PLASMA LEPTIN LEVELS AND A RESTRICTIVE LUNG IN OBESE THAI CHILDREN AND ADOLESCENTS

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Abstract. Morbid obesity, the most significant risk factor for development of several respiratory diseases, is linked to decreased pulmonary function. The aim of this study was to determine the relationships between pulmonary function and plasma levels of homeostasis model assessment-insulin resistance (HOMA-IR), insulin, leptin, hs-CRP and fasting glucose. Values were measured in 39 Thai children and adolescents, divided into three groups according to lung function (forced expiratory volume in one second, FEV_1); normal (Group A) FEV_1 ≥80% (*n*=19), obese normal (Group B) FEV_1 ≥80% (*n*=14) and obese (Group C) FEV_1 <80% (*n*=6). Body mass index was highest in group C. Groups A and B were comparable for FEV_1, forced vital capacity (FVC), maximal voluntary ventilation (MVV) and FEV_1/FVC, whereas Group C exhibited significantly reduced FEV_1, FVC and MVV but a normal FEV_1/FVC ratio. All values except the FEV_1/FVC ratio were significantly lower than in groups A and B. Group C had significantly higher levels of leptin, insulin, FG and HOMA-IR than Groups A and B (p<0.001). There was a significant negative correlation between FEV_1 and MVV with leptin, insulin and HOMA-IR, but not with high-sensitivity C-reactive protein (hs-CRP). We conclude that FEV_1 is reduced in obese children and adolescents and inversely correlates with plasma leptin, insulin and HOMA-IR levels. We have shown that the most important factor in inducing a restrictive lung in these patients may be related to leptin status.

Keywords: lung defect, leptin, obesity, Thai, children, adolescents

INTRODUCTION

The prevalence of obesity is steadily increasing in children, adolescents and adults around the globe (WHO, 2014) and has become a major public health problem (Peeters *et al*, 2003). Recently, we reported the prevalence of obesity and overweight in 12- to 18-year-old adolescents to be 4.9% and 9.5% using the sex-specific BMI-for-age, and 13.7% and 5.3% using the Thai standard-weight-for-height (Sengmeuang *et al*, 2010). Morbid obesity is well recognized as the most important risk factor for development of several respiratory diseases (Beuther *et al*, 2006; Deane and Thomson, 2006; Murugan and Sharma, 2008) which are associated with

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reduced lung function. Unlike adults, however, there have been few studies in children and adolescents with obesity and those that do exist demonstrate conflicting results in regard to pulmonary function (Marcus *et al*, 1996; Li *et al*, 2003; Ulger *et al*, 2006; Charususin *et al*, 2007).

It is well known that obese adults have high plasma leptin, C-reactive protein (CRP) and insulin levels. Lung function impairment has been shown to be associated with CRP levels (Aronson *et al*, 2006; Dahl *et al*, 2007) and insulin resistance (Paek *et al*, 2010). In fact, several studies in obese adults demonstrate that insulin resistance and fasting plasma insulin are inversely associated with FEV_1 and FVC (Lazarus *et al*, 1998; Engstrom *et al*, 2003; Walter *et al*, 2003; Lawlor *et al*, 2004). In obese children, hyperinsulinemia and insulin resistance have been demonstrated (Caprio, 2002; Shea *et al*, 2003). Moreover, adipose tissue seems to play a significant role in insulin resistance which is a common feature of obesity being mediated by high levels of leptin (Chiarelli and Marcovecchio, 2008). The only study in normal-weight adults revealed that those with impaired FEV_1 have high leptin levels. Higher levels of markers of systemic inflammation, *eg*, CRP, leukocytes, and fibrinogen have been observed in adults with restrictive rather than those with preserved lung function (Sin and Man, 2003). That is, FEV_1 was found to be inversely related to circulating leptin, and these authors suggested that reduced lung function could be related to high leptin levels.

These previous studies were carried out in countries other than Thailand. Recognising that pulmonary function depends on gender, age, weight, height and ethnicity, we therefore wished to evaluate the association between pulmonary function and leptin, CRP, fasting glucose, HOMA-IR and insulin in Thai children and adolescents in northeastern Thailand.

