Dose-dependent effect of capsaicin on endurance capacity in rats

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The present study was designed to determine the effects of various levels of capsaicin (CAP) on endurance capacity in forty-nine male Sprague–Dawley rats, aged 4 weeks, which were assigned to four groups. Rats were given orally either control (0) or 6, 10 or 15 mg CAP/kg body weight 2 h before exercise by stomach intubations using a round-ended needle. The rats in each group were divided into two subgroups for resting or swimming exercise. Swimming exercise was performed with a weight corresponding to 3 % body weight attached to the tail, and endurance capacity was evaluated by swimming time to exhaustion. The highest dose (15 mg/kg) of CAP significantly ($P < 0.05$) increased endurance performance time and caused plasma non-esterified fatty acid to rise significantly by about 1·4-fold compared with that of non-CAP treated rats at exhaustion ($P < 0.05$). The highest dose of CAP had no effect on liver and gastrocnemius muscle glycogen (white and red) in resting rats, but significantly increased glycogen contents of both muscles at exhaustion ($P < 0.05$). At rest, plasma noradrenalin levels of the rats receiving the highest dose were greater than that of non-CAP-treated rats and remained greater until exhaustion. The effects of CAP on endurance capacity have received little attention and have conveyed conflicting impressions. Kim *et al.* (1997) failed to show the maximal effect of 10 mg/kg doses of CAP on swimming endurance time in mice. The influences of various doses of CAP on endurance capacity were still unclear. Matsuo *et al.* (1996) reported that the intake of CAP have little sparing effect on glycogen in the liver and soleus muscles at rest and during exercise in rats previously fed a CAP-containing diet *ad libitum* for 1 week. Our present results suggest more than the highest dose of CAP (15 mg/kg) can cause the increase of endurance capacity, which might be induced through the sparing of muscle glycogen and the rise of non-esterfied fatty acids following the increase of circulating catecholamine.

Capsaicin: Catecholamine: Swimming: Endurance capacity: Non-esterified fatty acid

Capsaicin (CAP) is a pungent component of hot red peppers, which are widely used as an important spice for enhancing the palatability of food, and also utilized as a medicine for developing counter-irritation (Wachtel, 1999).

Kim *et al.* (1997) demonstrated that oral administration of CAP resulted in increased endurance time during prolonged work. These increases were associated with enhanced lipolysis and sparing of stored glycogen, which results in delaying complete glycogen depletion by an increase in circulating catecholamine. The enhanced availability of non-esterfied fatty acids (NEFA) was thought to cause greater fat metabolism in the active muscle, which in turn inhibited carbohydrate metabolism and resulted in increased exercise capacity. We have recently demonstrated that CAP added to a high-fat diet (0·14 g CAP/kg) raised serum NEFA concentrations in rats, which possibly led to an increase in fat utilization as a energy source (Oh, 2001). Kawada *et al.* (1986) reported that CAP administration transiently induced a higher RQ and then markedly lowered it to about 0·75.

The importance of the amount of glycogen stored in working muscles for the endurance capacity has been described (Leighton & Foot, 1995). Increased fat utilization during endurance exercise enables athletes to improve endurance capacity (Azevedo *et al.* 1998). Therefore, the increase of fatty acid utilization during exercise is supposed to improve endurance capacity.

There have been very few studies of exercise and different doses of CAP. Kim *et al.* (1997) failed to show any effects of 3–15 mg CAP/kg on endurance time during swimming exercise tests. However, there was no CAP effect at 15 mg/kg. The dose relationships of CAP and metabolism during exercise have not been studied in detail; none of the few studies measured plasma hormones. By examining the endurance time, metabolic and hormonal responses to various CAP doses, one could gain a deeper understanding of how the effects of CAP are mediated. In the present study, we investigated the effects of light, moderate and heavy doses of CAP on endurance capacity and glycogenolysis by exhaustive swimming exercise in rats.

