Pharmacology Biochemistry & Behavior, Vol. 29, pp. 33–37. © Pergamon Journals Ltd., 1988. Printed in the U.S.A. 0091-3057/87 $3.00 + .00

Nicotine Increases Thermogenesis in Brown Adipose Tissue in Rats[1]

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Received 5 September 1986

LUPIEN, J. R. AND G. A. BRAY. *Nicotine increases thermogenesis in brown adipose tissue in rats.* PHARMACOL BIOCHEM BEHAV **29**(1) 33–37, 1988.—This study has tested the hypothesis that nicotine might increase thermogenesis in rats by activating the sympathetic nervous system which supplies brown adipose tissue. Three hours after a single injection of nicotine, both the turnover of norepinephrine and the binding of the purine nucleotide, guanosine 5'-diphosphate (GDP) to mitochondria from brown adipose tissue were significantly increased. After 11 days of treatment with nicotine, the turnover of norepinephrine and the GDP binding to mitochondria from brown adipose tissue both remained elevated but weight gain was not different. These data are consistent with the hypothesis that nicotine may have part of its effect through changes in thermogenesis involving sympathetic nervous activation of peripheral thermogenic tissues such as brown adipose tissue.

GDP-Binding Sympathetic activity Norepineprhine turnover

THERE is substantial evidence that smokers, as a group, weigh less than non-smokers [2, 10–12, 21, 24]. When smokers give up the habit, their weight rises to a new plateau which is similar to that of people who never smoked [2–4, 6, 10, 11, 21, 24–26]. Both increased food intake [13–15] and decreased energy expenditure have been proposed as mechanisms for this effect [9,15]. Smokers have a higher resting metabolic rate than non-smokers, and smoking itself acutely increases energy expenditure [13,15]. Conversely, cessation of smoking appears to decrease the metabolic rate [9].

In experimental animals, treatment with nicotine produces a decrease in body weight or a slowed rate of weight gain. A reduction in food intake has been reported in some studies [12,18] but not others [12,17]. The consistent reduction in body weight without a concomitant reduction of food intake is reminiscent of the effects of thermogenic drugs which increase energy expenditure and slow weight gain without significantly reducing food intake [1, 19, 31]. One mechanism for this effect is thought to involve an activation of the sympathetic nervous system and an increase in heat production through activation of thermogenic mechanisms in brown adipose tissue [14, 20, 22]. Since nicotine can stimulate the sympathetic nervous system through its action on sympathetic ganglia it might act in this fashion to enhance thermogenesis [16]. To test this hypothesis we have performed acute and chronic experiments with nicotine and measured the turnover of norepinephrine brown adipose tissue and the binding of the purine nucleotide, guanosine 5'-diphosphate (GDP) to mitochondria from this tissue.

METHOD

Animals

The 61 rats used in these studies were purchased from Charles River Breeding Laboratories (Wilmington, MA). Animals were housed in a vivarium maintained at 22±1°C with lights on from 0600 to 1800 hr. Tap water was available ad lib. Food as Wayne Lablox (Chicago, IL) was available ad lib (experiments 2 and 3), or in a restricted schedule of 4 hours per day (experiment 1). All animals had access to food until the time of sacrifice.

Experimental Procedures

Two acute experiments and one chronic experiment were performed. In one acute experiment, rats were schedule fed by giving them access to food from 0900 to 1300 for 5 days. On the experimental day nicotine, 0.4 mg/kg, was given subcutaneously (SC) at the initiation of the feeding period at 0900. Animals had access to food until they were killed 4 hours later. For the acute measurements of norepinephrine turnover, 12 rats received nicotine 0.4 mg/kg SC and 12 received vehicle (neutralized 0.15 M NaCl). Subgroups were killed at 0, 3 and 6 hours after receiving alpha-methyl-p-tyrosine 80 mg/kg IP. All rats were fed ad lib. This dose of nicotine was based on previous literature [5]. In the chronic experiment 24 rats had ad lib access to food and water. Half of this group received nicotine 0.4 mg/kg SC and the other half vehicle. Twenty-four hours after the last dose of nicotine, animals were sacrificed for measurement of norepi-