MATERIALS AND METHODS

Participants

We randomly recruited 20 obese and 19 normal-weight children and adolescents, 12-18 years old, from an urban area of Khon Kaen Province using sex-specific, BMI-for-age growth charts that we have developed (Sengmeuang *et al*, 2010). All children and adolescents had no history of cardiopulmonary or any other diseases. The methods used in this study were reviewed and approved by the Khon Kaen University Ethics Committee for Human Research (HE490936).

Pulmonary function measurements

A Vitalograph Pneumotrac (Vitalograph Ireland) was used to measure pulmonary function which includes FVC, FEV_1, FEV_1/FVC ratio, peak expiratory flow (PEF), and forced expiratory flow (FEF) at 25%, 50%, 75%, 25%-75% of the FVC, and MVV. Each was read from the best of three recordings made in the standing position with a nose clip in place according to the ATS/ERS Standardization of Lung Function Testing (Miller *et al*, 2005). All values were expressed as percentages of the predicted normal values (%pred). Normal values were defined as ≥80% of the predicted value. The presence of a restrictive defect was defined as low FEV_1 and FVC, and a normal or slightly increased FEV_1/FVC ratio, and an obstructive abnormality was defined as low FEV_1 and FVC, and a reduced FEV_1/FVC ratio (Pellegrino *et al*, 2005).

Laboratory measurements

After a 12-hour fast, a venous blood sample, approximately 10 ml, was drawn

Table 1
Clinical and anthropometric characteristics of 19 normal-weight and 20 obese children and adolescents.

	Normal weight (Group A)	Obese	
		$FEV_1 \geq 80\%$ (Group B)	$FEV_1 < 80\%$ (Group C)
Age (years)	15.0±1.3	15.2±1.5	14.0±1.3
Gender (M/F)	4/15	4/10	3/3
Body weight (kg)	51.6±5.6	83.2±15.9[a]	96.6±8.9[a]
BMI (kg/m^2)	20.2±1.6	32.2±4.4[a]	36.8±5.9[a,b]
WHR	0.76±0.06	0.87±0.06[a]	0.93±0.07[a]

Values are mean±SD. BMI, body mass index; WHR, waist to hip ratio. [a]$p<0.001$, Group A&B *vs* C; [b]$p<0.01$, Group C *vs* B.

from each participant. Aliquots of the sampled blood were stored for subsequent analysis of biochemical markers. Plasma leptin and insulin levels were determined by immunoradiometric assay. Insulin resistance was estimated using the homeostasis model assessment (HOMA score) (Keskin *et al*, 2005). Insulin resistance was defined as a value for HOMA-IR greater than 3.16 (Keskin *et al*, 2005). Plasma CRP and glucose levels were determined using a high sensitivity assay (immunonephelometric assay on a BN ProSpec) and by a UV test (enzymatic reference method with hexokinase) (Roche/Hitachi Cobas C-system).

Statistical analysis

Data were expressed as mean±standard deviation (SD). The significance of differences between means was analyzed using one-way analysis of variance (ANOVA) or two-sample Wilcoxon rank-sum (Mann-Whitney) tests. The associations between hs-CRP, leptin, insulin, FG and HOMAR-IR, and pulmonary function were examined using a multiple linear regression model. Statistical analyses were made using STATA version 10.0 (StataCorp, College Station, TX). A $p<0.05$ was considered significant.

RESULTS

Clinical characteristics of the healthy normal-weight and obese children and adolescents are summarized in Table 1. Out of 39 subjects, 49% (n=19) were of normal weight with normal lung function, *eg*, FEV_1 80%pred (Group A), 36% (n=14) were obese with normal FEV_1, *eg*, FEV_1 ≥80%pred (Group B) and 15% (n=6) were obese with reduced FEV_1, *eg*, FEV_1<80%pred, consistent with a restrictive lung defect (Group C). Age and height were not significantly different among the three groups whereas weight, BMI and the waist to hip ratio (WHR) were significantly different ($p<0.001$). Furthermore, BMI was higher in Group C than Group B ($p<0.01$) (Table 1).

