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Final Report

Plasma adipokines before and after lifestyle intervention in obese Thai children

By Dr Sirinuch Chomtho

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Chulalongkorn University

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Abstract

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Abstract:

BACKGROUND: A low-glycemic index (GI) diet has been documented to be beneficial for improving insulin sensitivity. The effect of the low-GI diet may modulate plasma adipokines produced by adipocytes which link to insulin resistance and the metabolic syndrome.

METHODS: Obese children aged 9–16 y were randomly assigned either a low-GI diet or a low-fat diet (control group) for 6 mo. Changes of BMI z-score, body composition measured by dual energy X-ray absorptiometry, and HOMA-IR were compared between groups. Serum adipokines namely: leptin, adiponectin, resistin, and visfatin were measured by ELISA technique. The relationships between these clinical outcomes and adipokines were assessed using correlations and multivariate analysis.

RESULTS: Fifty-two participants completed the study (mean age: 12.0 ± 2.0 y, 35 boys); both groups showed significantly decreased BMI z-score, but subtle changes in fat and fat-free mass. The intervention group had significant changes in fasting insulin and HOMA-IR, whereas the control group did not. There were no significant differences in adipokines between the groups in visit 1 and visit 6. However, there were relationships between baseline leptin and the change of BMI z-score in both groups, as well as baseline resistin, baseline visfatin and the change of BMI z-score in the control group. There were significant positive associations between baseline leptin and the change of FMI in both groups. The intervention tended to weaken the negative relationship between adiponectin and the change of FFMI.

CONCLUSION: Serum leptin was positively correlated with the change of BMI z-score and FMI in both groups. These results indicate that circulating leptin levels may represent the change of BMI and adipose tissue mass in obese children, and could therefore be a useful indicator to predict the improvement in insulin resistance from an obesity intervention trial.

Keywords: obesity, adipokines, leptin, resistin, adiponectin, visfatin

บทคัดย่อ

รหัสโครงการ: MRG5580204

ชื่อโครงการ:ระดับอะดิโพคายน์ก่อนและหลังการปรับเปลี่ยนพฤติกรรมในเด็กอ้วนไทย

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บทคัดย่อ:

ที่มา มีงานวิจัยที่สนับสนุนว่าอาหารดัชนีน้ำตาลต่ำมีประโยชน์ในการทำให้ภาวะดื้อต่ออินซูลินในดีขึ้น ทั้งนี้อาหารดัชนีน้ำตาลต่ำอาจทำให้เกิดการเปลี่ยนแปลงของระดับอะดิโพคายน์ซึ่งผลิตจากอะดิโพซัยต์ที่อาจจะมีความสัมพันธ์กับภาวะดื้อต่ออินซูลิน

วิธีการศึกษา เด็กอ้วนอายุตั้งแต่ 9-16 ปีได้ถูกสุ่มให้ได้รับอาหารดัชนีน้ำตาลต่ำหรืออาหารไขมันต่ำ (กลุ่มควบคุม) เป็นเวลา 6 เดือน ผู้วิจัยทำการเปรียบเทียบและหาความสัมพันธ์ระหว่างการเปลี่ยนแปลงของดัชนีมวลกาย องค์ประกอบของร่างกาย ภาวะดื้อต่ออินซูลินและอะดิโพคายน์ต่างๆได้แก่ เลปติน, รีซิสติน, อะดิโพเน็กติน, วิสฟาตินซึ่งตรวจวัดโดยวิธีอีไลซ่า โดยใช้สถิติการหาความสัมพันธ์แบบตัวแปรเดียวและหลายตัวแปร

ผลการศึกษา มีเด็กอ้วนเข้าร่วมการศึกษาจนครบจำนวน 52 ราย(อายุเฉลี่ย 12±2 ปี, เพศชาย 35 ราย) ดัชนีมวลกายลดลงอย่างมีนัยสำคัญในทั้ง 2 กลุ่มแต่ดัชนีมวลไขมันและมวลปราศจากไขมันเปลี่ยนแปลงไม่ชัดเจน กลุ่มที่ได้รับอาหารดัชนีน้ำตาลต่ำมีการลดลงของภาวะดื้ออินซูลินอย่างชัดเจนในขณะที่กลุ่มควบคุมไม่มีการเปลี่ยนแปลง ไม่มีความแตกต่างของระดับอะดิโพคายน์ระหว่าง 2 กลุ่มทั้งช่วงเริ่มแรกและสิ้นสุดการศึกษาแต่อย่างไรก็ตามระดับเลปตินเริ่มต้นมีความสัมพันธ์กับการเปลี่ยนแปลงของดัชนีมวลกายในทั้ง 2 กลุ่มและระดับรีซิสตินและวิสฟาตินมีความสัมพันธ์กับการเปลี่ยนแปลงของดัชนีมวลกายในกลุ่มควบคุม ระดับเลปตินเริ่มต้นมีความสัมพันธ์เชิงบวกกับการเปลี่ยนแปลงของดัชนีมวลไขมันในทั้ง 2 กลุ่ม การได้รับอาหารดัชนีน้ำตาลต่ำทำให้ความสัมพันธ์เชิงลบของอะดิโพเน็กตินกับการเปลี่ยนแปลงของดัชนีมวลปราศจากไขมันอ่อนลง

สรุปผลการศึกษา เลปตินมีความสัมพันธ์เชิงบวกกับการเปลี่ยนแปลงของดัชนีมวลกายและดัชนีมวลไขมันในเด็กอ้วนที่เข้าร่วมการศึกษาทั้งสองกลุ่ม ผลการศึกษานี้แสดงให้เห็นว่าระดับเลปตินในซีรั่มอาจเป็นตัวแทนของการเปลี่ยนแปลงของมวลไขมันในเด็กอ้วนและอาจใช้เป็นตัวพยากรณ์ผลของการรักษาโรคอ้วนในเด็กในแง่มวลไขมันและภาวะดื้อต่ออินซูลินได้

คำหลัก : อ้วน, อะดิโพคายน์, เลปติน, รีซิสติน, อะดิโพเน็กติน, วิสฟาติน

Executive summary

Introduction to Research

Prevalence of childhood and adolescent obesity is increasing both in developed and developing countries. In Thailand, there is a trend towards a higher number of children with overnutrition rather than undernutrition (1). These obese children have several health problems, such as obstructive sleep apnea, deformity of bone and joints, and the metabolic syndrome similar to that of obese adults. Therefore, childhood obesity and the metabolic syndrome is an emerging health problem that needs early interventions with dietary control, increased physical activity and behavior modification. The metabolic syndrome consists of insulin resistance, hypertension, dyslipidemia (hypertriglyceridemia, low HDL-C) and central obesity (2). The pathogenesis and pathophysiology of the metabolic syndrome remains controversial issues (3). It is postulated that obese children have accumulated excess adipose tissue, especially those with central adiposity, which may release free fatty acids and deposit it in muscles, the liver and the pancreas. Recent knowledge shows that adipocytes play an important role as an endocrine organ to regulate metabolism and energy homeostasis, leading to the discovery of several adipokines, including leptin, visfatin, and resistin, and pro-inflammatory cytokines that cause chronic low-grade inflammatory state. On the other hand, adiponectin, which is one of the

adipokines and has an anti-inflammatory effect as well as increases insulin sensitivity, is suppressed. All these changes may disturb the insulin signaling and eventually cause insulin resistance (4).There is some recent evidence regarding the roles of adipokines in the pathogenesis of the metabolic syndrome in obese children and the association of these adipokines and body fat (in particular, visceral fat).

Literature review

Leptin is a hormone secreted by adipocytes. It acts through the hypothalamus, suppressing appetite and increasing energy expenditure. Most studies (5-7) in Caucasians and Asians alike, found that overweight and obese children have higher plasma leptin levels than do normal-weight children, especially obese children who have the metabolic syndrome. On the contrary, a few small studies showed that lifestyle intervention in obese children reduced leptin and increased soluble leptin receptor (8, 9). Adiponectin is an insulin-sensitizing hormone produced and released by adipocytes. It has been shown to decrease circulating glucose levels by suppressing gluconeogenesis in the liver and enhancing insulin signaling in the skeletal muscle (10). A previous study showed that serum adiponectin is inversely associated with obesity (11). In addition, increased serum adiponectin levels may play a protective role in metabolic syndrome. The body fat distribution influences adiponectin levels, with abdominal

obesity associated with lower concentrations (12, 13). The association between central adiposity and reduced adiponectin was seen with visceral fat accumulation in children (14). Resistin is a cytokine expressed in adipose tissue. Despite showing a strong linkage with obesity and insulin resistance in animal models, its role in obesity and insulin sensitivity in human is still controversial. One interventional study from Germany found no relationship between resistin, insulin resistance index, and weight status in childhood (15), while Elloumi et al. demonstrated an intriguing increase in resistin in boys after a 2-month weight loss program (16). Furthermore, visfatin is a newly discovered adipokine that mimics the action of insulin via a distinct binding site on the insulin receptor and, therefore, an increased risk for insulin resistance (14). It is produced mainly from visceral adipose tissue in animal models. Araki et al. (17) reported a good correlation between visceral adipose tissue and plasma visfatin from a cross-sectional study in 56 obese Japanese children. The researchers also demonstrated a significant difference between plasma visfatin between normal and obese children with and without metabolic syndrome. The same findings were also found in obese Caucasian children (18, 19). However, the data were conflicting whether visfatin has any association with insulin resistance and other components of the metabolic syndrome. So far, there is only one intervention study in

Europe reporting the effect of weight reduction program on visfatin level in obese adolescents (20).

However, the longitudinal and interventional study of the change in these adipokines after lifestyle interventions with the aim of body fat reduction is still scarce. This information could enlighten the pathophysiologic changes that occur in childhood obesity and the metabolic syndrome as well as proven causal relationship between these biomarkers and body fat. In our previous study on the effects of low-GI diet on insulin resistance, we found that the low-GI diet may improve insulin sensitivity in obese children with high baseline insulin (21).

Objectives

1. To compare plasma adipokine concentrations before and after a 6-month instruction of low-GI diet versus conventional management in obese Thai children.

2. To study a relationship between plasma adipokines and body composition as well as the metabolic syndrome risks during the 6-month studied period.

Research methodology

Study design

The protocol was approved by the Institutional Review Board of the Faculty of Medicine, Chulalongkorn University, Thailand. This study was a single-center prospective, randomized,

controlled trial. The methods were already published in the previous journal article (21). In brief, eligible participants for this study with a BMI higher than the International Obesity Task Force cut-off, corresponding to a BMI of thirty in adulthood (22), were enrolled and randomized to obesity clinic instruction or an intervention of a low-GI diet. For the intervention group, individual goals for weight management were set and instruction about low-GI foods was provided. A dietitian emphasized the selection of low-GI carbohydrates, which were adapted from the table by Foster-Powell et al. (23). The control group received conventional management instructions at the Nutrition Clinic about energy restriction, low-fat and high-fiber diet. Moreover, the participants in both groups received the same instruction about increasing physical activity. Both groups needed to maintain the monthly visits for six months. The adherence to the nutritional education and physical activity recommendation was evaluated by using three-day dietary records and a physical activity questionnaire at each visit. One blood sample was taken in the morning after 12 h of fasting. The main outcomes were adipokines that the participants were examined for at the first and sixth visits, and which included serum leptin, adiponectin, resistin, and visfatin levels. These adipokines were measured by ELISA KIT (R&D System Inc., Minneapolis, USA for leptin, adiponectin, and resistin, and Adipogen Life Sciences, Liestal, Switzerland) and analyzed by using software: Revelation Quicklink version 4.25. The secondary

outcomes were the correlations between the adipokines and BMI z-score that was calculated based on World Health Organization (WHO) 2007 growth reference using the WHO AnthroPlus program (24), and body composition that was evaluated by using DXA (Hologic QDR Discovery A) on the first and sixth visits.