Materials and methods

Animal care and experimental design

Forty-nine male Sprague–Dawley rats, aged 4 weeks and weighing 75–85 g, were purchased from Japan Charles

River Co., Ltd (Yokohara/Kanagawaken, Japan). The animals were individually housed in a room maintained at $24 \pm 2°C$ and $50 \pm 5\%$ humidity with a 12 h light–dark cycle. They were fed on a commercial chow diet (Oriental Yeast Co. Ltd., Chiba, Japan) *ad libitum* and allowed free access to water. Eight groups of animals were studied at this time: (1) 15 mg CAP/kg, killed after exercise to exhaustion (*n* 6); (2) 15 mg CAP/kg, killed at rest (*n* 6); (3) 10 mg CAP/kg, killed after exercise to exhaustion (*n* 6); (4) 10 mg CAP/kg, killed at rest (*n* 6); (5) 6 mg CAP/kg, killed after exercise to exhaustion (*n* 6); (6) 6 mg CAP/kg, killed at rest (*n* 6); (7) vehicle-treated, killed after exercise to exhaustion (*n* 6); (8) vehicle-treated, killed at rest (*n* 7).

Two weeks before the experiment, all the rats were allowed to swim for 30 min/d to accustom them to swimming and to being handled. Thereafter, rats were fasted overnight, and then both swimming and resting rats were given orally either vehicle or CAP at the doses of 6, 10 or 15 mg/kg body weight, 2 h before exercise, by stomach intubations using a round-ended needle (Fig. 1). During the days of experimentation, rats allocated to groups 1, 3, 5 and 7 swam until exhaustion. Groups 2, 4, 6 and 8 rested in their cage for the corresponding time. Swimming exercise was carried out in a circular tank (diameter 0·50 m, depth 0·55 m) filled with water maintained at a temperature of $36 \pm 2°C$. The water depth, 0·35 m, was set so that the rats could not rest by supporting the tail on the bottom of the tank. Each of the rats had a weight attached (3 % body weight) to the tail for the duration of the swim-to-exhaustion exercise. This exercise corresponds to an intensity of approximately four to five metabolic equivalents (Dawson & Horvath, 1970). The animals were assessed to be exhausted when they failed to rise to the surface of the water to breathe within 7 s. At this moment, the animals were removed from the tank. They were anaesthetized with sodium pentobarbitate and killed by exsanguination from the carotid artery before exercise or immediately after exhaustion. Blood plasma was collected and stored at −80°C in a deep freeze for
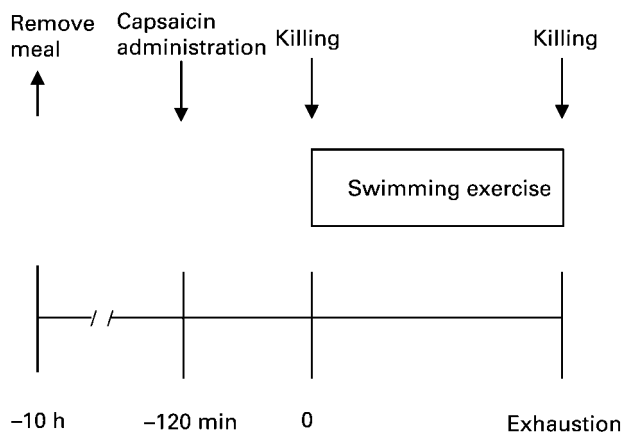
future analysis for catecholamine, glucose, NEFA, insulin and glucagon concentrations with commercial kits (Wako Pure Chemical Industries, Chu-ku Osaka, Japan). Liver and muscles (white and red gastrocnemius) were removed and then frozen in liquid N_2 to hold glycogen intact. Liver and muscle glycogen content was determined using the method of Lo *et al.* (1970). Briefly, portions of the muscle and liver were put in a tube containing 0·5 ml KOH (300 ml/l) saturated with Na_2SO_4 and immersed in a boiling water-bath for 20–30 min, before glycogen was assayed using a commercial kit (Glycogen Test Wako; Wako Pure Chemical Industries). To avoid circadian variations in physical activity, swimming was done from 10.00 to 17.00 hours, a period in which minimal variation of the endurance capacity has been confirmed in rats (Conlee *et al.* 1976).

