch test, unless  
211 otherwise stated.

212 Due to non-normality of results and residuals, we used linear robust regression by Huber's M method  
213 (30), for analyses including QoL and cortisol. To estimate the relative influence by rBO, AD and  
214 allergic sensitisation on QoL<sub>2</sub>, we calculated the percent point change equivalent to the difference in  
215 score for each QoL domain, given per nmol/L change in cortisol. For comparison, we calculated the  
216 difference in each QoL domain score that was attributed to a difference in salivary cortisol level of 95<sup>th</sup>  
217 versus 5<sup>th</sup> percentile (QoL score at the salivary cortisol level of 95<sup>th</sup> percentile minus QoL score at the  
218 5<sup>th</sup> percentile). Salivary cortisol was studied as a continuous variable, and presented graphically by  
219 quartiles.

220 Each atopic manifestation was included in robust regression models to assess their potential influence  
221 on both cortisol and QoL<sub>2</sub>, as well as the associations between the two (see Figure 2, hypothesis). For  
222 graphical presentations of QoL versus cortisol levels and cortisol levels versus atopic manifestations we  
223 used data unadjusted for age and gender. In line with previously demonstrated associations between  
224 morning salivary cortisol and age as well as gender (13), we decided a priori to analyse age and gender  
225 adjusted associations between cortisol and QoL as well between QoL and atopic manifestations. The

226 atopic manifestations were not considered to be confounders, as they could be causally associated with  
227 both cortisol and QoL<sub>2</sub>.

228 **Legend Figure 2:**

229 Directed acyclic graph showing hypothesised influence on cortisol and QoL<sub>24m</sub> by allergic diseases  
230 above the red line, and observed influence in the bronchiolitis group below the red line. The red line  
231 indicates the net result from the influence of allergic disease on the association between morning  
232 salivary cortisol and QoL<sub>24m</sub>

233

234 Using QoL<sub>2</sub> as dependent variable in two-way regression analyses, we tested for interactions between  
235 the group affiliation (bronchiolitis or controls) and cortisol, as well as between atopic manifestations  
236 and cortisol. Due to interactions between group affiliation and salivary cortisol as well as atopic  
237 dermatitis, analyses were stratified by group affiliation.

238 Possible confounding was assessed by robust regression and considered relevant if the outcome of the  
239 model was changed by at least 25% (31) by any of the possible confounders (socioeconomic factors,  
240 parental allergic disease, secondary smoke). Confounding by socioeconomic factors was tested by  
241 including these factors in multiple regression models, and eliminating the factors with highest p-values  
242 stepwise by Hosmer's procedure (31) until only factors with p-values < 0.05 remained, retaining age  
243 and gender.

244 The level of statistical significance was set to p<0.05 for all analyses.

245 Analyses were performed with the IBM SPSS Statistics 21 (IBM Corporation, Armonk, New York,  
246 USA), and the NumberCruncher Statistical System (NCSS Kaysville, Utah, USA), version 11.

## 248 RESULTS

249

### 250 Atopic manifestations and QoL<sub>2</sub>; bronchiolitis group vs. controls

251 Children in the bronchiolitis group were significantly more often affected by at least one atopic

252 manifestation at two years and had more often rBO than the controls, while AD was similar in the two

253 groups (Table 1).

254 The QoL<sub>2</sub> scores varied from 0-100 in five domains, with the smallest score range seen in the domain

255 Getting along (53.3), as shown in Table 2. The bronchiolitis group reported larger improvement in

256 health (Change in health), while controls scored significantly higher for Overall health and General

257 health (Table 2).

#### 258 Table 2

259 Unadjusted weighted means (95% CI) of QoL at two years of age (QoL<sub>24m</sub>) of children included at  
260 hospitalisation for acute bronchiolitis and control children

Domain	Previous bronchiolitis	Control children	All children
	Unadjusted weighted means (95% CI)		Median (min, max)
Overall health	83.4 (81.3, 85.5)**	88.7 (86.3, 91.1)	85.0 (0.0, 100.0)
Physical abilities	100.0 (100.0, 100.0)	100.0 (100.0, 100.0)	100.0 (0.0, 100.0)
Growth and development	94.7 (93.7, 95.6)	95.2 (94.1, 96.3)	97.2 (0.0, 100.0)
Bodily pain/ discomfort	80.5 (78.4, 82.6)	78.5 (76.0, 80.9)	75.0 (8.3, 100.0)
Temperament and moods	84.2 (83.0, 85.4)	83.0 (81.7, 84.4)	84.7 (36.8, 100.0)
General behaviour	84.5 (82.9, 86.0)	85.2 (83.4, 86.9)	85.4 (35.4, 100.0)
Overall behaviour	85.0 (85.0, 85.0)	85.0 (85.0, 85.0)	85.0 (30.0, 100.0)
Getting along	78.8 (77.6, 80.0)	78.5 (77.2, 79.9)	78.3 (45.0, 98.2)
General health	67.1 (65.0, 69.2)****	78.3 (75.9, 80.7)	75.0 (18.2, 100.0)
Change in health	65.2 (62.8, 67.8)****	56.9 (54.1, 59.7)	50.0 (0.0, 100.0)
Parental impact – emotions	91.3 (90.1, 92.6)	91.3 (89.9, 92.7)	92.9 (35.7, 100.0)
Parental impact – time	95.2 (94.2, 96.1)	94.2 (93.1, 95.3)	95.2 (28.6, 100.0)
Family cohesion	79.6 (77.3, 82.0)	80.8 (78.1, 83.5)	85.0 (0.0, 100.0)

