

# Effects of sucrose, caffeine, and cola beverages on obesity, cold resistance, and adipose tissue cellularity

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BUKOWIECKI, LUDWIK J., JEAN LUPIEN, NICOLE FOLLÉA, AND LAILA JAHJAH. *Effects of sucrose, caffeine, and cola beverages on obesity, cold resistance, and adipose tissue cellularity*. *Am. J. Physiol.* 244 (Regulatory Integrative Comp. Physiol. 13): R500–R507, 1983.—Rats consuming Coca-Cola and Purina chow ad libitum increased their total energy intake by 50% without excess weight gain. Their resistance to cold was markedly improved. These phenomena were characterized by significant increases in interscapular brown adipose tissue weight (IBAT) (91%), cellularity (59%), triglyceride content (52%), protein content (94%), and cytochrome oxidase activity (167%). In contrast, Coca-Cola consumption did not significantly affect the cellularity or triglyceride content of parametrial white adipose tissue (PWAT), although it slightly augmented PWAT weight. The effects of Coca-Cola on cold resistance, IBAT cellularity, and composition were entirely reproduced by sucrose, but not caffeine, consumption. Although caffeine also increased IBAT cellularity and composition, it significantly decreased the rate of body weight gain, PWAT weight, and adipocyte size. Moreover, it markedly inhibited adipocyte proliferation in PWAT thereby mimicking the effects of exercise training and food restriction (Bukowiecki et al., *Am. J. Physiol.* 239 (Endocrinol. Metab. 2): E422–E429, 1980). It is concluded *a*) that sucrose and Coca-Cola consumption improve the resistance of rats to cold, most probably by increasing brown adipose tissue cellularity, and *b*) that moderate caffeine intake might be useful for inhibiting proliferative activity in white adipose tissue, thereby preventing obesity.

rat; thermogenesis; adipocytes; cytochrome oxidase

IN RECENT YEARS, it has become increasingly evident that brown adipose tissue represents the principal effector of diet- and cold-induced thermogenesis in homeotherms (12, 29). Hyperphagia as well as cold exposure increase heat production in brown adipose tissue by activating the sympathetic nervous system and consequently enhancing the release of norepinephrine from sympathetic nerve endings surrounding the brown adipocytes (2, 9, 20). The interaction of the neurohormone with cellular  $\beta_1$ -adrenergic receptors triggers stimulation of respiration via activation of hormone-sensitive lipases (5, 6). Long-chain fatty acids released from triglyceride stores play a fundamental role in controlling brown adipose tissue oxidative metabolism by self-regulating lipolysis and by serving as the next messenger after cAMP in the stimulus-calorigenesis sequence (6).

It is now clear that the physiological function of diet-induced thermogenesis is to burn the excess of calories

ingested after overeating to prevent excessive weight gain. It follows that a malfunctioning of thermogenesis in brown adipose tissue should theoretically lead to a high metabolic efficiency resulting at long term in obesity (14). This proposition was initially based on the observations that genetically obese animals were very efficient in storing food energy because of a defect in the mechanisms regulating diet- as well as cold-induced thermogenesis (16). It has very recently received additional support by the demonstration that the sympathetic nervous system is defective not only in genetic obesity (*ob/ob* mice and Zucker rats) but also in hypothalamic obesity (19, 22, 30). On the other hand, it is known that rats possess the ability of adapting to cold as well as to hyperphagia by increasing their capacity for responding calorigenically to catecholamines (12, 25, 29). This phenomenon is characterized by a remarkable hyperplasia of brown adipose tissue that represents the physiological explanation for the hyperadrenergic response of cold-acclimated and/or hyperphagic animals to catecholamines (4).

The principal goal of the present studies was to test whether it would be possible to prevent obesity and/or increase cold resistance in rats by continuously stimulating the activity of the sympathetic nervous system with drugs or nutrients. The effects of sucrose and caffeine consumption were investigated for three reasons *a*) it has been demonstrated that both agents are very effective in stimulating the activity of the sympathetic nervous system when ingested orally (3, 20, 28), *b*) sucrose and caffeine are present in a wide variety of palatable food, often in combination with each other as in Cola beverages (13), and *c*) the comparison of the effects of these two compounds would make it possible to differentiate between the consequences of long-term stimulation of the sympathetic nervous system with a nutrient (sucrose) and a relatively safe drug (caffeine). Because it was found that sucrose and Coca-Cola consumption significantly increased the resistance of rats to cold, whereas caffeine intake inhibited body weight gain, the effects of these agents on adipocyte proliferation in interscapular brown adipose tissue (IBAT) and parametrial white adipose tissue (PWAT) were also evaluated.

## EXPERIMENTAL PROCEDURES

*Effects of sucrose, caffeine, and cola beverages on the resistance of rats to cold.* Female Wistar rats weighing 190–210 g were divided into six groups of 12 animals each.

The rats were housed in individual cages at 25°C and were submitted to a photoperiod of 12:12 LD. Each group of animals had free access to Purina chow and to one of the following beverages: tap water, Coca-Cola, sucrose 1 (sucrose dissolved in tap water at a concentration of 12 g/100 ml of water) (12%), sucrose 2 (32%), caffeine 1 (0.057%), and caffeine 2 (0.20%). After 9 wk, the resistance of rats to cold was measured. The animals were not fasted and were maintained under their normal housing conditions until 3–4 h before the experiment. The rats were lightly anesthetized with pentobarbital sodium (3 mg/100 g body wt ip) and were exposed to cold (4°C) for 5 h. Supplementary doses of anesthetic were given at approximately 90-min intervals. The core temperature was measured each hour with a thermocouple (Bailey Instruments, Saddle Brooke, NJ) inserted into the rectum. Shivering activity was measured in the warm (25°C) and 90 min after cold exposure (4°C) by electromyography (E and M Instrument, Houston, TX) with a needle electrode implanted in the femoral bicep muscle. During the measurements, the animals were lying on their left side on a laboratory table covered with a paper towel while the needle electrode was implanted in their right leg. At the end of the experiment, animals were returned to their normal experimental conditions. One week later, the rats were killed by cervical dislocation. IBAT and PWAT were carefully dissected, freed from all extraneous material, and weighed.

