MATERIALS AND METHODS

Animal Care

Eighty male rats of the Sprague-Dawley strain, weighing between 220-260 g, were obtained from the National Animal Center, Salaya Campus, Mahidol University. All animals were housed individually in rodent cages in the animal room where the temperature was maintained approximately at 24-25°C with 12:12 hours dark-light cycle. After being allowed to acclimatize for at least 5 days, the animals were matched as closely as possible for body weight and then randomly divided into 2 groups: high-carbohydrate diet (HC) and high-fat diet (HF) groups. The animal in HF group consumed a low carbohydrate diet consisting of 75%E fat, 20%E protein and 5%E carbohydrate by caloric value (3,970 Kcal), while the HC group received a high carbohydrate diet consisting of 0%E fat, 20%E protein, 80%E carbohydrate during the eight week training period. Based on caloric in normal diet, composition of diets used in the study were calculated (Conlee, *et al.*, 1990) (Table 1, A). Food and water were provided *ad libitum* and the body weight was measured weekly. The maximal oxygen consumption (Vo_{2max}) and endurance test were measured at the commencement of the first for the baseline control and every two weeks.

After measurement of endurance test at the 8th week, animals from each groups were assigned to one of four subgroups as follows:

Table 1. Composition of experiment diets.

A.

Ingredients	Normal Diet		HC Diet		HF Diet	
	g/Kg	%Kcal	g/kg	%Kcal	g/Kg	%Kcal
Cornstarch	680	68.51	746.60	79.85	76.36	5
Lard	50	11.34	0	0	508.14	74.85
Casein	200	20.15	187.70	20.15	307.77	20.15
Vitamins & Minerals	70	0	65.70	0	107.72	0

Diet ingredients and nutrient analyses were modified from Conlee, *et al.* (1990). HC: high carbohydrate diet; HF: high fat diet. Energy (Kcal) per gram: carbohydrate 4; fat 9; protein 4

B.

Ingredients	Formula I diet		Formula II diet		Formula III diet	
	g/Kg	%Kcal	g/Kg	%Kcal	g/Kg	%Kcal
Cornstarch	665.82	66.24	636.36	61.71	621.05	59.44
Lard	60.76	13.61	83.12	18.14	94.74	20.14
Casein	202.53	20.15	207.80	20.15	210.53	20.15
Vitamins & Minerals	70.88	0	72.72	0	73.68	0

High-fat diet

HF subgroup: The animals remained on the same control diet.

HFI subgroup: The animals were fed with the isocaloric formula I diet containing fat 14% of total of energy in the diet, (%E), carbohydrate 66%E and protein 20%E

HFII subgroup: The animals were fed with the isocaloric formula II diet containing fat 18% of total of energy in the diet (%E), carbohydrate 62%E and protein 20%E

HFIII subgroup: The animals were fed with the isocaloric formula III diet containing fat 20% of total of energy in the diet (%E), carbohydrate 59%E and protein 20%E

High-CHO diet

HC subgroup: The animals remained on the same control diet

HCI subgroup: The animals were fed with the same isocaloric formula I diet as HFI subgroup

HCII subgroup: The animals were fed with the same isocaloric formula II diet as HFII subgroup

HCIII subgroup: The animals were fed with the same isocaloric formula III diet as HFIII subgroup

Composition of isocaloric formula I, II and III diets is shown in Table 1B.

After being rested for 2 days, all animals were trained on a treadmill at the same intensity and duration of the 8th week for a further twelve days. On the final day of the experiment, one-half of animals in each of the four subgroups were run to exhaustion, whereas the remaining rats served as resting controls. After that, all animals were immediately killed for collection of blood samples.

Collection of Blood Sample

Collection of blood samples were performed before training for a baseline control, and then at the end of 8th week training. After the animals were anesthetized with diethyl ether, blood sample was collected from the tail vein. The tail was cleaned with 70% ethanol, then a small portion of the tail tip was cut off with a sharp blade. Aliquots of 0.5 ml volume of blood were collected in microcentrifuge tubes containing sodium fluoride (NaF). Plasma samples were collected after centrifugation (H-103 N series, Kokusan Ensinki Co., Ltd., Japan).and were analyzed for glucose concentration. Another 0.5 ml aliquot of blood, after 1-2 hrs standing at room temperature, was centrifuged to separate serum from the cellular constituents. The plasma and serum samples were stored at -18^{0}C until analysis.

Exercise Procedure

All animals were trained on a motorized rodent treadmill (Columbus instrument, USA) between 8.00-12.00 A.M. five days per week, in a similar fashion as described by Mokelke, *et al.* (1997). Initially, all animals ran at 20 m/min for 3 weeks and was increased to 24 m/min in the 4th week and then to the final speed of 28 m/min after 6 weeks. The running grade for the first 3 weeks during this adjustment period was 5% and was increased to 10% at the 5th week. Daily running duration was 10 min for the first week and was increased by 10 min every week until the maximal running duration was 60 min. By the end of the 6th week, the animals were running for 60 min/day, 28 m/min with a 10% grade, and were maintained for 2 more weeks.