261 \*\*\*\*p<0.0001

262

### 263 Quality of life and salivary cortisol at two years of age

264 In the bronchiolitis group, eight QoL<sub>2</sub> domains were significantly and positively associated with

265 morning salivary cortisol (p=0.0001-p=0.035), see Table 3 and Figure 3. The association between  
 266 Overall health and salivary cortisol was significant only in boys, (p<0.0001).

267 **Table 3**

268 The potential influence of recurrent bronchial obstruction (rBO), atopic dermatitis (AD) and allergic  
 269 sensitisation (AS) on the associations between Quality of Life (QoL<sub>2</sub>) and salivary cortisol at two years  
 270 of age is shown for 203 children who had moderate to severe acute bronchiolitis in infancy.

271 The influence by including each atopic manifestation (rBO, AD and AS) is shown as the percentage  
 272 change of QoL per 1 nmol/L change in salivary cortisol, adjusted for age and gender. Each column  
 273 includes all children with the observed atopic manifestation, and they are not mutually exclusive.

Domain (Mean domain score difference <sup>1</sup> by difference between 95 <sup>th</sup> and 5 <sup>th</sup> percentile of cortisol, 51.6 nmol/L)	Change in QoL <sub>2</sub> score per nmol/L unit salivary cortisol	rBO % change in association	AD	Allergic sensitisation
Overall health <sup>2</sup> boys (16.0)	0.31 (0.17, 0.45)****	-20.7	-2.1	-0.5
Overall health girls (-0.0)	-0.00 (-0.16, 0.16)			
Growth and development (3.8)	0.07 (0.02, 0.13)**	-1.4	1.7	0.6
Bodily pain/ discomfort (6.2)	0.12 (0.01, 0.23)*	-8.3	5.4	-2.3
Temperament and moods (6.1)	0.12 (0.06, 0.18)***	-6.9	1.4	-1.5
General behaviour (4.6)	0.09 (0.01, 0.17)*	-6.7	-1.5	1.3
Getting along (4.0)	0.08 (0.02, 0.13)**	-3.0	-0.3	0.9
General health (5.6)	0.11 (-0.00, 0.22) <sup>3</sup>	-26.9	0.4	0.3
Parental impact –Emotions (4.5)	0.09 (0.3, 0.15)**	-6.1	-5.1	1.2
Parental impact – Time (3.0)	0.06 (0.01, 0.10)*	-7.7	-0.1	0.9

274 <sup>1</sup>QoL score difference equals percentage point difference.

275 <sup>2</sup>Overall health was gender stratified due to interaction.

276

277 \*p<0.05 \*\*p<0.01 \*\*\* p<0.001 \*\*\*\*p<0.0001

278 <sup>3</sup>p=0.0517

279

280 **Legend Figure 3:**

281 Bronchiolitis group: QoL<sub>2</sub> scores for each domain, unadjusted, for each quartile of morning salivary  
 282 cortisol, 1<sup>st</sup> quartile lowest cortisol, 4<sup>th</sup> quartile highest. Due to interaction between gender and cortisol  
 283 for the Overall health domain, this domain was analysed separately for the genders. An association was  
 284 found only for boys for this domain. For Overall health, results for boys are shown. For the other  
 285 domains, results for both genders analysed together are shown.

286

287

288 In the controls, General health only was significantly associated with cortisol. The significant increase  
289 of 0.1 percentage point per nmol/L in cortisol level (95% CI 0.0, 0.2,  $p=0.046$ ) corresponded to a QoL<sub>2</sub>  
290 difference of five percent points between children having cortisol levels at the 5<sup>th</sup> vs 95<sup>th</sup> percentile (a  
291 difference of 51.6 nmol/L of salivary cortisol). No further analyses were performed in this group, with  
292 only one QoL domain significantly associated with salivary cortisol.

293 The hypothesised (top) and observed (bottom) influence of atopic manifestations on cortisol and QoL<sub>2</sub>  
294 are shown schematically in Figure 2. As shown in Table 2, the strongest influence on the associations  
295 between cortisol and QoL<sub>2</sub> was exerted by rBO, reducing the associations with 1.4 to 26.9 per cent,  
296 followed by changes related to AD ranging from -5.5 to 5.1 and less than 3 per cent changes by allergic  
297 sensitisation. However, all associations between QoL and cortisol remained significant after including  
298 rBO, AD and allergic sensitisation into the regression analyses.

299 Finally, we found no significant confounding effect of socioeconomic factors, parental ethnicity and  
300 second-hand smoke at two years of age, and these were consequently not included in the final  
301 multivariate analyses (see Supporting Information, Table 4 for details).