**Isolation of brown and white adipocytes.** Adipocytes were isolated from brown and white adipose tissue pieces by a collagenase method exactly as previously described (5). Triplicate samples of the washed cells were diluted with trypan blue stain (0.4% in normal saline), and the adipocytes were counted in Neubauer's hemacytometer. The percentage of cells resistant to trypan blue staining was usually higher than 98%.

**Cellularity of brown and white adipose tissue.** Triglycerides and cytochrome oxidase were used as markers for monitoring adipocyte recovery from PWAT and IBAT, respectively. Cytochrome oxidase activity and triglycerides were measured in cleaned tissue pieces and homogenized cells as previously described (5). The cellularity of IBAT, expressed in millions of brown adipocytes per total tissue was obtained by dividing total IBAT cytochrome oxidase activity by the cytochrome oxidase activity of one million of isolated brown adipocytes. Similarly, the cellularity of PWAT was obtained by dividing the total PWAT triglyceride content by the triglyceride content of 10<sup>6</sup> isolated white adipocytes (5, 8).

**Protein determination.** Proteins were estimated using Lowry's method (23).

**Statistics.** The statistical significance of the data was evaluated by analysis of variance and by Duncan's multiple *F* test (11, 27). When only two means were compared, the unpaired *t* test was used (27). In all tables and figures, *n* denotes the number of individual experiments performed on separate occasions and the symbols (\*), (†), or NS indicate that the differences between experimental and control values were significant (*P* < 0.01), highly significant (*P* < 0.05), or not significant, respectively.

**Materials.** Coca-Cola was bought five times concentrated from the local machine distributor company. It was diluted to its normal concentration with tap water but not gassed with carbon dioxide as it usually is. According to Coca-Cola Co., the beverage contains 11.5 g of sucrose and 10.5 mg caffeine/100 ml. Coca-Cola was given to rats in bottles of 500 ml as the animals were drinking more than 100 ml/day and fresh solutions were made each 2–3 days. Caffeine was bought from Sigma and other compounds were of analytic grade.

## RESULTS

**Effects of sucrose, caffeine, and cola beverages on rat growth and energy intake.** Preliminary experiments revealed that rats consume an enormous quantity of Coca-Cola if they are allowed to drink the beverage ad libitum. In fact, their mean Coca-Cola consumption over an experimental period of 9 wk reached approximately 150 ml/day (Table 1). This value represents five times the normal water intake of the control group. The animals drank twice as much Coca-Cola as sucrose dissolved in water at approximately the same concentration as in Coca-Cola (12%). Higher concentrations of sucrose (32%) did not result in higher energy consumption because the rats ingested less fluid. Rats offered Coca-Cola and Purina chow ad libitum during 9 wk increased their total energy intake by nearly 50% although they markedly decreased their laboratory chow consumption (Table 1). However, the total energy intake of rats drinking 12% sucrose was not statistically different from controls receiving water,

TABLE 1. *Effect of sucrose, caffeine, and Coca-Cola consumption on energy intake and body weight gain efficiency*

	Controls	Coca-Cola	Sucrose (12%)	Sucrose (32%)	Caffeine (0.057%)	Caffeine (0.20%)
Body wt gain, g/9 wk	91.8 ±3.5	97.0 ±4.9 (106%)	98.8 ±5.8 (108%)	83.9 ±5.4 (91%)	83.4 ±3.4 (91%)	64.5* ±2.8 (70%)
Fluid intake, ml/day	29.1 ±3.9	152.4* ±12.4 (523%)	82.9* ±8.2 (284%)	38.2* ±4.2 (131%)	31.2 ±4.0 (107%)	19.3* ±3.2 (66%)
Caffeine intake, mg/day		15.9 ±0.3			17.7 ±0.6 (111%)	38.6* ±1.7 (240%)
Sucrose intake, MJ/9 wk		18.4 ±0.4	10.5* ±0.3 (58%)	12.8* ±0.4 (70%)		
Lab chow intake, MJ/9 wk	17.7 ±0.4	7.7* ±0.3 (43%)	7.5* ±0.3 (42%)	6.1* ±0.3 (34%)	18.4 ±0.3 (104%)	17.1 ±0.4 (96%)
Energy intake, ‡ MJ/9 wk	17.7 ±0.4	26.1* ±0.4 (147%)	17.9 ±0.4 (101%)	19.2† ±0.6 (108%)	18.5 ±0.3 (105%)	17.1 ±0.4 (97%)
Body wt gain efficiency, g gain/MJ eaten	5.2 ±0.1	3.7† ±0.1 (71%)	5.5 ±0.3 (106%)	4.4† ±0.2 (85%)	4.5† ±0.1 (86%)	3.7† ±0.1 (71%)

Values are means ± SE (*n* = 10–12). Values in parentheses are % of control values. The statistical significance of the data was evaluated by variance analysis and completed by Duncan's multiple range test (11, 27). \* and †, Differences between the control and experimental groups that were statistically at the levels *P* < 0.01 and *P* < 0.05, respectively. ‡ Represents the total energy intake from sucrose and lab chow.

because the former group totally compensated its higher sucrose consumption by ingesting less Purina chow. Rats consuming 32% sucrose were only slightly hyperphagic. Thus, the high palatability of Coca-Cola represents an excellent physiological stimulus for increasing total calorie intake in rats. Paradoxically, ad libitum Coca-Cola drinking did not significantly enhance rat body weight gain (Fig. 1), resulting in a marked decrease in body weight gain efficiency (Table 1).