Statistical analysis

Descriptive statistics were used to summarize all quantitative data. Qualitative data were summarized using percentages. Continuous variables were reported as mean ± standard deviation (SD) and compared between the control and intervention groups by an independent sample t-test. Paired t-test for dependent samples was used to evaluate the changes within the groups before and after the six-month period. We evaluated the correlations between BMI z-score, waist circumference, body composition, insulin resistance, adipokines, and lipid profiles in each group and in all participants and present as table and forest plots. Statistical significance was defined as a *P*-value < 0.05. Analyses were completed using SPSS 23.0 (SPSS Inc., Chicago, IL).

Result

Seventy participants (47 boys, 23 girls) were enrolled and randomized into two groups: 35 in each control and intervention group as shown in the flow diagram in our previous study (21). There were 52 out of 70 participants who completed all six visits (27 in the control and 25 in the intervention groups). Mean age in the control group was 12.0 ± 2.1 y and 11.9 ± 1.9 y in the intervention group. The other baseline characteristics were shown in the previous study (21). All analyses followed the intention-to-treat principle. The intervention group had significantly increased daily consumption of the low-GI food and decreased total energy intake compared to the control group. Moreover, brisk walking was the common feature of exercise in both groups and around half of the participants in both groups were physically active.

There were significant differences in plasma insulin levels and HOMA-IR; however, no significant differences in adipokines between the two groups in visit 1 and visit 6 were observed (**Table 1**).

Table 1 Comparison of BMI z-score, waist circumference, body composition measured by DXA, fasting insulin, HOMA-IR, adipokines, and lipid profiles between the control and intervention groups (*n = 52*)

Parameters	Control (*n = 27*)	Intervention (*n = 25*)	*P*-value[a]
Visit 1			
BMI z-score[b]	3.6 ± 1.6[a]	3.7 ± 0.9	0.88
Waist circumference (cm)	103.1 ± 14.9	105.8 ± 7.7	0.40
% Fat[c]	41.1 ± 6.0	42.1 ± 4.8	0.56
FMI (kg/m^2)	14.0 ± 4.5	14.7 ± 3.8	0.56
FFMI (kg/m^2)	18.8 ± 2.9	19.1 ± 2.6	0.66
Laboratory test			
Fasting insulin (mU/L)	15 ± 8	22 ± 14	**0.035**
HOMA-IR	3.1 ± 1.7	4.8 ± 3.3	**0.035**
Leptin (ng/mL)	27.7 ± 15.6	28.8 ± 12.9	0.80
Adiponectin (ng/mL)	2,203.1 ± 1,934.8	2,077.3 ± 1,499.8	0.81
Resistin (ng/mL)	13.1 ± 6.6	12.6 ± 5.9	0.79
Visfatin (ng/mL)	11.1 ± 12.5	17.9 ± 15.9	0.13
Serum total cholesterol (mmol/L)	4.5 ± 0.8	4.7 ± 0.8	0.26
Serum triglyceride (mmol/L)	1.1 ± 0.5	1.2 ± 0.4	0.41
Serum HDL (C) (mmol/L)	1.2 ± 0.2	1.2 ± 0.4	0.45
Serum LDL (C) (mmol/L)	2.7 ± 0.7	3.1 ± 0.6	**0.029**
Visit 6			
BMI z-core	3.4 ± 1.3	3.4 ± 0.9	0.87
Waist circumference (cm)	104.2 ± 15.8	106.3±9.5	0.55
% Fat	40.2+8.7	43.1+6.1	0.55
FMI (kg/m^2)	14.4 + 4.5	15.2+4.4	0.59
FFMI (kg/m^2)	18.8+3.4	18.8+2.6	0.84
Laboratory test[d]			
Fasting insulin (mU/L)	14 ± 10	14 ± 11	0.88
HOMA-IR	3.2 ± 2.6	2.9 ± 2.3	0.69
Leptin (ng/ml)	30.1 ± 18.2	26.2 ± 15.5	0.41
Adiponectin (ng/ml)	2,424.1 ± 2,427.6	2,461.8 ± 1,703.3	0.94
Resistin (ng/ml)	13.2 ± 7.0	13.6 ± 9.8	0.89
Visfatin (ng/ml)	14.6 ± 12.5	14.5 ± 14	0.75
Serum total cholesterol (mmol/L)	4.4 ± 0.9	4.7 ± 0.7	0.39
Serum triglyceride (mmol/L)	1.1 ± 0.6	1.1 ± 0.4	0.81
Serum HDL (C) (mmol/L)	0.1 ± 0.2	1.3 ± 0.3	0.68
Serum LDL (C) (mmol/L)	2.7 ± 0.9	3 ± 0.7	0.26

C, cholesterol; DXA, dual-energy X-ray absorptiometry; FMI, fat mass index = fat mass (kg)/height (m^2); FFMI, fat-free mass index = fat-free mass (kg)/height (m^2); HOMA-IR, homeostatic model of assessment-insulin resistance = (FI × FPG)/22.5; FI, fasting insulin concentration (mU/l); FPG, fasting plasma glucose (mmol/l). [a]These data showed means ± SDs and independent sample t-test was used to evaluate continuous variables. [b]BMI z-score were calculated from WHO growth reference 2007 (24). [c]Percentage of fat was calculated from fat mass (kg)/body weight (kg) × 100. [d]There were data from 26 participants in the control group due to missing laboratory results from 1 participant. Bold text shows that intervention group was different from control group, $P < 0.05$.

Despite decreased BMI z-score between the first and the sixth visits in the intervention group ($P < 0.0001$), leptin and visfatin decreased while adiponectin increased, but these were not significantly different between the first and the last visit (**Table 2**).

Table 2 Comparison of body composition measured by DXA, fasting insulin, HOMA-IR, adipokines, and lipids profiles between the first and last visits during the 6-mo period (*n* = 52)

Group	Parameters	Visit 1	Visit 6	*P*-value[a]
Control	BMI z-score	3.6±1.6[b]	3.4±1.3	**0.007**
(*n* = 27)	Waist circumference (cm)	103.1±14.9	104.2±15.8	0.32
	% Fat	36.6+8.2	35.7+5.8	0.50
	FMI (kg/m^2)	12.4+5.2	12.3+4.0	0.84
	FFMI (kg/m^2)	20.8+3.4	21.5+3.6	0.14
	Fasting plasma insulin, mU/L	15±8	14±10	0.70
	HOMA-IR	3.1±1.7	3.2±2.6	0.91
	Leptin (ng/ml)	27.7 ± 15.6	30.1 ± 18.2	0.34
	Adiponectin (ng/ml)	2,203.1 ± 1,934.8	2,424.1 ± 2,427.6	0.33
	Resistin (ng/ml)	13.1 ± 6.6	13.2 ± 7.0	0.91
	Visfatin (ng/ml)	11.1 ± 12.5	14.6 ± 12.5	0.37
	Serum total cholesterol, mmol/L	4.5±0.8	4.4±0.9	0.51
	Serum triglyceride, mmol/L	1.1±0.5	1.1±0.6	0.67
	Serum HDL (C), mmol/L	1.2±0.2	0.1±0.2	0.35
	Serum LDL (C), mmol/L	2.7±0.7	2.7±0.9	0.89
Intervention	BMI z-score	3.7±0.9	3.4±1.0	**<0.0001**
(*n* = 25)	Waist circumference (cm)	105.8±7.7	106.3±9.5	0.53
	% Fat	42.1+4.8	43.1+6.1	0.06
	FMI (kg/m^2)	14.7+3.8	15.2+4.4	0.07
	FFMI (kg/m^2)	19.1+2.6	18.8+2.6	0.12
	Fasting plasma insulin, mU/L	22±14	14±11	**0.004**
	HOMA-IR	4.8±3.3	2.9±2.3	**0.007**
	Leptin (ng/ml)	28.8 ± 12.9	26.2 ± 15.5	0.35
	Adiponectin (ng/ml)	2,077.3 ± 1,499.8	2,461.8 ± 1,703.3	0.14
	Resistin (ng/ml)	12.6 ± 5.9	13.6 ± 9.8	0.57
	Visfatin (ng/ml)	17.9 ± 15.9	14.5 ± 14	0.37
	Serum total cholesterol, mmol/L	4.7±0.8	4.7±0.7	0.60
	Serum triglyceride, mmol/L	1.2±0.4	1.1±0.4	0.24
	Serum HDL (C), mmol/L	1.3±0.4	1.3±0.3	1.00
	Serum LDL (C), mmol/L	3.2±0.6	3.0±0.7	0.25

[a]Paired t-test was used to compare data between the first and sixth visits. [b]Means ± SDs (all such values). Bold text showed that visit 6 was significantly different from visit 1, $P < 0.01$. C, cholesterol; HOMAIR, homeostatic model of assessment-insulin resistance = (FI × FPG)/22.5; FI, fasting insulin concentration (mU/l); FPG, fasting plasma glucose (mmol/l).

Table 3 shows no significant differences in the changes of body composition, adipokines, and lipid profiles, even though fasting insulin and HOMA-IR significantly decreased from baseline to visit 6 in the intervention group (22.2 ± 14.3 vs. 13.7 ± 10.9 mU/l; P = 0.004 and 4.8 ± 3.3 vs. 2.9 ± 2.3; P = 0.007, respectively).

