

1 **Brief Report: Training load, salivary immunoglobulin A and**  
2 **illness incidence in elite paratriathletes**

3 **Original Investigation**

4 Ben T. Stephenson<sup>1</sup>, Eleanor Hynes<sup>2</sup>, Christof A. Leicht<sup>1</sup>, Keith Tolfrey<sup>1</sup> and Victoria L.  
5 Goosey-Tolfrey<sup>1</sup>

6  
7 <sup>1</sup>School of Sport, Exercise & Health Sciences, The Peter Harrison Centre for Disability Sport,  
8 Loughborough University, Loughborough, LE11 3TU, U.K.

9 <sup>2</sup>School of Sport & Exercise Sciences, University of Kent, Canterbury, Kent, CT2 7NZ, U.K.

10

11 **Corresponding author:**

12 Prof Victoria Goosey-Tolfrey

13 School of Sport, Exercise & Health Sciences,

14 The Peter Harrison Centre for Disability Sport

15 Loughborough University,

16 Loughborough,

17 LE11 3TU,

18 U.K.

19 Email: v.l.tolfrey@lboro.ac.uk

20 Phone +44 (0) 1509 226386

21

22 **Running head:** Training load, IgA & paratriathlon

23

24 Abstract word count: 249

25 Text-only word count: 1499

26 Figures: 1

27 Tables: 2

28 **Brief report: Training load, salivary immunoglobulin A and illness**  
29 **incidence in elite paratriathletes**

30

31

32 **Abstract**

33 *Purpose:* To gain an exploratory insight into the relationship between training load (TL),  
34 salivary secretory immunoglobulin A (sIgA) and upper respiratory tract illness (URI) in elite  
35 paratriathletes.

36 *Methods:* Seven paratriathletes were recruited. Athletes provided weekly saliva samples for the  
37 measurement of sIgA over 23 consecutive weeks (February - July) and a further 11 consecutive  
38 weeks (November – January). sIgA was compared to individuals' weekly training duration,  
39 external TL and internal TL, utilising time spent in pre-determined heart rate zones.  
40 Correlations were assessed via regression analyses. URI was quantified via weekly self-report  
41 symptom questionnaire.

42 *Results:* There was a significant negative relationship between athletes' individual weekly  
43 training duration and sIgA secretion rate ( $p = 0.028$ ) with changes in training duration  
44 accounting for 12.7% of the variance (quartiles: 0.2%, 19.2%). There was, however, no  
45 significant relationship between external or internal TL and sIgA parameters ( $p \geq 0.104$ ). There  
46 was no significant difference in sIgA when URI was present or not (101% vs 118% healthy  
47 median concentration;  $p \geq 0.225$ ); likewise, there was no difference in sIgA when URI occurred  
48 within two weeks of sampling or not (83% vs 125% healthy median concentration;  $p \geq 0.120$ ).

49 *Conclusions:* Paratriathletes' weekly training duration significantly affects sIgA secretion rate,  
50 yet we did not find a relationship between external or internal TL and sIgA parameters. Further,  
51 it was not possible to detect any link between sIgA and URI occurrence which throws into  
52 question the potential of using sIgA as a monitoring tool for early detection of illness.

53

54 **Key words:** mucosal immune function, disability, triathlon, monitoring, TRIMP

55 **Introduction**

56 Paratriathlon is a variant of triathlon modified for individuals with a physical impairment.<sup>1</sup> It  
57 has been shown that paratriathletes produce large training loads (TLs) to maximise beneficial  
58 adaptations.<sup>1</sup> However, there is a risk high TLs will increase the likelihood of illness,<sup>2</sup> most  
59 commonly upper respiratory tract illness (URI)<sup>3</sup> which can directly impair performance or limit  
60 training availability.<sup>2</sup>