Because the intake of Purina chow was significantly reduced in sucrose-consuming animals, one might wonder whether they were protein and/or vitamin deficient. Knowing the proportion of proteins in Purina chow (20.1 g/100 g or 23.5% expressed as % of total metabolizable energy), it can be calculated from the data in Table 1 that the proportion of energy value of proteins consumed by the rats in the cola, 12% sucrose, and 32% sucrose groups were 5.9, 8.4, and 6.4%, respectively. Thus, none of the groups can be considered protein deficient, the 12% sucrose group consumed approximately double (8.4%) of the minimum required (4.6%) to induce protein

deficiency in adult rats (25). Similarly, it can be calculated that the cola or sucrose-consuming rats were not deficient in any of the major vitamins (vitamins A, B<sub>12</sub>, D, niacin, riboflavin, thiamin, folic acid, biotin, choline, and pantothenate) (25).

Although it is unlikely that the animals were protein deficient, they were certainly on a low-protein diet. This might be important, because it has very recently been suggested that diets low in proteins and high in carbohydrates are more effective than low-protein high-fat diets for decreasing body weight gain efficiency (32). Presumably, low-protein high-carbohydrate diets would also represent an optimal combination for activating thermogenesis in brown adipose tissue, although this still remains to be directly demonstrated. This might explain the remarkable decrease in both weight gain efficiency induced by palatable sucrose solutions in the present studies (Table 1) and the failure to detect significant changes in this parameter in previous studies when female Wistar rats were fed high-fat low-protein "cafeteria" diets (4).

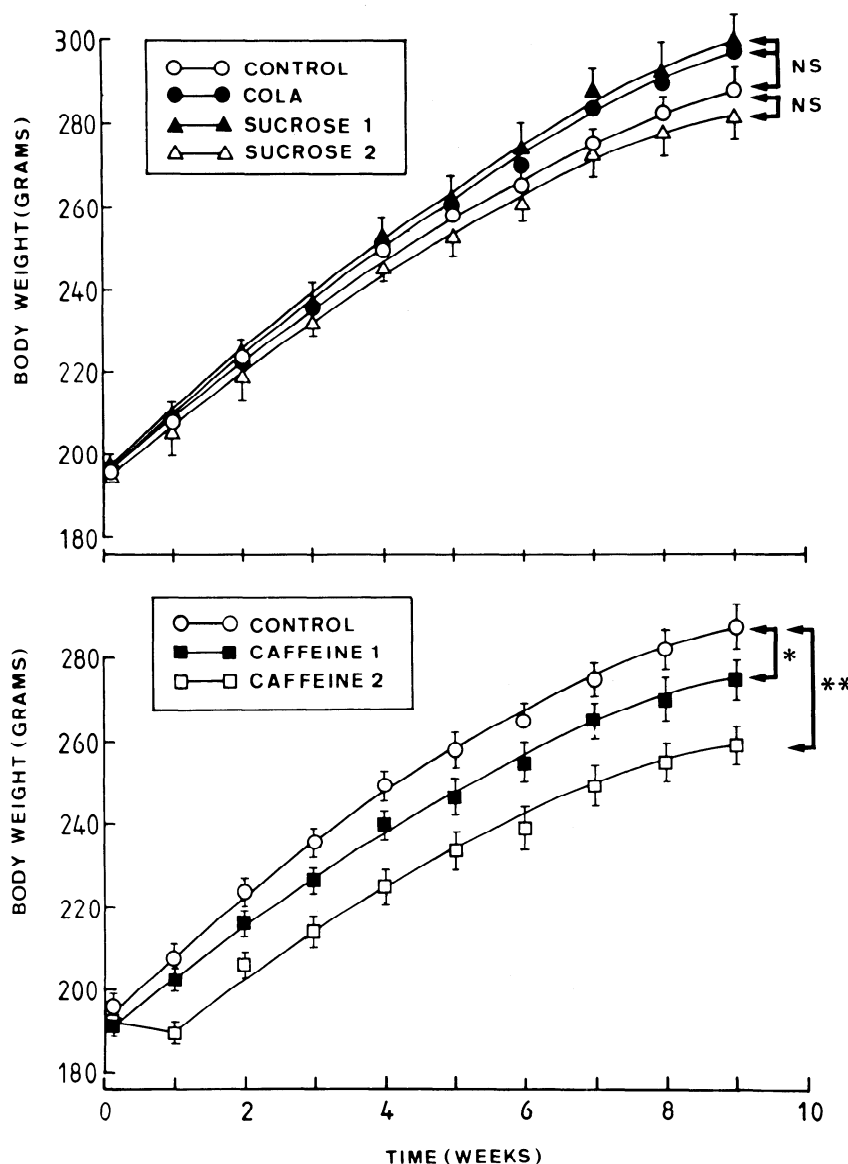


FIG. 1. Effects of sucrose, caffeine, and Coca-Cola consumption on rat growth. Composition of diets and animal housing conditions are described under EXPERIMENTAL PROCEDURES. Body weights were recorded weekly. Each point represents mean  $\pm$  SE of 12 animals. \*\* Differences between measurements that were significant at level  $P < 0.01$  (cf. EXPERIMENTAL PROCEDURES). After 9 wk, resistance of animals to cold was evaluated (Figs. 4 and 5). Animals were then returned to their respective experimental conditions during 1 wk before being killed for IBAT and PWAT analysis (Tables 1 and 2).

