Introduction

Cerebrospinal fluid (CSF) analysis is an important tool in the diagnostic work-up of many neurological disorders, but reference ranges for CSF glucose, CSF/plasma glucose ratio and CSF lactate based on studies with large numbers of CSF samples are not available. Our aim was to define age-specific reference values. In 1993 The Nijmegen Observational CSF Study was started. Results of all CSF samples that were analyzed between 1993 and 2008 at our laboratory were systematically collected and stored in our computerized database. After exclusion of CSF samples with an unknown or elevated erythrocyte count, an elevated leucocyte count, elevated concentrations of bilirubin, free hemoglobin, or total protein 9,036 CSF samples were further studied for CSF glucose (n = 8,871), CSF/plasma glucose ratio (n = 4,516) and CSF lactate values (n = 7,614). CSF glucose, CSF/plasma glucose ratio and CSF lactate were age-, but not sex dependent. Age-specific reference ranges were defined as 5–95th percentile ranges. CSF glucose 5th percentile values ranged from 1.8 to 2.9 mmol/L and 95th percentile values from 3.8 to 5.6 mmol/L. CSF/plasma glucose ratio 5th percentile values ranged from 0.41 to 0.53 and 95th percentile values from 0.82 to 1.19. CSF lactate 5th percentile values ranged from 0.88 to 1.41 mmol/L and 95th percentile values from 2.00 to 2.71 mmol/L. Reference ranges for all three parameters were widest in neonates and narrowest in toddlers, with lower and upper limits increasing with age. These reference values allow a reliable interpretation of CSF results in everyday clinical practice. Furthermore, hypoglycemia was associated with an increased CSF/plasma glucose ratio, whereas hyperglycemia did not affect the CSF/plasma glucose ratio.

Abstract

Cerebrospinal fluid (CSF) analysis is an important tool in the diagnostic work-up of many neurological disorders, but reference ranges for CSF glucose, CSF/plasma glucose ratio and CSF lactate based on studies with large numbers of CSF samples are not available. Our aim was to define age-specific reference values. In 1993 the Nijmegen Observational CSF Study was started. Results of all CSF samples that were analyzed between 1993 and 2008 at our laboratory were systematically collected and stored in our computerized database. After exclusion of CSF samples with an unknown or elevated erythrocyte count, an elevated leucocyte count, elevated concentrations of bilirubin, free hemoglobin, or total protein 9,036 CSF samples were further studied for CSF glucose (n = 8,871), CSF/plasma glucose ratio (n = 4,516) and CSF lactate (n = 7,614). CSF glucose, CSF/plasma glucose ratio and CSF lactate were age-, but not sex dependent. Age-specific reference ranges were defined as 5–95th percentile ranges. CSF glucose 5th percentile values ranged from 1.8 to 2.9 mmol/L and 95th percentile values from 3.8 to 5.6 mmol/L. CSF/plasma glucose ratio 5th percentile values ranged from 0.41 to 0.53 and 95th percentile values from 0.82 to 1.19. CSF lactate 5th percentile values ranged from 0.88 to 1.41 mmol/L and 95th percentile values from 2.00 to 2.71 mmol/L. Reference ranges for all three parameters were widest in neonates and narrowest in toddlers, with lower and upper limits increasing with age. These reference values allow a reliable interpretation of CSF results in everyday clinical practice. Furthermore, hypoglycemia was associated with an increased CSF/plasma glucose ratio, whereas hyperglycemia did not affect the CSF/plasma glucose ratio.
and were anonymously processed for the purpose of this study, the METC waived the need for patient informed consent. CSF samples that were analyzed between September 1993 and December 2008 (n = 23,618) were included. To define reference values for glucose and lactate in CSF obtained by lumbar puncture we have excluded (1) all CSF samples from patients from the neurosurgical ward or intensive care units (where CSF is often obtained from ventricular shunts); and CSF samples with (2) an erythrocyte count ≥200/μL or unknown erythrocyte count; (3) a bilirubin concentration ≥0.5 μmol/L and/or a free hemoglobin concentration ≥0.25 μmol/L; (4) an elevated leucocyte count; or (5) an elevated total protein concentration (Fig. 1). We have used strict exclusion criteria for leucocyte count and total protein concentration based on age-specific reference values as given in figure 1 [9–12]. In a previous study we identified all patients with GLUT1DS in our CSF database (n = 3) [13]. These patients were excluded from the present study. We finally included 9,036 CSF samples for further analyses. Patient population consisted of 49.7% males and 50.3% females. The population was predominantly Caucasian.