61 A key antibody in host defence is salivary secretory immunoglobulin A (sIgA). sIgA has  
62 been acknowledged as the first line of defence in mucosal immunity.<sup>4</sup> Several authors have  
63 shown that, over prolonged periods, depressions in sIgA, proposed to be modulated by high  
64 TLs with insufficient recovery,<sup>5</sup> increase the likelihood of URI.<sup>6,7</sup> To date, this research has  
65 focused on able-bodied athletes. However, some have studied Paralympic populations, whom  
66 may be at heightened risk of URI due to their propensity for excessive overload, and therefore  
67 TLs, caused by movement inefficiencies.<sup>8</sup> Whilst Leicht *et al.*<sup>9</sup> presented a negative correlation  
68 between TL and sIgA in Paralympic athletes, there is currently little consensus regarding the  
69 effects of TL on mucosal immunity in this population.<sup>10</sup> Furthermore, there has been a wide  
70 variety of methods of external (ETL) or internal (ITL) TL representation in the literature, which  
71 likely have differing degrees of association to mucosal immunity.

72 Therefore, the aim of this present study was to gain an exploratory insight into the effects  
73 of TL, quantified using objective measures, on sIgA and resultant URI incidence in  
74 paratriathletes.

75 **Methods**

76 *Participants*

77 Seven elite, mixed impairment, paratriathletes participated in this study (Table 1). All provided  
78 written informed consent and the procedures were approved by the University Ethical Advisory  
79 Committee. All had regularly competed at international level for 2 to 7 years, with six  
80 competing at the 2016 Paralympic Games and all reported being free from illness prior to the  
81 commencement of the study. Athletes' typical weekly training volume was  $11.2 \pm 3.9$  h.

82 *Study design*

83 Saliva samples were collected over 23 consecutive weeks (February – July) and a further 11  
84 consecutive weeks (November - January) whilst athletes undertook their normal training and  
85 competition regimes. Athletes visited the laboratory three times (February, July and November)  
86 for testing of parameters used in TL quantification.<sup>1</sup> Due to the variable nature of athletes'  
87 schedules it was not possible to collect samples from every athlete each week (11 to 31 samples  
88 per athlete).

89 *Saliva collection and analysis*

90 Samples were collected on the same weekday (06:00 – 08:00 h) every week, before training,  
91 10 minutes after last fluid intake, whilst fasted and before brushing their teeth. Athletes  
92 provided a timed, unstimulated saliva sample. Sample volume was estimated assuming a saliva  
93 density of  $1.00 \text{ g}\cdot\text{ml}^{-1}$ .<sup>11</sup> Saliva flow rate was calculated from sample volume and collection  
94 time. Upon sample provision, athletes completed a questionnaire capturing illness symptoms  
95 (14 in total) for the preceding seven days. Athletes recorded the number of days they  
96 experienced each symptom and the severity of each symptom on a three-point scale. The  
97 number of days each symptom persisted was multiplied by the severity rating and summed to  
98 provide an overall quantitative symptom score; a score  $\geq 12$  indicated the presence of URI.<sup>11</sup>  
99 Athletes also reported if training availability was affected. IgA concentration was determined  
100 by ELISA.<sup>9</sup> Individuals' healthy median sIgA concentration was calculated as the median of

101 concentrations when URI was not present.<sup>7</sup> Secretion rate (SR) was calculated from sIgA  
102 concentration and saliva flow rate.<sup>11</sup>

### 103 *Laboratory testing and training load quantification*

104 Athletes performed both a cycling/handcycling and running/racing-wheelchair incremental  
105 exercise test for the determination of heart rate (HR) associated with aerobic (AeLT) and  
106 anaerobic (AnLT) lactate thresholds.<sup>1</sup>

107 Training was represented as total weekly duration for swim, cycle and run training.  
108 Resistance training was not included due to its small contribution to total weekly training.  
109 Further, ETL accounting for differences in the relative stress of triathlon modalities was  
110 calculated (Equation 1).<sup>12</sup> ITL was calculated from an adaptation of the methods of Cejuela-  
111 Anta and Esteve-Lanao<sup>12</sup>, incorporating the time spent in pre-determined zones (Equation 2),  
112 based on the HR associated with lactate thresholds.<sup>1</sup> Due to the inability to record HR during  
113 swim training, this was represented solely by swim duration. Training duration, ETL and ITL  
114 were relativised to the highest individual weekly value recorded during the study period.