Table 3 Comparison of changes in anthropometry, body composition measured by DXA and laboratory tests between the control and intervention groups during the 6-month period (*n = 52*)

Changes in outcomes[a]	Control (*n* = 27)	Intervention (*n* = 25)	*P*-value[b]
BMI z-score	-0.3 ± 0.5[c]	-0.3 ± 0.2	0.99
Waist circumference, cm	1.1 ± 5.6	0.5 ± 4.1	0.66
FMI, kg/m^2	0.3 ± 1.6	0.8 ± 3.5	0.45
FFMI, kg/m^2	0.2 ± 0.8	0.8 ± 3.9	0.44
% Fat[d]	0.1 ± 2.8	0.1 ± 3.0	0.35
Fasting plasma insulin, mU/L	-0.8 ± 11.3	-8.5 ± 13.5	**0.032**
HOMA-IR	0.1 ± 2.8	-1.9 ± 3.2	**0.019**
Leptin, ng/ml	2.5 ± 12.7	-2.6 ± 12.9	0.18
Adiponectin, ng/ml	221 ± 1,120	384.5 ± 1,164.9	0.63
Resistin, ng/ml	0.1 ± 5.1	0.9 ± 7.5	0.66
Visfatin, ng/ml	3.4 ± 18.9	-2.9 ± 16.3	0.23
Serum total cholesterol, mmol/L	-0.1 ± 0.6	-0.1 ± 0.7	0.98
Serum triglyceride, mmol/L	0 ± 0.4	-0.1 ± 0.4	0.26
Serum HDL (C), mmol/L	0 ± 0.2	0 ± 0.3	0.55
Serum LDL (C), mmol/L	0 ± 0.5	-0.1 ± 0.5	0.38

[a]Changes in outcomes were the average of the difference between visit 6 and visit 1. [b]Independent sample t-test was used to compare the data between the two groups. [c]Means ± SDs (all such values). [d]Percentage of fat = fat mass (kg) × 100/body weight (kg). Bold text showed that intervention group was different from control group, P < 0.05. C, cholesterol; DXA, dual-energy X-ray absorptiometry; FFMI, fat-free mass index = fatfree mass (kg)/height (m^2); FMI, fat mass index = fat mass (kg)/height (m^2); HOMA-IR, homeostatic model of assessment-insulin resistance = (FI × FPG)/22.5; FI, fasting insulin concentration (mU/l); FPG, fasting plasma glucose (mmol/l).

The correlations between adipokines and the other parameters show in **Table 4** and **Figure 1-4**. There were significant relationships between baseline leptin and the change of BMI

z-score in the control and intervention groups (95% CI 0.16, 0.74, p < 0.001 and 95% CI 0.09, 0.75, p < 0.001, respectively) as well as baseline resistin, baseline visfatin and the change of BMI z-score (95% CI 0.12, 0.74, p = 0.013 and 95% CI 0.10, 0.72, p = 0.015, respectively) in the control group. There were significant associations between baseline leptin and the change of FMI in the control and intervention groups (95% CI 0.48, 0.87, p = < 0.001 and 95% CI 0.19, 0.79, p < 0.001, respectively). The intervention tended to weaken the negative relationship between adiponectin and the change of FFMI. There were no significant correlations between baseline adipokines and the changes of insulin and HOMA-IR in either group.

Table 4 Correlations between change of adipocytokines and change of anthropometry, body composition, and lipid profiles in the control group, intervention group, and all of the participants

Change of outcomes	Control (*n* = *27*)				Intervention (*n* = *25*)			
	Leptin	Adiponectin	Resistin	Visfatin	Leptin	Adiponectin	Resistin	Visfatin
BMI z-score	0.55**	-0.16	0.51**	0.48*	0.51**	-0.04	0.18	-0.01
% Fat	0.53**	-0.17	0.10	0.23	0.35	0.13	-0.06	-0.45*
FMI, kg/m^2	0.73**	-0.23	0.25	0.29	0.54**	-0.09	0.17	-0.48*
FFMI, kg/m^2	0.36	-0.41	0.13	0.23	0.23	-0.31	0.41	0.07
Insulin, mU/L	0.34	0.19	0.08	-0.04	0.19	-0.37	0.25	-0.14
HOMA-IR	0.24	0.09	-0.05	0.00	0.20	-0.33	0.24	-0.12
Leptin, ng/mL	1	0.14	0.25	0.14	1	0.07	0.25	-0.52**
Adiponectin, ng/mL	0.14	1	-0.07	-0.42*	0.07	1	-0.59**	-0.01
Resistin, ng/mL	0.25	-0.07	1	0.53**	0.25	-0.59**	1	-0.02
Visfatin, ng/mL	0.14	-0.42*	0.53**	1	-0.52**	-0.01	-0.02	1
TC, mmol/L	0.12	0.16	0.20	0.16	0.07	0.21	-0.29	-0.15
TG, mmol/L	0.21	0.29	0.15	0.03	0.39	0.25	0.09	-0.17
HDL (C), mmol/L	-0.32	0.02	-0.08	0.19	0.02	0.42	-0.15	-0.06
LDL (C), mmol/L	0.37	0.05	0.28	0.22	0.44**	0.11	-0.30	-0.42

*Correlation is significant at the 0.05 level.

**Correlation is significant at the 0.01 level.

C, cholesterol; FFMI, fat-free mass index = fat-free mass (kg)/height (m2); FMI, fat mass index = fat mass (kg)/height (m2); HOMA-IR, homeostatic model of assessment-insulin resistance = (FI × FPG)/22.5; FI, fasting insulin concentration (mU/l); FPG, fasting plasma glucose (mmol/l); TC: Serum total cholesterol; TG: Serum triglyceride

Figure 1 Forest plots of the correlations between baseline leptin and the changes of BMI z-score, FMI, FFMI, insulin, and HOMA-IR in control and intervention groups

Leptin

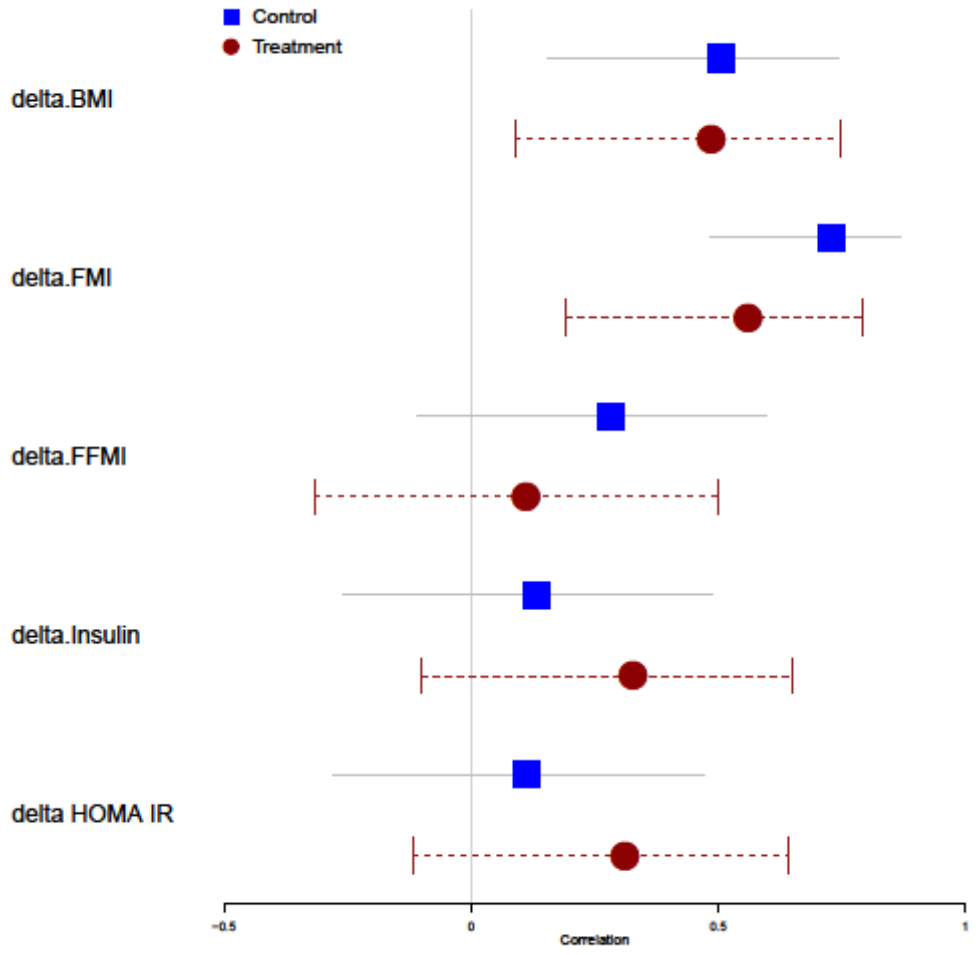


Figure 2 Forest plots of the correlations between baseline adiponectin and the changes of BMI z-score, FMI, FFMI, insulin, and HOMA-IR in control and intervention groups

Adiponectin

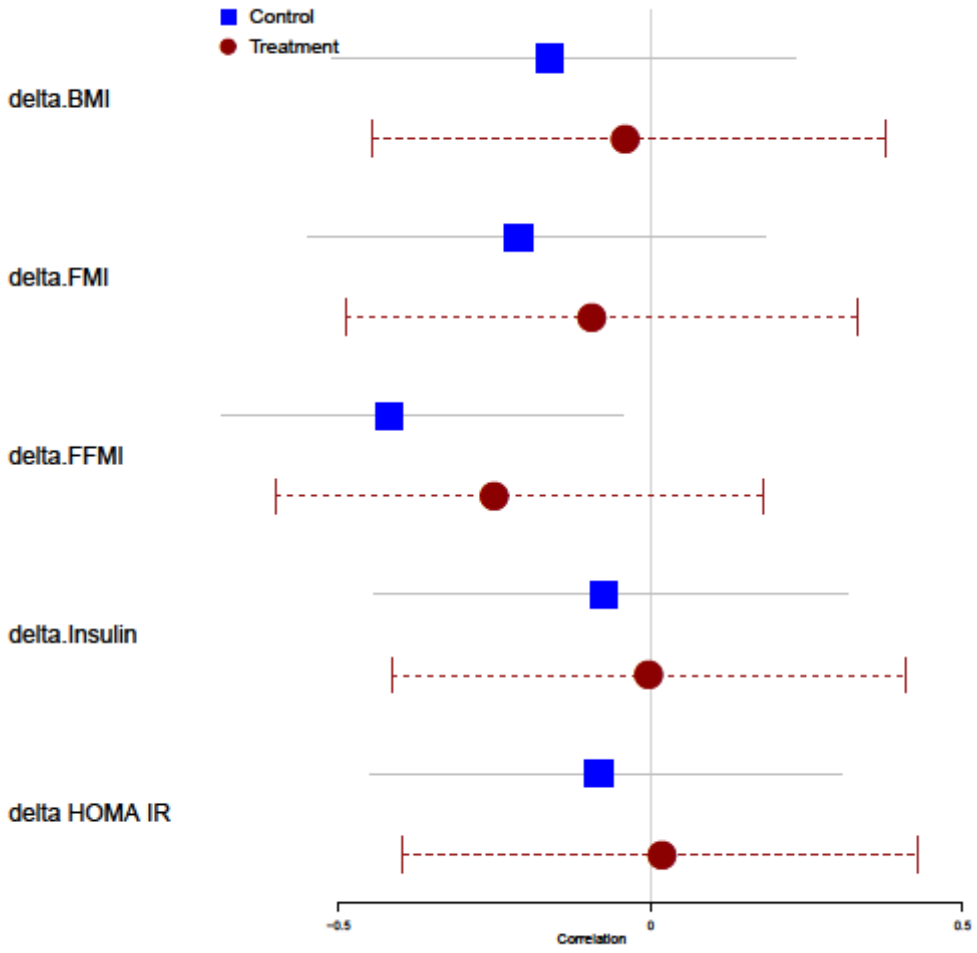


Figure 3 Forest plots of the correlations between baseline resistin and the changes of BMI z-score, FMI, FFMI, insulin, and HOMA-IR in control and intervention groups