Statistical methods

Results are expressed as mean values with their standard errors. The statistical significance ($P < 0.05$) of differences was determined by ANOVA followed by a Fisher Protected Least Significant Difference (PLSD) *post hoc* analysis.
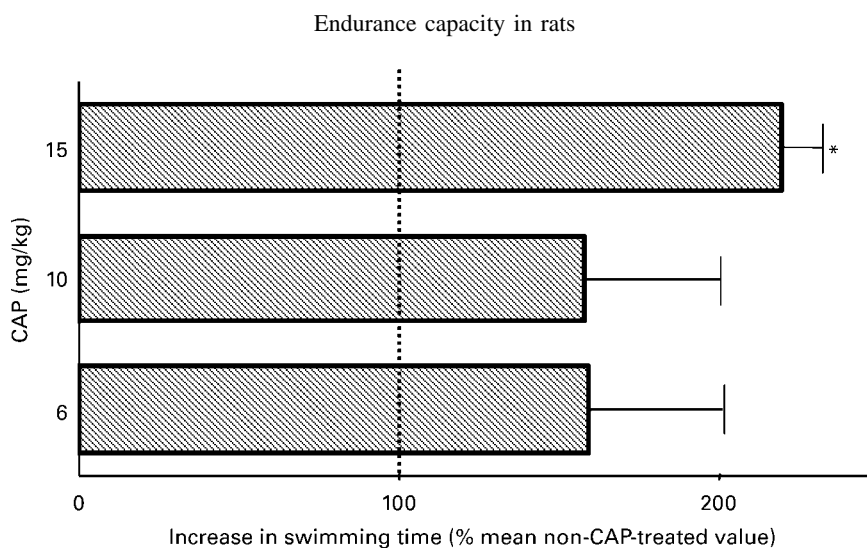
Results

Endurance swimming time

The groups treated with 15 mg CAP/kg showed a significant increase in swimming time to exhaustion of 219 % compared with non-CAP-treated groups (156 (SE 38) min, $P < 0.05$). At the 6 and 10 mg/kg levels, no clear effect of CAP was observed (Fig. 2).

Blood measurements

Plasma adrenalin levels in both 10 and 15 mg CAP/kg groups were significantly higher at exhaustion compared with that at rest ($P < 0.05$) (Fig. 3(a)). Plasma noradrenalin levels in all the groups treated with CAP were significantly higher at exhaustion compared with those at rest ($P < 0.05$). At exhaustion, plasma noradrenalin level in 15 mg CAP/kg group was significantly ($P < 0.01$) higher than that in non-CAP-treated group, though at rest, plasma noradrenalin level in 15 mg CAP/kg group was significantly ($P < 0.05$) higher than non-CAP-treated group (Fig. 3(b)). In the rest groups, plasma NEFA was not significantly increased by CAP administration. However, at exhaustion, plasma NEFA was significantly higher in the 15 mg/kg group than in the non-CAP-treated group ($P < 0.05$) and it was significantly higher in all the groups treated with CAP at exhaustion than in the rest groups ($P < 0.05$) (Fig. 4(a)). Plasma glucose was not increased by CAP administration in the rest groups. However, at exhaustion, plasma glucose was significantly higher in the 15 mg/kg group than that in the non-CAP-treated counterpart ($P < 0.05$) and significantly lower than in the rest groups ($P < 0.05$) (Fig. 4(b)). The exercise until exhaustion caused a significant reduction of plasma insulin and an increment of glucagon in the all groups including the non-CAP-treated groups ($P < 0.05$) (Fig. 5).

Fig. 1. Protocol for swimming exercise in the rats. The swimming exercise was performed until exhaustion with a weight (3 % body weight) attached to the rat's tail. Animals were assessed to be fatigued when they failed to rise to the surface of the water to breathe within a 7 s period. For details of procedures, see pp. 515–516.