#### 115 *Equation 1*

$$116 \quad ETL = 0.75(\text{swim duration}) + 0.5(\text{cycling duration}) + (\text{run duration})$$

#### 117 *Equation 2*

$$118 \quad ITL = [0.75(\text{swim duration})] + [0.5(TIZ1_C + 2[TIZ2_C] + 3[TIZ3_C])] + [(TIZ1_R +$$
  
$$119 \quad 2[TIZ2_R] + 3[TIZ3_R])]$$

120 TIZ<sub>nC</sub> – weekly time (min) spent in zone *n* during cycling. TIZ<sub>nR</sub> – weekly time  
121 (min) spent in zone *n* during running. Zone 1 – below AeLT. Zone 2 – above AeLT,  
122 below AnLT. Zone 3 – above AnLT.

### 123 *Statistical analyses*

124 Statistical analyses were conducted using SPSS Statistics 23.0 (IBM, New York, USA);  
125 statistical significance was set at  $p < 0.05$ . Coefficients of variation were calculated for all  
126 saliva variables. Each salivary variable was matched to the athlete's individual TL/training  
127 duration for the preceding seven days. A logarithmic transformation was applied to salivary  
128 variables to weight increases by a certain factor the same as decreases by the same factor.<sup>9</sup>  
129 Slopes of linear regression lines between salivary variables and TL/training duration were  
130 calculated for each athlete and compared, as a group, to a fixed zero with a Wilcoxon statistic.  
131 All salivary data were grouped, represented as relative deviation from individuals' healthy  
132 median value and compared, via paired-samples t-test or Mann-Whitney U test, to elucidate to  
133 the likelihood URI occurrence within two weeks of sample provision. Similarly, salivary  
134 variables when URI were present were compared to samples when healthy (> 2 weeks to/from  
135 URI).

### 136 **Results**

137 132 saliva samples were collected. The between- and within-individual variability in sIgA  
138 concentration was 70% and 40%, respectively, whilst sIgA SR variability was 88% and 46%,  
139 respectively. Athletes' mean sIgA concentration and SR were  $162 \pm 127 \mu\text{g}\cdot\text{ml}^{-1}$  and  $78 \pm 76$   
140  $\mu\text{g}\cdot\text{min}^{-1}$ , respectively.

141 There was a significant negative relationship between athletes' total training duration  
142 and sIgA SR ( $p = 0.028$ ; Figure 1). The amount of variance in sIgA SR explained by changes  
143 in training duration was 12.7% (quartiles 0.2%, 19.2%). There was no significant relationship  
144 between ETL ( $p \geq 0.398$ ) or ITL ( $p \geq 0.104$ ) and sIgA SR or concentration.

145 Six athletes reported at least one URI occurrence (range: 0 to 9 URI per athlete) with a  
146 total of 22 separate URI episodes. On average, athletes presented with URI every seven weeks  
147 and every 8 samples. During 50% of URI episodes, athletes had to reduce or suspend training.  
148 There was no significant difference in relative deviation from individual median sIgA  
149 concentration, SR or saliva flow rate between weeks with URI and when healthy ( $p \geq 0.228$ )  
150 or between samples with URI within two weeks and samples without URI within two weeks  
151 ( $p \geq 0.120$ ) (Table 2).