[1]Supported in part by grants RO1 DK 32018 from the National Institutes of Health and the Quebec Research Council.
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TABLE 1
EFFECT OF NICOTINE ON BROWN ADIPOSE TISSUE*

	Saline	Nicotine (0.4 mg/kg)
Body weight (g)	249.7 ± 9.3 (n=6)	245.5 ± 3.5 (n=7)
Intrascapular brown adipose tissue mass (mg)	148.8 ± 8.6	169.4 ± 9.4
Total protein content (mg)	21.84 ± 1.19	23.35 ± 0.88
Mitochondrial protein (mg)	2.74 ± 0.18	2.71 ± 0.28
Food intake (g of lab chow eaten after 3 hr feeding period)	10.25 ± 2.05	10.80 ± 1.12
Specific GDP binding (pmol/mg protein)	93.91 ± 7.49	147.61 ± 10.86‡
Total GDP binding	258.22 ± 25.79	389.81 ± 35.56†

*Rats were trained to eat meals between 0900–1300 during 5 days prior to the experiment. The day of the experiment, they were injected at 0900 and sacrificed at 1200, and fed ad lib. Results are mean values ± SEM.
†$p<0.05$, ‡$p<0.01$.

nephrine turnover and determination of guanosine 5'-diphosphate (GDP) binding to mitochondria from brown adipose tissue.

Measurement of Guanosine 5'-Diphosphate (GDP) Binding to Mitochondria From Brown Adipose Tissue

At the termination of each experiment, the interscapular brown adipose tissue (IBAT) was removed and carefully cleaned of all fat. The tissue for GDP binding was homogenized at 0°C in a medium containing 0.25 M sucrose, 0.2 mM EDTA (potassium salt) and 1 mM HEPES buffer, pH 7.2, using a glass motor-driven homogenizer. The binding of GDP to mitochondria from interscapular brown adipose tissue was performed as previously described [17]. The mitochondria were isolated by centrifugation, and the binding of GDP to mitochondria from brown adipose tissue was performed by incubating isolated mitochondria (0.5 mg of protein/ml) for 10 minutes at room temperature (20°C) in a medium containing 10 μM ^{3}H-GDP (1.25 μCi/ml), ^{14}C-sucrose (0.1 μCi/ml), 100 mM sucrose, 20 mM K-TES (pH 7.1), 1 mM K-EDTA, 10 mM choline chloride and 5 μM rotenone. The mitochondria with the radioactive GDP bound to them were isolated by collecting the mitochondria on millipore filters and counting the filters.

Measurement of Norepinephrine

Measurement of norepinephrine in homogenates of brown adipose tissue, heart and spleen was performed as previously described [32], with modifications described below. Beginning 4 hours after the injection of nicotine in experiment 2 and 24 hours after injecting nicotine in the chronic experiment, 12 control and 12 experimental animals received an intraperitoneal injection of the methyl ester of alpha-methyl-p-tyrosine at a dose of 80 mg/kg IP. This drug blocks

