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Influence of capsaicin, curcumin and ferulic acid in rats fed high fat diets*

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Abstract. Three compounds capsaicin, curcumin and ferulic acid showing hypolipidemic activity have been tested in adult Wistar rats fed high fat diets. Capsaicin (0·20 mg%) fed to female rats along with a 30% saturated fat diet lowered the rate of weight gain, liver and serum triglycerides. In male rats it lowered only the liver and serum total and very low density and low density lipoprotein triglycerides whether fed continuously for 13 or 8 weeks after interchanging the control and test diets from the 5th week onwards. Capsaicin fed to female rats in 30% mixed fat diet increased the rate of weight gain, lowered liver and serum triglycerides, lowered adipose tissue lipoprotein lipase, elevated the hormone sensitive lipase and serum free fatty acids. Capsaicin in 30% saturated fat diet lowered both the enzyme activities to a much lesser extent. Curcumin and ferulic acid (both at 25 mg%) in 30% saturated fat diet tended to lower the rate of weight gain, liver total lipids and serum triglycerides. It is of significance that a common dietary compound 'capsaicin' in the range of human intake triggers lipid lowering action in rats fed high fat diets.

Keywords. Capsaicin; curcumin; ferulic acid; lipids; lipoprotein lipase; hormone sensitive lipase.

Introduction

Red pepper or chilli is one of the spices being evaluated in this Institute for their beneficial or adverse effects. Investigations on this common spice and its active principle 'capsaicin' (trans-8-methyl-N-vanillyl-6-nonanamide) were prompted by a report about the latter impairing fat absorption in rats leading to growth retardation (Nopanitaya, 1973). Fat absorption was found to be not affected by red pepper (5 g%) or equivalent capsaicin (15 mg%) in rats fed low or high fat diets (Sambaiah *et al.,* 1978, 1984). A significant observation in rats fed red pepper/equivalent capsaicin or a synthetic analogue (N-vanillyl nonanamide) along with the high fat diets was the mobilisation of triglycerides (TG) from liver to serum (Sambaiah *et al.,* 1978). This was further confirmed in rats fed choline deficient diet and in those administered $CC1_4$, ethionine, or ethanol (Sambaiah, K. and Satyanarayana, M. N., unpublished results). Besides the lipotropic effect, a hypolipidemic effect was also observed with red pepper/capsaicin (Sambaiah and Satyanarayana, 1980, 1982). More recently 6 other spice constituents and a natural plant component 'ferulic acid' [3-(4-hydroxy 3-methoxyphenyl) propeonic acid] having a related structure showed promise as

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Abbreviations used: BSA, Bovine serum albumin; TG, triglyceride; E I-III, experiments I-III; LPL, lipoprotein lipase; HSL, hormone sensitive lipase; FFA, free fatty acids; LDL, low density lipoproteins; HDL, high density lipoproteins; VLDL, very low density lipoproteins; PDE, phosphodiesterase.

hypolipidemic agents (Srinivasan, M. R. and Satyanarayana, M. N., unpublished results). The findings about red pepper/capsaicin lowering liver, serum and even carcass lipids (Sambaiah and Satyanarayana, 1982) and other compounds mentioned above showing a hypolipidemic effect indicated their likely value as anti-obesity agents. The present communication describes the results of capsaicin, curcumin and ferulic acid in rats fed high fat diets.

Materials and methods

The Wistar rats used were from the Institute Stock Colony. They were fed 21% casein synthetic diet for 4 weeks prior to feeding of the experimental diets. The procedures for maintenance of rats, monitoring of food intake, collection of samples of liver, perirenal adipose tissue, serum, analysis of total lipids, triglycerides, cholesterol, free fatty acids, lipoproteins and statistical evaluation were as described earlier (Srinivasan and Satyanarayana, 1986).

The sources of chemicals were as indicated below:
Capsaicin (synthetic analogue, Fluka-AG, Switzerland), Curcumin (Flavours and Essences Limited, Mysore), trans-Ferulic acid, bovine serum albumin (BSA), Triolein, Triton X-100 (Sigma, USA), Heparin (Biological E. Ltd., Hyderabad) and Glycerol (1-^{14}C) triolein (Radio Chemical Centre, Amersham, England). All other chemicals used were of analytical grade. The solvents were distilled before use.