Fasting insulin, HOMA-IR, hs-CRP and leptin levels in plasma were significantly higher in Groups B and C than Group A ($p<0.001$) whereas fasting glucose was not significantly different (Fig 1).

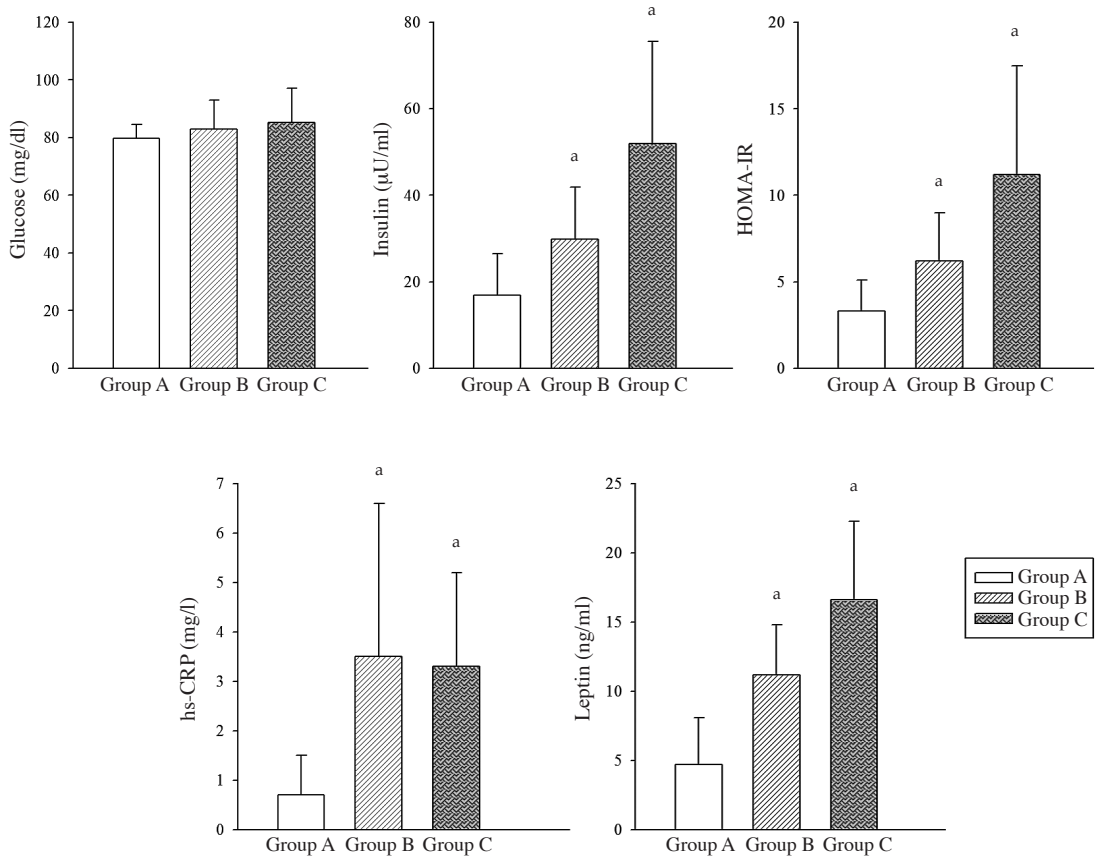

Fig 1–Comparisons between glucose, insulin, hs-CRP, HOMA-IR and leptin in 19 normal-weight children (Group A, FEV_1 ≥80%pred) and in 20 obese children and adolescents (Group B, FEV_1 ≥80%pred, *n*=14 and Group C FEV_1<80%pred, *n*=6). Values are mean±SD. [a]*p*<0.001, Group A & B *vs* C and Group A *vs* B. No significance in hs-CRP between Group B and Group C. HOMA-IR, homeostasis model assessment-insulin resistance; hs-CRP, high-sensitivity C-reactive protein; FEV_1, forced expiratory volume in 1 second.