To study the effects of caffeine on rat metabolism, the drug was dissolved in tap water at low (0.057%) and high (0.20%) concentrations and was given to two additional groups of animals. Considering that rats consumed five times more Coca-Cola than water (Table 1), caffeine concentration in water had to be approximately increased five times over its normal concentration in Coca-Cola (10.5 mg/100 ml) to reach an equivalent daily consumption of 15.9 mg of caffeine/rat. Animals receiving high concentrations of caffeine (0.20%) decreased their mean fluid intake by 35%. Nevertheless, they consumed approximately twice as much caffeine per day as rats having 0.057% caffeine or Coca-Cola. Rats drinking 0.20% caffeine stopped growing during the 1st wk of the experiment and started to regain some weight only during the 2nd wk but never caught up with the controls. An early reduction in body weight gain was not observed in animals consuming the low-caffeine solution (0.057%). These animals did not gain weight as rapidly as the controls.

Weekly averages of fluid and food consumption are given in Figs. 2 and 3. Rats given Coca-Cola progressively increased their fluid intake from  $800 \pm 45$  ml during the 1st wk to  $1,200 \pm 60$  ml during the 4th wk ( $P < 0.01$ ) and then maintained such an elevated fluid consumption during the following 5 wk. This suggests that the animals progressively learned to recognize the taste of Coca-Cola and gradually increased their fluid intake because they were repeatedly rewarded with sensory satisfaction (31).

On the other hand, rats receiving the high-caffeine solution (0.20%) drank significantly less fluid than controls, especially during the 1st wk. Nevertheless, no signs of dehydration were observed, and their rectal temperature was normal (Fig. 4). Indeed, the lowest fluid intake registered during the 1st wk in these animals ( $60.5 \text{ ml} \cdot 100 \text{ g}^{-1} \cdot \text{wk}^{-1}$ ) was still higher than that required to

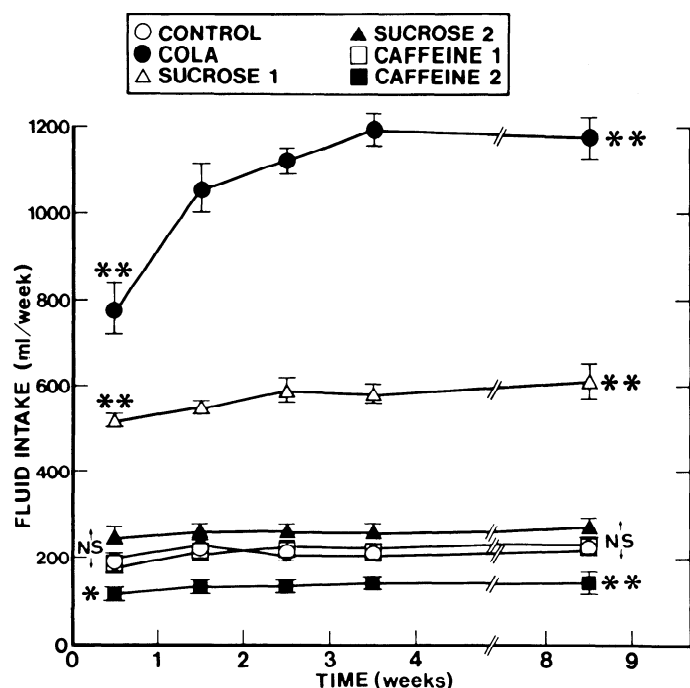


FIG. 2. Effects of sucrose, caffeine, and Coca-Cola consumption on weekly fluid intake. Animals and other experimental details were as in Fig. 1.

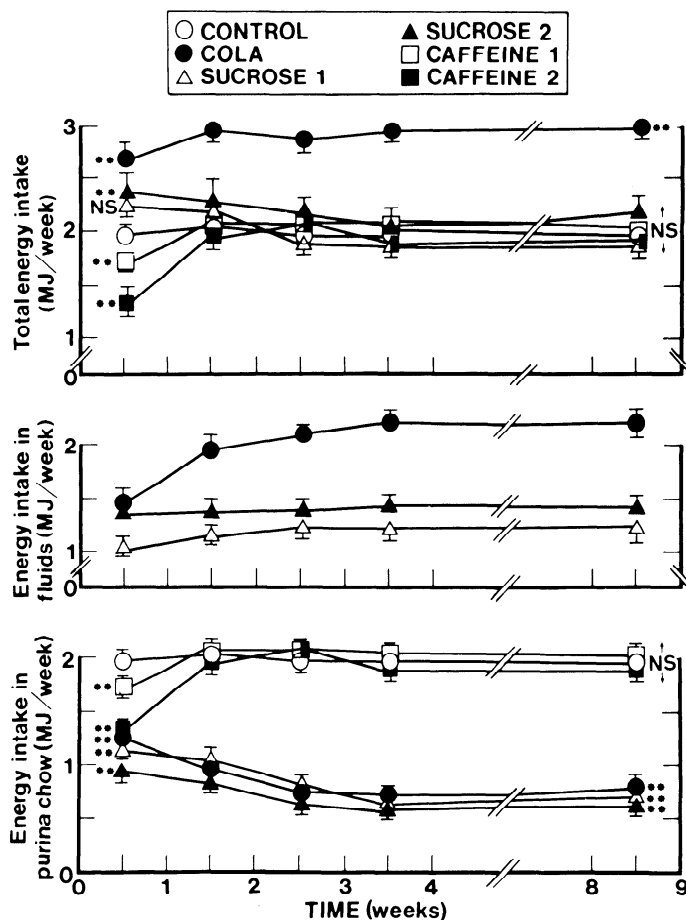


FIG. 3. Effects of sucrose, caffeine, and Coca-Cola consumption on weekly energy intake. Animals and other experimental details were as in Fig. 1.