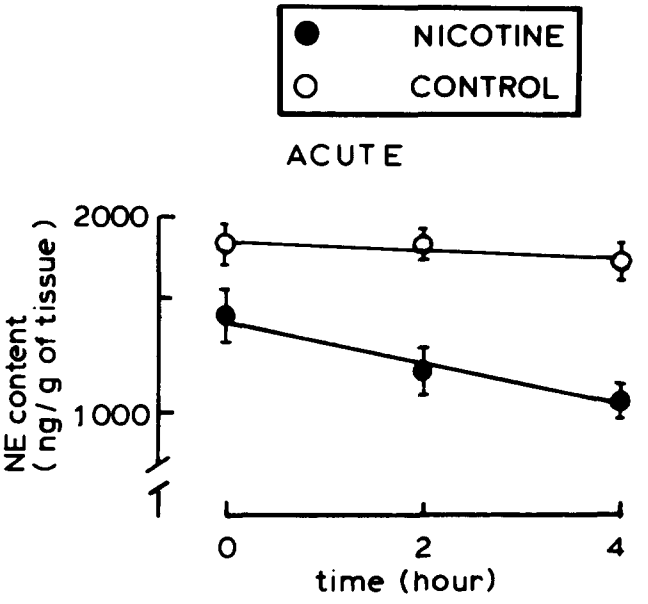

FIG. 1. Effects of acute treatment with nicotine on the turnover of norepinephrine in interscapular brown adipose tissue. Each animal received the methyl ester of alpha-methyl-para-tyrosine 80 mg/kg IP at 4 hr. At the times indicated, the interscapular brown adipose tissue from 4 rats in each group was dissected free and divided in half with the half for norepinephrine being rapidly frozen until assay, usually within 2 weeks. The fractional turnover (k) of norepinephrine in IBAT of the control rats was 1.4±1.6%/hr and the turnover rate was 25.6 ng/g/hr. In the nicotine treated animals the fractional turnover (k) was 7.3±2.7%/hr and the turnover rate was 100 ng/g/hr. Data are expressed as the mean±SEM with N=4 animals per time point.

tyrosine hydroxylase and prevents reaccumulation of norepinephrine which is released in response to neural or drug stimuli. Subgroups of 4 animals from each treatment group were killed at 0, 2 and 4 hr after injecting alpha-methyl-p-tyrosine. The interscapular brown adipose tissue was dissected as described above, and frozen on dry ice and stored at −70°C for later determination of norepinephrine. Usually within 1 week, the frozen tissues were weighed, homogenized in ice-cold 0.1 N perchloric acid and reduced glutathione in a Brinkman polytron (Brinkman Instruments Inc., Westbury, NY) and centrifuged at 0°C. Aliquots of the supernatant were neutralized with 0.1 N KOH and the supernatant obtained by centrifugation applied to an HPLC and the eluates detected in an electrochemical detector.

Reagents

Norepinephrine standards and the methyl ester of alpha-methyl-p-tyrosine were purchased from Sigma Chemicals (St. Louis, MO). Nicotine was obtained from the same supplier, dissolved in saline and neutralized to pH 7.2±0.2 with NaOH. Chemicals for the HPLC were obtained from Rainin (Emeryville, CA).

Data Analysis

All data are expressed as the mean±SEM. Statistical analysis for drug effects was performed by analysis of variance. For the acute experiments a one-way ANOVA was used, but for food intake and body weight a two-way

TABLE 2

EFFECT OF NICOTINE ON THE BINDING OF GDP TO BROWN ADIPOSE TISSUE*

	Control	Nicotine
Body weight (g)	242.4 ± 4.6	245.5 ± 3.9
Intrascapular brown (mg) adipose tissue mass	133.8 ± 8.2	152.6 ± 9.9
Total protein content (mg)	21.58 ± 1.79	23.07 ± 1.72
Mitochondrial protein (mg)	2.75 ± 0.10	2.91 ± 0.11
Specific GDP binding (pmole/mg of mitochondrial protein)	105.60 ± 4.50	133.46 ± 5.78‡
Total GDP binding (pmole/depot)	291.29 ± 18.73	386.62 ± 23.66†
n	12	12

*Rats fed ad lib received an injection of nicotine 0.4 mg/SC measurement after 3 hours.
Results are mean values ± SEM.
†$p<0.05$, ‡$p<0.01$.

ANOVA with one repeat measure was used. Post-hoc comparisons were by the Newman-Keuls multiple range test. The norepinephrine concentrations were plotted semilogarithmically and the slope of decline was calculated by the method of least squares.

