Salivary leptin concentrations in Bruneian secondary school children

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ABSTRACT

Objective: To investigate the association between leptin levels and body mass index in Bruneian secondary school students.

Methods: The body mass index of Bruneian secondary school students (aged 11–18 years) was determined before collecting their saliva using the passive drool technique. Salivary leptin concentration was determined by ELISA.

Results: Correlation and partial correlation (controlled for age) analyses showed no significant differences between the levels of salivary leptin of normal weight and overweight students.

Conclusions: No significant increase in the salivary leptin concentration was observed in overweight compared with normal weight Bruneian secondary school students.

1. Introduction

Obesity is a growing problem worldwide and is associated with several health-related conditions, including cancer [1], and cardiovascular diseases [2]. In Southeast Asia, the emerging problem of rising rates of childhood obesity is alarming [3]. In Brunei, a 1995–1996 national survey showed a prevalence of obesity in males and females at 11.2% and 12.8%, respectively [4]. However, the prevalence rates have increased over the past decade. A 2008 World Health Organisation report on body mass index (BMI) showed that Brunei had the highest obesity rate among Southeast Asia countries [5].

Leptin, important in lipid metabolism, has been suggested to be a biomarker for obesity and abnormal metabolic profile. Obesity is defined as excessive growth of adipose tissue [6]. In humans, circulating leptin correlates with body weight and fat mass [7], and therefore, leptin is a good predictor for adiposity. The relationship between plasma leptin levels and body composition has been studied in adolescents from non-Southeast Asia [8] and Southeast Asia countries including Malaysia [9], Thailand [10] and the Philippines [11]. In all these studies, a positive association between BMI and plasma leptin was observed. However, all these studies measured leptin in the blood plasma compartment. Saliva, on the other hand, has major advantages over peripheral blood for the study of obesity in children because sample collection is non-invasive and rapid. However, few investigations involving children have used saliva as a model biological fluid to measure leptin levels in obesity [12].

To date, there are no reports on leptin levels and obesity in the Bruneian population and secondary school students.
2. Materials and methods

2.1. Ethical approval

This project received ethical clearance from the PAPRSB Institute of Health Sciences, Universiti Brunei Darussalam Ethics Committee.

2.2. Subjects

This study using the multi-stage cluster sampling method involved secondary schools in the Brunei-Muara District. The projected sample size was 374 students, but only 169 secondary school students aged between 11 and 18 years agreed to participate in the study (45% response rate). Students were asked to fill in questionnaires, and anthropometric measurements (height and weight) were performed, and saliva samples were then collected. No physical examination was performed during data collection. School children were from five secondary schools in the Brunei-Muara District, Brunei Darussalam. Consent was obtained from all participants and their parents. According to the World Health Organisation criteria for 5–19 years old children and adolescents, a BMI between 15 and 22 kg/m² was considered as normal weight. A BMI of > 21.5 kg/m² was considered as overweight for girls, and BMI of 15–21.5 kg/m² as normal weight. For boys, a BMI ≥ 22 kg/m² was considered as overweight. The BMI was calculated as weight (kg) divided by height squared (m²). Inclusion criteria of participants were students without any non-communicable diseases. Students were advised not to consume carbonated drinks 1 h prior to saliva collection.

2.3. Saliva collection

Saliva from participants was collected by trained research personnel using the passive drool technique as described in the guidelines for Salimetrics saliva collection methods (Salimetrics, Carlsbad, CA, USA). Participants were required to drool through a 15 mL sterile centrifuge tube to obtain a volume of approximately 1 mL of saliva. Saliva was centrifuged at 3000 r/min for 15 min before storing at −80 °C.

2.4. Salivary leptin concentration measurement

Saliva was thawed at room temperature before the ELISA on salivary leptin was performed in duplicate for each sample. The concentration of salivary leptin (pg/mL) was measured using a leptin ELISA kit (Ray Biotech, Inc., Norcross GA, USA) according to the manufacturer’s specifications.

2.5. Statistical analysis

Comparison of salivary leptin concentration, BMI and age between Brunei secondary school males and females were conducted by t-test. Correlations between BMI and leptin concentrations were conducted for the various subgroups of students. Partial correlations were also conducted to control for age. Data were analysed using IBM SPSS Version 20.0 (SPSS Institute, Chicago, IL, USA).

3. Results

Out of the 169 school children saliva samples, 40 were excluded from the analysis based on the poor percentage coefficient of variation (CV) (> 10%). Another 34 samples were excluded because the salivary leptin measured was below the detection threshold (2 pg/mL). Therefore, a total of 95 student samples were selected for analysis, of which 37 were boys and 58 girls (Table 1). Of the boys, 54% (n = 20) had BMIs ≥ 22 kg/m² (overweight), while 31% (n = 18) of the girls had BMIs > 21.5 kg/m² (overweight). Most of the students were native Malay ethnicity (> 95%). No significant correlation was found between the levels of salivary leptin of normal weight and overweight children, irrespective of sex when controlled for age (Table 2 and Figure 1). Salivary leptin levels were found to be similar between boys and girls (Figure 1B,C). To discount the possibility that the 40 samples initially excluded from the correlation analysis because of the poor % CV underestimated the data, we included these samples for analysis and found that the correlation between salivary leptin levels of normal and overweight individuals was not significant (Table 3). Overall, the data shown that there was no correlation between BMI and salivary leptin levels in the school children tested.