The normal and high fat diets consisted of 10% refined groundnut oil (Postman brand) and 30% hydrogenated fat (Dalda brand) or 30% mixed fat (1:1 groundnut oil and hydrogenated fat) besides the following ingredients in g%: casein, 21; sucrose, 10; salt mixture (Bernhart and Tomarelli, 1966) 4; vitamin mixture (National Academy of Sciences, Washington, 1965); and corn starch to make upto 100. In experiment-III ingredients including the fat in liquid form were mixed and sucrose was added last as a syrup which facilitated preparation of a uniform water slurry. The test compounds were dissolved or dispersed in the fat portion before final mixing with the normal and high fat diets.

Experiments were carried out (E I–III) on the influence of I capsaicin (0·2 mg%), curcumin [l,7-Bis(4-hydroxy,3-methoxyphenol) 1–6 heptadiene, the colouring principle of turmeric, 25 mg%] and ferulic acid (25 mg%) in adult female rats fed 30% saturated fat, II capsaicin (0·17 mg%) in adult female rats fed 30% mixed fat and III capsaicin (0·20 mg%) in adult male rats fed 30% hydrogenated fat (i) continuously for 13 weeks and (ii) for 8 weeks after interchanging the control and test diets from the fifth week onwards. For comparison diets containing 10% groundnut oil diet without and with capsaicin have been fed to rats in I and II.

The enzymes lipoprotein lipase (LPL) (Schotz *et al.,* 1970) and hormone sensitive lipase (HSL) (Khoo and Steinberg, 1975) in perirenal adipose tissue were assayed as follows. The tissue was homogenized in 4 parts of 0·05 M Tris-HCl pH 8·2 containing 1 IU heparin/ml and spun at 8,000 *g* for 30 min. The fat-free clear solution was assayed for activity. The substrate was prepared by sonicating 0·125 mmol triolein, 1 μCi labelled triolein (54 mCi/m mol), 1 part of 1% (w/v) BSA (repeatedly extracted with solvent to remove 75 % of the initial free fatty acids (FFA) content) in Tris-HCl buffer pH 8·2, 1 part of 1% (w/v) Triton X-100, 8 parts of Tris-HCl buffer pH 8·2 containing 1 IU/ml heparin (5000 IU/ml). Prior to assay 3 parts of

the above emulsion were activated with 1 part of an over-night fasted rat serum by incubation at 37°/20 min in a Dubnoff shaking incubator (60 cycles/min).

The assay medium consisting of 1 part of the enzyme and 4 parts of the activated substrate with or without sodium chloride (1 M final concentration) was incubated at 37°C for 40 min in the shaking incubator. The zero hour control was the sample without incubation. The reaction was stopped by adding a mixture of isopropanol: 3 N H_2SO_4 (40:1). The labelled FFA released was extracted with hexane and solubilized with 0·1 N KOH. Suitable aliquots of the latter were counted using Bray's medium in a Beckmann liquid scintillation counter and the activity expressed as μmol FFA released per gram weight of wet tissue per hour.

The activity obtained without sodium chloride addition was the sum of LPL and HSL referred to as total activity. Subtracting from this the activity observed with NaCl addition represents LPL activity. The HSL activity measured at the optimum pH of 6·4 in 0·05 M phosphate buffer in place of the Tris HCl buffer, pH 8·2 was similar indicating that the activity measured at this pH represented the true value. All assays were conducted using Tris-HCl buffer, pH 8·2.

Results

Table 1 shows the initial and final weights, the food intake, the liver and adipose tissue weights of rats in the 3 experiments. The food intake data in E-I is not listed as it was not possible to monitor it in the usual practice in vogue at this Institute. The rats were fed the diet in the form of a slurry in water to avoid spillage of the dry diet. The high fat content led to the separation of fat as a layer at the top when a water slurry was made. The mixed fat did not cause such a problem. The problem with the saturated fat was overcome in E-III as described earlier. The food intake was not affected by capsaicin in high fat diets in E-II or E-III, whereas there was a lowering observed in the high fat fed groups without or with capsaicin when compared with those of the corresponding 10% fat fed groups in E-II. The liver and adipose tissue weights were not affected by the test compounds.

The rate of weight gain increased with the 30% fat fed group over that of the 10% fat in E-I at the 4th, 5th and 7th weeks. Capsaicin inclusion increased the rate of weight gain initially at the 2nd, 3rd and 4th week followed by a decrease at the 5th and 7th weeks. Curcumin showed a tendency to lower it from the 4th week onwards. Ferulic acid had no influence in the early stages but lowered at the 5th and 7th weeks. Capsaicin in the 30% mixed fat showed an increase over that of the 10% fat at the 6th and 7th weeks in E-II. No change in the rate of weight gain was observed in either of the groups fed continuously or otherwise in E-III (figure 1).