RESULTS

Experiment 1

In the first acute experiment, animals had been trained to eat their food between 0900 and 1300 during the 5 days prior to the injection of nicotine. On the experimental day nicotine was injected at 0900 and the animals sacrificed 3 hours later with their usual access to food (Table 1). Food intake over 3 hours was the same in both groups, but there was a significant increase in both the specific GDP binding (pmol/mg protein $p<0.01$) and in the total GDP binding ($p<0.05$) obtained by multiplying the specific binding by the mitochondrial protein.

Experiment 2

In the second acute experiment, animals had ad lib access to food. Nicotine was injected at 0900, and three hours later the alpha-methyl-p-tyrosine was injected and animals sacrificed at 6, 8 and 10 hr after giving nicotine for measurement of norepinephrine turnover. The turnover of norepinephrine in brown adipose tissue following a single injection of nicotine is shown in Fig. 1 (data on heart and spleen also available but not shown). The concentration of norepinephrine in the interscapular brown adipose tissue at the beginning of the turnover study was significantly lower in the animals acutely treated with nicotine. The rate of release of norepinephrine was also significantly faster in the tissue from nicotine-treated rats. The turnover of norepinephrine, which is the product of the initial tissue concentration of norepinephrine times the rate of turnover, was thus markedly increased in the nicotine-treated rats. Indeed, in the 3 hr between the injection of nicotine and the first measurements more than 20% of the norepinephrine had been released from IBAT. As shown in Table 2, pretreatment of rats for 3 hours with nicotine significantly increased the specific GDP binding, as well as the total GDP binding to mitochondria. Tissue weight and mitochondrial protein, on the other hand, were unaffected. Addition of nicotine in vitro to mitochondria from control rats had no effect on the binding of GDP to the mitochondria.

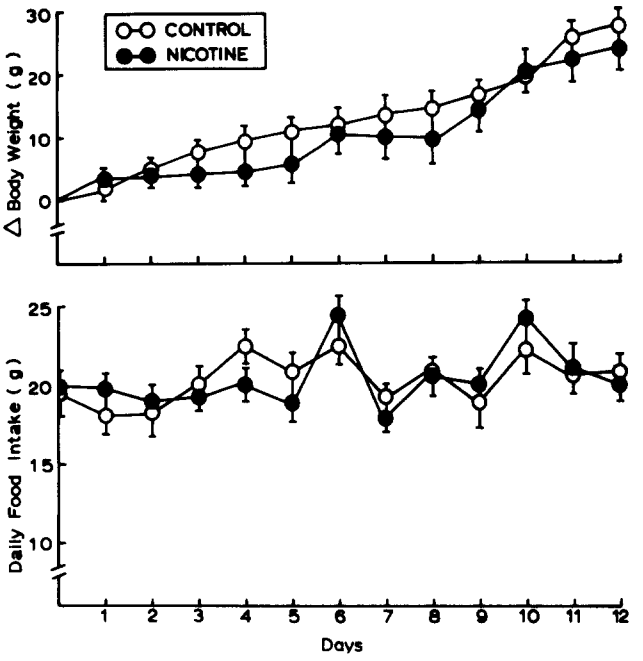

FIG. 2. Body weight and food intake of nicotine-treated rats. The data are the mean±SEM for 13 rats in each group. There were no differences between groups.

Experiment 3

In the chronic experiment, rats were treated with nicotine for 11 days (Figs. 2 and 3). Neither body weight nor food intake were significantly different between control and nicotine-treated rats (Fig. 2). Mean weight gain in the treated animals was not significantly different from that in the vehicle-treated controls [23.9±2.8 (N=13) vs. 27.3±2.5 (NE=13)]. Food intake was also not different in the nicotine-treated rats. As shown in Fig. 3, chronic treatment with nicotine significantly increased the turnover of norepinephrine in the brown adipose tissue ($p<0.05$). In contrast to the acute study, however, the initial values of norepinephrine in IBAT were the same. Thus, after chronic treatment, the enhanced turnover reflects the higher fractional turnover rats (k). The binding of GDP to mitochondria from interscapular brown adipose tissue at the end of the 11 days of chronic treatment with nicotine is shown in Table 3. The weight of IBAT and its protein content were not different. Specific GDP-binding, however, was significantly increased after chronic treatment with nicotine.