initiate dehydration in adult rats ( $52.5 \text{ ml} \cdot 100 \text{ g}^{-1} \cdot \text{wk}^{-1}$ ) (10). Fluid consumption in the animals receiving the low-caffeine solution was normal.

Except for the 1st wk, the total energy intake remained remarkably constant throughout the entire experimental period in all groups of animals (Fig. 3). The progressive increase of energy intake from Coca-Cola during the first 4 wk was only partially compensated by a parallel decrease of calorie consumption from Purina chow, the net result being an increased energy intake of 40–50% during the entire experimental period. In contrast, animals consuming sucrose at approximately the same concentration as in Coca-Cola (12%) were not hyperphagic, whereas those taking 32% sucrose were only slightly hyperphagic.

Rats on the high-caffeine solution (0.20%) decreased their food intake during the 1st wk only, indicating either that caffeine acted as a true anorectic agent or that rats decreased their food intake because they initially disliked the taste of caffeine and therefore reduced their fluid intake [rats restricted in fluid intake usually decrease solid chow intake (10)]. In any case, the decreased body weight gain during the 1st wk (Fig. 1) did not result solely from the decreased calorie intake because caffeine significantly decreased body weight gain efficiency during this week ( $7.0 \pm 0.45$  for controls vs.  $-4.4 \pm 2.6$  for the high-caffeine group,  $P < 0.01$ ) as well as during the entire experimental period (Table 1). These results suggest that

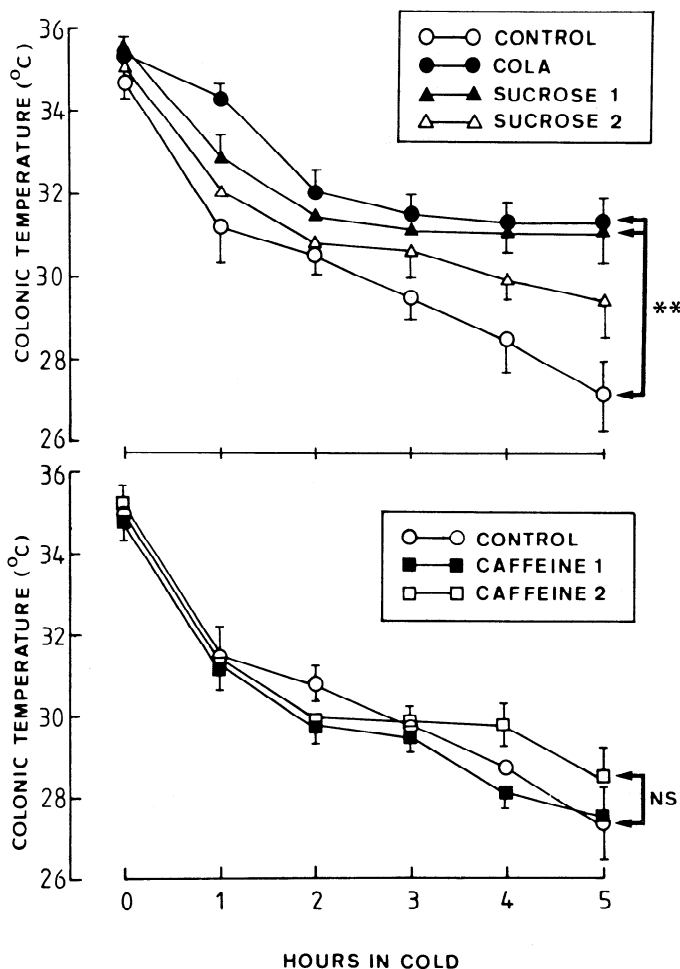


FIG. 4. Effects of sucrose, caffeine, and Coca-Cola consumption on rat resistance to cold. Animals were lightly anesthetized, placed in a cold room at 4°C, and their colonic temperature was measured as described under EXPERIMENTAL PROCEDURES. Each point represents mean  $\pm$  SE of measurements made on 8–10 animals.

caffeine might transiently act both as an anorectic and as a thermogenic drug, especially when given at high concentration.

**Effects of sucrose, caffeine, and cola beverages on the resistance of rats to cold.** Two parameters were used to test the resistance to cold: the decrease in rectal temperature after exposure to cold (4°C) (Fig. 4) and the shivering activity assessed from the electromyographic activity of the femoral biceps muscles (Fig. 5). Coca-Cola and sucrose consumption significantly reduced the drop in rectal temperature after cold exposure over a period as long as 5 h. In the same animals, shivering activity was also reduced, particularly in Coca-Cola-drinking rats (Fig. 5). Although it has been reported that caffeine displays short-term thermogenic effects in humans as well as in rats (1, 17, 28), caffeine consumption over a period of 9 wk did not significantly affect cold resistance. It should be emphasized that rats were exposed to cold at least 3 h after they had the last opportunity to drink and that, thereafter, cold resistance was assessed during 5 h. Thus, only the long-term adaptive effects of sucrose, caffeine, and Coca-Cola consumption were measured under the present experimental conditions.