Resistin

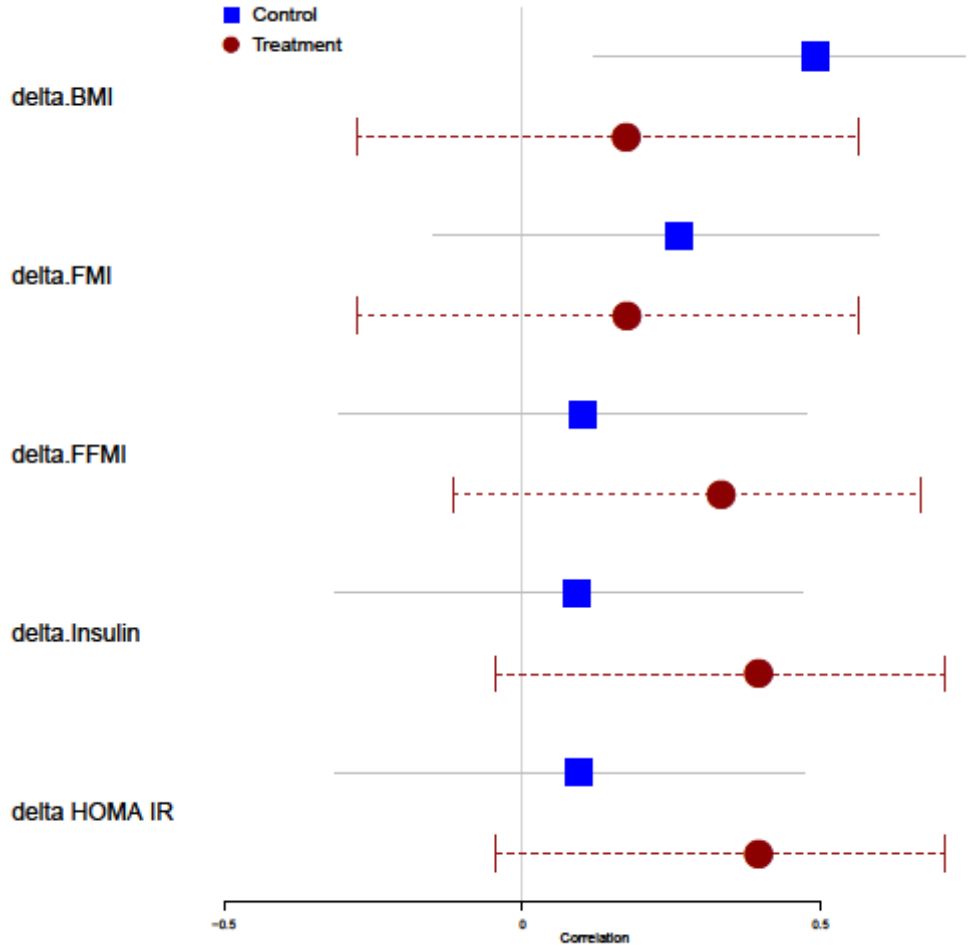
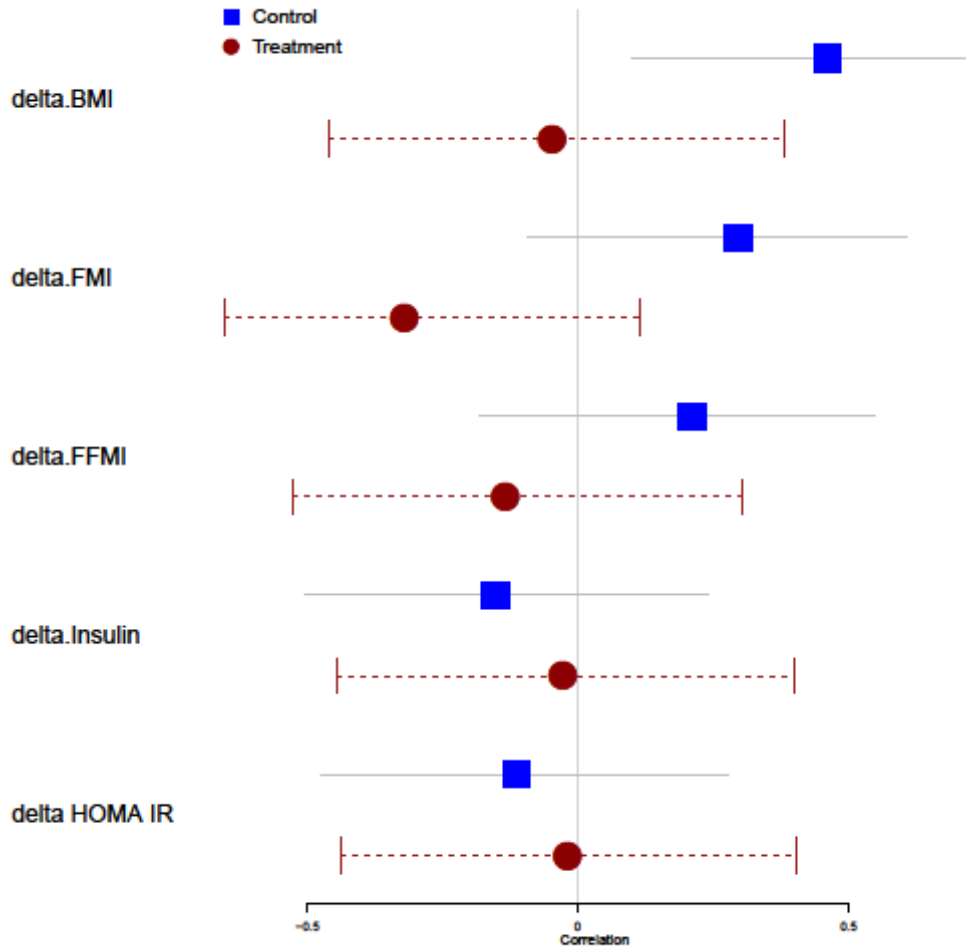


Figure 4 Forest plots of the correlations between baseline visfatin and the changes of BMI z-score, FMI, FFMI, insulin, and HOMA-IR in control and intervention groups

Visfatin



Conclusion and Discussion

To our knowledge, this is the first study to investigate the levels of adipokines -- leptin, adiponectin, resistin, and visfatin -- in obese children and adolescents, and their correlations with anthropometric indices, body composition, and metabolic parameters after receiving low-GI diet instruction compared to those receiving conventional instruction.

Leptin levels in obese individuals are typically elevated, which can lead to leptin resistance (25). The results in our study show that serum leptin levels were strongly and linearly related to BMI z-score, WC, and adiposity in both groups. In agreement with previous reported findings, the expression and secretion of leptin was positively correlated with BMI, WC, and FM (26-29). Leptin levels in obese children and adolescents significantly reduced after the weight reduction program because leptin levels reflect the amount of adipose tissue (30), although there was no evidence for a strong relation of serum leptin with measures of either subcutaneous or visceral adiposity in our study, the same result found in an earlier study (31). However, no significant differences in serum leptin between the first and the sixth visits and between the two groups were observed. This might be because the BMI z-score was not significantly different between the groups and the percentage of decreased BMI z-score from the baseline in both groups was not enough to detect a change of leptin levels during the 6-

month studied period. The results from the study suggest that in order to see the most favorable effects on leptin, a >10% weight loss along with a >10% visceral fat loss is most likely necessary (32). Additionally, no significant correlation between leptin and insulin resistance was found in our study. More recently, a study in 4- to 17-year-old children showed that BMI explained most of the variability in leptin and concluded that in obesity, total adiposity, but not insulin, is the main determinant of leptin levels (33). Fasting insulin is an indirect measure of insulin resistance. Moreover, the use of insulin to assess the relation between leptin and insulin resistance may not be optimal because fasting insulin is highly related to adiposity (34). However, some studies demonstrated an association between leptin levels and insulin as well as HOMA-IR (35, 36). While the effect of GI on insulin resistance has been evaluated previously, reported results have been inconsistence. Several studies demonstrated a beneficial effect of low-GI diets on insulin resistance among children and adolescents, as did our previous study (21, 37, 38). Low-GI foods are absorbed and digested slowly and therefore provide a prolonged full state in which free fatty acid release is suppressed. Thus, improvements observed in glucose tolerance, insulin sensitivity, and leptin may be observed following a low-GI diet (39). This beneficial effect has also been observed following a hypocaloric high-GI diet (40). Based on those reported results, it is probable that the effect of caloric restriction is stronger

than dietary GI and the effect of low-GI instruction from our study may not be strong enough to lead to a significant change of serum leptin and make a difference between groups after receiving the interventions.

Like leptin, adiponectin is a hormone secreted by adipocytes. Previous studies reported that there was an inverse association between body weight and circulating adiponectin, and adiponectin was decreased in overweight and obese children and adults (21, 41). Additionally, the comparison between 2 meals with different GI showed that serum adiponectin increased following consumption of a low-GI meal (42). Other studies also demonstrated that lower adiponectin was associated with higher dietary GI and glycemic load (GL) (43, 44) and suggest that dietary modifications may improve adiponectin. Based on the correlation of GI and GL diet with adiponectin, as mentioned above, and a tendency of dependence of adiponectin on the absolute values of lean mass parameters, which may indicate that distribution of this adipokine depends on localized soft lean mass, low GI diet may be able to de-couple the association between adiponectin and FFMI when the participants receive the low GI instruction rather than conventional instruction, as our research study shows. Adiponectin is believed to improve insulin sensitivity through a number of different mechanisms. Moreover, adiponectin has potent anti-apoptotic effects which are believed to prevent lipid-induced pancreatic beta cell apoptosis

(45). Both of these actions would be expected to be protective against diabetes. Unlike those studies, changes in serum adiponectin between the first and the last visits within groups were not observed. In addition, change in serum adiponectin concentration were not significantly different between the two studied groups, and association between adiponectin and insulin sensitivity indices were not found. The results were similar to another randomized clinical trial studied overweight and obese adolescents receiving a low-GI diet instruction for 10 weeks (46). The possible reason may be because adiponectin did not respond to this degree of BMI z-score change and not enough FM was lost and/or FFM was increased over the course of the trial; the literature also described the degree of weight loss related to serum adiponectin concentration and suggests that a decrease of >10% in visceral fat may be necessary to increase adiponectin level (32, 47, 48).

Resistin is one of the adipokines, a protein hormone that seems to be involved in the process of blood glucose regulation and has been linked to obesity, type 2 diabetes, inflammation, and atherosclerosis (49, 50). In our study, there was no significant difference in resistin level within groups and between groups during the 6-month trial. However, resistin was significantly correlated with the change BMI z-score in the control group. Furthermore, this adipocytokine was inversely associated with adiponectin in the intervention group. These results

probably indicate that resistin may represent a link between obesity and insulin resistance. However, the results were a bit inconsistent in that adipose tissue does not appear to play a role in the production of this protein and it is possible that adiposity and resistin generate cardiovascular and metabolic syndrome risks via different pathways (51). The physiologic role of resistin on obesity and insulin resistance is unclear and not conclusive. Therefore, the role of resistin as an adipocytokine in children might need to be reevaluated.