302

303 **Table 4**

304 Bronchiolitis group: Change of associations between salivary morning cortisol at and QoL24m at two  
 305 years of age by socioeconomic factors, including age, gender, and the following socioeconomic factors:  
 306 mother's education, father's education, mother's income, father's income, ethnicity of father and of  
 307 mother (Caucasian or not) and secondhand smoke exposure at two years of age. The socioeconomic  
 308 factors have been eliminated by Hosmer's stepdown procedure, finally retaining factors with  $p < 0.05$ .  
 309 Age and gender have been retained in the models

Adjusted for/domain	Change of QoL score per nmol/L changed salivary cortisol after adjustment	% influence on change of QoL score by adjustment	Socioeconomic factors retained in the model
Overall Health	0.15 (0.05, 0.26)**	-16.7%	Caucasian father <sup>1</sup> ****
Growth and Development	0.07 (0.02, 0.13)*	-3.7%	Caucasian father <sup>1</sup> *
Bodily Pain/ Discomfort	0.12 (0.01, 0.23)*		All factors insignificant; eliminated from model
Temperament and Moods	0.11 (0.05, 0.17)***	-8.1%	Caucasian mother <sup>1</sup> **
General Behaviour	0.08 (0.00, 0.16)*	-10.9%	Caucasian mother <sup>1</sup> **
Getting Along	0.06 (0.01, 0.12)*	-18.8%	Education mother <sup>1</sup> ** , education father <sup>2</sup> ** , Caucasian mother <sup>1</sup> **
Parental Impact -Emotions	0.08 (0.02, 0.14)*	-11.7%	Income father <sup>1</sup> * , Caucasian mother <sup>1</sup> ****
Parental Impact - Time	0.05 (0.01, 0.10)*	-13.1%	Caucasian mother <sup>1</sup> * Caucasian father <sup>1</sup> **

310

311 <sup>1</sup> positively associated with QoL domain

312 <sup>2</sup> negatively associated with QoL domain

313 \* $p < 0.05$  \*\* $p < 0.01$  \*\*\* $p < 0.001$  \*\*\*\* $p < 0.0001$

314

315 **Salivary cortisol in bronchiolitis group vs. controls, and atopic manifestations**

316 The age and gender adjusted salivary cortisol levels at two years were similar in the bronchiolitis group  
317 and controls. Weighted mean difference was -0.70 (95% CI -3.7, 2.3) nmol/L.

318 Salivary cortisol was significantly lower in children with rBO vs. children without rBO for the  
319 bronchiolitis group and controls together (weighted mean difference -4.1 (95%CI -7.3,-1.0) nmol/L), as  
320 shown schematically in Figure 2, and in unadjusted analysis in Figure 4. Neither AD nor allergic  
321 sensitisation was significantly associated with morning salivary cortisol at two years of age (Figures 1  
322 and 3).

323 **Legend Figure 4:**

324 Morning salivary cortisol in children of the bronchiolitis group and controls together, without recurrent  
325 bronchial obstruction (rBO), defined as at least three wheeze episodes, compared to with rBO, no atopic  
326 dermatitis (AD) vs. with AD as well as with no or any positive skin prick test (SPT) to common inhalant  
327 and food allergens.

328

329 **QoL<sub>2</sub> and atopic manifestations**

330 The QoL<sub>2</sub> was significantly associated with rBO and AD in the bronchiolitis group, and with rBO as  
331 well as allergic sensitisation in the controls, as shown in Table 5, referring to absolute changes (=   
332 percentage point changes) in QoL<sub>2</sub> scores by the atopic diseases.

333



334 **Table 5**

335 **The impact of allergic diseases on QoL<sub>2</sub> is given for each domain as mean (95% CI), adjusted for age and**  
 336 **gender, given for infants with moderate to severe bronchiolitis in infancy compared to controls.**

337 As an example; the negative association of General health (GH) with rBO is stronger in the bronchiolitis group  
 338 than among controls, both being statistically significant.