Obese children and adolescents had insulin resistance and leptin resistance. That is, Group C had fasting insulin levels of 174% and 309% compared to Groups B and A, respectively (*p*<0.001). Similarly, HOMA-IR in Group C was 180% and 341% compared to Groups B and A (*p*<0.001). Additionally, leptin was 149% and 351% compared to Groups B and A (*p*<0.001). Group C had higher leptin, insulin and HOMA-IR than Group B (*p*<0.01). Plasma hs-CRP was similar between Groups B and C but was higher (448%) than in Group A (*p*<0.001).

Values of PEF, FEF at 25%, 50%, 75% and 25%-75% of the FVC in Group C were significantly lower than in Groups A (*p*<0.001) and B (*p*<0.05, *p*<0.05, *p*<0.01, *p*<0.01, *p*<0.01, respectively) (Fig 2). The mean predicted values for FVC, FEV_1 and MVV among the three groups revealed significant differences (Fig 3). Groups A and B had normal values. In contrast, Group C had impaired FEV_1

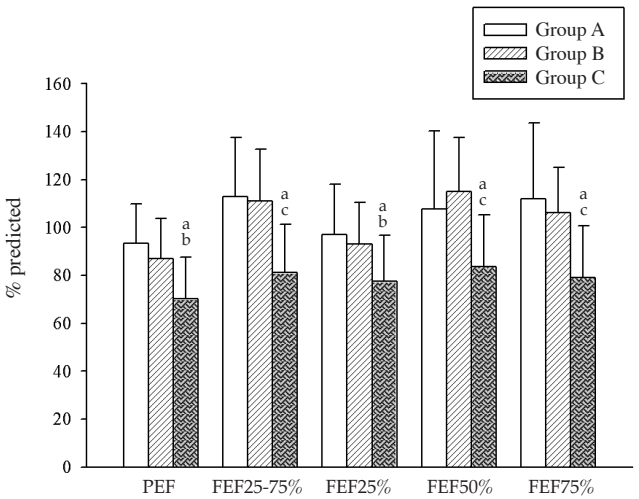

Fig 2–PEF, FEF25-75%, FEF25%, FEF50% and FEF75% in 19 normal-weight (Group A, FEV_1≥80%pred) and in 20 obese children and adolescents (Group B, FEV_1≥80%pred, *n*=14 and Group C, FEV_1<80%pred, *n*=6). Values are mean±SD. [a]*p*< 0.001, Group A & B *vs* C; [b]*p*<0.05 and [c]*p*< 0.01, Group B *vs* Group C. PEF, peak expiratory flow; FEF, forced expiratory flow; FEV_1, forced expiratory volume in 1 second.

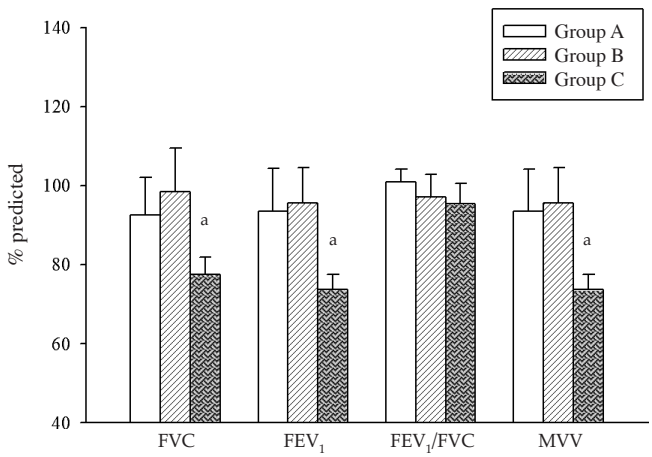

Fig 3–FVC, FEV_1, FEV_1/FVC and MVV in 19 normal-weight (Group A, FEV_1≥80%pred) and in 20 obese children and adolescents (Group B, FEV_1≥80%pred, *n*=14 and Group C, FEV_1<80%pred, *n*=6). Values are mean±SD. [a]*p*<0.001, Groups A & B *vs* Group C. FVC, forced expiratory capacity; MVV, maximal voluntary ventilation.

