

The thermogenic properties of ephedrine/methylxanthine mixtures: animal studies^{1,2}

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ABSTRACT An over-the-counter preparation containing ephedrine, caffeine, and theophylline was examined for thermogenic anti-obesity properties. Administration of the methylxanthines to MSG-induced obese mice for 6 wk had no effect on energy balance or body composition. In contrast, treatment with ephedrine alone caused losses of 14% in body weight and 42% in body fat, effects brought about mainly by a 10% increase in energy expenditure. These changes were accentuated when ephedrine was administered together with one or both methylxanthines: energy expenditure was increased by a further 10%, and led to a reduction of about 25% in body weight and 75% in body fat, while the total food intake and body protein were unaltered. These results indicate that dietary methylxanthines like caffeine and theophylline, although alone have little effect on energy balance, can nevertheless markedly potentiate the thermogenic anti-obesity effect of ephedrine and normalize the body composition of the obese to the lean levels. *Am J Clin Nutr* 1986;43:388-394.

KEY WORDS Thermogenesis, obesity, sympathomimetic, energy balance, energy expenditure

Introduction

The use of thermogenic drugs for the treatment of obesity has enormous pharmaceutical potential and provides a rational basis for treatment on the assumption that obesity is due to a metabolic defect. Much of the evidence of a defective thermogenesis in human obesity is circumstantial (1), but in animal models there is overwhelming evidence for an elevated energetic efficiency in the etiology of the various genetic, dietary, and hypothalamic types of obesity (2, 3).

Recent studies have implicated an important role for the sympathetic nervous system (SNS) in the control of diet-induced thermogenesis (4-7), and the reports of reduced noradrenaline (NA) turnover rates in various tissues of several obese types (8-12) have strengthened the case for a search for drugs that would mimic the effect of NA in stimulating thermogenesis, and could thus lead to reductions in body fat.

Already a number of novel sympathetic stimulants with specificity for brown adipose tissue (13) as well as common sympathomimetics currently used for other treatments (14, 15) have been shown to possess thermogenic

properties. In particular, the ability of ephedrine to stimulate metabolic rate and thus cause fat losses in various animal models of obesity has been established (16-18). On the other hand, methylxanthines like theophylline are only effective as thermogenic anti-obesity agents at high doses, and only in some obese models (14). We now report the findings of studies conducted on obese mice showing that although methylxanthines like caffeine and theophylline have little long-term influence on energy balance, they can nevertheless markedly potentiate the thermogenic anti-obesity effect of ephedrine and lead to a normalization of the body weight and body composition of the obese to that of the lean.

Materials and methods

Mice of the CFLP strain were made obese by chemical lesioning of the hypothalamus following injections of

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monosodium glutamate (MSG) during the first week of life. Both MSG-obese mice and lean controls were weaned and maintained on a stock diet (CRM, Christopher Hill Group, London) for about 3 mo. Energy balance studies over a period of 6 wk were then conducted on male MSG-obese mice. At the start of the experiment, 42 obese mice, aged 15–16 wk, were divided into seven groups ($n = 6$) with similar mean body weights. One group (the Initial control group) was killed at the beginning to provide the initial carcass energy. Another group, the No-drug (ND) control group, was fed a powdered form of the stock diet, while the remaining five groups were also fed the same diet into which was incorporated the following drug(s): ephedrine hydrochloride (Thornton and Ross, Linthwaite Laboratories, UK); caffeine anhydrous (BDH Chemicals Ltd, Poole, UK); theophylline sodium glycinat (Riker Laboratories, Loughborough, UK). The ephedrine/methylxanthine(s) combinations were administered in the same dose ratios as found in an over-the-counter preparation “for the relief of bronchial coughs, wheezing, breathlessness and to clear the chest” (Do-Do tablets, product of Ciba Laboratories, Horsham, West Sussex, UK). The drug doses (g/kg diet) administered to the various groups are shown below in parentheses: Group C + T, caffeine (1.36) and theophylline (2.27); Group E, ephedrine (1.0); Group E + C + T, ephedrine (1.0), caffeine (1.36), and theophylline (2.27); Group E + C, ephedrine (1.0) and caffeine (3.63); Group E + T, ephedrine (1.0) and theophylline (3.63). It should be pointed out that although differences in thermogenic potency between caffeine and theophylline have not been reported *in vivo*, the authors do not claim that all methylxanthines are equally potent.