CSF glucose was determined in 8,871 CSF samples, CSF/plasma glucose ratio in 4,518 samples and CSF lactate concentrations in 7,614 CSF samples. To determine whether multiple lumbar punctures from the same patient influenced our results, we have in second instance also studied the CSF glucose (n = 7,245) and CSF lactate (n = 6,856) concentrations if only the first CSF sample of each patient was included.

In second instance, the 5–95th percentile ranges were determined after exclusion of (1) CSF samples from patients with hypoglycaemia (defined as plasma glucose <3.0 mmol/L), hyperglycaemia (defined as plasma glucose >7.8 mmol/L) or unknown plasma glucose level at the moment of lumbar puncture (Fig. 1); (2) CSF samples with a clearly elevated CSF lactate (arbitrarily defined as >3000 μmol/L) and CSF glucose concentrations were age-dependent with remarkably low 5th percentile values at the age of 6 months (1.7–2.0 mmol/L) (Fig. 2A and Table S1). After the age of 6 months the CSF glucose 5th percentile values gradually increased over the entire age range studied (from 2.4 to 2.9 mmol/L). CSF glucose 95th percentile values were highest in neonates (5.6 mmol/L) and lowest in patients aged 3–6 months (3.9 mmol/L) and 3–4 years (3.8 mmol/L). Above the age of 4 years a gradual increase in the CSF glucose 95th percentile was seen (from 4.0 to 6.1 mmol/L). After exclusion of CSF samples from patients with an abnormal or unknown plasma glucose level at the moment of lumbar puncture, the CSF glucose 5th percentile value did not show a change of >10% of the original value. CSF glucose 95th percentile values, however, did change more than 10% in patients aged 3–5 weeks, 3–12 months and ≥60 years after the application of these exclusion criteria. Numbers were unaffected in the other age groups.

CSF/ plasma glucose ratio

Plasma glucose was measured in 4,516 out of 9,036 (50%) CSF samples. CSF glucose ranged from 1.0 to 11.9 mmol/L (mean 3.42 mmol/L; SD 0.769). CSF glucose concentrations were age-dependent with remarkably low 5th percentile values until the age of 6 months (1.7–2.0 mmol/L) (Fig. 2A and Table S1). After the age of 6 months the CSF glucose 5th percentile values gradually increased over the entire age range studied (from 2.4 to 2.9 mmol/L). CSF glucose 95th percentile values were highest in neonates (5.6 mmol/L) and lowest in patients aged 3–6 months (3.9 mmol/L) and 3–4 years (3.8 mmol/L). Above the age of 4 years a gradual increase in the CSF glucose 95th percentile was seen (from 4.0 to 6.1 mmol/L). After exclusion of CSF samples from patients with an abnormal or unknown plasma glucose level at the moment of lumbar puncture, the CSF glucose 5th percentile value did not show a change of >10% of the original value. CSF glucose 95th percentile values, however, did change more than 10% in patients aged 4–5 weeks, 3–12 months and ≥60 years after the application of these exclusion criteria. Numbers were unaffected in the other age groups.

Statistical analysis

Statistical analyses were performed using SPSS version 20.0 (SPSS INC, Chicago, IL, USA). Patients were divided into age groups which are commonly used in daily clinical practice (as used by MeSH, Pubmed) (Table 1). We included all 9,036 CSF samples of which 8,871 samples were studied for CSF glucose, 4,516 samples for CSF/plasma glucose ratio and 7,614 samples for CSF lactate values. Reference values for CSF glucose, CSF/plasma glucose ratio and CSF lactate were based on the 5–95th data-observed percentile ranges. 95% Confidence Intervals were based on Bootstrap Percentiles (based on 1000 bootstrap samples).

Results

Literature study

Reference ranges for CSF glucose, CSF/plasma glucose ratio and CSF lactate based on studies including large numbers of CSF samples were not available. Most studies had been performed in newborn or young infants and were based on a small number of patients (i.e. <350 patients) and only a few had been performed in patients without an explicit neurological disorder. We have summarized the results of our literature study in Table 2. In all studies CSF glucose was measured by the hexokinase method and CSF lactate was determined enzymatically.