339

	Recurrent bronchial obstruction		Atopic dermatitis		Allergic sensitisation	
	bronchiolitis group	controls	bronchiolitis group	controls	bronchiolitis group	controls
<b>OH</b>	-12.3 (-16.5, -8.2)****	-6.6 (-13.9, 0.7)	-5.5 (-11.7, 0.6)	-6.3 (-13.6, 0.9)	-0.7 (-8.6, 7.2)	-11.7 (-21.6, -1.9)*
<b>PA</b>	-1.2 (-2.1, -0.4)**	Not done <sup>3</sup>	-0.0 (-0.0, 0.0)	-2.1 (-3.2, -1.0)***	0.0 (-0.2, 0.2)	Not done <sup>7</sup>
<b>GD</b>	-3.5 (-5.8, -1.2)**	-1.3 (-4.3, 1.8)	-2.5 (-5.8, 0.7)	-1.2 (-4.2, 1.8)	-0.1 (-4.3, 4.0)	-1.9 (-6.4, 2.6)
<b>BD</b>	-6.5 (-10.8, -2.1)**	-7.3 (-14.7, 0.1)	-7.2 (-13.4, -0.9)*	-1.7 (-8.6, 5.3)	4.2 (-3.6, 12.1)	-6.4 (-16.8, 4.0)
<b>TM</b>	-3.1 (-5.5, -0.7)*	-4.1 (-7.8, -0.5)*	-5.1 (-8.4, -1.8)**	1.0 (-2.6, 4.5)	-2.5 (-1.8, 6.8)	-1.3 (-6.5, 3.9)
<b>GB</b>	-4.7 (-7.9, -1.4)**	-0.7 (-5.5, 4.1)	-5.3 (-9.9, -0.7)*	-4.1 (-8.8, 0.6)	-0.8 (-6.8, 5.2)	-7.0 (-13.7, -0.3)*
<b>OB</b>	-0.0 (-0.0, 0.0)	Not done <sup>4</sup>	0.0 (0.0, 0.0)	Not done <sup>5</sup>	0.0 (-0.0, 0.0)	Not done <sup>7</sup>
<b>GA</b>	-1.9 (-4.2, 0.4)	-4.0 (-7.9, -0.0)*	-6.2 (-9.3, -3.0)***	-0.9 (-4.8, 2.9)	-0.4 (-4.6, 3.8)	-8.0 (-13.6, -2.4)**
<b>GH</b>	-13.8 (-17.8, -9.8)****	-6.8 (-12.9 -0.7)*	-5.8 (-12.0, 0.3)	-7.0 (-13.0, -1.0)*	0.1 (-7.9, 8.1)	-12.2 (-20.5, -3.9)**
<b>CH</b>	6.9 (0.8, 13.0)*	6.8 (-0.6, 14.2)	7.8 (-0.9, 16.4)	3.1 (-3.3, 9.6)	-1.0 (-12.3, 10.3)	7.3 (-2.8, 17.4)
<b>PE</b>	-4.0 (-6.4, -1.5)**	-2.3 (-5.9, 1.4)	-5.7 (-9.2, -2.2)**	-1.6 (-5.2, 2.0)	-0.2 (-4.7, 4.3)	-5.8 (-10.7, -1.0)*
<b>PT</b>	-2.5 (-4.5, -0.5)*	-2.1 (-5.0, 1.0)	-1.9 (-4.8, 1.0)	-1.9 (-4.9, 1.0)	-0.0 (-3.7, 3.7)	-4.3 (-8.3, -0.2)*
<b>FC</b>	-0.1 (-4.7, 4.5)	0.3 (-7.4, 8.0)	-5.4 (-11.9, 1.1)	0.7 (-6.7, 8.1)	-0.0 (-3.7, 3.7)	-1.8 (-12.3, 8.7)

340

341 <sup>3</sup>Not done because gender is highly correlated with other X's <sup>4</sup>Not done because rBO is highly correlated with  
 342 other X's <sup>5</sup>Not done because AD is highly correlated with other X's <sup>6</sup>Not done, procedure call invalid <sup>7</sup>Not  
 343 done because AS is highly correlated with other X's

344

345 OH = Overall health; PA = Physical abilities; GD = Growth and development; BD = Bodily pain/ discomfort; TM  
 346 = Temperament and moods; GB = General behaviour; OB = Overall behaviour; GA = Getting along; GH =  
 347 General health; CH = Change in health; PE = Parental impact – emotions; PT = Parental impact – time; FC =  
 348 Family cohesion

349

350 **Association between QoL<sub>1</sub> and salivary cortisol at two years**

351 In the bronchiolitis group only, QoL<sub>1</sub> was significantly and positively associated with morning salivary  
 352 cortisol at two years of age in age and gender adjusted analysis for 8/13 domains, as shown in Table 6.

353

354 **Table 6**

355 Associations between QoL<sub>1</sub> (quality of life at a mean age of 14 months) scores and subsequent cortisol  
356 at visit 2, at a mean age of 24 months, bronchiolitis group only, adjusted for age at the two year old visit  
357 and gender.

	Change in cortisol nmol/L per QoL14m score change
358 Overall health	0.17 (0.02, 0.32)*
359 Physical abilities	0.92 (0.41, 1.43)***
360 Growth and development	0.34 (0.09, 0.60)**
361 Temperament and moods	0.35 (0.12, 0.59)**
362 General health	0.17 (0.01, 0.33)*
363 Parental impact - emotions	0.45 (0.19, 0.71)***
364 Parental impact - time	0.28 (0.11, 0.46)**

366

367

368 \*p<0.05 \*\*p<0.01 \*\*\*p<0.001

369

## 370 DISCUSSION

371 Quality of life was significantly associated with morning salivary cortisol at two years of age for most  
372 domains among children hospitalised with acute bronchiolitis in infancy, i.e. low cortisol was associated  
373 with low QoL, and high cortisol with high QoL. Significant associations were observed for the General  
374 Health domain only in controls. The associations remained significant, but were weakened by rBO, with  
375 only limited or no significant influence by AD or allergic sensitisation. The QoL in one-year old  
376 children was associated with salivary cortisol at two years of age.