The animals were housed in a metabolism room maintained at $25 \pm 1^\circ\text{C}$ with a 12:12 light/dark cycle, and weighed at about the same time on alternate days. Food intake was measured weekly and for all weeks, and metabolizable energy (ME) was determined by the method of Miller and Payne (19). At the end of the experiment all obese animals as well as a group of lean controls ($n = 6$) were killed by cervical dislocation. The skull, thoracic, and abdominal cavities were incised, after which the carcasses were dried in an oven at 105°C for 48 h. Each dried carcass was then homogenized in a grinder, and triplicate samples were analyzed for energy content by bomb calorimetry (19). Carcass fat was measured by the soxhlet fat extraction method (20) and carcass protein was calculated from a general formula relating the energy derived from fat, the total energetic value of the carcass, and the energy derived from protein (6). Body energy gain was calculated from the difference between the final body energy content and the energy content of the initial control group killed at the beginning of the experiment. Energy expenditure (total heat production) over the entire 6-wk experimental period was determined as the difference between the ME intake and the energy gain—ie by the comparative carcass method (6, 7). Oxygen consumption over periods of 24 h was also determined at $25 \pm 1^\circ\text{C}$ using twin open-circuit calorimeters described previously (6, 7); the measurements were carried out on individual animals over two separate 24 h periods during weeks 3–6 of the experiment.

The data on energy balance and body composition of drug-treated and nontreated obese animals were analyzed using one-way analysis of variance (values for lean controls were not included in this analysis). Also calculated is a

critical difference which is the minimum difference between means that would achieve a significant difference at the 5% level.

The United Kingdom Home Office Regulations and Guide for the Care and Use of Laboratory Animals were followed.

Results

The growth curves of the various groups of obese animals are shown in **Figure 1**. The ME intake calculated over periods of 2 wk are represented in the form of bar-charts. The No-drug (ND) control group maintained a constant body weight throughout the experiment. Administration of caffeine and theophylline (C + T) caused a transient loss in body weight over the first week, an effect that is mostly accounted for by a decrease in food intake. Over the next few weeks, the C + T group gradually regained its former body weight while on a level of food intake similar to that of the ND-control group. At the end of the experiment, there were no significant differences in body weight between the C + T group and ND-control group.

Ephedrine (E) treatment caused a reduction in body weight of the order of 14% after 2 wk, and this was at least partially accounted for by a 6% reduction in food intake. However, this initial anorectic effect was compensated for by a 10% increase in food intake over the next 2 wk, but the E-treated group did not regain any previous loss in body weight. During the last 2 wk, the lower body weight of the E-treated group was maintained during periods when their food intake was similar to that of the ND-controls. Thus, at the end of the 6-wk study, the body weight of the E-treated group was significantly reduced by 16% (**Table 1**), but the total ME intake over that same period was no different from that of the controls (**Table 2**).

The effects of treatments with E + C + T, E + C, or E + T on the pattern of weight losses were similar to each other and much more pronounced than that caused by treatment with E alone (**Figure 1**). Over the first 2 wk, the 25% reduction in body weight in these groups was at least partly due to reductions of between 15–23% in food intake compared to the ND-controls. Over the next 2 wk, however, the food intake of the E + C + T, E + C, and

