INTRODUCTION

Regulation of energy balance is essential to avoid metabolic disorders, such as obesity, metabolic syndrome, and diabetes. The composition of macronutrients in a meal is important in influencing individuals to regulate appropriate energy balance. The potency of different macronutrients to stimulate satiety and to suppress subsequent food intake is not equal. The most potent satiating macronutrient is protein, followed by carbohydrates, then fat (Jequier and Bray, 2002). It has been shown that a high-fat diet lowers the thermic effects of the meal, which may result in body weight gain (Nagai et al, 2005).

The majority of Asians especially Thais, are habitual high-carbohydrate consumers. A proper Thai meal is made up of rice and side dishes. Although it has been recommended, that the caloric ratio of macronutrients in meals for Thais should be 50% carbohydrates, 30% fat and 20% protein (Sirijakawal et al, 1999), the energy distribution of traditional Thai foods is 70%, 17% and 13%, respectively (Kosulwat, 2002). Prepared Thai foods are easily purchased on the sidewalk and have become popular, especially among those with a busy lifestyle. According to an FAO report, 20,000 street food vendors provide city residents in Bangkok with 40% of their overall energy intake (Food and Agriculture Organization, 2001). Unfortunately, most of this food is rich in fat and protein to make them more palatable. Thus, depending on prepared foods may play a crucial role in the development of obesity among Thais.

Leptin, produced from white-adipose tissue, is a well-known hormone affecting weight homeostasis and obesity by limiting food intake and increasing energy expenditure (Auwerx and Staels, 1998). In the central con-
control of satiation, leptin activates specific leptin receptors on satiety areas in the hypothalamus, or promotes down-regulation of neuropeptide Y, which stimulates appetite and feeding (Smith et al, 1996). Peripherally, leptin increases fatty acid oxidation in the muscle and liver (Havel, 2004). Leptin and insulin provide important negative feedback signals to the central nervous system to regulate food intake and energy balance, proportional to peripheral energy stores and coupled with catabolic circuits (Benoit et al, 2004). A high-carbohydrate meal, compared to a high-fat meal, of equal energy causes a higher increase in plasma leptin levels due to greater increments in plasma glucose and insulin (Romon et al, 1999).

This study aims to investigate the changes in leptin, insulin and glucose in Thai subjects after having a single high carbohydrate, low fat, low protein meal (HC-LFLP) in comparison to a low carbohydrate, high fat, high protein meal (LC-HFHP). The hormonal responses to a single meal with different macronutrient composition may give some information to help select suitable foods for proper energy among Thais and other Asians with similar food habits.

MATERIALS AND METHODS

Subjects

Forty healthy volunteers (20 men and 20 women), aged 20 to 30 years with a body mass index (BMI) ranging from 18.50 to 24.99 kg/m² participated in this study (Table 1). Subjects reported they were habitually consuming >55-65% carbohydrates with each meal and rarely consumed a high-fat, high-protein meal. None of them had taken medication or hormone tablets within the past two months and their body weight had been stable during the previous six months prior to the study.

Before entering the study, a single 12-hour overnight fasting blood sample was analyzed for complete blood count and blood chemistry (glucose, triglycerides, total cholesterol, blood urea nitrogen and creatinine). Exclusion criteria for the study included those with diabetes mellitus, liver disease, kidney, heart or other serious health problems. All the volunteers gave informed consent and the study was approved by the Human Ethics Committee of the Faculty of Medicine Siriraj Hospital, Mahidol University, Thailand.

Study design

Before the study, volunteers refrained from caffeine and smoking for 2 days and fasted for at least 12 hours overnight. On the morning of the experiment day, after resting for 15 minutes, the first blood sample was drawn at 08:00 AM via a retained venous catheter that was kept patent with a heparin lock. Fasting was continued until 11:00 AM (≥15 hours) then a second blood sample was collected before the test meal was served.

Subjects were divided into two groups according to the test meal; each group contained 10 men and 10 women. The HC-LFLP meal was composed of 70% carbohydrates, 15% fat and 15% protein. The LC-HFHP meal was composed of 20% carbohydrates, 50% fat and 30% protein. Both meals (HC-LFLP and LC-HFHP) were prepared by a Siriraj Hospital nutritionist (Table 2). The energy allowance from each meal was the same at 41.86 kJ (10 kcal/kg) body weight. Subjects were asked to finish the test meal within 15 minutes.