Table 1

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>37</td>
<td>58</td>
</tr>
<tr>
<td>Age span (year)</td>
<td>12–17</td>
<td>12–17</td>
</tr>
<tr>
<td>Age (year)</td>
<td>14.42 ± 1.655</td>
<td>14.48 ± 1.227</td>
</tr>
<tr>
<td>Leptin (pg/mL)</td>
<td>24.79 ± 18.80</td>
<td>32.74 ± 22.08</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>24.17 ± 5.80</td>
<td>24.17 ± 5.38</td>
</tr>
</tbody>
</table>

*: Data were expressed as mean ± SD.

Table 2

<table>
<thead>
<tr>
<th>Children</th>
<th>Correlation</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>All children</td>
<td>−0.091</td>
<td>0.399</td>
</tr>
<tr>
<td>All children (controlled for age)</td>
<td>−0.084</td>
<td>0.438</td>
</tr>
<tr>
<td>Girls only</td>
<td>−0.203</td>
<td>0.157</td>
</tr>
<tr>
<td>Girls only (controlled for age)</td>
<td>−0.197</td>
<td>0.175</td>
</tr>
<tr>
<td>Boys only</td>
<td>0.107</td>
<td>0.523</td>
</tr>
<tr>
<td>Boys only (controlled for age)</td>
<td>0.115</td>
<td>0.497</td>
</tr>
</tbody>
</table>

Figure 1. No significant difference in the salivary leptin levels between normal and overweight school children was found. A: School children; B: Boys only; C: Girls only. Scatter plots show the spread of the data between normal and overweight. The horizontal line represents the mean and the error bars represent the SD.
in adults, not children [13]. Therefore it is possible that
years) [14]. Although we did not measure blood plasma leptin
levels in our study, previous findings have shown a strong
correlation between salivary and plasma leptin in adolescents
[14], suggesting that salivary leptin values in our study could
indeed reflect circulating blood leptin levels. Contrary to our
findings, Goodson et al. found that salivary leptin was three
times higher in obese compared to healthy normal weight
Kuwaiti children aged between 10 and 12 years [12]. However,
it is important to note that the saliva samples from this
previous study were collected from children who were not
ethnically from the Southeast Asia region [12]. Ethnicity is an
important factor to consider in our analysis. A previous
investigation has shown that South Asian women and men
had significantly higher blood plasma leptin concentrations than all
other groups tested (European, Chinese and Aboriginals) [15].
Although this study measured plasma leptin levels in adults
(aged 35–75 years), it is possible that the levels of salivary
leptin in the Bruneian adolescents are different from those of
non-Asian adolescents.

In conclusion, we found that male Bruneian secondary school
students were more overweight than the females. Our analysis of
leptin levels in the saliva of the students showed no significant
difference between normal and overweight children. A follow up
study is needed to investigate the resting levels of salivary and
plasma leptin in young boys and girls to understand the differen-
tial regulation of salivary leptin.

4. Discussion

This is the first study to investigate leptin concentrations in
Bruneian school children by examining the association of BMI
with leptin concentrations in the salivary compartment. Our re-
sults showed no significant correlation between salivary leptin
levels of normal and overweight individuals (Table 2 and
Figure 1). Also, the salivary leptin levels between male and
female students were not significantly different (Figure 1). It is
known that salivary leptin and plasma leptin concentrations are
significantly higher in females than males [13], suggesting that
there may be differential regulation of salivary leptin between
males and females. However, Randeva et al. measured leptin
levels in the saliva of the students showed no signifi-
cant difference between normal and overweight children. A follow up
study is needed to investigate the resting levels of salivary and
plasma leptin in young boys and girls to understand the differen-
tial regulation of salivary leptin.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgments

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References

[1] Ungefroren H, Gieseler F, Fliedner S, Lehnert H. Obesity and
Nitsch D. Urbanization and non-communicable disease in South-
est Asia: a review of current evidence. Public Health 2014; 128:
886-95.
Bandar Seri Begawa: Community Nutrition Division, Ministry of
www.wpro.who.int/nutrition/documents/docs/bru.pdf [Accessed
on 6th October, 2015]
Available from: http://apps.who.int/iris/bitstream/10665/128038/1/
9789241507509_eng.pdf?ua=1 [Accessed on 6th October, 2015]
resistance and diet-induced obesity: central and peripheral actions
plasma levels in healthy Spanish children and adolescents,
children with obesity, and adolescents with anorexia nervosa and
[9] Fan SH, Say YH. Leptin and leptin receptor gene polymorphisms
and their association with plasma leptin levels and obesity in a multi-ethnic
Viroonudomphol D, Supawan V, et al. Leptin concentration in
relation to body mass index (BMI) and hematological measure-
ments in Thai obese and overweight subjects. Southeast Asian J
[12] Goodson JM, Kantarci A, Hartman ML, Denis GV, Stephens D,
Hasturk H, et al. Metabolic disease risk in children by salivary
[13] Randeva HS, Karteris E, Lewandowski KC, Sailesh S, O’Hare P,
Hillhouse EW. Circadian rhythmicity of salivary leptin in healthy
et al. A comparison of leptin and ghrelin levels in plasma and saliva
et al. Ethnic variation in adiponectin and leptin levels and their
association with adiposity and insulin resistance. Diabetes Care
2010; 33: 1629-34.