Visfatin is the other adipocytokine which has been suggested as a marker of adipocity and as a protein with possible insulin-like function (14). We found no significant differences in visfatin within groups and between groups during the 6-month studied period, and no relationship with either BMI z-score or FMI was found. The studies about visfatin in obese children were controversial. Davutoglu et al. showed that log visfatin levels were significantly positively correlated to weight, BMI (like the results in our study), and WC in the obese children group (52). Additionally, another case-control study found that visfatin levels were significantly associated with total abdominal fat, visceral fat, subcutaneous visceral fat, and HOMA-IR (53). Nonetheless, the cross-sectional study in 175 overweight and obese children showed no relationship between serum visfatin levels and BMI or a high WC (19). Visfatin was also not associated with insulin and HOMA-IR in the study. It was inconsistent with another study of

Mitra et al. (54). They found a correlation of visfatin with insulin resistance indices and metabolic syndrome, and visfatin levels were higher in the obese children and adolescents, and in obese children with metabolic syndrome, compared to obese subjects without metabolic syndrome. In our study, the obese children with metabolic syndrome were not enrolled at the baseline; therefore, the association of visfatin and insulin resistance indices might not be strong enough to be detected at the end of the study. From the study, serum visfatin may not be a specific marker for insulin metabolism compatible with another study (17).

In addition, lipid profiles were not correlated with any adipokines in the study. These relationships were inconsistent in previous literature. This discrepancy may be attributable to differences in the degrees of fatty acid saturation within the triglyceride molecule, as a consequence of dietary differences between the different participants studied to date (55). Despite the RCT study, the low-GI diet intervention, the same time of anthropometric and adipocytokine examining, and the outcomes we evaluated, the limitations of this study are from the dietary intake data: the actual energy intake from both groups was higher than that were instructed, even with the significant changes in the amount of low-GI foods consumed in the intervention group. Therefore, this might result in subtle changes in body composition and adipokines. Moreover, the effect size of BMI z-score difference of 0.78 may be too large for this

kind of intervention, which might bring about less change in body composition and adipokines. Although we wanted to study the effects of realistically achievable low-GI diet on all of the outcomes in the participants' daily lives, this intention-to-treat approach may underestimate the efficacy of the low-GI diet on adipokines.

In conclusion, serum leptin levels were strongly correlated with the change of BMI z-score, WC, and FMI in both groups. These results indicate that circulating leptin levels may represent the change of BMI and adipose tissue mass in obese children, and could therefore be a useful indicator to predict the developing or improving of insulin resistance and/or metabolic syndrome. There seems to be insufficient evidence to consider other adipokines as a marker of adiposity and metabolic syndrome in obese children and adolescents. Identification of the receptor system for leptin and resistin, relevant signaling pathways, and their sensitivity state are needed for a complete evaluation of the role of adipokines in obese children. Other inflammatory markers may be considered to use for evaluating the effect of obesity intervention trial on inflammation among this population.

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Appendix

Effects of low-glycemic index diet on plasma adipokines in obese children

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ABSTRACT

BACKGROUND: A low-glycemic index (GI) diet has been documented to be beneficial for improving insulin sensitivity. The effect of the low-GI diet may modulate plasma adipokines produced by adipocytes which link to insulin resistance and the metabolic syndrome.

METHODS: Obese children aged 9–16 y were randomly assigned either a low-GI diet or a low-fat diet (control group) for 6 mo. Changes of BMI z-score, body composition measured by dual energy X-ray absorptiometry, and HOMA-IR were compared between groups. Serum adipokines namely: leptin, adiponectin, resistin, and visfatin were measured by ELISA technique.The relationships between these clinical outcomes and adipokines were assessed using correlations and multivariate analysis.

RESULTS: Fifty-two participants completed the study (mean age: 12.0 ± 2.0 y, 35 boys); both groups showed significantly decreased BMI z-score, but subtle changes in fat and fat-free mass. The intervention group had significant changes in fasting insulin and HOMA-IR, whereas the control group did not. There were no significant differences in adipokines between the groups in visit 1 and visit 6. However, there were relationships between baseline leptin and the change of BMI z-score in both groups, as well as baseline resistin, baseline visfatin and the change of BMI z-score in the control group. There were significant positive associations between baseline leptin and the change of FMI in both groups. The intervention tended to weaken the negative relationship between adiponectin and the change of FFMI.

CONCLUSION: Serum leptin levels were positively correlated with the change of BMI z-score and FMI in both groups. These results indicate that circulating leptin levels may represent the change of BMI and adipose tissue mass in obese children, and could therefore be a useful indicator to predict the improvement of insulin in an obesity intervention trial.

(word count 286)

INTRODUCTION

Prevalence of childhood and adolescent obesity is increasing both in developed and developing countries. In Thailand, there is a trend towards a higher number of children with overnutrition rather than undernutrition (1). These obese children have several health problems, such as obstructive sleep apnea, deformity of bone and joints, and the metabolic syndrome similar to that of obese adults. Therefore, childhood obesity and the metabolic syndrome is an emerging health problem that needs early interventions with dietary control, increased physical activity and behavior modification. The metabolic syndrome consists of insulin resistance, hypertension, dyslipidemia (hypertriglyceridemia, low HDL-C) and central obesity (2). The pathogenesis and pathophysiology of the metabolic syndrome remains controversial issues (3). It is postulated that obese children have accumulated excess adipose tissue, especially those with central adiposity, which may release free fatty acids and deposit it in muscles, the liver and the pancreas. Recent knowledge shows that adipocytes play an important role as an endocrine organ to regulate metabolism and energy homeostasis, leading to the discovery of several adipokines, including leptin, visfatin, and resistin, and pro-inflammatory cytokines that cause chronic low-grade inflammatory state. On the other hand, adiponectin, which is one of the adipokines and has an anti-inflammatory effect as well as increases insulin sensitivity, is suppressed. All these changes may disturb the insulin signaling and eventually cause insulin resistance (4).There is some recent evidence regarding the roles of adipokines in the pathogenesis of the metabolic syndrome in obese children and the association of these adipokines and body fat (in particular, visceral fat).

Leptin is a hormone secreted by adipocytes. It acts through the hypothalamus, suppressing appetite and increasing energy expenditure. Most studies (5-7) in Caucasians and Asians alike, found that overweight and obese children have higher plasma leptin levels than do normal-weight children, especially obese children who have the metabolic syndrome. On the contrary, a few small studies showed that lifestyle intervention in obese children reduced leptin and increased soluble leptin receptor (8, 9). Adiponectin is an insulin-sensitizing hormone produced and released by adipocytes. It has been shown to decrease circulating glucose levels by suppressing gluconeogenesis in the liver and enhancing insulin signaling in the skeletal muscle (10). A previous study showed that serum adiponectin is inversely associated with obesity (11). In addition, increased serum adiponectin levels may play a protective role in metabolic syndrome. The body fat distribution influences adiponectin

levels, with abdominal obesity associated with lower concentrations (12, 13). The association between central adiposity and reduced adiponectin was seen with visceral fat accumulation in children (14). Resistin is a cytokine expressed in adipose tissue. Despite showing a strong linkage with obesity and insulin resistance in animal models, its role in obesity and insulin sensitivity in human is still controversial. One interventional study from Germany found no relationship between resistin, insulin resistance index, and weight status in childhood (15), while Elloumi et al. demonstrated an intriguing increase in resistin in boys after a 2-month weight loss program (16). Furthermore, visfatin is a newly discovered adipokine that mimics the action of insulin via a distinct binding site on the insulin receptor and, therefore, an increased risk for insulin resistance (14). It is produced mainly from visceral adipose tissue in animal models. Araki et al. (17) reported a good correlation between visceral adipose tissue and plasma visfatin from a cross-sectional study in 56 obese Japanese children. The researchers also demonstrated a significant difference between plasma visfatin between normal and obese children with and without metabolic syndrome. The same findings were also found in obese Caucasian children (18, 19). However, the data were conflicting whether visfatin has any association with insulin resistance and other components of the metabolic syndrome. So far, there is only one intervention study in Europe reporting the effect of weight reduction program on visfatin level in obese adolescents (20).

However, the longitudinal and interventional study of the change in these adipokines after lifestyle interventions with the aim of body fat reduction is still scarce. This information could enlighten the pathophysiologic changes that occur in childhood obesity and the metabolic syndrome as well as proven causal relationship between these biomarkers and body fat. In our previous study on the effects of low-GI diet on insulin resistance, we found that the low-GI diet may improve insulin sensitivity in obese children with high baseline insulin (21). Therefore, the objective of the present study was to compare plasma adipokine concentrations before and after a 6-month instruction of low-GI diet versus conventional management in obese Thai children. Additionally, we aimed to study a relationship between plasma adipokines and body composition as well as the metabolic syndrome risks during the 6-month studied period.

METHODS

Study design

The protocol was approved by the Institutional Review Board of the Faculty of Medicine, Chulalongkorn University, Thailand. This study was a single-center prospective, randomized, controlled trial. The methods were already published in the previous journal article (21). In brief, eligible participants for this study with a BMI higher than the International Obesity Task Force cut-off, corresponding to a BMI of thirty in adulthood (22), were enrolled and randomized to obesity clinic instruction or an intervention of a low-GI diet. For the intervention group, individual goals for weight management were set and instruction about low-GI foods was provided. A dietitian emphasized the selection of low-GI carbohydrates, which were adapted from the table by Foster-Powell et al. (23). The control group received conventional management instructions at the Nutrition Clinic about energy restriction, low-fat and high-fiber diet. Moreover, the participants in both groups received the same instruction about increasing physical activity. Both groups needed to maintain the monthly visits for six months. The adherence to the nutritional education and physical activity recommendation was evaluated by using three-day dietary records and a physical activity questionnaire at each visit. One blood sample was taken in the morning after 12 h of fasting.

The main outcomes were adipokines that the participants were examined for at the first and sixth visits, and which included serum leptin, adiponectin, resistin, and visfatin levels. These adipokines were measured by ELISA KIT (R&D System Inc., Minneapolis, USA for leptin, adiponectin, and resistin, and Adipogen Life Sciences, Liestal, Switzerland) and analyzed by using software: Revelation Quicklink version 4.25. The secondary outcomes were the correlations between the adipokines and BMI z-score that was calculated based on World Health Organization (WHO) 2007 growth reference using the WHO AnthroPlus program (24), and body composition that was evaluated by using DXA (Hologic QDR Discovery A) on the first and sixth visits.

Statistical analysis

Descriptive statistics were used to summarize all quantitative data. Qualitative data were summarized using percentages. Continuous variables were reported as mean ± standard deviation (SD) and compared between the control and intervention groups by an independent sample t-test. Paired t-test for dependent samples was used to evaluate the changes within the groups before and after the six-month period. We evaluated the correlations between BMI z-score, waist circumference, body composition, insulin resistance, adipokines, and lipid profiles in each group and in all participants

and present as forest plots. Statistical significance was defined as a *P*-value < 0.05. Analyses were completed using SPSS 23.0 (SPSS Inc., Chicago, IL).