## 152 **Discussion**

153 Training duration explained 12.7% of the variance in sIgA SR, albeit with large inter-individual  
154 variability. The variability is likely due to the individualised nature of which training affected  
155 mucosal immunity and we also acknowledge genetic, nutritional and psychological factors that  
156 may have an influence.<sup>9</sup> There was, however, no significant relationship between ETL or ITL  
157 and sIgA. Relationships between TL and sIgA have been shown elsewhere in able-bodied  
158 sport,<sup>7</sup> yet, Leicht *et al.*<sup>9</sup> are the only researchers to note this interaction in a Paralympic  
159 population. This study, however, was conducted in an intermittent ball sport with solely spinal  
160 cord injured athletes, thus, was disparate from the multi-impairment endurance sport of  
161 paratriathlon. Further, the aforementioned study relied upon subjective measures of TL  
162 quantification rather than objective parameters such as HR.

163 Here, athletes averaged one URI episode every seven weeks, significantly greater than  
164 the four URI episodes annually previously reported.<sup>6</sup> This may be due to Paralympic athletes'  
165 vulnerability to illness as a consequence of movement inefficiencies increasing the likelihood  
166 of excessive overload.<sup>8</sup> Nonetheless, in 50% of URI incidences, athletes stated that their  
167 training had been impaired. This highlights the desirability for identifying athletes at risk of  
168 illness prior to decrements in training or competitive performance.<sup>6</sup> The present study,  
169 however, noted no relationship between salivary variables and URI incidence. A lack of  
170 relationship between sIgA and URI incidence has been reported elsewhere.<sup>2,9</sup> Although sIgA  
171 plays a major role in mucosal immunity,<sup>4</sup> there are many mechanisms responsible for host  
172 defence and insufficiencies in any, not merely sIgA, are likely to heighten the risk of illness.<sup>7</sup>

173 Training duration was the only training measure significantly related to salivary  
174 parameters. This may signify a failing of the methods of Cejuela-Anta and Esteve-Lanao<sup>12</sup> to  
175 adequately quantify ETL in its relation to URI incidence. As such, it is likely this method does  
176 not truly characterise the stress imposed on the mucosal immune system. Nonetheless, it was  
177 surprising that ITL did not relate to sIgA as this likely better represented the physical stress  
178 imposed by training than ETL.

## 179 **Practical Applications**

180 This exploratory study found depressions in sIgA SR during periods of high weekly training  
181 duration. Due to this, and Paralympic athletes' propensity for illness, we recommend that  
182 measures are put in place to minimise the likelihood for missed or impaired training. This  
183 includes structuring and accurately monitoring TL to maximise recovery whilst lessening  
184 exposure to physical, environmental or psychological stressors.<sup>7</sup>

185 A limitation to the current study was the lack of mechanistic data to elucidate the  
186 relationship between training duration or TL and sIgA. Measurement of cortisol, a potential  
187 modulating factor in sIgA suppression,<sup>2,4</sup> may have provided further insight into the causes of  
188 variation of sIgA.

189 **Conclusions**

190 Paratriathletes' weekly training duration is negatively correlated with sIgA SR, yet there is no  
191 significant relationship between ETL or ITL and sIgA parameters. Furthermore, it was not  
192 possible to detect any link between sIgA and URI occurrence.