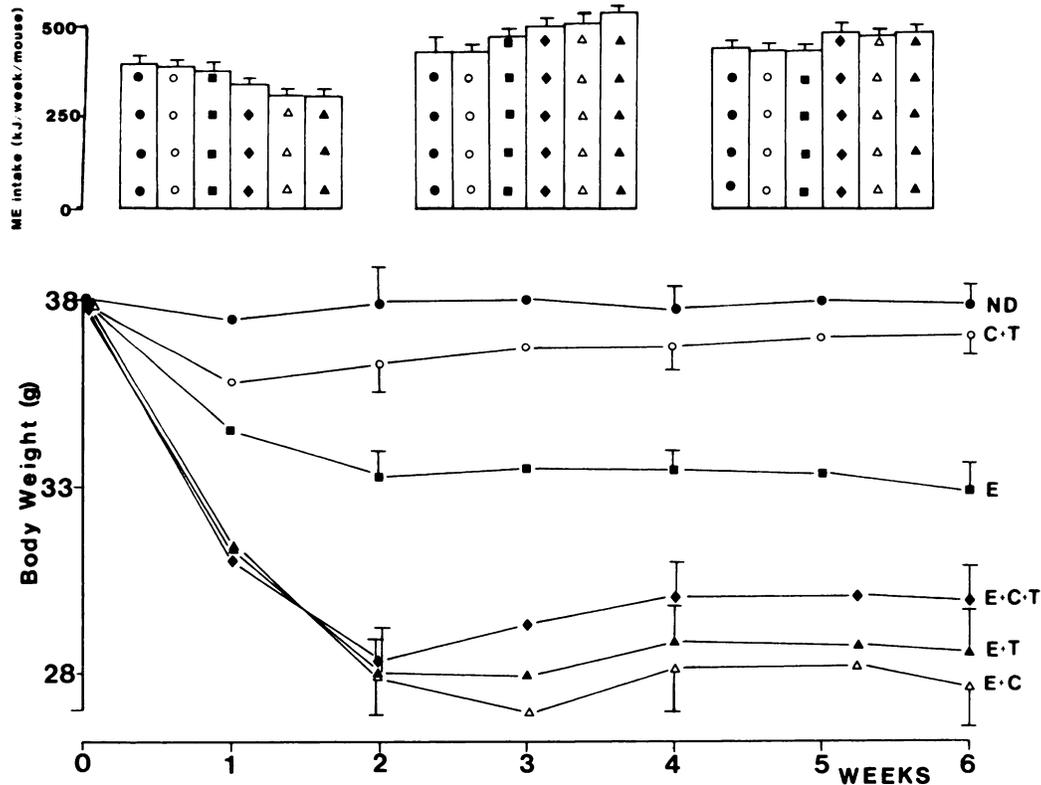


FIG 1. Body weight and food intake (ME) of obese mice during treatment for 6 wk with various combinations of ephedrine (E), and the methylxanthines caffeine (C) and theophylline (T), compared with the no-drug (ND) controls. Vertical bars represent the standard errors of mean values.

E + T groups was significantly higher than that of the ND control group (by 13%, 15%, and 24%, respectively), and these increases compensated for the reductions found during the first 2 wk. Thus after 4 wk of treatment with E + C + T, E + C, or E + T, the total food intake of these groups was similar to that of the ND control group, and yet they weighed nearly 25% less than the controls. Their post-obese body weights were maintained in the last 2 wk despite the fact that food intake over that period was still higher by about 10% compared to the ND controls (Figure 1).

Energy expenditure (Table 2), calculated over the entire experiment by the comparative carcass method was found to be significantly higher by about 10% as a result of treatment with E, and between 18–22% higher by treatment with E + C + T, E + C, or E + T, whereas the C + T treatment had no effect on metabolic rate. The effects of ephedrine alone

or in combination with caffeine and/or theophylline on metabolic rate did not alter body protein, but the losses of body fat were 42% with E alone, and nearly 75% with the mixtures E + C + T, E + C, or E + T (Table 1). Measurements of 24 h oxygen consumption (Table 2) during the second half of the experiment confirm the energy balance data that the thermogenic effect of ephedrine is potentiated by caffeine or theophylline: daily oxygen consumption, expressed per animal, was increased by 8% with E and by 20–24% with E + C + T, E + C, or E + T. If the data on oxygen consumption are expressed as a function of body weight (eg per $\text{kg}^{0.75}$), then daily metabolic rate was increased by about 14% with E alone, and by nearly 50% with the mixtures E + C + T, E + C, or E + T. This potentiation of the thermogenic anti-obesity effects of ephedrine by the caffeine and/or theophylline led to a normalization of the body

TABLE 1

Final body weight and body composition of obese mice treated with ephedrine alone or in combination with caffeine and/or theophylline, and comparison with lean controls