Maximal Oxygen Consumption (Vo_{2max})

Vo_{2max} was determined on the treadmill by Oxymax (Columbus Instrument, USA). Treadmill was placed in an airtight chamber. Each test chamber required a controlled source of fresh air. This air supply came from an air pump. Fresh air was presented to the test chamber system which the flow to the chamber was controlled and measured. Flow rate of the air was continuously maintained at 5 l/min. The system pump drawed air sample from each test chamber. The air sample was dried and presented to the oxygen sensors for analysis. The system junction cabinet readed the various electronic signals from the system hardware

and presented them to the system computer via the system interface carel.

The procedure of Vo_{2max} measurement was modified from the method of Bedford, *et al.* (1979). As shown in Table 2, the treadmill testing procedure consisted of a 3 min warm-up at 8 m/min and 0% grade, the speed and/or grade were stepwise increased every 3 min. Vo_{2max} was defined as the point at which O_2 consumption (Vo_2) did not increase with further increase in workload or when the rat was unable or unwilling to continue running.

Table 2. Description of treadmill testing procedure

Stage	Grade, degrees	Speed, m/min	Duration, min
1	0	8	3
2	5	15	3
3	10	19	3
4	10	24	3
5	10	30	3
6	10	35	3
7	15	35	3
8	15	40	3
9	15	44	3
10	15	48	3

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Endurance Test

The endurance test was performed during 1.00-4.00 P.M to negate any possible effects of diurnal variation in glycogen concentration,. The endurance test protocol was modified from the method of Mercier, *et al.* (1995). Rats were run on the treadmill with speed of 28 m/min at 10% grade until they could no longer keep pace with the treadmill or they came in contact with electric grid at the rear of the treadmill five times in two minutes. At that point, exhaustion time of the exercise was determined. All rats were run by the same investigator to avoid any variation in judgement of exhaustion that may exist among investigators.

Measurement of Systolic Blood Pressure and Heart Rate

Systolic blood pressure and pulse rates in conscious rats were measured in conscious rats by means of the tail cuff method using a Blood Pressure Recorder (Model 8006 UGO Basile, Italy). The principle of the method is to detect the pulse in the caudal artery of the rat's tail with temperamental transducers. The rats were trained to acclimatize to the apparatus in order to reduce stress prior to measurement. The systolic blood pressure and pulse rate were monitored 3 times and reported as the average of the measurements.

Measurement of systolic blood pressure and heart rate in conscious rats was performed prior training and then on the final day of 8th week of training.

Animal Sacrifice and Blood Collection

All animals were killed after being anesthetized with Pentobarbital sodium (50 mg/Kg body wt.) administered intraperitoneally. The blood was drawn about 2.5 ml from the abdominal aorta. A 0.9 ml aliquot of blood, for measurement of triglyceride, was collected in a glass tube, and the serum was then separated, after 2 hr. standing, by centrifugation at 2000 g. for 10 min. Other aliqouts of blood, 0.8 ml for measurement of glucose and lactate, were collected in two microcentrifuge tubes, containing sodium fluoride or potassium oxalate anticoagulant, respectively. After centrifugation, plasma were separated. The samples were kept frozen at -18^{0}C until analysis.

Biochemical Assays

Blood concentration of glucose, triglyceride, cholesterol and lactate were achieved by facility from Central Diagnostic Laboratory, Maharaj Nakron Chiangmai hospital.

Cholesterol : The serum cholesterol was measured by enzymatic method by an automatic analyzer (Merck Co. Ltd., Germany).

Glucose: The plasma glucose was analyzed by O-toluidine method (Lewandowski,1987). As modified from the method of Lapachet, *et al.* (1996), the blood glucose utilization rate was calculated as follow:

Average rate of blood glucose combustion (mg/dl/min)

$$= \frac{\text{(Resting plasma glucose - Exhausting plasma glucose)}}{\text{Endurance time.}}$$

Triglyceride: The serum triglyceride was measured by enzymatic method by an automatic analyzer (Merck Co. Ltd., Germany). The blood triglyceride utilization rate was calculated as follow:

Average rate of blood triglyceride combustion (mg/dl/min)

$$= \frac{\text{(Resting serum triglyceride - Exhausting serum triglyceride)}}{\text{Endurance time.}}$$

Lactate: The plasma lactate was measured by enzymatic method by an automatic analyzer (Merck Co. Ltd., Germany). The blood lactate production rate was calculated as follow:

Average rate of blood lactate combustion (mmol/min)

$$= \frac{\text{(Resting plasma lactate - Exhausting plasma lactate)}}{\text{Endurance time.}}$$

These calculated values represented the average utilization rates of blood glucose, triglyceride and average production rate of blood lactate over time.

Statistical Analysis

The data from the experiments were expressed as means $\pm$ standard error (means $\pm$ SE) which were calculated by statistical method. One way analysis of variance were used to compare differences among group. The significance in each group difference were tested using the student's t-test. For all statistical analyses, a level of $p<0.05$ was considered to be statistically significant.

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