**Effects of sucrose, caffeine, and cola beverages on**

**brown adipose tissue cellularity and composition.** Coca-Cola and sucrose consumption at low (12%) or high (32%) concentrations nearly doubled IBAT weight, cellularity, and protein content (Table 2). IBAT hypertrophy was accompanied by an even greater augmentation of total tissue cytochrome oxidase activity that increased by as much as 176% in the group receiving Coca-Cola. Because mitochondrial proteins represent approximately 40% of total tissue protein in brown adipose tissue (26), the results indicate that IBAT mitochondrial oxidative capacity is markedly enhanced by Coca-Cola and sucrose consumption. In contrast, sucrose-containing beverages did not significantly affect IBAT total triglyceride content or the triglyceride content per adipocyte. It can, therefore, be concluded that IBAT hypertrophy mainly results from a proliferation of brown adipocytes rather than from an increased cellular storage of triglycerides.

Although caffeine obtunded cellular proliferation in PWAT (vide infra), it slightly increased the total number of IBAT adipocytes without significantly modifying other measured parameters.

**Effects of sucrose, caffeine, and cola beverages on white adipose tissue cellularity and composition.** Coca-Cola and sucrose consumption significantly increased PWAT weight but barely affected tissue cellularity (Table 3). The latter parameter was increased by only 25% in the group receiving sucrose at high concentration (32%). The increased PWAT weight after sucrose consumption appears to result from an increased tissue triglyceride content associated with slight increases in cellularity and water content.

On the other hand, the weight and triglyceride content of PWAT were markedly decreased after caffeine consumption (Table 3). This resulted from a reduction in

TABLE 2. Effects of sucrose, caffeine, and Coca-Cola consumption on interscapular brown adipose tissue composition

	Controls	Coca-Cola	Sucrose (12%)	Sucrose (32%)	Caffeine (0.057%)	Caffeine (0.20%)
IBAT wt, mg	323 $\pm 29$	617* $\pm 29$ (191%)	718* $\pm 37$ (221%)	782* $\pm 39$ (242%)	323 $\pm 22$ (100%)	328 $\pm 30$ (102%)
Total number of adipocytes $\times 10^6$	31.1 $\pm 1.3$ (159%)	49.4* $\pm 3.6$ (159%)	47.3† $\pm 3.5$ (152%)	47.2† $\pm 3.4$ (152%)	37.6 $\pm 3.7$ (121%)	48† $\pm 3.7$ (154%)
Total triglyceride content, mg	83.1 $\pm 10$ (152%)	126.4† $\pm 9.8$ (152%)	139.8† $\pm 11.1$ (168%)	153.5* $\pm 17.3$ (185%)	69.1 $\pm 7.7$ (83%)	70.4 $\pm 12.1$ (85%)
Triglyceride content per adipocyte, ng	2.7 $\pm 0.4$ (100%)	2.7 $\pm 0.2$ (100%)	2.8 $\pm 0.2$ (104%)	3.2 $\pm 0.4$ (119%)	1.8 $\pm 0.2$ (67%)	1.5† $\pm 0.4$ (55%)
Total protein content, mg	20.7 $\pm 1.3$ (194%)	40.1* $\pm 3.4$ (194%)	52.6* $\pm 5.7$ (256%)	46.1* $\pm 5.1$ (223%)	21.8 $\pm 2.8$ (105%)	22.1 $\pm 3.7$ (107%)
Total cytochrome oxidase content $\mu\text{mol} \cdot \text{O}_2^{-1} \cdot \text{min}^{-1}$	90.3 $\pm 8.9$ (267%)	241.1* $\pm 31.9$ (267%)	196.3* $\pm 24.6$ (217%)	208.7* $\pm 25.3$ (231%)	115.4 $\pm 12.8$ (127%)	98.5 $\pm 9.7$ (109%)

Values are means  $\pm$  SE ( $n = 10$ –12). Values in parentheses are % of control values. The statistical significance of the data was evaluated by variance analysis and completed by Duncan's multiple range test (11, 27). \* and †, Differences between the control and experimental groups that were statistically significant at the levels  $P < 0.01$  and  $P < 0.05$ , respectively.