Measure from the time of starting the meal, blood samples were collected every 30 minutes for 2 hours until 01:00 PM. All the subjects stayed in the experiment room and had no exertion. Blood was collected in an EDTA treated tube and in a separate plain tube for clotting. All blood samples were centrifuged within 2 hours at 1,400 rpm at 4°C for 10 min-
utes. Analysis of glucose in the EDTA plasma was immediately performed using the glucose oxidase method with a Hitachi 917 Automatic Analyzer. Sera were separated and kept at -20°C until assayed for insulin and leptin using the commercial RIA kits (Linco Research, St Charles, MO).

Statistical analysis

Differences in glucose, insulin and leptin at different times for each diet were compared using one-way ANOVA followed by the Fisher PSLD post hoc analysis. The Student's t-test was used for comparing parameters between the two groups. The 2-hour integrated areas under the curves were calculated for blood leptin, insulin and glucose concentrations minus their respective values at 11:00 AM (DAUC) using the trapezoidal rule. Significance was set at p≤0.05. Data are presented as mean ± SEM in the text, tables, and figures.

RESULTS

The levels of blood glucose, insulin and changes in leptin concentrations in response to HC-LFLP or LC-HFHP meals are shown in Fig 1. During the 3-hour prolongation of the fast, leptin declined in both meal-groups. Leptin continued to decrease following the LC-HFHP meal, but not in the group receiving the HC-LFLP meal. A significant difference in serum leptin between the two test meals was detected at 1-2 hours after the meal. Plasma glucose and insulin levels after 3-hour additional fasting were not different, but their re-

Table 1
Characteristics of studied subjectsa.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>24.20 ± 0.71</td>
<td>24.80 ± 0.55</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>60.90 ± 1.72</td>
<td>49.00 ± 1.11b</td>
</tr>
<tr>
<td>Height, cm</td>
<td>169.3 ± 1.62</td>
<td>156.2 ± 1.21b</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>21.18 ± 0.35</td>
<td>20.07 ± 0.32b</td>
</tr>
<tr>
<td>Waist:hip ratio</td>
<td>0.81 ± 0.005</td>
<td>0.75 ± 0.015b</td>
</tr>
<tr>
<td>Triceps skin fold, mm</td>
<td>13.28 ± 0.99</td>
<td>23.63 ± 1.40b</td>
</tr>
</tbody>
</table>

a Values are means ± SEM, n = 20/group
b Significant difference from males at p < 0.05 (unpaired t- test)

Table 2
Nutrient composition and samples of HC-LFLP and LC-HFHP meals.

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>HC-LFLP</th>
<th>LC-HFHP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (kg)</td>
<td>50</td>
<td>60</td>
</tr>
<tr>
<td>Energy, kJ (kcal)</td>
<td>2,093 (500)</td>
<td>2,512 (600)</td>
</tr>
<tr>
<td>Carbohydrate, % (g)</td>
<td>70 (87.5)</td>
<td>70 (105)</td>
</tr>
<tr>
<td>Fat, % (g)</td>
<td>15 (8.3)</td>
<td>15 (10)</td>
</tr>
<tr>
<td>Protein, % (g)</td>
<td>15 (18.8)</td>
<td>15 (22.5)</td>
</tr>
<tr>
<td>Meal sample</td>
<td>Fried rice:</td>
<td>Rice with roast pork:</td>
</tr>
<tr>
<td></td>
<td>Cooked rice, 240 g</td>
<td>Cooked rice, 300 g</td>
</tr>
<tr>
<td></td>
<td>Chicken (thigh), 15 g</td>
<td>Roast pork, 30 g</td>
</tr>
<tr>
<td></td>
<td>1 Egg, 50g</td>
<td>1/2 Egg</td>
</tr>
<tr>
<td></td>
<td>Vegetable oil, 2 tsp</td>
<td>Sweet sauce, 2 tsp</td>
</tr>
<tr>
<td></td>
<td>1-2 small cucumber</td>
<td>1-2 small cucumber</td>
</tr>
<tr>
<td></td>
<td>1-2 spring onion</td>
<td>1-2 spring onion</td>
</tr>
<tr>
<td></td>
<td>Coriander</td>
<td>Coriander</td>
</tr>
</tbody>
</table>

1-2 small cucumber
1-2 spring onion
Coriander

1/2 Egg
Sweet sauce, 2 tsp
1/4 cup
Fried egg
POSTPRANDIAL LEPTIN RESPONSE TO THAI MEALS

Fig 1—Blood glucose, insulin and \( \Delta \) leptin responses in a group consuming a high carbohydrate, low fat, low protein meal (HC-LFLP, \( n=20 \)) and a low carbohydrate, high fat and high protein meal (LC-HFHP, \( n=20 \)).