193 **References**

- 194 1. Mujika I, Orbañanos J, & Salazar H. Physiology and training of a world-champion  
195 paratriathlete. *Int J Sports Physiol Perform.* 2015;10:927-930.
- 196 2. Cunniffe B, Griffiths H, Proctor W, Davies B, Baker JS, Jones KP. Mucosal immunity and  
197 illness incidence in elite rugby union players across a season. *Med Sci Sports Exerc.*  
198 2011;4:388-397.
- 199 3. Derman W, Schweltnus MP, Jordaan E, et al. Sport, sex and age increase risk of illness at  
200 the Rio 2016 Summer Paralympic Games: a prospective cohort study of 51 198 athlete  
201 days. *Br J Sports Med.* 2018;52, 17-23.
- 202 4. Walsh NP, Gleeson M, Shephard RJ, et al. Position statement part one: Immune function  
203 and exercise. *Exerc Immunol Rev.* 2011;17:6-63.
- 204 5. Pyne DB, Gleeson M. Effects of intensive exercise training on immunity in athletes. *Int J*  
205 *Sport Med.* 1998;19:S183-S194.
- 206 6. Gleeson M, McDonald WA, Pyne DB, Cripps AW, Francis JL, Fricker PA, Clancy RL.  
207 Salivary IgA levels and infection risk in elite swimmers. *Med Sci Sports Exerc.*  
208 1999;31:67-73.
- 209 7. Neville V, Gleeson M, Folland JP. Salivary IgA as a risk factor for upper respiratory  
210 infections in elite professional athletes. *Med Sci Sports Exerc.* 2008;40, 1228-1236.
- 211 8. Blauwet CA, Brook EM, Tenforde AS, et al. Low energy availability, menstrual  
212 dysfunction, and low bone mineral density in individuals with a disability: implications for  
213 the para athlete population. *Sports Med.* 2017;47:1697-1708.
- 214 9. Leicht CA, Bishop NC, Paulson TAW, Griggs KE, Goosey-Tolfrey V. Salivary  
215 immunoglobulin A and upper respiratory symptoms during 5 months of training in elite  
216 tetraplegic athletes. *Int J Sports Physiol Perform.* 2012;7:210-217.
- 217 10. Edmonds R, Burkett B, Leicht A, McKean M. Effect of chronic training on heart rate  
218 variability, salivary IgA and salivary alpha-amylase in elite swimmers with a disability.  
219 *PLoS One.* 2015;10:e0127749.
- 220 11. Gleeson M, Bishop N, Oliveira M, McCauley T, Tauler P, Muhamad AS. Respiratory  
221 infection risk in athletes: association with antigen-stimulated IL-10 production and salivary  
222 IgA secretion. *Scand J Med Sci Sports.* 2012;22:410-417.
- 223 12. Cejuela-Anta R, Esteve-Lanao J. Training load quantification in triathlon. *J Human Sport*  
224 *Exerc.* 2011;6:1-15.