DISCUSSION

The present studies have made two original observations. First, both acute and chronic treatment with nicotine significantly increased the turnover of norepinephrine. Second, the

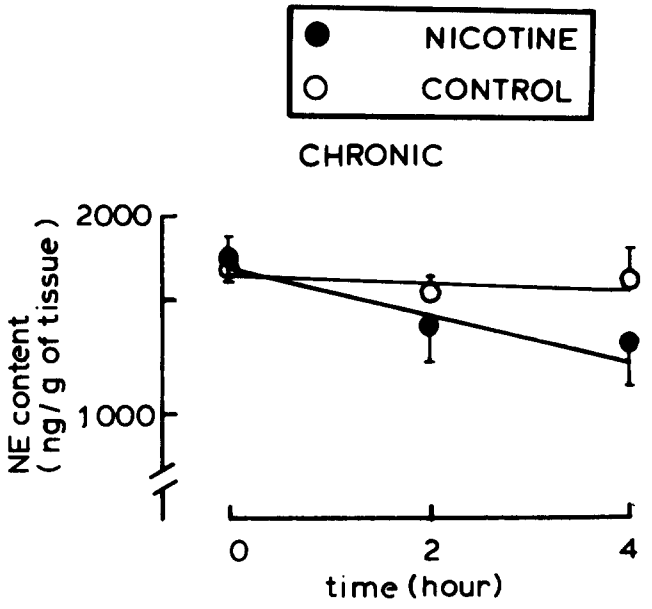

FIG. 3. Effects of chronic treatment with nicotine on the turnover of norepinephrine. Animals received daily injections of vehicle or nicotine (0.4 mg/kg SC). Twenty-four hours after the last injection the turnover of norepinephrine was measured. The fractional turnover (k) of norepinephrine in IBAT of vehicle-treated rats was 25.9%/hr and the turnover rate was 25.9 ng/g/hr. In the nicotine treated rats the fractional turnover (k) was 8.6±3.5%/hr and the fractional rate was 145 ng/g/hr. The data are expressed as mean±SEM for N=4 animals per time point.

TABLE 3

EFFECT OF CHRONIC TREATMENT WITH NICOTINE ON THE BINDING OF GDP TO BROWN ADIPOSE TISSUE*

	Saline	Nicotine
Body weight (g)	260.3 ± 3.3†	257.9 ± 2.6
Interscapular brown (mg) adipose tissue	198.8 ± 15.3	202.0 ± 11.5
Total protein content (mg)	29.74 ± 1.10	27.25 ± 1.35
Mitochondrial protein (mg)	3.79 ± 0.23	2.65 ± 0.18
Specific GOP binding (pmole/mg of mitochondrial protein)	93.37 ± 6.01	118.96 ± 6.66‡
Total BDP binding (pmole/depot)	352.47 ± 27.22	436.26 ± 41.88

*Animals received daily doses of 0.4 mg/kg of nicotine for 11 days. The last dose of nicotine was 24 hr before sacrifice.
†Mean ± SEM.
‡$p<0.05$ (Unpaired Student's t-test).

the binding of the purine nucleotide guanosine 5'-diphosphate (GDP) to mitochondria from this tissue increased, whereas food intake was unchanged.