Values are means \( \pm \) SEM; *Different between test meals, \( p < 0.05 \); Values of times without # are different from values at 11:00 AM (0 hour) within the same group, \( p < 0.05 \).

Fig 2—Area under the curves (AUC) for \( \Delta \) glucose, \( \Delta \) insulin and \( \Delta \) leptin from values in groups consuming different test meals (\( n=20 \) in each group).

Values are means \( \pm \) SEM; *Difference from another test meal, \( p < 0.05 \).

DISCUSSION

We report that a single low carbohydrate, high fat, high protein meal leads to lower postprandial leptin levels compared to a meal high in carbohydrates, low in fat and protein. Our responses at each sampling in the study period found the HC-LFLP group had higher glucose and insulin levels than the LC-HFHP group. The 2-hour AUC in postprandial values for blood leptin, insulin, and glucose were statistically different between the test meals for the whole group of study subjects (\( N=20 \)). Women had higher fasting plasma leptin levels than men (10.37 \( \pm \) 1.48 vs 4.23 \( \pm \) 1.33 ng/ml at 08:00 AM). However, the meal-induced changes in leptin, insulin and glucose were not significantly different between sexes (\( p>0.05 \)).
data shows there was a significant difference in glucose and insulin levels between the two meal types. The higher leptin levels in the carbohydrate-rich meal appeared to be mediated by increasing serum insulin levels.

Our results are in line with previous studies in which protein was 15% of the test meal. The results of previous studies indicate that leptin is suppressed more by fat-rich foods than carbohydrate-rich foods during the first 3-6 hours after meal (Evans et al, 2001; Raben et al, 2003). High-fat diets (65% fat, 12% protein) and high-protein diets (31% fat, 32% protein) decrease plasma leptin levels more than high-carbohydrate diets (65% carbohydrates, 12% protein) (Raben et al, 2003). Thus, the high fat and high protein levels (50% fat, 30% protein) in our test meal may acted together to inhibit leptin levels.

Decreased leptin concentrations may relate to an increased prevalence of obesity after a longer period of high-fat consumption. Although, measurement of leptin levels for 2 hours after a meal did not predict the daily hormonal output in our subjects, low-fat diets are widely accepted to be an effective tool in eliciting weight loss or slowing weight gain (Jequier and Bray, 2002).

It has been shown that infusion of insulin antibody into rats increased meal size. Infusion of low doses of insulin into the cerebroventricular system of rats causes a reduction of food intake and body weight (Chavez et al, 1996). This suggests that the presence of insulin may be related to meal termination. Thus, a meal high in fat may be less effective for the suppression of appetite and to decrease food intake because of the lower insulin and leptin levels that they induce.

Decreased leptin secretion during prolonged fasting shown in our subjects supported leptin's role in homeostatic regulation of energy balance. However, circadian fluctuations of plasma leptin with a trough in the morning and an increase nocturnally have been found both in fasting and non-fasting subjects (Wolthers et al, 1999; Elimam and Marcus, 2002). Diurnal variation of leptin secretion should be taken into consideration in clinical studies. Gender differences in blood leptin concentrations were found in our subjects and in other studies, (Ostlund et al, 1996; Rosenbaum et al, 1996). However, meal-induced changes in plasma leptin concentrations did not differ between sexes.

In conclusion, we demonstrate a postprandial decrease in leptin after a single meal low in carbohydrates and high in fat and protein (LC-HFHP), but does not decrease after a high carbohydrate meal low in fat and protein (HC-LFHP). In our opinion, food high in fat and protein is not suitable in terms of an energy balance compared to the traditional low-fat, low-protein diet of Asian people. The long-term outcomes of a high-fat, high-protein diet on the hormones affecting food intake together with a satiety rating and measurement of caloric intake should be further studied in the Asian population.

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