CSF glucose concentrations

CSF glucose concentrations were measured in 8,871 out of 9,036 (96%) CSF samples. CSF glucose ranged from 1.0 to 11.9 mmol/L (mean 3.42 mmol/L; SD 0.769). CSF glucose concentrations were age-dependent with remarkably low 5th percentile values until the age of 6 months (1.7–2.0 mmol/L) (Fig. 2A and Table S1). After the age of 6 months the CSF glucose 5th percentile values gradually increased over the entire age range studied (from 2.4 to 2.9 mmol/L). CSF glucose 95th percentile values were highest in neonates (5.6 mmol/L) and lowest in patients aged 3–6 months (3.9 mmol/L) and 3–4 years (3.8 mmol/L). Above the age of 4 years a gradual increase in the CSF glucose 95th percentile was seen (from 4.0 to 6.1 mmol/L). After exclusion of CSF samples from patients with an abnormal or unknown plasma glucose level at the moment of lumbar puncture, the CSF glucose 5th percentile value did not show a change of >10% of the original value. CSF glucose 95th percentile values, however, did change more than 10% in patients aged 4–5 weeks, 3–12 months and ≥60 years after the application of these exclusion criteria. Numbers were unaffected in the other age groups.

CSF/plasma glucose ratio

Plasma glucose was measured in 4,516 out of 9,036 (50%) paired samples and ranged from 0.80 to 40.4 mmol/L (mean 5.5 mmol/L; SD 1.98). An elevated plasma glucose (>7.8 mmol/L) was found in 338 samples (7.5%). Plasma glucose was elevated in 13.5% of the samples from patients older than 50 years of age, compared to 3.7% in samples from patients younger than 50 years.
Figure 1. Flow chart of inclusion of CSF samples. N – number of CSF samples.
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95th percentile values were particularly high in neonates (children) (0.86–1.14 mmol/L) and increased with age. CSF lactate concentrations were, like CSF glucose, remarkably low in (young) groups. Above the age of 4 years the CSF/plasma glucose 5th percentile values were 10% lower compared to the original value in patients below the age of 6 months (0.36–0.42) and in children aged 3–6 months. Whereas below the age of 12 months (1.03–1.38). Reference range after exclusion of CSF samples from patients with abnormal plasma glucose (<3.0 mmol/L or >7.8 mmol/L) at the moment of lumbar puncture (only represented if >10% different from to the original value). Numbers between brackets represent values without correction for plasma glucose.

### Table 1. Age-specific reference values for CSF glucose, CSF/plasma glucose ratio and CSF lactate.

<table>
<thead>
<tr>
<th>Age</th>
<th>CSF glucose (mmol/L)</th>
<th>CSF/plasma glucose ratio</th>
<th>CSF lactate (mmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neonate</td>
<td>0–4 weeks</td>
<td>1.9–5.6</td>
<td>0.42–1.10 (1.38)*</td>
</tr>
<tr>
<td>Infant</td>
<td>4–8 weeks</td>
<td>1.7–5.6 (5.1)*</td>
<td>0.36–1.20</td>
</tr>
<tr>
<td></td>
<td>2–6 months</td>
<td>1.9–4.9 (3.9)*</td>
<td>0.39–1.10</td>
</tr>
<tr>
<td></td>
<td>6–12 months</td>
<td>2.4–4.9 (4.3)*</td>
<td>0.44–1.05</td>
</tr>
<tr>
<td>Toddler</td>
<td>1–3 years</td>
<td>2.4–4.2</td>
<td>0.44–0.84</td>
</tr>
<tr>
<td>Preschool child</td>
<td>3–4 years</td>
<td>2.4–3.8</td>
<td>0.43–0.86</td>
</tr>
<tr>
<td>School child</td>
<td>4–10 years</td>
<td>2.5–4.0</td>
<td>0.45–0.84</td>
</tr>
<tr>
<td>(Pre)adolescent</td>
<td>10–18 years</td>
<td>2.6–4.3</td>
<td>0.47–0.83</td>
</tr>
<tr>
<td>Adult</td>
<td>18–30 years</td>
<td>2.7–4.4</td>
<td>0.46–0.90</td>
</tr>
<tr>
<td>Middle aged</td>
<td>30–50 years</td>
<td>2.8–4.4</td>
<td>0.46–0.88</td>
</tr>
<tr>
<td>Aged</td>
<td>50–60 years</td>
<td>2.8–4.2 (4.8)*</td>
<td>(0.43) 0.48–0.87*</td>
</tr>
<tr>
<td></td>
<td>60–80 years</td>
<td>2.9–4.4 (5.6)*</td>
<td>(0.42) 0.46–0.84*</td>
</tr>
<tr>
<td>≥80 years</td>
<td>2.9–4.5 (6.1)*</td>
<td>(0.35) 0.42–0.81*</td>
<td>1.4–2.7</td>
</tr>
</tbody>
</table>