RESULTS

Seventy participants (47 boys, 23 girls) were enrolled and randomized into two groups: 35 in each control and intervention group as shown in the flow diagram in our previous study (21). There were 52 out of 70 participants who completed all six visits (27 in the control and 25 in the intervention groups). Mean age in the control group was 12.0 ± 2.1 y and 11.9 ± 1.9 y in the intervention group. The other baseline characteristics were shown in the previous study (21). All analyses followed the intention-to-treat principle. The intervention group had significantly increased daily consumption of the low-GI food and decreased total energy intake compared to the control group. Moreover, brisk walking was the common feature of exercise in both groups and around half of the participants in both groups were physically active (21).

There were significant differences in plasma insulin levels and HOMA-IR; however, no significant differences in adipokines between the two groups in visit 1 and visit 6 were observed (Table 1).

Despite decreased BMI z-score between the first and the sixth visits in the intervention group (P < 0.0001), leptin and visfatin decreased while adiponectin increased, but these were not significantly different between the first and the last visit. Table 2 shows no significant differences in the changes of body composition, adipokines, and lipid profiles, even though fasting insulin and HOMA-IR significantly decreased from baseline to visit 6 in the intervention group (22.2 ± 14.3 vs. 13.7 ± 10.9 mU/l; P = 0.004 and 4.8 ± 3.3 vs. 2.9 ± 2.3; P = 0.007, respectively). The correlations between adipokines and the other parameters show in Figure 1. There were significant relationships between baseline leptin and the change of BMI z-score in the control and intervention groups (95% CI 0.16, 0.74, p < 0.001 and 95% CI 0.09, 0.75, p < 0.001, respectively) as well as baseline resistin, baseline visfatin and the change of BMI z-score (95% CI 0.12, 0.74, p = 0.013 and 95% CI 0.10, 0.72, p = 0.015, respectively) in the control group. There were significant associations between baseline leptin and the change of FMI in the control and intervention groups (95% CI 0.48, 0.87, p = < 0.001 and 95% CI 0.19, 0.79, p < 0.001, respectively). The intervention tended to weaken the negative relationship between adiponectin and the change of FFMI. There were no correlations between baseline adipokines and the changes of insulin and HOMA-IR in either group.

DISCUSSION

To our knowledge, this is the first study to investigate the levels of adipokines -- leptin, adiponectin, resistin, and visfatin -- in obese children and adolescents, and their correlations with anthropometric indices, body composition, and metabolic parameters after receiving low-GI diet instruction compared to those receiving conventional instruction.

Leptin levels in obese individuals are typically elevated, which can lead to leptin resistance (25). The results in our study show that serum leptin levels were strongly and linearly related to BMI z-score, WC, and adiposity in both groups. In agreement with previous reported findings, the expression and secretion of leptin was positively correlated with BMI, WC, and FM (26-28). Leptin levels in obese children and adolescents significantly reduced after the weight reduction program because leptin levels reflect the amount of adipose tissue (29), although there was no evidence for a strong relation of serum leptin with measures of either subcutaneous or visceral adiposity in our study, the same result found in an earlier study (30). However, no significant differences in serum leptin between the first and the sixth visits and between the two groups were observed. This might be because the BMI z-score was not significantly different between the groups and the percentage of decreased BMI z-score from the baseline in both groups was not enough to detect a change of leptin levels during the 6-month studied period. The results from the study suggest that in order to see the most favorable effects on leptin, a >10% weight loss along with a >10% visceral fat loss is most likely necessary (31). Additionally, no significant correlation between leptin and insulin resistance was found in our study. More recently, a study in 4- to 17-year-old children showed that BMI explained most of the variability in leptin and concluded that in obesity, total adiposity, but not insulin, is the main determinant of leptin levels (32). Fasting insulin is an indirect measure of insulin resistance. Moreover, the use of insulin to assess the relation between leptin and insulin resistance may not be optimal because fasting insulin is highly related to adiposity (33). However, some studies demonstrated an association between leptin levels and insulin as well as HOMA-IR (34, 35). While the effect of GI on insulin resistance has been evaluated previously, reported results have been inconsistence. Several studies demonstrated a beneficial effect of low-GI diets on insulin resistance among children and adolescents, as did our previous study (21, 36). Low-GI foods are absorbed and digested slowly and therefore provide a prolonged full state in which free fatty acid release is suppressed. Thus, improvements observed in glucose tolerance, insulin sensitivity, and leptin may be observed following a low-GI diet (37). This

beneficial effect has also been observed following a hypocaloric high-GI diet (38). Based on those reported results, it is probable that the effect of caloric restriction is stronger than dietary GI and the effect of low-GI instruction from our study may not be strong enough to lead to a significant change of serum leptin and make a difference between groups after receiving the interventions.

Like leptin, adiponectin is a hormone secreted by adipocytes. Previous studies reported that there was an inverse association between body weight and circulating adiponectin, and adiponectin was decreased in overweight and obese children and adults (39). Additionally, the comparison between 2 meals with different GI showed that serum adiponectin increased following consumption of a low-GI meal (40). Other studies also demonstrated that lower adiponectin was associated with higher dietary GI and glycemic load (GL) (41) and suggest that dietary modifications may improve adiponectin. Based on the correlation of GI and GL diet with adiponectin, as mentioned above, and a tendency of dependence of adiponectin on the absolute values of lean mass parameters, which may indicate that distribution of this adipokine depends on localized soft lean mass, low GI diet may be able to de-couple the association between adiponectin and FFMI when the participants receive the low GI instruction rather than conventional instruction, as our research study shows. Adiponectin is believed to improve insulin sensitivity through a number of different mechanisms. Moreover, adiponectin has potent anti-apoptotic effects which are believed to prevent lipid-induced pancreatic beta cell apoptosis (42). Both of these actions would be expected to be protective against diabetes. Unlike those studies, changes in serum adiponectin between the first and the last visits within groups were not observed. In addition, change in serum adiponectin concentration were not significantly different between the two studied groups, and association between adiponectin and insulin sensitivity indices were not found. The results were similar to another randomized clinical trial studied overweight and obese adolescents receiving a low-GI diet instruction for 10 weeks (43). The possible reason may be because adiponectin did not respond to this degree of BMI z-score change and not enough FM was lost and/or FFM was increased over the course of the trial; the literature also described the degree of weight loss related to serum adiponectin concentration and suggests that a decrease of >10% in visceral fat may be necessary to increase adiponectin level (31, 44).

Resistin is one of the adipokines, a protein hormone that seems to be involved in the process of blood glucose regulation and has been linked to obesity, type 2 diabetes, inflammation, and atherosclerosis (45). In our study, there was no significant difference in resistin level within groups and

between groups during the 6-month trial. However, resistin was significantly correlated with the change BMI z-score in the control group. Furthermore, this adipocytokine was inversely associated with adiponectin in the intervention group. These results probably indicate that resistin may represent a link between obesity and insulin resistance. However, the results were a bit inconsistent in that adipose tissue does not appear to play a role in the production of this protein and it is possible that adiposity and resistin generate cardiovascular and metabolic syndrome risks via different pathways (46). The physiologic role of resistin on obesity and insulin resistance is unclear and not conclusive. Therefore, the role of resistin as an adipocytokine in children might need to be reevaluated.

Visfatin is the other adipocytokine which has been suggested as a marker of adipocity and as a protein with possible insulin-like function (14). We found no significant differences in visfatin within groups and between groups during the 6-month studied period, and no relationship with either BMI z-score or FMI was found. The studies about visfatin in obese children were controversial. Davutoglu et al. showed that log visfatin levels were significantly positively correlated to weight, BMI (like the results in our study), and WC in the obese children group (47). Additionally, another case-control study found that visfatin levels were significantly associated with total abdominal fat, visceral fat, subcutaneous visceral fat, and HOMA-IR (48). Nonetheless, the cross-sectional study in 175 overweight and obese children showed no relationship between serum visfatin levels and BMI or a high WC (19). Visfatin was also not associated with insulin and HOMA-IR in the study. It was inconsistent with another study of Mitra et al. (49). They found a correlation of visfatin with insulin resistance indices and metabolic syndrome, and visfatin levels were higher in the obese children and adolescents, and in obese children with metabolic syndrome, compared to obese subjects without metabolic syndrome. In our study, the obese children with metabolic syndrome were not enrolled at the baseline; therefore, the association of visfatin and insulin resistance indices might not be strong enough to be detected at the end of the study. From the study, serum visfatin may not be a specific marker for insulin metabolism compatible with another study (17). In addition, lipid profiles were not correlated with any adipokines in the study. These relationships were inconsistent in previous literature. This discrepancy may be attributable to differences in the degrees of fatty acid saturation within the triglyceride molecule, as a consequence of dietary differences between the different participants studied to date (50).

Despite the RCT study, the low-GI diet intervention, the same time of anthropometric and adipocytokine examining, and the outcomes we evaluated, the limitations of this study are from the

dietary intake data: the actual energy intake from both groups was higher than that were instructed, even with the significant changes in the amount of low-GI foods consumed in the intervention group. Therefore, this might result in subtle changes in body composition and adipokines. Moreover, the effect size of BMI z-score difference of 0.78 may be too large for this kind of intervention, which might bring about less change in body composition and adipokines. Although we wanted to study the effects of realistically achievable low-GI diet on all of the outcomes in the participants' daily lives, this intention-to-treat approach may underestimate the efficacy of the low-GI diet on adipokines.

In conclusion, serum leptin levels were strongly correlated with the change of BMI z-score, WC, and FMI in both groups. These results indicate that circulating leptin levels may represent the change of BMI and adipose tissue mass in obese children, and could therefore be a useful indicator to predict the developing or improving of insulin resistance and/or metabolic syndrome. There seems to be insufficient evidence to consider other adipokines as a marker of adiposity and metabolic syndrome in obese children and adolescents. Identification of the receptor system for leptin and resistin, relevant signaling pathways, and their sensitivity state are needed for a complete evaluation of the role of adipokines in obese children. Other inflammatory markers may be considered to use for evaluating the effect of obesity intervention trial on inflammation among this population.