Group	Final body wt g	Carcass composition		
		Fat g	Protein g	Water g
ND control	38.0 ± 0.5	13.2 ± 0.5	5.3 ± 0.6	17.8 ± 0.3
C + T	36.8 ± 0.4	12.9 ± 0.4	5.5 ± 0.5	16.2 ± 0.5
E	32.9 ± 0.7	7.6 ± 0.8	5.3 ± 0.3	19.1 ± 0.4
E + C + T	30.0 ± 0.9	3.1 ± 0.4	5.2 ± 0.3	20.2 ± 1.0
E + C	27.7 ± 1.1	2.7 ± 0.4	5.8 ± 0.2	18.1 ± 1.2
E + T	28.6 ± 1.5	3.0 ± 0.3	4.9 ± 0.4	19.6 ± 1.0
Lean	30.7 ± 0.4	2.4 ± 0.2	6.0 ± 0.2	21.3 ± 0.7
Significance of <i>F</i>	P < 0.001	P < 0.001	NS	P < 0.01
Critical difference	2.6	1.4	1.2	2.1

Values are means ± SE (n = 6).

Each parameter was assessed using one-way analysis of variance (lean values excluded).

Critical difference is the least (p < 0.05) difference between means.

Groups = ND: no-drug treated; E: ephedrine; C: caffeine; T: theophylline.

weight, fat, and protein of the obese to similar levels as that of the lean controls (Table 1).

Discussion

The ability of the sympathetic stimulant ephedrine to sustain an increase in metabolic rate and to cause substantial loss in the body fat of several animal models of obesity is now well established (14–18). In contrast, there is virtually no published data about the long-term influence of caffeine on the energy balance of the obese, although several acute met-

abolic rate studies in man have shown that it is thermogenic (21–23). On the other hand, theophylline, which is a very similar compound to caffeine, is effective in raising the metabolic rate and lowering the body fat in dietary-induced obese rodents and in the genetically obese fafa rats, but not in the genetic ob/ob obese mice nor in the hypothalamic (MSG)-obese mice (14). The present study confirms the poor effect of chronic administration of methylxanthines in the MSG-induced obese mice, but indicates that either caffeine or theophylline or both in combina-

TABLE 2

Six weeks energy balance and 24 h oxygen consumption of obese mice treated with ephedrine alone, or in combination with caffeine and/or theophylline

Group	Total ME intake kJ/mouse	Body energy gain kJ/mouse	Total energy expenditure kJ/mouse	24 h Oxygen consumption	
				(L O ₂ /mouse)	(L O ₂ /kg ^{0.75})
ND control	2502 ± 62	+109 ± 8	2393 ± 66	2.80 ± 0.06	33.2 ± 0.4
C + T	2462 ± 29	+99 ± 7	2363 ± 25	2.71 ± 0.05	32.7 ± 0.6
E	2530 ± 57	-106 ± 24	2636 ± 42	3.03 ± 0.04	38.0 ± 0.7
E + C + T	2604 ± 15	-283 ± 13	2887 ± 14	3.52 ± 0.06	50.0 ± 1.2
E + C	2535 ± 81	-284 ± 16	2820 ± 80	3.40 ± 0.12	49.7 ± 0.9
E + T	2627 ± 73	-296 ± 15	2923 ± 75	3.43 ± 0.12	49.4 ± 1.4
Significance of <i>F</i>	NS	p < 0.001	p < 0.001	p < 0.001	p < 0.001
5% critical difference	177	50	173	0.24	2.8

Values are mean ± SE (n = 6). Critical difference is the least significant (p < 0.05) difference between means as assessed by analysis of variance. ND: no-drug, E: ephedrine, C: caffeine, T: theophylline.

tion can markedly potentiate the thermogenic anti-obesity effects of ephedrine, such that within a period of 4–6 wk, the body composition of the treated obese animals is normalized to that of lean controls.