377 We are not aware of other studies comparing QoL and morning salivary cortisol in children. Our  
378 findings that after moderate to severe acute bronchiolitis in infancy, QoL<sub>2</sub> was associated with salivary  
379 cortisol at two years of age, is to our knowledge novel. The results could not be confirmed in the  
380 population based controls. We have previously found that infants with acute bronchiolitis have higher  
381 morning salivary cortisol than controls (13), indicating acute stress. Others have found other signs of  
382 acute stress in acute bronchiolitis with respiratory syncytial virus, differing from other infections and  
383 acute diseases (32). Reduced QoL, found after acute bronchiolitis (5, 33), may partly be expressions of  
384 chronic stress, not only physical, but possibly psychological stress. Concerns of the parents of the  
385 children of the bronchiolitis group, as indicated by the Parental impact – emotions and Parental impact –  
386 time domains in the present study, seem to be associated with the children's cortisol levels. The  
387 associations that we found after acute bronchiolitis between cortisol and QoL in domains reflecting  
388 expressions of pain, moods and behaviour, i.e. Bodily pain/ discomfort, Temperament and moods,  
389 General behaviour and Getting along, partly influenced by rBO and AD, may also indicate a role of  
390 psychological stress in the development of atopic disease. Other studies have shown that pre- and  
391 postnatal maternal distress and negative life events are associated with allergic disease in children (19,  
392 34). This may suggest long term effects on QoL of severe lower respiratory infection in infancy,  
393 possibly through chronic stress which has been negatively associated with morning cortisol (18, 35).  
394 The 16 percentage point difference in Overall health in boys with low versus high salivary cortisol is  
395 likely to be clinically relevant as they are comparable to the eight percentage point General health

396 differences between children with and without asthma-like symptoms reported from the Generation R  
397 study (36).

398 The observed association between Overall health and morning salivary cortisol at two years of age was  
399 significant among both genders analysed together, but only in boys by gender stratified analyses. This  
400 may be explained by our significantly higher salivary cortisol levels in girls compared to boys in the  
401 Bronchiolitis ALL study reported previously (13).

402 The influence by rBO, and to a lesser extent AD, on the associations between QoL and salivary cortisol  
403 in children who were hospitalised for acute bronchiolitis in infancy in our study point to complex  
404 associations between allergic diseases, QoL and salivary cortisol. This is in line with attenuated  
405 regulation of the HPA axis previously shown in children with chronic atopic diseases, with a reduced  
406 cortisol after acute stress provocation, indicating chronic stress (16, 19, 35, 37) and reduced morning  
407 salivary cortisol observed in adults (23) and children (18) with poor asthma control. Our previous  
408 findings of increased morning salivary cortisol during acute bronchiolitis in infancy (13) and reduced  
409 QoL nine months later (7), were extended in the present study, showing that rBO, but not one episode of  
410 bronchiolitis (13) was associated with lower morning salivary cortisol at two years of age. This may  
411 indicate that repeated episodes of bronchial obstruction are necessary to affect cortisol levels, possibly  
412 through chronic stress (13, 15, 19), irrespective of current use of inhaled corticosteroids (23). On the  
413 other hand, our study was not designed to identify the reverse possibility of a potential causal role of  
414 low salivary cortisol levels outside acute respiratory disease for the development of recurrent bronchial  
415 obstruction.

416 The influence of AD on the associations between QoL and cortisol in our study was less clear, possibly  
417 because most children with AD had mild disease. However, supporting our findings of some influence  
418 on the cortisol-QoL association, Stenius et al. reported higher morning salivary cortisol in two year old  
419 children with atopic dermatitis as opposed to children with recurrent wheeze who had a non-significant  
420 tendency to lower salivary cortisol (22). The possible opposite association between rBO and AD with  
421 cortisol in early childhood is not clear, since both diseases have been associated with reduced QoL.

422 The lack of significant associations between allergic sensitisation and QoL in the bronchiolitis group  
423 and allergic sensitisation and salivary cortisol may have several explanations. In our study less than 10  
424 per cent of the subjects were sensitised to at least one allergen, limiting the likelihood of observing  
425 significant associations. On the other hand, allergic sensitisation may not affect QoL before allergen  
426 exposure causes symptoms, which for inhalant allergens occur more frequently with increasing age (38).

427 Our finding that reduced QoL about one year of age was associated with lower salivary cortisol at two  
428 years of age is to our knowledge also novel. We recently showed in the same study population that in  
429 addition to having been hospitalised for acute bronchiolitis, disease severity and asthma risk factors as  
430 well as AD were associated with reduced QoL at 14 months of age (7, 8).

431 The direct clinical implications of our findings remain unclear at present. The influence by our asthma  
432 proxy of rBO dominated the association between QoL and salivary cortisol, weakening most of the  
433 associations by more than five per cent. This finding is supported by a stronger influence by the total  
434 number of wheeze episodes, as shown in the Supporting Information. The maintenance of statistical  
435 significance of the influence of cortisol on QoL after including rBO in the regression model also  
436 indicates an additive negative effect of low cortisol and rBO on QoL. The implications in terms of the  
437 Overall health domain could be shown by a 24-months-old boy with rBO and low salivary cortisol, at  
438 the 5<sup>th</sup> percentile, having an estimated 23.1 percentage point lower QoL than a boy without rBO who  
439 had a high salivary cortisol level, at the 95<sup>th</sup> percentile. However, our study suggests that in addition to  
440 rBO and to some extent AD, also acute moderate to severe infant bronchiolitis may play a role in the  
441 association between future salivary cortisol and QoL. Although acute infant bronchiolitis per se may not  
442 lead to future low cortisol levels, QoL was reduced both at 14 and 24 months of age compared to  
443 controls. Although the influence of the asthma proxy of at least three episodes of bronchial obstruction  
444 in our study dominated the association between cortisol and QoL at two years, the associations were  
445 significant also among children in the bronchiolitis group who did not go on to develop rBO, as shown  
446 in Supporting Information. Together these observations suggest that children who have acute  
447 bronchiolitis in infancy and reduced QoL some months later may be at increased risk of chronic stress,