Fig. 2. Comparison of swimming times until exhaustion among three different doses of capsaicin (CAP). Values are means with their standard errors shown by bars (n 6–7 per group). For details of procedures, see pp. 515–516. Mean value was significantly different from that of the non-CAP-treated group: *$P<0.05$.

Glycogen contents

Glycogen contents in liver and gastrocnemius muscles (red, white) were not affected by CAP (15 mg/kg) administration at rest, but both muscle glycogen contents were significantly higher in the CAP (15 mg/kg) group than in the non-CAP group at exhaustion ($P<0.05$) (Table 1).

Discussion

CAP is well known to stimulate lipolysis and/or fat oxidation during exercise; the related hormonal and metabolic responses with enhanced lipolysis to its graded dosages have not yet been sufficiently investigated. In the present study, we examined the physiological responses to various dose levels of CAP in rats. The glycogen-sparing effect provides an important survival advantage for improving endurance performance time, because glycogen depletion is associated with physical exhaustion and slower utilization of glycogen results in improved endurance capacity.

The results of the present study showed that oral administration of >15 mg CAP/kg affects the glycogen content in the liver and muscle (white and red gastrocnemius) as well as plasma catecholamine, glucose, NEFA, insulin, glucagon at rest and swimming time to exhaustion. This conclusion is similar to that of Kim *et al.* (1997) following oral administration of 10 mg CAP/kg.