Capsaicin lowered (i) the liver total lipids, liver and serum TG in 30% fat fed groups in E-I, E-II and E-III, (ii) very low density lipoprotein (VLDL), LDL and HDL TG in E-II, and (iii) VLDL, LDL TG in E-III (figures 2 and 3). Capsaicin elevated and lowered respectively HDL TG in the 10 and 30% fat fed rats in E-II. An elevation in HDL TG was observed earlier in rats fed high sucrose diet (Srinivasan, M. R. and Satyanarayana, M. N., unpublished results).

In E-I capsaicin lowered the total adipose tissue lipolytic activity and particularly the LPL activity which was higher in the 30% fat fed basal group, whereas curcumin and ferulic acid tended to lower the total activity. In E-II, capsaicin lowered and

Table 1. Influence of test compounds on food intake, body, liver and adipose tissue weights.

Groups	Body weight (g) Initial	Body weight (g) Final	Food intake (g)	Liver weight g/100 g body wt.	Adipose tissue weight g/100 g body wt.
		Experiment I			
10% Fat	172·7 ± 4·1	192·3 ± 3·4	—	2·28 ± 0·08	1·42 ± 0·13
30% Fat (hydrogenated)	172·9 ± 4·2	210·3 ± 7·0	—	2·89 ± 0·22	1·77 ± 0·19
30% Fat + capsaicin (0·2 mg%)	173·6 ± 4·4	200·5 ± 4·3	—	3·00 ± 0·08	1·53 ± 0·19
30% Fat + curcumin (25 mg%)	173·4 ± 4·1	198·7 ± 8·8	—	3·07 ± 0·14	1·69 ± 0·16
30% Fat + trans-ferulic acid (25 mg%)	173·1 ± 4·5	197·8 ± 5·4	—	3·32 ± 0·12	1·53 ± 0·12
Statistical significance: 2 ~ 1		0·05		0·05	
		Experiment II			
10% Fat	176·6 ± 3·4	195·6 ± 4·8	484·9 ± 12·9	2·32 ± 0·048	1·16 ± 0·117
10% Fat + capsaicin (0·17 mg%)	176·7 ± 3·0	196·6 ± 3·3	490·3 ± 4·2	2·17 ± 0·063	1·28 ± 0·101
30% Mixed fat	176·7 ± 4·3	201·6 ± 6·9	413·1 ± 16·7	2·66 ± 0·170	1·93 ± 0·126
30% Mixed fat + capsaicin (0·17 mg%)	176·4 ± 5·7	215·1 ± 8·5	441·3 ± 18·1	2·45 ± 0·071	2·13 ± 0·152
Statistical significance:					
1 ~ 3			0·01		0·001
2 ~ 4			0·05	0·02	0·001
		Experiment III			
		5 weeks 13 weeks			
30% Hydrogenated fat	162·3 ± 5·61	273·3 ± 9·83 347·5 ± 13·12	1083·7 ± 29·7	3·82 ± 0·11	2·09 ± 0·18
30% Hydrogenated + capsaicin (0·20 mg%)	162·5 ± 6·11	266·2 ± 8·90 334·3 ± 10·80	1077·5 ± 10·9	3·71 ± 0·07	2·41 ± 0·10
30% Hydrogenated fat*	162·1 ± 5·39	271·5 ± 10·4 346·0 ± 11·36	1077·7 ± 16·6	4·03 ± 0·07	2·62 ± 0·14
30% Hydrogenated + capsaicin (0·20 mg%)*	162·1 ± 6·05	275·0 ± 8·45 346·2 ± 11·31	1099·7 ± 28·6	3·80 ± 0·10	2·53 ± 0·22
Statistical significance:					
1 ~ 3					0·05

*At 5th week diets were inter-changed and fed for 8 weeks.

Values are mean ± SEM for 7 rats fed for 7 weeks in experiments I, II, and for 13 weeks in experiment III.