(73.8 ± 3.70%pred), FVC (77.5 ± 4.46%pred), MVV (73.7 ± 3.82%pred) but normal FEV_1/FVC ratios (95.5 ± 5.08%pred) which is indicative of a restrictive defect. Furthermore, all pulmonary function variables in Group C except FEV_1/FVC were significantly lower than those of Groups A and B (*p*<0.001).

Fig 4 summarizes correlations between HOMA-IR, insulin, leptin, hs-CRP and FEV_1 in children and adolescents. Using a multiple regression analysis model, FEV_1 was found to be strongly and inversely correlated with leptin (R^2=0.560, *p*=0.007), insulin (R^2=0.453; *p*=0.002) and HOMA-IR (R^2=0.421, *p*=0.003), but not with hs-CRP (R^2=0.286, *p*=0.807). Similarly, MVV was inversely correlated with leptin (R^2=0.582, *p*<0.01). It was also inversely correlated with insulin (R^2=0.493, *p*<0.05) and HOMA-IR (R^2=0.367, *p*<0.05) but not with hs-CRP (Fig 4).

DISCUSSION

The present study demonstrates reductions in FEV_1, FVC, PEF, FEF and MVV with evidence of a restrictive pulmonary defect in six out of 20 obese children and adolescents. This is in agreement with an earlier study from the USA which reported a restrictive defect, although only in one out of 17 obese children and adolescents (Marcus *et al*, 1996). However, it is inconsistent with other studies (from Hong Kong and

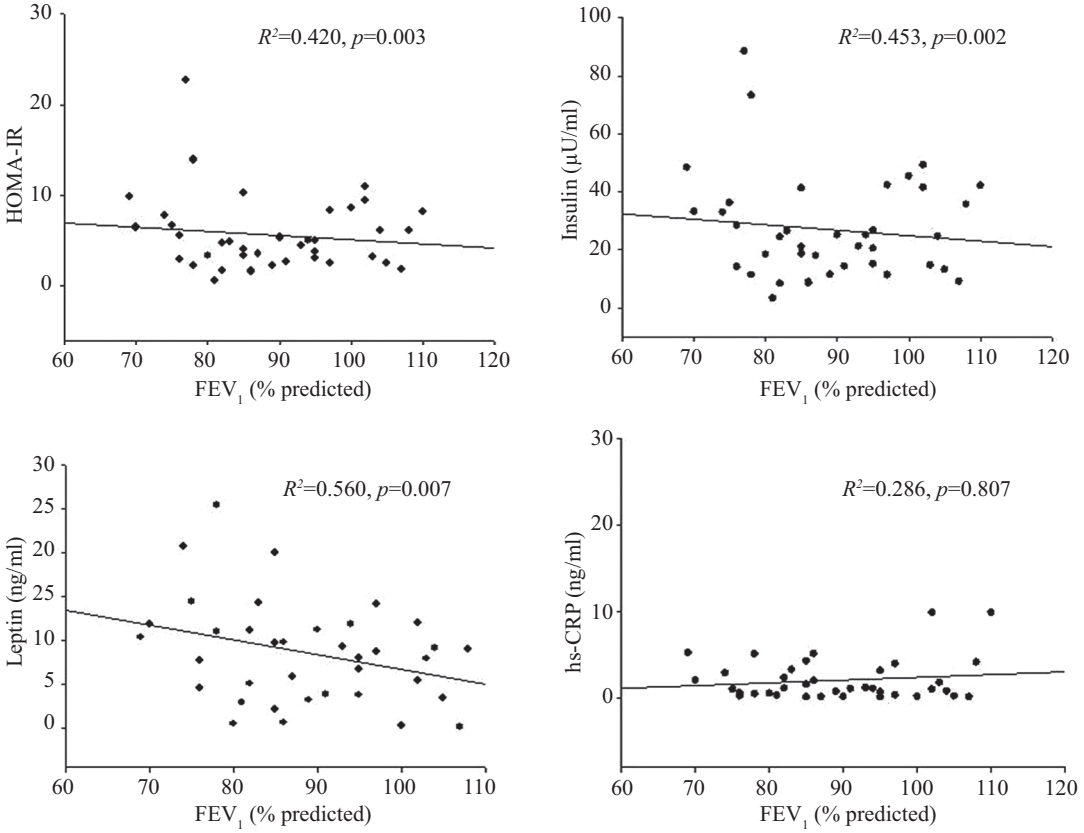

Fig 4–Correlations between HOMA-IR, insulin, leptin, hs-CRP and FEV_1 in 39 Thai children and adolescents. HOMA-IR, homeostasis model assessment-insulin resistance; hs-CRP, high-sensitivity C-reactive protein; FEV_1, forced expiratory volume in 1 second.