225

226

227

228

229

230

231

232

233

234

235

236

237

238

239

240

241

242

243

244

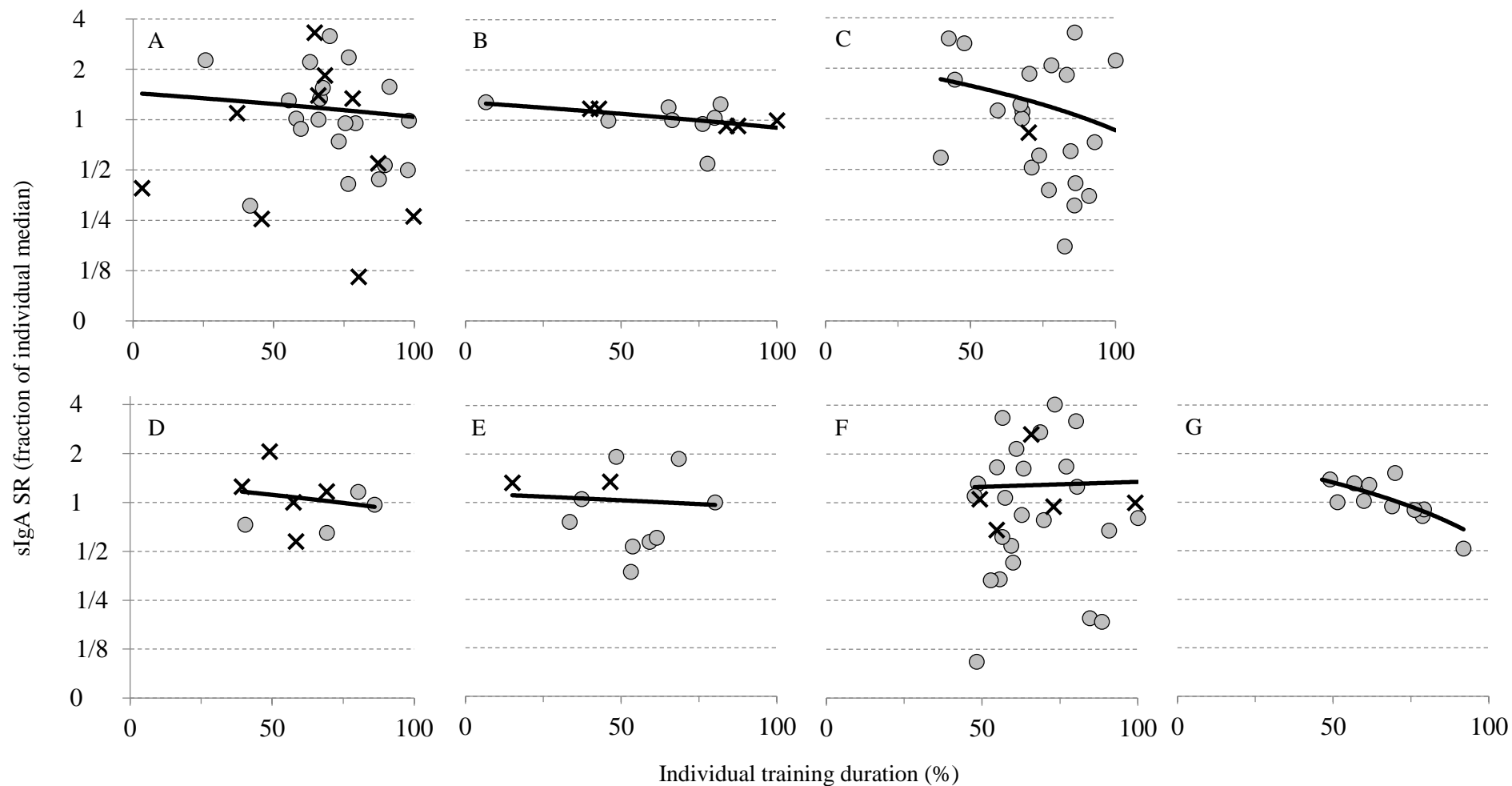


Figure 1 Individual athletes' salivary secretory immunoglobulin A (sIgA) secretion rate (SR) plotted against individual training duration with regression lines (lines are distorted by logarithmic scale). × Samples when URI was present. A-G are participant codes.



*Table 1 Participant characteristics. Values mean  $\pm$  standard deviation, where appropriate.*

Age (y)	30 $\pm$ 10
Body mass (kg)	69.5 $\pm$ 6.5
Cycling $\dot{V}O_{2\text{peak}}$ (l $\cdot$ min <sup>-1</sup> )	4.06 $\pm$ 0.61
Sex	6 male, 1 female
Impairment	1 SCI (T6i), 1 unilateral transfemoral amputation, 1 hemiplegia cerebral palsy, 3 unilateral transradial amputation, 1 lower leg impairment

$\dot{V}O_{2\text{peak}}$  – Peak rate of oxygen uptake. SCI – Spinal cord injury. T6i – Incomplete lesion at the 6<sup>th</sup> thoracic vertebra.

Table 2 Relationship between URI state and URI occurrence within two weeks of sampling date on individual deviation of saliva data, median (quartiles).

<b>URI state</b>	sIgA conc. (% indiv. median)	<i>P</i>	sIgA SR (% indiv. median)	<i>P</i>	Saliva flow rate (% indiv. median)	<i>P</i>
URI	101 (94, 117)	0.225	113 (98, 128)	0.494	107 (87, 131)	0.478
Healthy	118 (95, 139)		98 (85, 127)		94 (77, 117)	
<b>URI within two weeks?</b>						
Yes	83 (54, 151)	0.120	76 (49, 142)	0.255	100 (84, 105)	0.937
No	125 (107, 147)		111 (96, 141)		95 (74, 113)	

URI – Upper respiratory tract illness. sIgA – Salivary secretory immunoglobulin A. SR – Secretion rate.