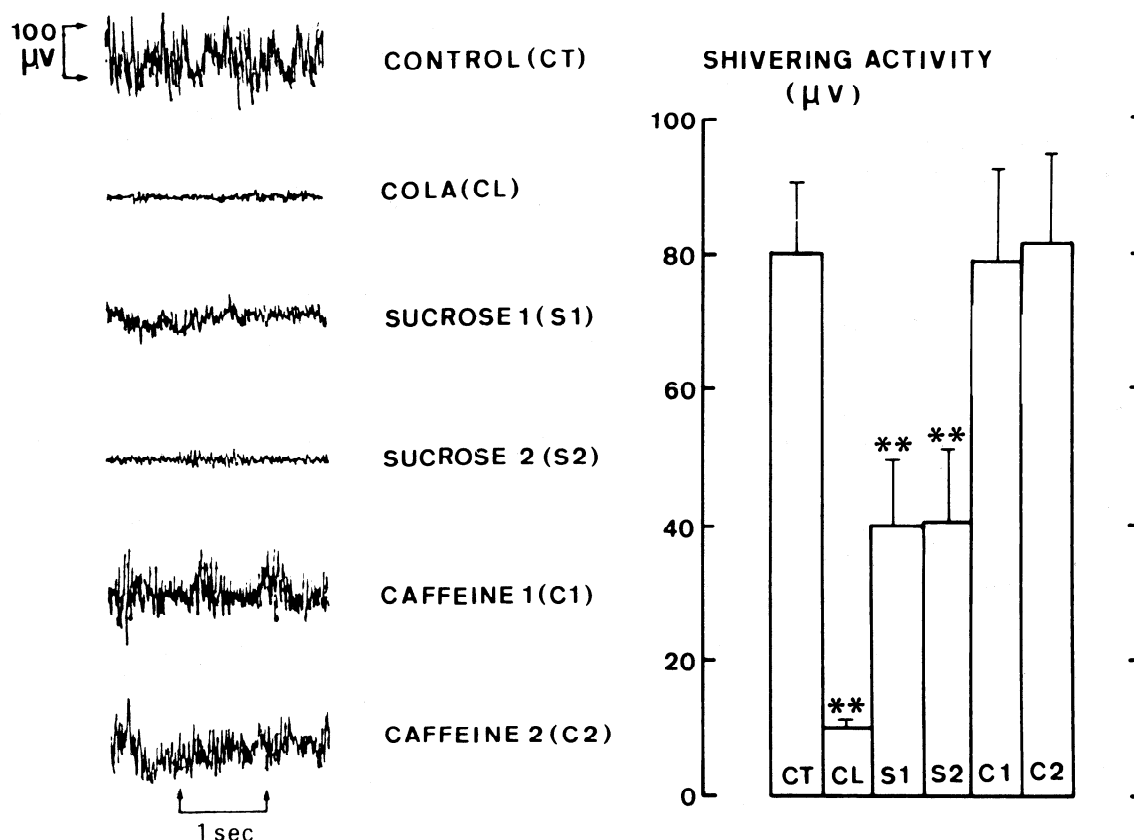


FIG. 5. Effects of sucrose, caffeine, and Coca-Cola consumption on shivering activity of rats exposed to cold. Animals and experimental conditions were same as in Fig. 4. Shivering was recorded as described under EXPERIMENTAL PROCEDURES. Left part shows examples of elec-

tromyographs directly photographed from recording sheets. The mean shivering activity of each rat shown to the right, was evaluated from amplitude of 300 consecutive spikes. Bars represent means  $\pm$  SE of measurements performed on 8-10 animals.

TABLE 3. Effects of sucrose, caffeine, and Coca-Cola consumption on parametrial white adipose tissue composition

	Con- trols	Coca- Cola	Sucrose (12%)	Sucrose (32%)	Caffeine (0.057%)	Caffeine (0.20%)
PWAT wt, g	6.6 $\pm 0.3$	8.7 $\dagger$ $\pm 0.6$ (131%)	8.8 $\dagger$ $\pm 0.6$ (133%)	8.9* $\pm 0.5$ (135%)	4.5* $\pm 0.4$ (68%)	3.4* $\pm 0.3$ (52%)
Total number of adipocytes $\times$ $10^6$	20.4 $\pm 0.9$	24.9 $\pm 1.6$ (122%)	24.3 $\pm 2.1$ (119%)	25.5 $\pm 1.6$ (125%)	14.0* $\pm 1.3$ (69%)	12.7* $\pm 0.9$ (62%)
Total triglyceride content, g	3.2 $\pm 0.3$	4.0 $\pm 0.3$ (125%)	4.3 $\dagger$ $\pm 0.4$ (134%)	4.2 $\dagger$ $\pm 0.3$ (131%)	1.7* $\pm 0.2$ (53%)	1.4* $\pm 0.1$ (44%)
Triglyceride content/ adipocyte, ng	158.2 $\pm 9.3$	160.8 $\pm 4.8$ (102%)	179.3 $\pm 12.1$ (113%)	166.2 $\pm 8.1$ (105%)	118.6* $\pm 5.4$ (75%)	108.8* $\pm 4.5$ (69%)

Values are means  $\pm$  SE ( $n = 10-12$ ). Values in parentheses are % of control values. The statistical significance of the data was evaluated by variance analysis and completed by Duncan's multiple range test (11, 27). \* and  $\dagger$ , Differences between the control and experimental groups that were statistically significant at the levels  $P < 0.01$  and  $P < 0.05$ , respectively.

adipocyte triglyceride content (or size) associated with a decrease in the total number of adipocytes. It therefore appears that caffeine consumption exerts a dual action on PWAT by reducing adipocyte size and by concurrently inhibiting adipocyte proliferation that normally occurs in this particular fat depot during growth of young rats (8).

## DISCUSSION

A few years ago, it was generally believed that adipose tissue hyperplasia in rats was complete between 9 and 14 wk of life and that further increase in adipose tissue mass was principally achieved through cellular hypertrophy (15). However, most of the early studies were done on the epididymial fat pads that, as far as hyperplasia is concerned, are not representative of other fat depots. In 1972, Lemmonier et al. (21) clearly showed that, even in adult animals, fat cell number is not fixed in every major adipose depot, and that fat cell hyperplasia represents an important way of tissue growth in perirenal, parametrial, or subcutaneous fat depots. Other studies have now demonstrated that adipocyte size and number can be modified in several adipose depots of adult rats, in particular by diet, food restriction, and exercise training (8, 18). In contrast, few laboratories have investigated the regulation of brown adipose tissue cellularity. It is known, however, that this thermogenic tissue is fully differentiated at birth, and that it is progressively replaced by white adipose tissue during rat growth at room temperature. The regression of brown adipose tissue is accompanied by a decrease in the calorogenic response of rats to catecholamines (26). Nevertheless, brown adipose tissue can regenerate in adult rats if they are exposed to cold for several days (4, 12, 26) or if they are fed palatable diets such as "cafeteria" food (28). Rothwell and Stock (29) have pointed out several similarities between diet- and cold-induced thermogenesis. In particular, they have