448 observed by lower salivary cortisol and reduced QoL at two years of age. Thus, our study support a role  
449 of chronic stress indicated by lower cortisol levels in development of asthma, but with unclear links to  
450 atopic dermatitis and allergic sensitisation in young children.

451 In line with previous studies finding marginally lower cortisol in adolescents with low socioeconomic  
452 status (39), we included socioeconomic data as well as second-hand smoking into regression analyses.  
453 However, none of these factors were found to be significant confounders, possibly reflecting the  
454 overriding effects by atopic diseases in the children, as well as a low frequency of second-hand smoke  
455 in our cohort.

#### 456 *Strengths and limitations*

457 The study strengths include a prospective design of a reasonably large group of children included in  
458 infancy with and without acute bronchiolitis and atopic disease, a high rate of follow-up investigations,  
459 repeated measurements and stringent clinical characterisation of the subjects. Also, the findings appear  
460 robust, as the associations remained significant after relevant adjustments.

461 The lack of significant associations between QoL and salivary cortisol in the control group may be due  
462 to the relatively few subjects with recurrent bronchial obstruction, shown to be most consistently  
463 associated with reduced QoL and salivary cortisol, and that the control children may be more  
464 heterogeneous, possibly with a lower risk of future asthma development, or that the control children in  
465 general had a higher QoL.

466 As previously reported (7, 8), we decided a priori not to adjust for multiple analyses, as the QoL  
467 domains were not independent from each other. Also, the associations with the different QoL domains  
468 point in the same direction, limiting the likelihood of incidental findings. The only domain pointing in  
469 the opposite direction was Change in health, where a lower score reflects less improvement from an  
470 earlier time point. This domain has been shown to be higher in children with chronic diseases than  
471 general population children (5), and was the only domain with higher scores in the bronchiolitis than the  
472 control group in our study.

473 The rate of AD and allergic sensitisation was high among the controls, possibly reflecting that parents  
474 with some atopic manifestation were more likely to enrol their child into the study.

475 The use of a single morning salivary cortisol measurement to improve feasibility of a high data  
476 collection may be a limitation of our study. However, previous studies of single morning measurements  
477 (18) and the lack of significant day-to-day variation between three samples taken at 4- to 8-day intervals  
478 (40), suggest that single measures may reflect the habitual morning cortisol state. Also, we sampled as  
479 soon as possible after the first awakening after 6:00 a.m. (13), to encompass a possible morning  
480 awakening response and the top circadian morning value (25).

## 481 **Conclusion**

482 At two years of age QoL was positively associated with morning salivary cortisol in children who had  
483 undergone moderate to severe acute bronchiolitis in infancy. The associations were influenced by  
484 recurrent bronchial obstruction and, to a limited extent, by atopic dermatitis. The QoL in one-year-old  
485 children was associated with salivary cortisol 10 months later.