Treatment with nicotine has been reported to reduce weight gain in rats [12,18], but food intake showed little change. This implies that nicotine is activating a thermogenic process. The increased thermogenic activity of brown adipose tissue after treatment with nicotine may be one explanation for the reduced body fat and body weight of tobacco smokers [2, 10, 11, 21, 24]. Westfall and Watt [37] reported that the inhalation of cigarette smoke causes a release of epinephrine from the adrenal medulla, resulting in a significant increase of this amine in the peripheral blood of dogs. Later the same authors [38] showed that both epinephrine and total catecholamine excretion increased significantly above control levels during periods of heavy smoking in man. The possibility that nicotine could enhance the activity of the sympathetic nervous system is supported by the increased turnover of norepinephrine in the present experiment (Figs. 1 and 3) and by the rise in norepinephrine and epinephrine concentrations in the blood following smoking [7]. Urinary norepinephrine excretion also increased along with metabolic expenditure during the 24 hour period of smoking as opposed to a 24 hour period of abstinence [15]. In experimental animals, nicotine has been reported to increase the weight of brown adipose tissue [27]. When nicotine was combined with caffeine, Wellman *et al.* [28] reported an increase in temperature of brown adipose tissue. In our studies nicotine increased GDP binding to mitochondria from brown adipose tissue in acutely treated animals whether fed or fasted. GDP binding was also increased in chronically treated animals.

Drugs which increase the activity of the sympathetic nervous system have been shown to lower body weight and body fat in experimental animals without lowering food intake [7, 19, 31]. This suggests that increasing the activity of the sympathetic nervous system may increase energy expenditure which either concomitantly suppresses food intake or fails to give rise to signals which would increase food intake. Thus, nicotine may both increase energy expenditure and inhibit a rise in food intake through its effects on the activity of the sympathetic nervous system.

REFERENCES

1. Arch, J. R. S., A. T. Ainsworth, R. D. M. Ellis, V. Piercy, V. E. Thody, P. L. Thurlby, C. Wilson, S. Wilson and P. Young. Treatment of obesity with thermogenic beta-adrenocepter agonists: Studies on BRL 26830A rodents. *Int J Obesity* **8:** Suppl 1, 1–11, 1984.
2. Blitzer, P. H., A. A. Rimm and E. E. Giefer. The effect of cessation of smoking on body weight in 57,032 women: Cross-sectional and longitudinal analyses. *J Chron Dis* **30:** 415–429, 1977.
3. Brozek, J. and A. Keys. Changes in body weight in normal men who stop smoking cigarettes. *Science* **125:** 1203, 1957.
4. Carney, R. M. and A. P. Goldberg. Weight gain after cessation of cigarette smoking. *N Engl J Med* **310:** 614–616, 1984.
5. Clarke, B. S. and R. Kumar. Characterization of the locomotor stimulant action of nicotine in tolerant rats. *Br J Pharmacol* **80:** 587–594, 1983.
6. Comstock, G. W. and R. W. Stone. Changes in body weight and subcutaneous fatness related to smoking habits. *Arch Environ Health* **24:** 271–276, 1972.
7. Cryer, P. E., M. W. Haymond, J. V. Santiago and S. D. Shah. Norepinephrine and epinephrine release and adrenergic mediation of smoking-associated hemodynamic and metabolic events. *N Engl J Med* **295:** 573–577, 1976.