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Table 1 Comparison of BMI z-score, waist circumference, body composition measured by DXA, fasting insulin, HOMA-IR, and adipokines between the control and intervention groups (*n = 52*)

Parameters	Control (*n = 27*)	Intervention (*n = 25*)	*P*-value[a]
Visit 1			
BMI z-score[b]	3.6 ± 1.6[a]	3.7 ± 0.9	0.88
Waist circumference (cm)	103.1 ± 14.9	105.8 ± 7.7	0.40
% Fat[c]	41.1 ± 6.0	42.1 ± 4.8	0.56
FMI (kg/m^2)	14.0 ± 4.5	14.7 ± 3.8	0.56
FFMI (kg/m^2)	18.8 ± 2.9	19.1 ± 2.6	0.66
Laboratory test			
Fasting insulin (mU/L)	15 ± 8	22 ± 14	**0.035**
HOMA-IR	3.1 ± 1.7	4.8 ± 3.3	**0.035**
Leptin (ng/mL)	27.7 ± 15.6	28.8 ± 12.9	0.80
Adiponectin (ng/mL)	2,203.1 ± 1,934.8	2,077.3 ± 1,499.8	0.81
Resistin (ng/mL)	13.1 ± 6.6	12.6 ± 5.9	0.79
Visfatin (ng/mL)	11.1 ± 12.5	17.9 ± 15.9	0.13
Visit 6			
BMI z-core	3.4 ± 1.3	3.4 ± 0.9	0.87
Waist circumference (cm)	104.2 ± 15.8	106.3±9.5	0.55
% Fat	40.2+8.7	43.1+6.1	0.55
FMI (kg/m^2)	14.4 + 4.5	15.2+4.4	0.59
FFMI (kg/m^2)	18.8+3.4	18.8+2.6	0.84
Laboratory test[d]			
Fasting insulin (mU/L)	14 ± 10	14 ± 11	0.88
HOMA-IR	3.2 ± 2.6	2.9 ± 2.3	0.69
Leptin (ng/ml)	30.1 ± 18.2	26.2 ± 15.5	0.41
Adiponectin (ng/ml)	2,424.1 ± 2,427.6	2,461.8 ± 1,703.3	0.94
Resistin (ng/ml)	13.2 ± 7.0	13.6 ± 9.8	0.89
Visfatin (ng/ml)	14.6 ± 12.5	14.5 ± 14	0.75

DXA, dual-energy X-ray absorptiometry; FMI, fat mass index = fat mass (kg)/height (m2); FFMI, fat-free mass index = fat-free mass (kg)/height (m2); HOMA-IR, homeostatic model of assessment-insulin resistance = (FI × FPG)/22.5; FI, fasting insulin concentration (mU/l); FPG, fasting plasma glucose (mmol/l). [a]These data showed means ± SDs and independent sample t-test was used to evaluate continuous variables. [b]BMI z-score were calculated from WHO growth reference 2007(24). [c]Percentage of fat was calculated from fat mass (kg)/body weight (kg) × 100. [d]There were data from 26 participants in the control group due to missing laboratory results from 1 participant. Bold text shows that intervention group was different from control group, P < 0.05.

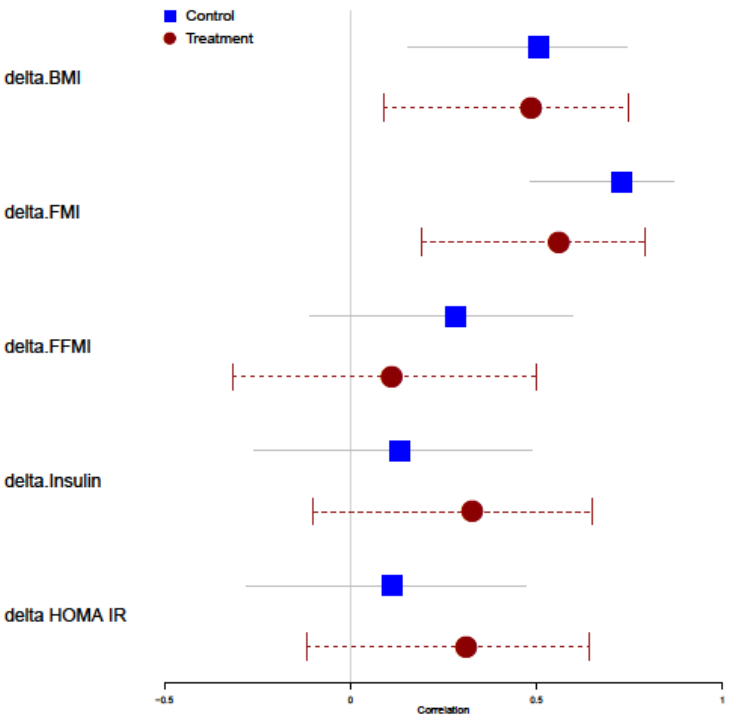
Table 2 Comparison of changes in anthropometry, body composition measured by DXA and laboratory tests between the control and intervention groups during the 6-month period (*n = 52*)

Changes in outcomes[a]	Control (*n = 27*)	Intervention (*n = 25*)	*P*-value[b]
BMI z-score	-0.3 ± 0.5[c]	-0.3 ± 0.2	0.99
Waist circumference, cm	1.1 ± 5.6	0.5 ± 4.1	0.66
FMI, kg/m^2	0.3 ± 1.6	0.8 ± 3.5	0.45
FFMI, kg/m^2	0.2 ± 0.8	0.8 ± 3.9	0.44
% Fat[d]	0.1 ± 2.8	0.1 ± 3.0	0.35
Fasting plasma insulin, mU/L	-0.8 ± 11.3	-8.5 ± 13.5	**0.032**
HOMA-IR	0.1 ± 2.8	-1.9 ± 3.2	**0.019**
Leptin, ng/ml	2.5 ± 12.7	-2.6 ± 12.9	0.18
Adiponectin, ng/ml	221 ± 1,120	384.5 ± 1,164.9	0.63
Resistin, ng/ml	0.1 ± 5.1	0.9 ± 7.5	0.66
Visfatin, ng/ml	3.4 ± 18.9	-2.9 ± 16.3	0.23

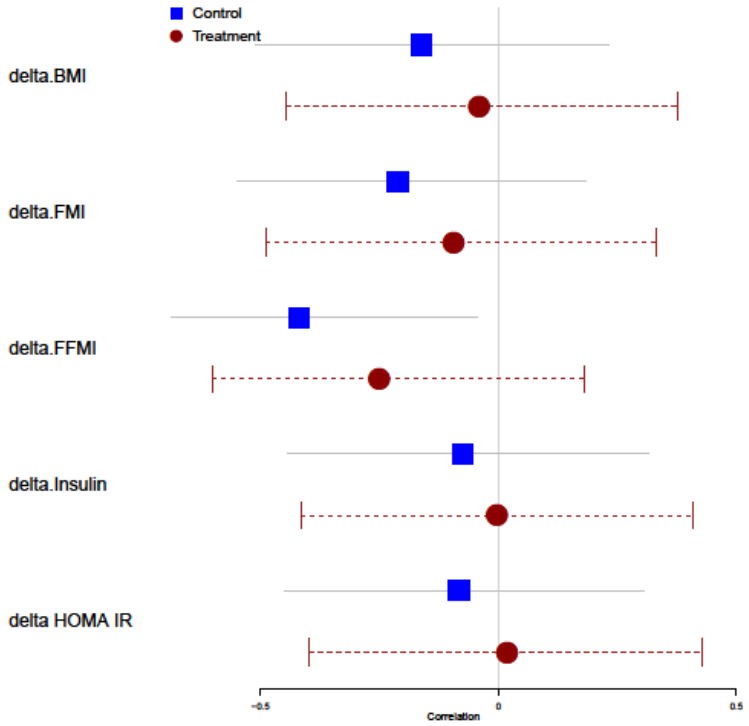
[a]Changes in outcomes were the average of the difference between visit 6 and visit 1. [b]Independent sample t-test was used to compare the data between the two groups. [c]Means ± SDs (all such values). [d]Percentage of fat = fat mass (kg) × 100/body weight (kg). Bold text showed that intervention group was different from control group, P < 0.05. DXA, dual-energy X-ray absorptiometry; FFMI, fat-free mass index = fatfree mass (kg)/height (m2); FMI, fat mass index = fat mass (kg)/height (m2); HOMA-IR, homeostatic model of assessment-insulin resistance = (FI × FPG)/22.5; FI, fasting insulin concentration (mU/l); FPG, fasting plasma glucose (mmol/l).

Figure 1 Forest plots of the correlations between baseline adipokines and the changes of BMI z-score, FMI, FFMI, insulin, and HOMA-IR in control and intervention groups

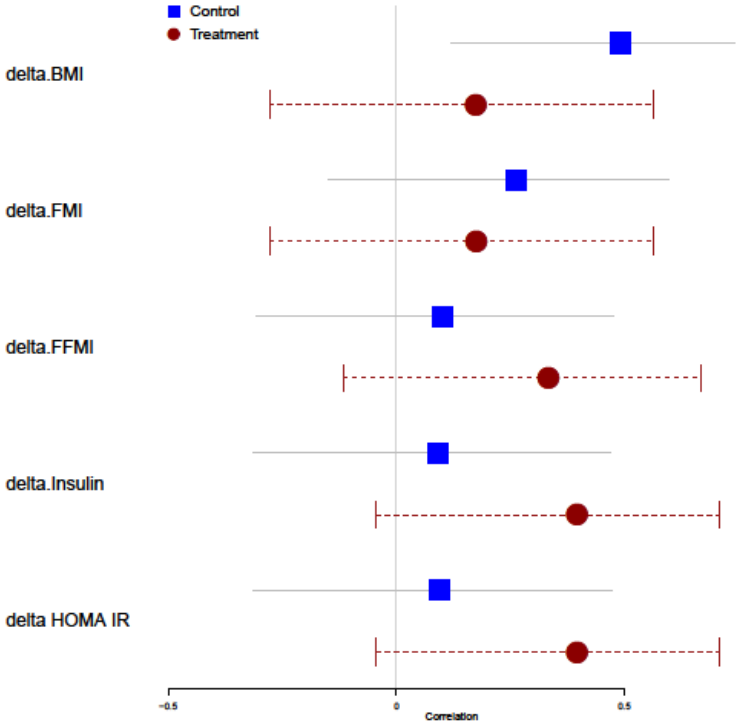
Leptin



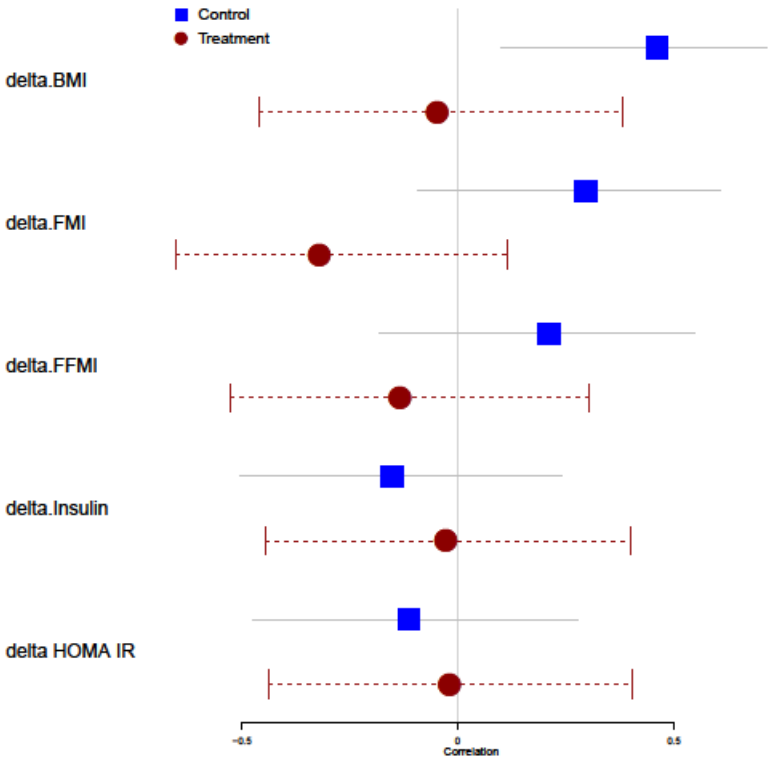
Adiponectin



Resistin



Visfatin



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Output

1. International Journal Publication

The manuscript is in the process of submitting to Pediatric Research Journal (Impact factor 2.761)

2. Research Utilization and Application

The research results demonstrate new insight into the adipokines especially leptin changes in an obesity intervention trial. Serum leptin was positively correlated with the change of BMI z-score and FMI during the 6-month intervention period, indicating that circulating leptin levels may represent the change in adipose tissue mass in obese children. Therefore, it could be a useful indicator to predict the improvement in fat mass and insulin resistance after the intervention. For example, an obese individual who had high baseline leptin may not response to lifestyle intervention as well as the one who had lower baseline leptin. This finding is potentially useful in clinical care setting for general pediatrician who takes care of obese children and could be used for further obesity research.

