

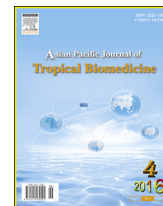
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## Asian Pacific Journal of Tropical Biomedicine

journal homepage: [www.elsevier.com/locate/apjtb](http://www.elsevier.com/locate/apjtb)Original article <http://dx.doi.org/10.1016/j.apjtb.2016.01.003>

## Salivary leptin concentrations in Bruneian secondary school children

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## ARTICLE INFO

## Article history:

Received 23 Oct 2015

Received in revised form 3 Nov 2015

Accepted 22 Nov 2015

Available online 8 Jan 2016

## Keywords:

Obesity

Saliva

Leptin

Brunei

Adolescents

Body mass index

## 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.

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The study protocol was performed according to the Helsinki declaration and approved by the PAPRSB Institute of Health Sciences, Universiti Brunei Darussalam Ethics Committee. Informed written consent was obtained from all participants and their parents.

Peer review under responsibility of Hainan Medical University. The journal implements double-blind peer review practiced by specially invited international editorial board members.

Foundation Project: Supported by the Brunei Research Council (Grant No. BRC4).

Therefore, our study investigated the BMI and salivary leptin levels in Bruneian 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/m<sup>2</sup> was considered as normal weight. A BMI of > 21.5 kg/m<sup>2</sup> was considered as overweight for girls, and BMI of 15–21.5 kg/m<sup>2</sup> as normal weight. For boys, a BMI ≥ 22 kg/m<sup>2</sup> was considered as overweight. The BMI was calculated as weight (kg) divided by height squared (m<sup>2</sup>). 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/m<sup>2</sup> (overweight), while 31% (*n* = 18) of the girls had BMIs > 21.5 kg/m<sup>2</sup> (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**

Characteristics of subjects selected for analysis.

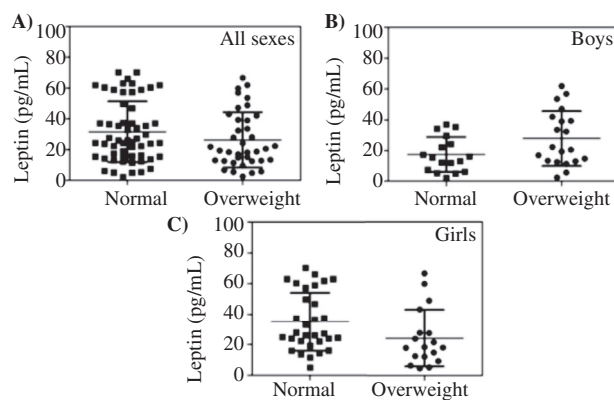
Characteristics	Male	Female
Number	37	58
Age span (year)	12–17	12–17
Age (year)	14.42 ± 1.65 <sup>a</sup>	14.48 ± 1.22 <sup>a</sup>
Leptin (pg/mL)	24.79 ± 18.80 <sup>a</sup>	32.74 ± 22.08 <sup>a</sup>
BMI (kg/m <sup>2</sup> )	24.17 ± 5.80 <sup>a</sup>	23.17 ± 5.38 <sup>a</sup>

<sup>a</sup>: Data were expressed as mean ± SD.

**Table 2**

Correlation analysis between BMI and leptin concentration and partial correlations controlled for age (excluding samples with poor % CV).

Children	Correlation	<i>P</i> value
All children	–0.091	0.399
All children (controlled for age)	–0.084	0.438
Girls only	–0.203	0.157
Girls only (controlled for age)	–0.197	0.175
Boys only	0.107	0.523
Boys only (controlled for age)	0.115	0.497



**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.

**Table 3**

Correlation analysis between BMI and leptin concentration and partial correlations controlled for age (including samples with poor % CV).

Children	Correlation	P value
All children	−0.072	0.423
All children (controlled for age)	−0.059	0.512
Girls only	−0.164	0.145
Girls only (controlled for age)	−0.150	0.176
Boys only	0.128	0.400
Boys only (controlled for age)	0.140	0.352

#### 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 results 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, Randeve *et al.* measured leptin in adults, not children [13]. Therefore it is possible that salivary leptin levels between males and females vary between adults and children.

Saliva serves as a good source of potential disease biomarkers; given its non-invasive and rapid collection process, it is less difficult to collect saliva compared to peripheral blood especially in children. Although salivary leptin levels were reported to be much lower than plasma leptin levels, there is a strong correlation between salivary and plasma leptin for both adults (aged 22–38 years) [13] and adolescents (aged 11–18 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 Aborigines) [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 differential regulation of salivary leptin.

#### Conflict of interest statement

We declare that we have no conflict of interest.

#### Acknowledgments

This project was funded by the Brunei Research Council (Grant No. BRC4). We would like to acknowledge the casual laboratory assistants under BRC4 who were involved in the logistics of the saliva collection. We also specially thank Heli Vitala for assisting in the distribution of the questionnaires to the participants.

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