8. Dallasso, H. M. and W. P. T. James. The role of smoking in the regulation of energy balance. *Int J Obesity* **8:** 365–375, 1984.
9. Glauser, S. C., E. M. Glauser, M. M. Reidenberg, B. F. Rusy and R. J. Tallarida. Metabolic changes associated with the cessation of cigarette smoking. *Arch Environ Health* **20:** 377–381, 1970.
10. Goldbourt, U. and J. H. Medalie. Characteristics of smokers, non-smokers and ex-smokers among 10,000 adult males in Israel. *Am J Epidemiol* **105:** 75–86, 1977.
11. Gordon, T., W. B. Kannel, T. R. Dawber and D. McGee. Changes associated with quitting cigarette smoking. The Framingham Study. *Am Heart J* **90:** 322–328, 1975.
12. Grunberg, N. E., D. J. Brown and D. E. Morse. Effects of nicotine on body weight and food consumption in rats. *Psychopharmacology (Berlin)* **83:** 93–98, 1984.
13. Hiestand, W. A., H. J. Ramsey and D. M. Hale. The effects of cigarette smoking on metabolic rate, heart rate, oxygen pulse, and breathing rate. *J Lab Clin Med* **25:** 1013–1017, 1940.
14. Himms-Hagen, J. Brown adipose tissue thermogenesis in obese animals. *Nutr Rev* **41:** 261–267, 1983.
15. Hofstetter, A., Y. Schutz, E. Jequier and J. Wahren. Increased 24-hour energy expenditure in cigarette smokers. *N Engl J Med* **314:** 79–82, 1986.
16. Lichtensteiger, W., M. Schlumpf and M. D. Davis. Catecholamines and nicotine in early neuroendocrine organization. *Monogr Neural Sci* **9:** 213–224, 1983.
17. Lupien, J. R. and G. A. Bray. Effect of fenfluramine on GDP-binding to brown adipose tissue mitochondria. *Pharmacol Biochem Behav* **23:** 509–513, 1985.
18. McNair, E. and R. Bryson. Effects of nicotine on weight change and food consumption. *Pharmacol Biochem Behav* **18:** 341–344, 1983.
19. Meier, M. K., L. Alig, M. E. Burgi-Saville and M. Muller. Phenethanolamine derivatives with calorigenic and antidiabetic qualities. *Int J Obesity* **8:** Suppl 1, 215–225, 1984.
20. Nicholls, D. G. and R. M. Locke. Thermogenic mechanisms in brown fat. *Physiol Rev* **64:** 1–40, 1984.
21. Noppa, H. and C. Bengtsson. Obesity in relation to smoking: A population study of women in Goteborg, Sweden. *Prevent Med* **9:** 534–543, 1980.
22. Rothwell, N. J. and M. J. Stock. Dietary obesity. *Clin Endocrinol Metab* **13:** 437–448, 1984.
23. Schechter, M. D. and P. G. Cook. Nicotine-induced weight loss in rats without an effect on appetite. *Eur J Pharmacol* **38:** 63–69, 1976.
24. Stamford, B. A., S. Matter, R. D. Fell, S. Sady, M. K. Cresanta and P. Papanek. Cigarette smoking, physical activity, and alcohol consumption: Relationship to blood lipids and lipoproteins in premenopausal females. *Metabolism* **33:** 585–590, 1984.
25. Stamford, B. A., S. Matter, R. D. Fell, S. Sady, P. Papanek and M. Cresanta. Cigarette smoking, exercise and high density lipoprotein cholesterol. *Atherosclerosis* **52:** 73–83, 1984.
26. Stubbe, I., J. Eskilsson and P. Nilsson-Ehle. High-density lipoprotein concentrations increase after stopping smoking. *Br Med J* **284:** 1511–1513, 1984.
27. Wager-Srdar, S. A., A. S. Lenne, J. E. Morley, J. R. Huidal and D. E. Niewoehner. Effects of cigarette smoke and nicotine on feeding and energy. *Physiol Behav* **32:** 289–295, 1984.
28. Wellman, P. J., M. M. Marmon, S. Reich and J. Ruddle. Effects of nicotine on body weight, food intake and brown adipose tissue thermogenesis. *Pharmacol Biochem Behav* **24:** 1605–1609, 1986.
29. Westfall, T. C. and D. T. Watts. Effect of cigarette smoke on epinephrine secretion in the dog. *Proc Soc Exp Biol Med* **112:** 843–847, 1963.
30. Westfall, T. C. and D. T. Watts. Catecholamine excretion in smokers and nonsmokers. *J Appl Physiol* **19:** 40–42, 1964.
31. Yen, T. T., M. M. McKee and N. B. Stamm. Thermogenesis and weight control. *Int J Obesity* **8:** Suppl 1, 65–78, 1984.
32. Yoshida, T., J. W. Kemnitz and G. A. Bray. Lateral hypothalamic lesions and norepinephrine turnover in rats. *J Clin Invest* **72:** 919–927, 1983.