the USA, respectively) which observed three children out of 48 (Li *et al*, 2003) and three out of 80 (Mallory *et al*, 1989) having obstructive defects. Interestingly, there is a study from Turkey reporting pulmonary restriction in three out of 17 and obstruction in eight out of 17 obese children (Boran *et al*, 2007). Our finding that FEV_1, FVC, and MVV were reduced is comparable to previous studies in obese children (Marcus *et al*, 1996; Li *et al*, 2003; Ulger *et al*, 2006). Other reports of obese children have found a decrease in pulmonary function such as FEV_1/FVC ratio and FEF25%-75% in boys and FVC and FEV_1 in girls in native US Americans and US Americans (Harik-Khan *et al*, 2001; Eisenmann *et al*, 2007). The only study in Thai obese children (aged 10 to 12 years) showed no reductions in FEV_1, VC and FVC (Charususin *et al*, 2007). In the present study, we found that six (three boys and three girls) out of 20 obese children and adolescents with impaired lung function had significantly higher BMI than that of obese children and adolescents with normal pulmonary function. An inverse correlation of FEV_1 with BMI has been shown in adults (Chen *et al*, 1993). The decreased pulmonary function in children and adolescents with obesity in previous studies could be due to several

mechanisms. Abdominal fat deposition may directly obstruct the descent of the diaphragm (Marcus *et al*, 1996) whereas fat deposition in the chest wall may diminish rib cage movement and thoracic compliance (Li *et al*, 2003), both leading to a restrictive pulmonary impairment (Mallory *et al*, 1989; Marcus *et al*, 1996; Boran *et al*, 2007).

We also found increased concentration of insulin, HOMA-IR, hs-CRP and leptin in obese children and adolescents compared to normal-weight counterparts. Furthermore, the obese subjects had insulin and leptin resistance. This finding is consistent with other studies demonstrating insulin resistance and hyperinsulinemia in children and adolescents with moderate to severe obesity (Caprio, 2002; Shea *et al*, 2003). Moreover, children with higher plasma leptin levels have been reported to have significantly higher insulin levels and insulin resistance than children with low leptin levels (Chu *et al*, 2000). There is evidence suggesting that obese children have higher leptin and CRP plasma levels than normal-weight children (Chiarelli and Marcovecchio, 2008). Furthermore, the elevation of leptin and CRP levels in adults have been reported to be associated with impaired FEV_1 (Sin and Man, 2003).

To our knowledge, this is the first study showing that leptin, insulin and HOMA-IR are significantly and inversely correlated with FEV_1 in obese children and adolescents. For the first time, our study on Thai children and adolescents has shown that fasting serum insulin levels are negatively correlated with FEV_1 which has been shown previously in adults (Lazarus *et al*, 1998). Furthermore, plasma leptin levels are elevated in adults of normal weight with impaired lung function (Sin and Man, 2003). A reduction in leptin levels might be a predictor of improvement in lung function (Leao da Silva *et al*, 2012). Increased leptin levels are strongly and independently associated with lung function impairment (Schols *et al*, 1999). An inverse association between pulmonary function and CRP in healthy adults has been demonstrated (Aronson *et al*, 2006). Therefore, we suggest that leptin may play a significant role in the impaired lung function observed in our obese subjects. However, lung tissue inflammation due to high levels of CRP and insulin could also be responsible for reduced lung function as well.

In conclusion, we found six out of 20 obese Thai children and adolescents had evidence of pulmonary restriction. High levels of, especially, leptin, insulin resistance and CRP are probably responsible for impaired lung function in these subjects.

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