The total ME intake was not altered by treatment with ephedrine alone or in combination with caffeine and/or theophylline. However, a closer examination of the food intake data shows that an anorectic effect over the first 2 wk no doubt contributed to the rapid loss in body weight. This appetite suppressant effect was only transient and was more than compensated for by an increase in food intake over the next 2 wk, followed by a level of food intake similar to that of the nontreated obese controls during the last 2 wk. The much lowered body weight however was not regained, and measurements of 24 h oxygen consumption during the second half of the experiment clearly indicate the potent thermogenic effects of ephedrine especially in combination with caffeine and/or theophylline. Similar transient anorectic effects are common with sympathetic stimulants in some obese animal models but not in others, and indicate that there are important differences in the response of the different types of obesity to the drugs: for example, while tranlycypromine and ephedrine have initial, albeit transient, anorectic effects in the genetically (*ob/ob* and *fafa*) and hypothalamically (MSG) obese animals, they have no effect on the appetite of dietary-induced obese rodents nor in lean animals (14, 15). No doubt, the classification of drugs as anorectic or thermogenic would require long-term energy balance studies in several animal models. It is not surprising that some of the most successful anorectic drugs, eg mazindol, phentermine, fenfluramine, and diethylpropion also have secondary thermogenic properties (24–27) given that these compounds are also potent stimulants of the SNS: the contribution of increased thermogenesis to weight loss by these compounds could well prove to be significant. But clearly in the case of ephedrine alone or in combination with caffeine and/or theophylline, the thermogenic effects are of primary importance, especially in maintaining the post-obese state.

The present study demonstrates the ability of the ephedrine/caffeine/theophylline mix-

ture to normalize the body weight and body composition of the MSG-obese mice, and also indicates that the potentiation of the thermogenic effect of ephedrine could be attributed to the general class of methylxanthine compounds. The exact pharmacological mechanisms by which noradrenaline (NA) controls whole body thermogenesis is unknown. However, it is generally accepted that at least part of the metabolic and thermogenic responses to NA are mediated by an increase in intracellular concentrations of cAMP following β -adrenergic stimulation of the membrane bound enzyme adenylate cyclase. Within the cell itself, cAMP is broken down by the enzyme phosphodiesterase, and in addition there is also some evidence that adenosine released from the cells in response to sympathetic stimulation, can inhibit NA release and/or act on specific receptors to inhibit the accumulation of cAMP (28, 29). Methylxanthines, in particular caffeine and theophylline, have the ability to inhibit both the effects of adenosine and phosphodiesterase, ie properties that would lead to prolongation of the effects of sympathetic stimulation. Such mechanisms of actions of methylxanthines on sympathetic activity could underlie the pharmacological basis by which they can increase energy expenditure and induce fat losses in some obese models. On the other hand, the failure of methylxanthines alone to have any thermogenic anti-obesity effect in the MSG-induced obese mice is compatible with the concept of a diminished sympathetic activity as the underlying cause for the elevated energetic efficiency that occurs following chemical lesioning of the hypothalamus. In the absence of sufficient sympathetically released NA, both the activities of adenosine and phosphodiesterase would be relatively low, and consequently, their inhibition by methylxanthines is unlikely to cause any marked change in sympathetic tone and thermogenesis. In contrast, ephedrine by its ability to enhance NA secretion (30) and also to stimulate adrenergic receptors (31) would increase sympathetic activity and metabolic rate. This increased NA secretion and consequent increase in intracellular cAMP levels would be sustained by the ability of the methylxanthines to inhibit phosphodiesterase. This could provide a possible

mechanism by which methylxanthines can potentiate the thermogenic effects of ephedrine: the combined effects of ephedrine and a methylxanthine could be said to be that of a long-acting NA which corrects the defective thermogenesis of animals that become obese following lesions in the hypothalamus.

The present findings that the MSG-obese animals are responsive to the thermogenic effects of ephedrine with or without methylxanthines are in agreement with our previous report of an unimpaired thermogenic response to different doses of NA in this obese model (32), and support our suggestion that obesity is not due to an insensitivity to NA, but rather to a deficiency of NA. This concept that the primary cause of the thermogenic defect in obesity results from a diminished SNS activity is further supported by the demonstration of a reduced NA turnover rate in young MSG-obese mice (12) and also in other animal obese models (8–11).

An important role of the SNS in energy balance regulation and thermogenesis is also evidenced by the fact that in lean mice, sympathetic denervation of brown fat (6), or chronic treatment with sympathetic inhibitor drugs (7) results in an elevated efficiency of food utilization and increased body fat. Thus in animals made obese by hypothalamic lesions, the central outflow of SNS activity is impaired, but the secretion of NA and thermogenesis may be restored by a combination of ephedrine and methylxanthine which normalizes the body composition of the obese to that of the lean. 

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