The results suggests that oral administration of >15 mg CAP/kg is involved in the increase of the endurance
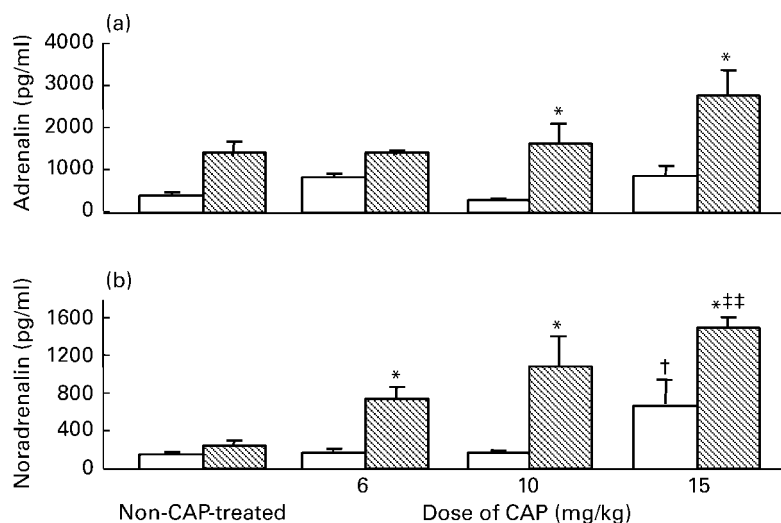
Fig. 3. Effects of various doses of capsaicin (CAP) and swimming to exhaustion on plasma adrenalin (a) and noradrenalin (b). □, Rest; ▨, exhaustion. Values are means with their standard errors shown by vertical bars (n 6–7 per group). For details of procedures, see pp. 515–516. Mean values were significantly different from those of the same group at rest: *$P<0.05$. Mean values were significantly different from those of the non-CAP-treated group: †$P<0.05$. Mean values were significantly different from those of the non-CAP-treated group after swimming to exhaustion: ‡‡$P<0.01$.
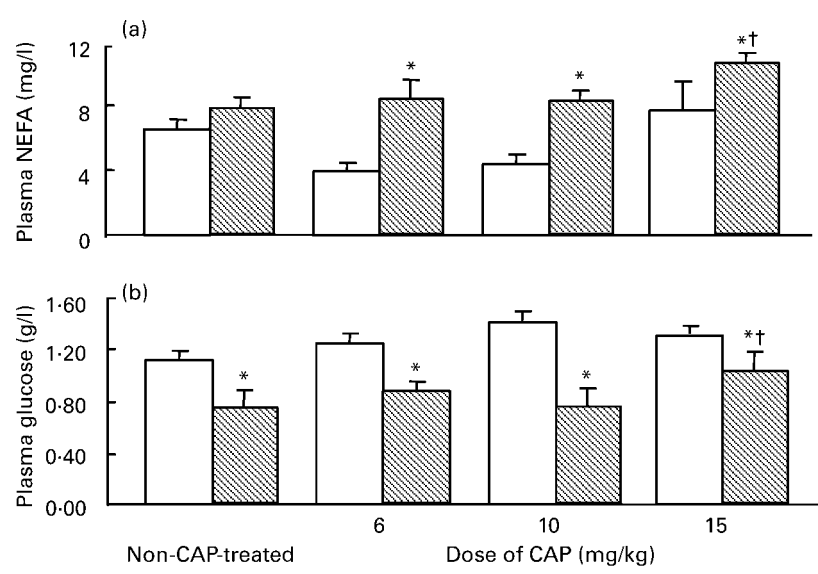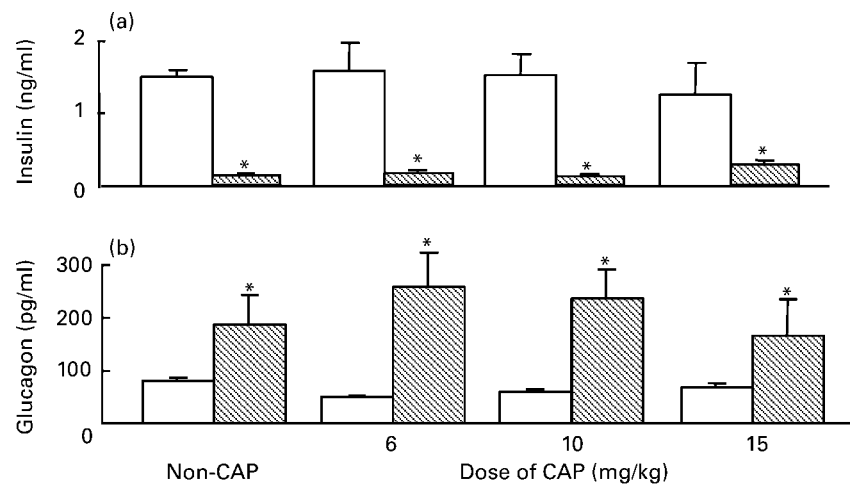
Fig. 4. Effects of various doses of capsaicin (CAP) and swimming to exhaustion on plasma non-esterified fatty acids (NEFA) (a) and glucose (b). □, Rest; ▨, exhaustion. Values are means with their standard errors shown by vertical bars (n 6–7 per group). For details of procedures, see pp. 515–516. Mean values were significantly different from those of the same group at rest: *$P<0.05$. Mean values were significantly different from those of the non-CAP-treated group: †$P<0.05$.

capacity after exercise to exhaustion. However, administration of only 10 mg CAP/kg resulted in a higher muscle glycogen after swimming (Kim *et al.* 1997). It was thus concluded: 'The maximal effect was observed at a dose of 10 mg/kg while more than 15 mg/kg had no effect.' We disagree with this conclusion because they used a short time (30 min) and this was insufficient to create a significant increase in plasma catecholamine levels.

Winder (1986) reported that caffeine elevating the plasma concentration of NEFA decreased the rate of glycogen depletion during exercise and would result in boosting lipid oxidation and depressing muscle glycogen utilization. Fat oxidation is enhanced with increased NEFA concentration. Klein *et al.* (1993) reported that about half of the NEFA mobilized from adipose tissue was utilized in contractive muscles during moderate intensity endurance exercise. In the present study, a significant increase of NEFA was observed at various doses of CAP groups. These results suggest that the glycogen thus saved could become an available energy source for the following phases of exercise, which delays onset of fatigue.