Reference values are based on the 5th to 95th percentile values. The subgroups from the original data (see Tables S1, S2, S3) were clustered into age groups which are commonly used in daily clinical practice (as used by MeSH, Pubmed).

Relation between plasma glucose, CSF glucose and CSF/plasma glucose ratio

A linear relation between plasma glucose and CSF glucose was found (Figure 3A). Hypoglycemia was associated with an increased CSF/plasma glucose ratio in most patients, whereas hyperglycemia was associated with only a minimal decrease of the CSF/plasma glucose ratio in the majority of the samples (Figure 3B).

### Discussion

Our study allowed for the first time to define age-specific reference values for CSF glucose, CSF lactate and CSF/plasma glucose for children and adults based on a large number of CSF samples. Reference ranges did not differ between males and females, which is in line with previous findings [15]. To our best knowledge, no studies are available reporting differences in CSF glucose or lactate based on race. We therefore believe that our data, mainly based on CSF samples from Caucasians, can be generalized to non-Caucasians.
Table 2. Summary of literature search for reference ranges of CSF glucose, CSF/plasma glucose ratio and CSF lactate.

<table>
<thead>
<tr>
<th>Reference</th>
<th>N</th>
<th>Inclusion criteria</th>
<th>Age</th>
<th>CSF glucose concentration (mmol/L)</th>
<th>CSF/plasma glucose</th>
<th>CSF lactate concentration (mmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Knight et al., 1981</td>
<td>314</td>
<td>Healthy children</td>
<td>1 wk–16 yrs</td>
<td>Mean 3.63; P2.5-97.5: 2.56–5.54</td>
<td>2.72–4.54</td>
<td>Mean: 1.75; P2.5-97.5: 0.98–3.17</td>
</tr>
<tr>
<td>Vamosi et al., 1983</td>
<td>144</td>
<td>Cisternal CSF specimens of fasted adults free from organic brain disease</td>
<td>16–69 yrs</td>
<td>-</td>
<td>-</td>
<td>Mean ± 2SD: 0.68–2.1</td>
</tr>
<tr>
<td>Ahmed et al., 1992</td>
<td>108</td>
<td>Noninfected term neonates</td>
<td>0–30 dys</td>
<td>Mean ± SD: 2.8±0.7</td>
<td>1.65–3.95</td>
<td>Mean: 0.62</td>
</tr>
<tr>
<td>Bonadio et al., 1992</td>
<td>75</td>
<td>Term febrile infants with negative cultures</td>
<td>0–8 wks</td>
<td>Mean ± SD: 2.56±0.56</td>
<td>1.64–3.48</td>
<td>-</td>
</tr>
<tr>
<td>Cameron et al., 1993</td>
<td>100</td>
<td>Children with normal CSF glucose, protein and cell count</td>
<td>Children (not further specified)</td>
<td>Range: 3.3–5.5</td>
<td>-</td>
<td>Range: 0.5–3.2</td>
</tr>
<tr>
<td>Benoist et al., 2003</td>
<td>197</td>
<td>Hospitalized children</td>
<td>0–15 yrs</td>
<td>-</td>
<td>-</td>
<td>Median: 1.5; SD 0.255 Range: 0.6–2.2</td>
</tr>
</tbody>
</table>

N - number of CSF samples included in study; wk(s) – week(s); dys – days; yrs- years; P – percentile.

doi:10.1371/journal.pone.0042745.t002
Since it seems impossible to obtain CSF from healthy volunteers (especially children) under standardized conditions (e.g., after fasting), we included CSF samples from our computerized database of patients with a medical indication for a lumbar puncture, which reflects the daily clinical practice. Because of the large number of samples it was not feasible to obtain clinical information from the patients. In order to exclude as many as possible CSF samples of patients with overt neurological disorders, we have therefore used strict exclusion criteria based on other CSF parameters (Fig. 1). Furthermore, since the concentrations of CSF glucose and CSF lactate in ventricular CSF differ from lumbar CSF values, we have excluded all ventricular CSF samples.