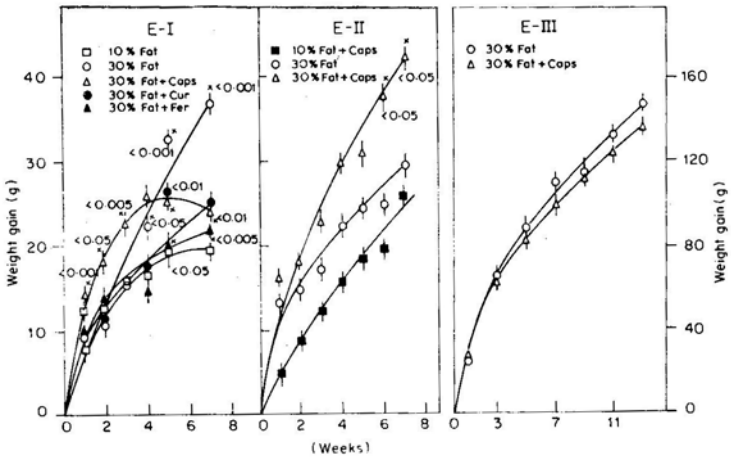

Figure 1. Rates of weight gain in E-I, E-II and E-III. The levels of significance in E-I are between 2~1, 2~3, 2~4, 2~5; in E-II between 1 ~3 (the rates of 10% fat and 10% fat + capsaicin almost similar). E-III, corresponding curves after interrupted feeding almost similar. CAPS, capsaicin; CUR, curcumin; Fer, ferulic acid.

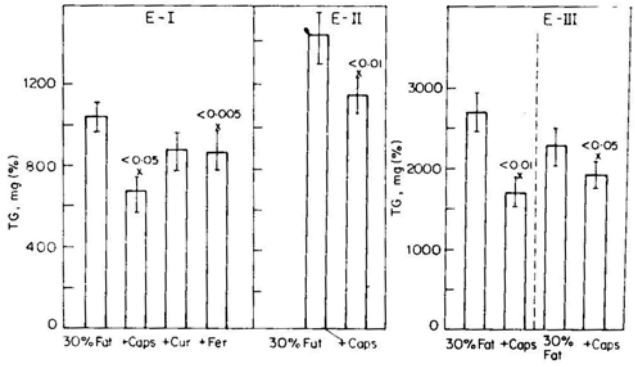

Figure 2. Liver TG in E-I, E-II and E-III. Levels of significance denoted between 30% fat and test compounds in E-I, between 30% fat and 30% fat+capsaicin in E-II, between 30% fat and 30% fat+capsaicin in E-III with continued and interrupted feeding (indicated by the broken line).

elevated respectively the adipose tissue LPL and HSL, only in the higher fat fed group. A corresponding increase in serum FFA by capsaicin was also observed (figure 4).

In E-I the serum cholesterol was lowered in the higher basal group but was not influenced by any of the test compounds. In E-II capsaicin lowered serum total cholesterol in both 10 and 30% fat fed rats. In E-III an increase in VLDL, LDL cholesterol and a lowering of HDL cholesterol was observed in the continuously fed group (table 2).

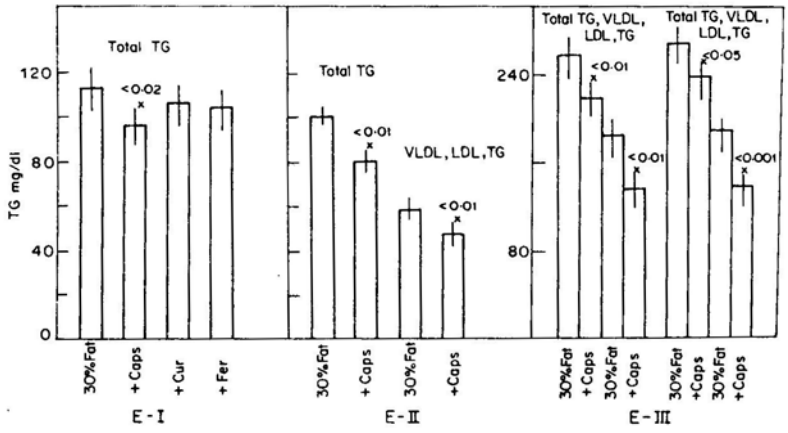

Figure 3. Serum triglycerides in E-I, E-II and E-III levels of significance between 30% fat and 30% fat + capsaicin.