3. Others

Poster presentations

3.1 Visuthranukul C, Lohawijarn N, Sirimongkol P, Prachansuwan A, Chomtho S. Effects of a low-glycemic index diet on body composition, insulin resistance, and plasma adiponectin in obese Thai children: a randomized controlled trial. Poster presentation of International Poster Showcase at the Clinical Nutrition Week of A.S.P.E.N., Savannah, GA, USA, January 18th-21st, 2014.

3.2 Chomtho, S, Sirimongkol, P, Prachansuwan, A, Visuthranukul, C, Poovorawan, Y. Plasma adipokines before and after lifestyle intervention in obese Thai children. Poster presentation at the TRF 14th New scholar meeting, Ambassador City, Jomtien, Chonburi, October 23th-25th, 2014.

Effects of a Low Glycemic Index Diet on Body Composition, Insulin Resistance, and Plasma Adiponectin in Obese Thai Children: A Randomized Controlled Trial

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INTRODUCTION

- The low glycemic index (GI) diet which has GI less than 55 can blunt postprandial glucose response, slow down insulin secretion and lead to longer satiety hence it may be beneficial for weight reduction and improve insulin resistance.[1]
- We aimed to compare the effectiveness of a low GI diet and a low calorie-low fat diet for the management of childhood obesity.

METHODS

- Obese children aged 9-16 years were randomized to receive either the instruction for a low GI diet (intervention group) or a low calorie-low fat diet (control group) and followed-up every month for 6 months.
- The low GI group received a small class teaching with parental participation, cooking demonstration of a low GI dishes with a variety of menus (Figure 1).
- The control group received the instructions about low-calorie (~1200-1300 kcal/day), low-fat (25% of total calories from fat) and high fiber diet.
- Body composition and metabolic syndrome risk changes were measured at the 1st and last visits.

Figure 1 Low GI group

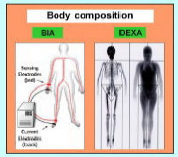

Figure 2 Body composition measurements, BIA every 1 month, DEXA at baseline and the 6th month

RESULTS

- A total of 70 obese children participated in the study and 74% completed the 6-month follow-up period.
- Their baseline characteristics were shown in table 1.
- Body mass index (BMI) z-score significantly decreased from 3.7±0.9 to 3.4±1.0 (p<0.0001) in the low GI group and from 3.6±1.6 to 3.4±1.3 (p=0.015) in the control group during the 6-month period.
- The changes in FMI and FFMI were similar in both groups (Figure 3).
- After 6 months, no significant differences in the changes of fasting plasma glucose and lipid profiles between the two groups were observed.
- Compared with the control group, the low GI group showed a significant decrease in fasting insulin (-0.8±11.3 mU/L vs -8.5±13.5 mU/L, p=0.032) and HOMA-IR (0.1±2.8 vs -1.9±3.2, p=0.019) (Table 2).
- Changes in plasma adiponectin followed the same trend which might have reflected better insulin sensitivity in the low GI group (Table 2).

	Control (n=35)	Low GI (n=35)	p-value
Age (years)	12.2±2.0	12.0±1.9	0.647
Gender male (n,%)	23(65.7)	24(68.6)	1.000
BMI z-score	3.5±1.4	3.6±0.9	0.726
Waist circumference (cm)	102.0±13.7	105.5±10.4	0.382
Fasting plasma glucose (mg/dl)	85.6±8.6	86.4±7.4	0.674
Total cholesterol (mg/dl)	170.8±31.8	178.1±30.9	0.368
Triglyceride (mg/dl)	101.8±49.2	108.0±43.7	0.606
HDL (mg/dl)	45.0±9.4	47.4±13.6	0.423
LDL (mg/dl)	103.6±25.8	118.1±24.9	0.034
Fasting insulin (mU/L)	15.9±9.1	21.0±13.0	0.065
HOMA-IR	3.2±1.9	4.5±3.0	0.032
Adiponectin (ng/ml)	1,988.3±1,766.8	1,927.6±1,356.7	0.877

Table 1 Baseline characteristics of obese children at the 1st visit

Changes in outcomes (visit 6-visit1)	Control (n=27)	Low GI (n=25)	p-value
Fasting plasma glucose (mg/dl)	1.0±7.8	-1.5±8.1	0.269
Total cholesterol (mg/dl)	-3.1±23.6	-2.9±27.8	0.978
Triglyceride (mg/dl)	2.9±35.1	-8.5±35.9	0.259
HDL (mg/dl)	1.7±8.9	0±10.9	0.549
LDL (mg/dl)	0.5±19.9	-4.2±17.6	0.379
Fastting insulin (mU/L)	-0.8±11.3	-8.51±13.5	0.032
HOMA-IR	0.1±2.8	-1.9±3.2	0.019
Adiponectin (ng/ml)	221.0±1,120.1	384.5±1,164.9	0.626

Table 2 Comparison of changes in fasting plasma glucose, lipid profiles, fasting insulin, HOMA-IR and adiponectin between the control and intervention groups. HOMA-IR: homeostasis model assessment-estimated insulin resistance = (fasting plasma glucose (mmol/L) x fasting insulin (mU/L))/22.5.[2]

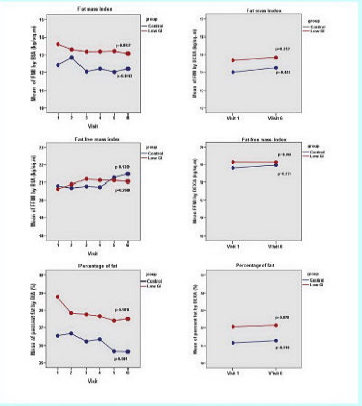

Figure 3 Body composition measured by BIA (A) and DEXA (B). The control group is represented by the blue line, the low GI group is represented by the red line. FM: fat mass index = fat mass (kg)/height (m^2), FFMI: fat-free mass index = fat-free mass (kg)/height (m^2), percentage of fat = fat mass (kg) x 100/body weight (kg).[3]

CONCLUSIONS

- Obese children who received the low GI diet had a significant decrease in fasting insulin and HOMA-IR compared with those followed the conventional low-calorie, low-fat diet.
- Despite subtle effects on body composition, the low GI diet might improve insulin sensitivity in obese children who had high baseline insulin.

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None of the authors has any conflict of interest.

Plasma Leptin, Resistin, and Visfatin after a Low Glycemic Index Diet in Obese Thai Children

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Background: Recent evidence suggesting the roles of adipokines in the pathogenesis of the metabolic syndrome in obese children; however, the longitudinal study of the change in these adipokines after lifestyle interventions is still scarce.

Objectives: 1. To study the change in plasma adipokines (leptin, visfatin, resistin) after a 6-month lifestyle intervention with low glycemic index diet in obese Thai children
2. To study the relationship between the change in these adipokines and body composition as well as the metabolic syndrome

Methods: Obese children aged 9-16 years from King Chulalongkorn Memorial Hospital were randomized to receive instruction either for a low-GI diet or a low-energy, low-fat diet (control group) to explore the effectiveness of a low-GI diet for weight management program. Both groups were followed-up every month for six months. Plasma adipokines were analyzed by ELISA technique (Quantikine, R&D system, Inc for leptin and resistin; Adipogen for visfatin)

References:
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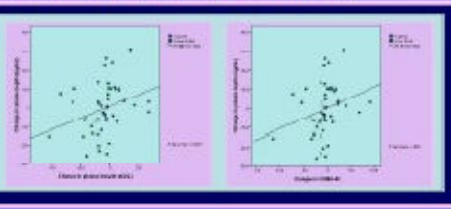
Results: A total of 70 obese children participated in the study with 26% drop-out. Daily consumption of the low-GI foods increased significantly in the low GI group (5.1±1.1 (range 3-8) vs. 1.5±0.8 (0-3) items/day) whereas no change was observed in the control group.

Table 1 Baseline characteristics	Control (n = 27)	Low GI diet (n = 25)	P value
Age, y	12.0±2.1	11.9±1.9	0.84
Male gender, n (%)	19 (70.3)	16 (64.0)	0.67
BMI, kg/m²	33.1±6.8	34.2±5.8	0.51
BMI z-score	3.6±0.8	3.7±0.8	0.88
Waist circumference, cm	103.1±14.9	105.8±7.7	0.40
FMI, kg/m²	14.0±4.5	14.7±3.8	0.56
FFMI, kg/m²	19.0±2.9	19.5±2.6	0.66
Fasting plasma glucose, mmol/L	5±1	5±0	0.91
Fasting plasma insulin, mU/L	15±8	22±14	0.032*
HOMA-IR	3.3±1.7	4.8±3.3	0.035*
Leptin, ng/mL	27.5±15.2	28.8±12.6	0.88
Resistin, ng/mL	13.8±6.1	17.8±5.8	0.16
Visfatin, ng/mL	12.2±13.5	16.8±16.3	0.032*

Table 2 Changes after 6 months	Control (n= 27)	Low GI diet (n = 25)	P value
BMI z-score	-0.3±0.5	-0.3±0.2	0.98
FMI, kg/m²	0.3±1.6	0.8±3.5	0.45
FFMI, kg/m²	0.2±0.8	0.8±2.0	0.44
Fasting plasma glucose, mmol/L	0.1±0.4	0.1±0.4	0.26
Fasting plasma insulin, mU/L	-0.8±11.3	-8.5±13.5	0.032*
HOMA-IR	0.1±2.8	-1.9±3.2	0.019*
Leptin, ng/mL	1.7±17.5	-3.1±9.8	0.39
Resistin, ng/mL	-0.1±4.3	0.8±1.4	0.17
Visfatin, ng/mL	3.1±18.2	-4.8±7.6	0.90

Results: The reduction in BMI z-score were significantly correlated with the alteration in plasma leptin (r=0.47, p 0.001), resistin (r=0.32, p 0.032), and visfatin (r=0.31, p 0.03). However, the decline in fasting insulin and HOMA-IR were weakly associated with the change in plasma leptin (r =0.30, p 0.04 and r =0.26, p 0.07, respectively).

Figure 1



Conclusion: Obese children who followed the low-GI diet program have shown some improvement in insulin sensitivity, reflecting through the change in plasma leptin.