The reference range for CSF glucose is generally set at 2.5–4.4 mmol/L, but ranges with a lower limit of 2.0 mmol/L or an upper limit of 6.5 mmol/L are used as well [1,16]. We found an age-dependent pattern for CSF glucose with 5th percentile values ranging from 1.7 to 2.9 mmol/L and 95th percentile values from 4.5 to 5.6 mmol/L. Age-dependency of CSF glucose had been described before in children [17]. CSF glucose 5th percentile values were remarkably low until the age of 6 months (1.7–2.0 mmol/L), which is in line with previous studies [9,18] (Table 2). The 95th percentile value in our study was especially high in neonates (3.6 mmol/L), which is higher than previously
described (3.5–4.0 mmol/L) [18]. Low CSF glucose 5th percentile values and high 95th percentile values in neonates in our study might be related to the high frequency of hypoglycemia and hyperglycemia in neonates, especially in preterm infants and neonates with a low birth weight [19,20].

After exclusion of CSF samples from patients with abnormal or unknown plasma glucose the 95th percentile values in infants aged 4 to 8 weeks and 3 to 12 months increased more than 10% compared to the original value. This was due to a skewed distribution most likely caused by the low number of CSF samples in these subgroups after exclusion of samples with abnormal or unknown CSF glucose (Table S2).

CSF glucose 5–95th percentile values were relatively high in patients older than 50 years of age. This was probably related to the increasing frequency of hyperglycemia in patients aged 50 years and older (13.5% compared to 3.7% in patients younger than 50 years of age). In a study comparing the CSF/blood glucose ratio from patients with diabetes mellitus and noninflammatory neurological conditions with patients with bacterial meningitis, the CSF glucose ranged from 2.78 to 25 mmol/L in patients with diabetes, with CSF/blood glucose ratio ranging from 0.20–3.46 [21]. To our best knowledge no studies are available on CSF glucose and lactate values in patients with diabetes without neurological disorders. In order to correct the reference range for CSF glucose and CSF/plasma glucose ratio for patients with abnormal plasma glucose, we have in second instance excluded all patients with abnormal (defined as >7.8 mmol/L or <3.0 mmol/L) or unknown plasma glucose. Reference ranges for CSF glucose before and after correction for plasma glucose are described in Table 1.

The reference value for CSF/plasma glucose ratio is typically about 0.6 and is generally set at 0.5–0.8 [15]. Our study confirms this reference range, although a slight age-dependent pattern for CSF/plasma glucose ratio was found. Below the age of 12 months relatively low 5th percentile values were seen (0.36–0.44), whereas 95th percentiles were high in patients younger than 12 months (1.05–1.20). A high CSF/plasma glucose ratio in newborns is thought to be related to the higher cerebral blood flow in newborns in combination with a smaller brain that utilizes less glucose [22,23]. Remarkably, the upper reference limit of CSF/plasma glucose values in patients under the age of 12 months in our study exceeds 1.0. Most likely such high ratios are caused by a decline in plasma glucose in the hours before the lumbar puncture, while the concentration in the cerebrospinal fluid follows only gradually. It is known that the equilibration of glucose between plasma and CSF takes approximately two to four hours [24]. Figure 3A shows that a CSF/plasma glucose ratio exceeding 1.0 is predominantly found in patients with hypoglycemia.

A linear correlation was found between plasma glucose (range 2.2–23.3 mmol/L) and CSF glucose (range 1.2–11.0 mmol/L), with CSF glucose values exceeding 8 mmol/L in patients with hyperglycemia. Whereas hypoglycemia was associated with an increase of the CSF/plasma glucose ratio, hyperglycemia barely affected the CSF/plasma glucose ratio (Fig. 3). This finding is in contradiction with the general assumption that CSF glucose concentrations tend to plateau at high blood glucose levels since glucose is transported from blood into CSF by facilitated diffusion and exhibits saturation kinetics [25]. Our study demonstrates that in clinically relevant levels of hyperglycemia (i.e. plasma glucose 7.8–25 mmol/L), CSF glucose is linearly correlated with plasma glucose.