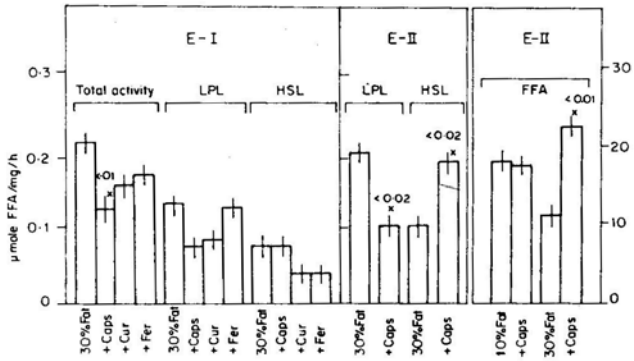

Figure 4. Lipolytic activity and serum FFA in E-I and E-II levels of significance denoted between 30% fat and 30% fat + test compounds in E-I, between 30% fat and 30% fat + capsaicin in E-II. In the latter the serum FFA in 30% fat is lowered significantly ($P < 0{\cdot}001$) in comparison with 10% fat diet.

Discussion

The results of E I-III show that the test compounds exert a significant influence in rats fed high fat diets. Although it was not possible to measure the food intake in E-I, the reasons such as the following lead to the conclusion that it is not affected adversely by any of the test compounds from the (i) normal appearance and behaviour of rats, (ii) absence of any gross difference in the final weights, (iii) earlier studies with capsaicin (1·5 or 0·15 mg%) showing no lowering of food intake when fed with 10% fat, 30% fat or high sucrose diets, and (iv) curcumin and ferulic acid which are devoid of pungent taste or/flavour unlike capsaicin, not lowering food intake even at higher levels (450 mg%) but showing an increase in rats fed high sucrose diets (Srinivasan, M. R. and Satyanarayana, M. N., unpublished results).

The lowering or the tendency to lower the rate of body weight gain in adult rats by

Table 2. Influence of capsaicin on serum lipids.

Groups	Total cholesterol	VLDL-LDL cholesterol	HDL cholesterol	HDL-TG
		Experiment II		
10% Fat	68·50 ± 1·92	39·10 ± 2·69	29·34 ± 2·40	13·86 ± 2·71
10% Fat + capsaicin (0·17 mg%)	58·08 ± 2·17	34·83 ± 2·77	23·26 ± 2·40	22·15 ± 4·49
30% Mixed fat	52·22 ± 2·26	30·03 ± 2·71	22·19 ± 1·50	40·97 ± 2·41
30% Mixed fat + capsaicin (0·17 mg%)	45·46 ± 1·80	24·77 ± 0·81	20·09 ± 2·09	32·41 ± 2·06
Statistical significance:				
1 ~ 2	0·01			
3 ~ 4	0·05			0·05
1 ~ 3	0·001	0·05	0·05	
2 ~ 4	0·01	0·01		
		Experiment III		
30% Hydrogenated fat	84·59 ± 5·97	48·97 ± 3·90	35·62 ± 2·70	70·70 ± 3·71
30% Hydrogenated fat + capsaicin (0·20 mg%)	78·65 ± 4·59	60·71 ± 3·49	18·18 ± 1·06	83·62 ± 7·52
30% Hydrogenated fat*	81·62 ± 4·05	51·57 ± 1·57	30·05 ± 3·76	81·13 ± 9·86
30% Hydrogenated fat + capsaicin (0·20 mg%)*	80·14 ± 5·57	57·51 ± 4·20	22·63 ± 1·57	98·55 ± 6·16
Statistical significance:				
1 ~ 2		0·05	0·001	
2 ~ 4			0·05	

Values are mean ± SEM for 7 rats as in table 1 and refer to mg/dl serum.

In experiment I, only total cholesterol determined. The values (mean±SEM) were respectively for groups 1–5: 64·77 ± 3·32, 52·52 ± 3·07, 47·86 ± 2·20, 54·49 ± 1·18 and 47·44 ± 4·29.

*Same notations as in table 1.

the test compounds observed in a period of 7 weeks shows the potential value of test compounds in minimising TG levels when fed to young rats along with the diet for longer periods. The effect is also likely to be more with increasing dosage. The experiments with male rats show the effect of capsaicin in preventing the elevation in liver and serum TGs when fed along with the high fat diet and in countering the elevated levels when the capsaicin containing diet is re-introduced. The two aspects both prevention and countering of lipid elevation, are of practical value. Feeding of the compounds from the weanling stage is likely to have a greater effect in countering the elevation. The lowering of (i) liver and serum TG by capsaicin (0·17 and 0·20 mg%) in the present experiments, (ii) serum total and VLDL, LDL TG by 0·19 and 0·32 g% red pepper with equivalent capsaicin 0·45 and 0·75 mg% in normal 10% fat diet (Srinivasan and Satyanarayana, 1986) and even carcass lipids by 5 g% red pepper and equivalent capsaicin 15mg% in a normal 10% fat diet (Sambaiah and Satyanarayana, 1982) indicate a dose dependent effect. Red pepper 0·5 g/kg body weight/day and capsaicin 50 mg/kg body weight/day fed to rats by stomach tube along with normal food and water increased the food intake but lowered the body weight at the end of 40 days feeding without causing any gross pathological changes (Monsereenusorn, 1983).