Catecholamine is well known to play a crucial role in the prevention of hypoglycaemia during exercise (Wasserman *et al.* 1984). CAP significantly increased circulating catecholamine levels with successively improved endurance performance (Lim *et al.* 1997). Graham & Spriet (1991) also found that CAP increased circulating catecholamine levels during exercise, but they did not observe the different dose effects of CAP on plasma catecholamine level at exhaustion. As shown in Fig. 3, the highest dose of CAP (15 mg/kg) increased plasma noradrenalin concentrations at exhaustion.

Fig. 5. Effects of various doses of capsaicin (CAP) and swimming to exhaustion on plasma insulin (a) and glucagon (b). □, Rest; ▨, exhaustion. Values are means with their standard errors shown by vertical bars (n 6–7 per group). For details of procedures, see pp. 515–516. Mean values were significantly different from those of the same group at rest: *$P<0.05$.

Table 1. Effect of 15 mg capsaicin/kg on liver and muscle glycogen‡
(Mean values with their standard errors for four to seven rats per group)

	Rest		Exhaustion	
	Mean	SE	Mean	SE
Liver (mg/g)				
Non-CAP	55·4	5·0	32·9**	6·0
CAP	69·7	20·0	34·1**	10·0
Gastrocnemius (mg/g)				
White				
Non-CAP	4·04	0·2	0·79**	0·3
CAP	3·66	0·2	1·90**†	1·0
Red				
Non-CAP	4·02	0·3	1·15**	0·4
CAP	3·87	0·1	1·52**†	0·5

CAP, capsaicin.

Mean values were significantly different from those at rest for the same treatment group: ** $P < 0.01$.

Mean values were significantly different from those of the corresponding non-CAP-treated group: † $P < 0.05$.

‡ For details of treatments and procedures, see pp. 515–516.

The metabolic effect of CAP on increasing endurance performance supported the notion of the sparing of muscle glycogen by oral administration of CAP 2 h before exercise (Kim *et al.* 1997). The glycogen saved becomes an available energy source for the following phase of exercise, which increase endurance capacity. Glycogen concentrations in muscle, as shown in Table 1, were reduced more slowly during exercise in CAP (15 mg/kg) group than in the non-CAP group. These results suggest that NEFA mobilized by CAP administration is used as a main energy source during exercise to exhaustion in rats, which enhances exercise time to exhaustion.

Costill *et al.* (1978) reported increased time to exhaustion with caffeine ingestion. Ivy *et al.* (1979) also reported enhanced exercise performance and found a significant difference in lipid oxidation. In the present study, endurance time to exhaustion was significantly higher in the highest dose CAP group compared with the non-CAP-treated group ($P < 0.05$). Decreased insulin secretion and increased glucagon secretion are involved in the precise matching of increased glucose production with increased glucose utilization that normally prevents hypoglycaemia during exercise (Issekutz & Vranic, 1980; Wolfe *et al.* 1986; Wasserman *et al.* 1989*a*). As shown in Fig. 5, plasma insulin secretion was significantly higher ($P < 0.05$) in the group at rest compared with the exhaustion, but plasma glucagon secretion was significantly higher after exhaustion compared with resting.

In summary, >15 mg CAP/kg 2 h prior to exercise enhances endurance performance. We propose that the >15 mg CAP/kg is a lipolytic food for its significant effects on glycogen sparing for two reasons: first, >15 mg CAP/kg enhances lipolysis activity and this maintains higher NEFA concentrations during endurance exercise; second, glycogen spared by >15 mg CAP/kg can be used as an important energy source at the latter stage of exercise, especially when the exercise intensity is increased at that point. Therefore, a high dose of CAP is a useful ergogenic acid when given 2 h prior to endurance exercise

in rats. Further work to elucidate the mechanism by which CAP promotes sparing of glycogen will be clearly warranted. The results presented here show that a high dose of CAP can dramatically alter many normal physiological and metabolic responses to endurance performance.

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