486

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493

494

495

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Figure 2

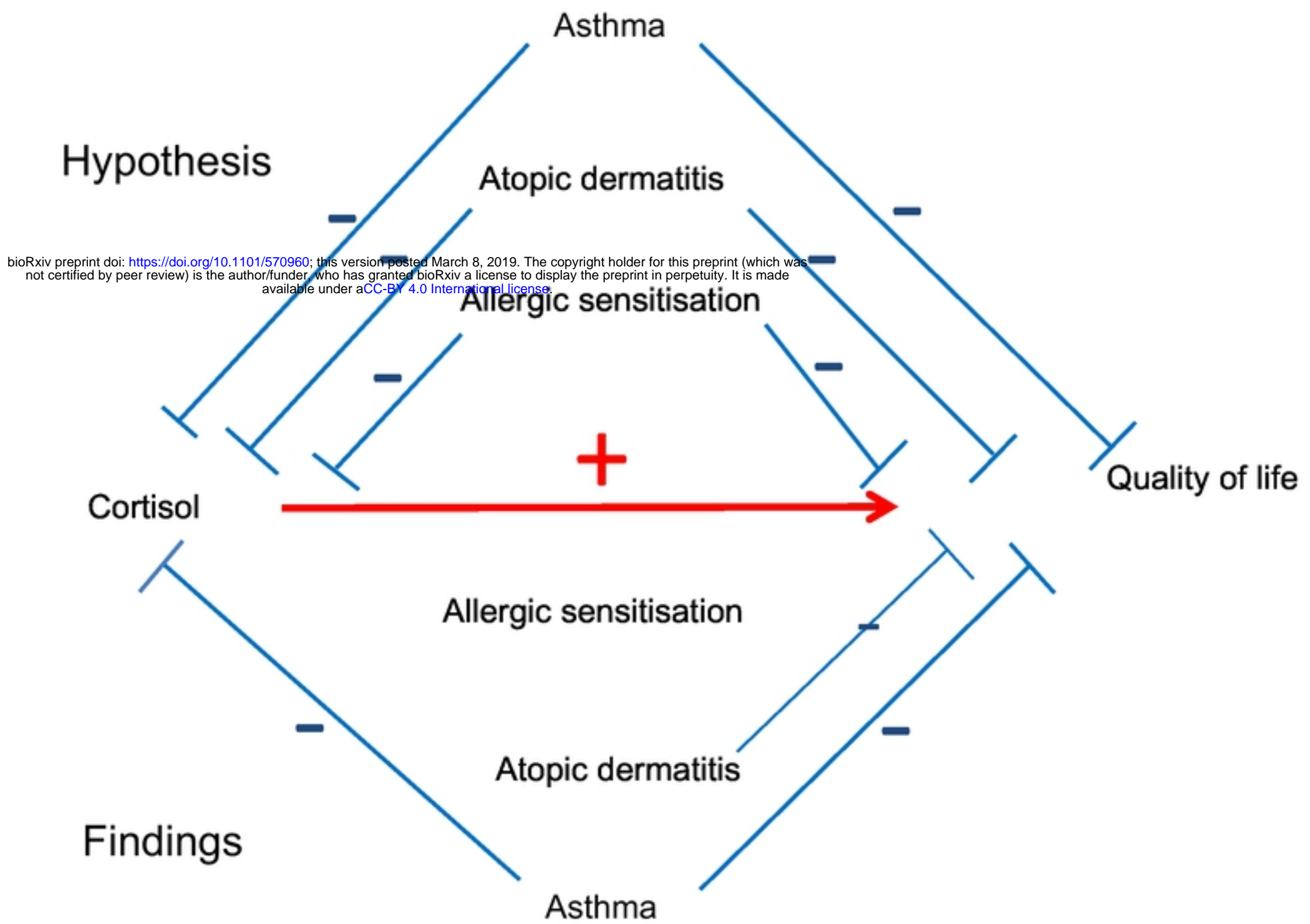
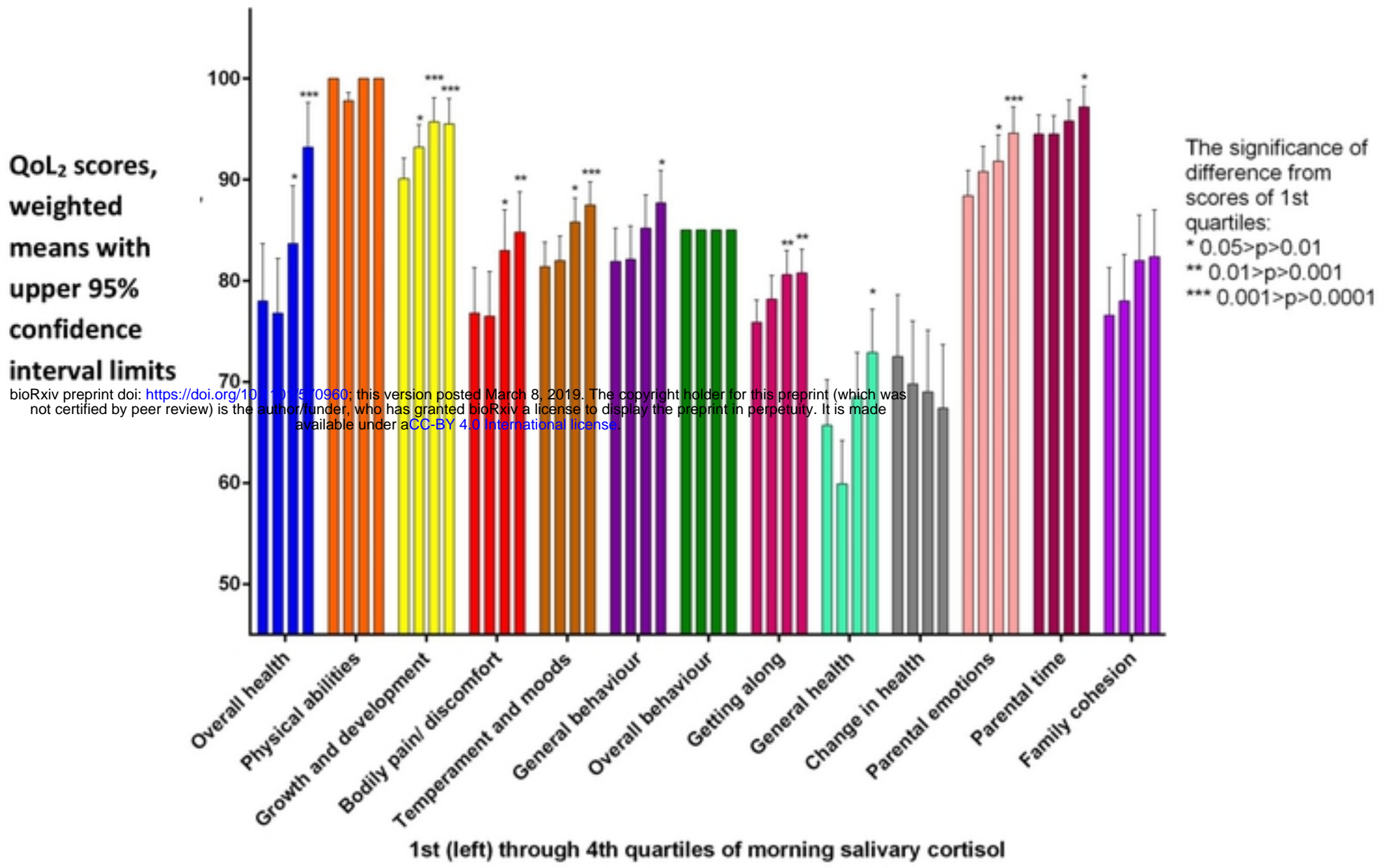