The problem with capsaicin is its pungent and irritant quality precluding its use at higher levels for greater effectiveness. It is of significance to note that the other natural compounds, curcumin and ferulic acid which exert a similar effect can be tested at higher levels safely in view of their innocuous nature.

The mechanism by which liver, serum and carcass lipids are lowered by dietary or other agents is of interest. The calories in excess of the requirement of the normal animal or man are known to be stored in the adipose tissue. The LPL and HSL of the adipose tissue responsible for the uptake of triglycerides and mobilization in the fed and starved states respectively and skeletal muscle LPL seem to determine the level of serum triglycerides. A related aspect is the role of substrate cycle between TG and FFA between adipose tissue and liver in determining TG levels in liver, serum and adipose tissue (Newsholme and Crabtree, 1976).

TG secretion from liver to serum is reported to be reduced in rats fed high fat diets (Kalopissis *et al.,* 1980). It is of significance that capsaicin (0·2 mg%) stimulates TG secretion (Sambaiah and Satyanarayana, 1987) and also elevates skeletal muscle LPL (Srinivasan, M. R. and Satyanarayana, M. N., unpublished results) both of which lead to lowering of liver and serum TG.

The elevation of HSL by capsaicin in the mixed fat diet is perhaps explainable by the reported specific diet induced transitions in membrane phospholipid fatty acid composition paralelled by changes in glucagon stimulated adenylate cyclase activity. The decreased cAMP formation reported in rats fed high fat diets (Neelands and Clandinin, 1983) is likely to be overcome by the inhibition of phosphodiesterase (PDE) by capsaicin, indication for which is observed in *in vitro* and *in vivo* experiments (Bharathi Salimath and Satyanarayana, M. N., unpublished results). The increase in the rate of weight gain in the higher mixed fat diet and triggering of the TG lowering process by capsaicin irrespective of the type of fat fed at higher levels need further study.

While these data were ready for publication, a report appeared about the lowering of serum TG and perirenal fat pad weight by 7, 14 and 21 mg% capsaicin in a dose

dependent manner in rats fed 30% lard for 10 days. An increase in LPL only at 14 mg% capsaicin without affecting HSL was also observed (Kawada *et al.,* 1986a). Our observations were a reduction in the rate of weight gain, a consistent lowering of both liver and serum TG even in rats already elevated by high fat feeding and a significant influence on the levels of adipose tissue, LPL, HSL, and skeletal muscle LPL brought about by capsaicin at much lower levels (0·17 and 0·20 mg%) fed along with 30% mixed fat diets. The lard diet closer to the 30% mixed fat diet (Singh *et al*., 1972; Waterman, 1951) causing an increase in LPL is in sharp contrast to our findings. The liver TG being not affected in their studies is perhaps due to the shorter period of feeding although at much higher concentrations. The dose dependent effect observed is similar to our observations. The capsaicin induced *ß*-adrenergic action on energy metabolism (Kawada *et al.,* 1986b), stimulation of catecholamine secretion from adrenal medulla (Watanabe *et al.,* 1987), and our observations on stimulation of adipose tissue HSL and inhibition of cAMP PDE indicate similarity of action.

The undue emphasis on the adverse effects of red pepper/capsaicin (Anon., 1986) in the human context appears out of place in view of (i) the tolerance and adaptation to them over centuries of use as a dietary ingredient, (ii) absence of epidemiological evidence of adverse effects from any part of the world, (iii) the growing popularity of their taste and appetizing but self-limiting quality (Govindarajan, 1985, 1986a, b; Govindarajan *et al.,* 1987; Govindarajan, V. S., Rajalakshmi, D. and Nagin Chand, unpublished results), (iv) their established safety in the normal range of human intake (Srinivasan *et al.,* 1980), and (v) particularly the lipid lowering and other favourable effects (Wang *et al.,* 1985) as revealed by investigations.

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