shown that hyperphagia and cold exposure both enhance the calorogenic response of rats to catecholamines. More recently, using radioautographic techniques, we demonstrated that acclimation to cold as well as adaptation to hyperphagia are characterized by a remarkable proliferation of brown adipocytes from precursor cells (interstitial cells and brown preadipocytes) (4). In fact, everything happens as if brown adipose tissue possesses the ability to grow and regress in accordance with the need for thermogenesis.

The data reported herein demonstrate that ad libitum feeding of sucrose or Coca-Cola along with Purina chow increases the cellularity of brown adipose tissue and improves the resistance of rats to cold while decreasing shivering activity in skeletal muscles (Figs. 4 and 5). Thus, it is possible to induce brown adipose tissue growth, not only by exposing rats to cold but, more simply, by allowing them to drink sucrose beverages. It is very likely that brown adipose tissue hyperplasia occurred in consequence of increased sympathetic activity, since it has been demonstrated that sucrose consumption is very effective in activating the sympathetic nervous system in rats (20). In fact, one of the reasons for investigating the effects of caffeine was based on the reports that caffeine mimics the activity of the sympathetic nervous system either by increasing the release of norepinephrine from sympathetic nerve endings or by inhibiting intracellular phosphodiesterases (1, 3, 28). As expected, caffeine consumption increased IBAT cellularity, but, contrary to sucrose and Coca-Cola, decreased body weight gain efficiency without increasing food intake (Table 1). Although there is some controversy over the effects of caffeine on food intake, one of the best-documented effects of caffeine consists in the stimulation of catecholamine secretion associated with a rise in the metabolic rate (1, 17). Under the present experimental conditions, caffeine acted only transiently as an anorectic agent when given at high concentrations (Fig. 3) and most probably reduced body weight gain efficiency via its thermogenic effects (Table 1). The slimming action of caffeine (Fig. 1) was characterized by a remarkable decrease of PWAT weight, adipocyte size, and cellularity (Table 3). In this respect, caffeine mimicked the effects of exercise training, food restriction (5), and cold acclimation (Bukowiecki et al., unpublished research), three other physiological conditions in which energy expenditure exceeds energy gain. It is important to point out that caffeine consumption, exercise training, food restriction, and cold acclimation all decreased PWAT cellularity by inhibiting the normal proliferation of adipocytes during rat growth. When the present experiments were started, young female Wistar rats weighed 190–210 g and contained  $8\text{--}10 \times 10^6$  of adipocytes in their parametrial fat pads (5). Nine weeks later, when the animals weighed approximately 300 g, PWAT cellularity was increased to  $20 \times 10^6$  in the controls, whereas it only reached  $14 \times 10^6$  in the animals consuming caffeine (Table 3). Whether caffeine consumption or long-term stress also enhanced the turnover of mature brown adipocytes is now known

and deserves further studies.

In contrast to caffeine, sucrose and Coca-Cola consumption slightly increased PWAT weight, adipocyte size, and cellularity but did not significantly affect total animal body weights (Table 1). This was particularly striking in rats drinking Coca-Cola that consumed nearly 50% more calories than controls without gaining weight (Table 1). The most likely explanation for the important reduction in body weight gain efficiency in the Coca-Cola group is an enhanced capacity of brown adipose tissue for energy expenditure by diet-induced thermogenesis. Indeed, the data in Tables 1–3 suggest that sucrose and caffeine act synergistically in increasing brown adipose tissue cellularity, and that this results in a decreased body weight gain. Caffeine or other methylxanthines might therefore represent a potential family of drugs that, if proven safe, might be useful for stimulating brown adipocytes proliferation while simultaneously preventing the synthesis of white adipocytes and thereby reducing the capacity for storing food energy.

Contrary to sucrose, that is a nutrient, caffeine markedly reduced tissue cellularity, adipocyte size, and triglyceride stores in PWAT and presumably also in other fat depots (Table 3). This might explain the failure of the methylxanthine to reproduce the effects of sucrose on cold resistance (Fig. 2) and shivering (Fig. 3).

Although it is well established that caffeine ingestion can transiently elevate oxygen consumption in humans (1, 17), its effects on adipocyte proliferation still remain to be evaluated. If caffeine acts in a similar fashion in humans as it does in rats, it should be more effective in preventing obesity during youth and adolescence, when adipocyte hyperplasia normally occurs, but might be also valuable in fighting maturity-onset obesity, especially that of the hyperplastic-hypertrophic type. In this context, a recent clinical study revealed that caffeine when combined with ephedrine, another thermogenic drug (7), significantly reduced body weights of obese patients as compared with obese controls on a placebo treatment, both groups receiving 1,200 kcal/day (24). It would therefore appear that in humans, as in rats, obesity can be reduced by thermogenic drugs mimicking the dissipative effects of diet-induced thermogenesis.

The possibility that particular combinations of drugs or nutrients, such as caffeine and ephedrine [as in "Elsinore Pills" (24)] or caffeine and sucrose (as in cola beverages), might act synergistically on the sympathetic nervous system and consequently affect thermogenesis and obesity, will certainly represent an exciting avenue for further research.

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