Figure 3

QoL scores of quartiles of morning salivary cortisol, unadjusted



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Figure 4

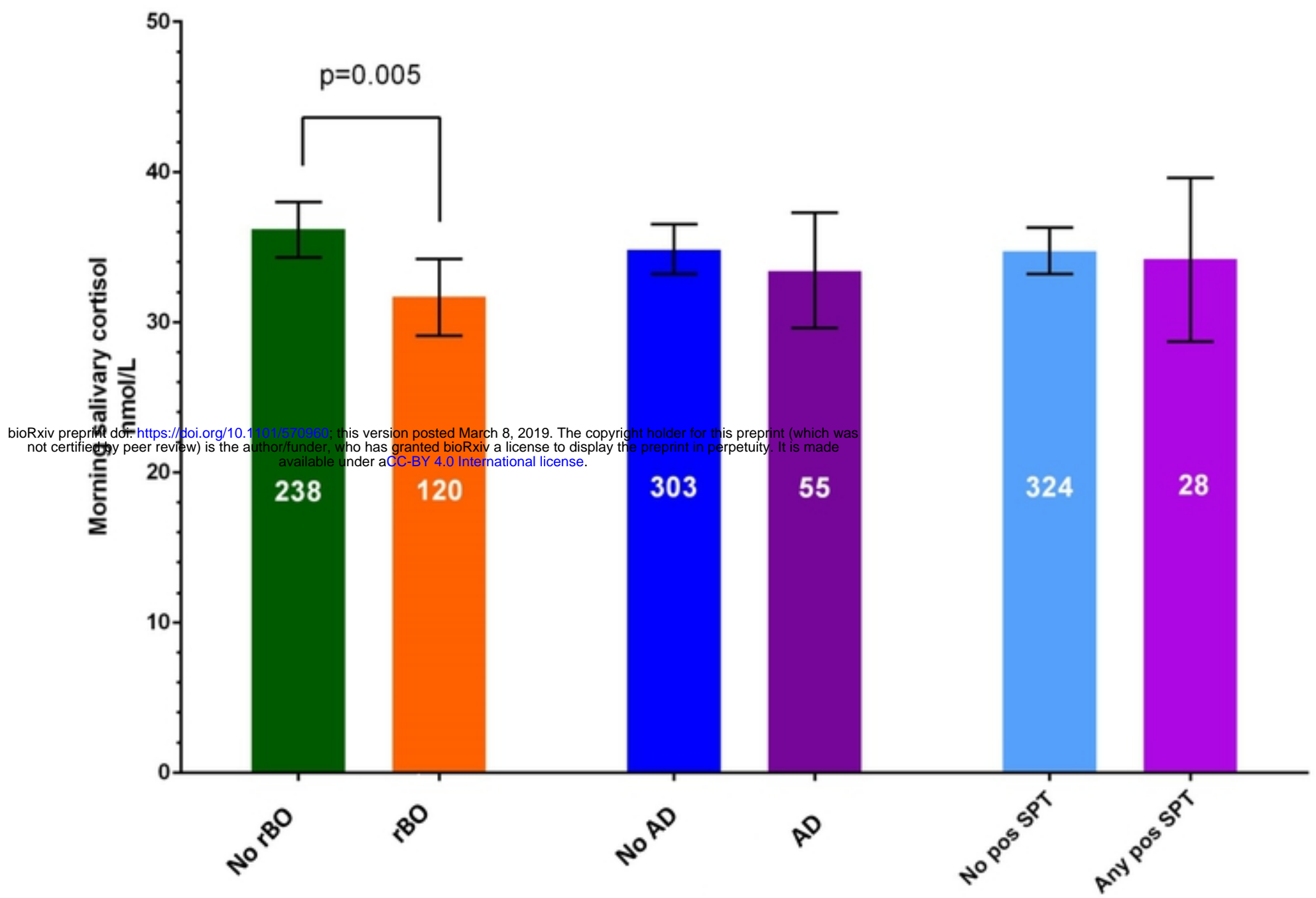


Figure 1