The reference range for CSF lactate is generally set at 1.2–2.1 mmol/L, but ranges from 0.6 to 3.1 mmol/L are used as well (Table 2) [1]. We found a clear age-dependent pattern for CSF lactate. A slight variation with age of the CSF lactate has been described before in a study of children aged 0–15 years [26], with 95th percentile values around 1.95 mmol/L. Higher 95th percentiles for children under the age of 6 months were found in the present study (2.01–2.64 mmol/L). Furthermore, we observed increasing 95th percentile values above the age of 40 years. The tendency for CSF lactate to increase in adulthood (after the age of 54 years) has been described before in a study on cisternal CSF specimens [15].

It is remarkable that after exclusion of all CSF samples with an elevated erythrocyte or leucocyte count, bilirubin, free hemoglobin, or total protein concentration, 149 CSF samples with a lactate concentrations >3000 μmol/L were found. CSF lactate concentrations can be raised in association with seizures, bacterial, fungal of tuberculous meningitis or encephalitis, cerebral ischemia, neurosarcoïdosis, malignancy and metabolic disorders [27]. In most of these disorders, however, other CSF parameters such as leucocyte count and protein count are raised as well. It is possible that some CSF samples from patients with a mitochondrial disorder have been included, which will increase the 95th percentile range of the reference range. We have therefore excluded CSF samples with a CSF lactate concentration >3000 μmol/L in second instance. This, however, did not change the 5–95th percentile value more than10% compared to the original value, except for >10% lower 95th percentile value in patients younger than 4 weeks and patients aged 3–6 months. For these patients we have therefore based the reference range for CSF lactate on the CSF samples with CSF lactate <3000 μmol/L (Table 1).

CSF results for glucose and lactate can be especially helpful in the diagnostic work-up of GLUT1 deficiency syndrome (GLUT1DS), a neurometabolic disorder caused by defective glucose transport into brain. With the exception of rare cases [28–30], the biochemical hallmark of GLUT1DS uniformly consists of low CSF glucose and lactate, and low CSF/plasma ratio for glucose [5,13]. The availability of detailed, age-related reference values as presented here will allow appropriate interpretation of CSF and thereby identification of patients who suffer from this treatable disorder.

In conclusion, we have defined age-specific reference values for CSF glucose, CSF/plasma glucose ratio and CSF lactate. They allow a reliable interpretation of CSF results in everyday clinical practice.

**Supporting Information**

**Table S1** N (total) – total number of CSF samples. N – number of CSF samples with measured CSF glucose concentration. 95% CI – 95% Confidence Interval based on Bootstrap Percentiles (based on 1000 bootstrap samples). Numbers and CSF glucose concentrations between brackets represent the results after exclusion of CSF samples with hypoglycemia (blood glucose <3.0 mmol/L), hyperglycemia (blood glucose >7.8 mmol/L), or unknown blood glucose at the moment of lumbar puncture (only shown if >10% different from the original value).

**Table S2** N (total) – total number of CSF samples. N – number of CSF samples with measured CSF glucose concentration. 95% CI – 95% Confidence Interval based on Bootstrap Percentiles (based on 1000 bootstrap samples). Sample size too small for bootstrapping. Numbers and CSF/blood glucose values between brackets represent the results after exclusion of CSF samples with hypoglycemia (blood glucose <3.0 mmol/L), hyperglycemia (blood glucose >7.8 mmol/L), or unknown blood glucose at the moment of lumbar puncture (only shown if >10% different from the original value).
Table S3  N (total) – total number of CSF samples. N – number of CSF samples with measured CSF glucose concentration. 95% CI – 95% Confidence Interval based on Bootstrap Percentiles (based on 1000 bootstrap samples). Numbers and CSF lactate concentrations between brackets represent the results after exclusion of CSF samples with CSF lactate >3000 μmol/L (only shown if >10% different from the original value).

Author Contributions
Conceived and designed the experiments: WGL MAW RAW MMV. Performed the experiments: MMV. Analyzed the data: WGL. Contributed reagents/materials/analysis tools: MMV. Wrote the paper: WGL. Interpretation of data: WGL MAW RAW MMV. Revising of the article for intellectual content: WGL MAW RAW MMV.

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