Note

**Caffeine Activates Brown Adipose Tissue Thermogenesis
and Metabolic Rate in Mice**

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Summary To clarify the effect of caffeine on brown adipose tissue (BAT) thermogenesis, we measured guanosine-5'-diphosphate (GDP) binding, a thermogenic indicator of BAT, and oxygen consumption in BAT mitochondria as well as BAT temperature and resting metabolic rate (RMR) in mice. Intraperitoneal injection of caffeine (60 mg/kg) significantly elevated BAT temperature with less effect on core temperature, and increased significantly GDP binding and oxygen consumption in BAT mitochondria, and RMR. These results suggest that caffeine activates BAT thermogenesis, which may contribute to the increase of RMR.

Key Words caffeine, brown adipose tissue (BAT), GDP binding, oxygen consumption, resting metabolic rate (RMR), BAT temperature

Caffeine is a naturally consumed substance that is widely contained in beverages such as coffee, tea, cocoa, and some cola drinks. It has been well known that caffeine increases metabolic rate in man (1, 2). A possible explanation for the thermic effect of caffeine is that it promotes the release of catecholamines (3, 4).

Brown adipose tissue (BAT) is a heat-producing tissue, recognized as diet-induced thermogenesis (5) and non-shivering thermogenesis (6). BAT thermogenesis is controlled primarily by the sympathetic nervous system (SNS) (7). Bukowiecki *et al.* (8) found that caffeine-treated animals showed increased BAT cellularity and protein content in BAT. Wellman and Marmon (9) also found that intraperitoneal injection of caffeine elevated BAT temperature. To date, however, direct biochemical quantification of BAT function after caffeine injection has not been reported. Therefore, we examined the effect of caffeine on BAT thermogenesis by measuring guanosine-5'-diphosphate (GDP) binding (a thermogenic indicator of BAT) and oxygen consumption in BAT mitochondria as well as measuring BAT temperature and resting metabolic rate (RMR) in mice.

BAT temperature. Under anesthesia by intraperitoneal injection of urethane

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(500 mg/kg), mice were placed in a box kept at $33 \pm 3^\circ\text{C}$. Small thermistors (IT-21, Sensortex, N. J., USA) were inserted under the interscapular BAT pad and 3 cm into the rectum of 15 female CRJ-ICR mice (aged 12 weeks, weighing about 30 g, Charles River Japan, Tokyo) as described elsewhere (10). After a stable baseline had been achieved (about 30 min), caffeine (1,3,7-trimethylxanthine) (Wako Chemical Industries, Osaka) in saline solution, was injected intraperitoneally (i.p.) at a dose of 40, 60, or 80 mg/kg. Saline only was injected as a control. BAT (T_{BAT}) and core (T_{core}) temperature were monitored for 60 min after the administration.

RMR and BAT thermogenesis. RMR was estimated using a closed-circuit metabolic system (Enviro-nics, USA). After a stable baseline had been achieved (about 30 min), caffeine was injected (i.p.) at a dose of 40 or 60 mg/kg, or saline injected as control. RMR was measured for 60 min after the injection.

BAT thermogenesis was assessed by measuring GDP binding and oxygen consumption in BAT mitochondria. Thirty minutes after the injection of caffeine (40 and 60 mg/kg) or saline, mice were killed by cervical dislocation at 0900 h. Interscapular BAT (IBAT) was rapidly removed and dissected from connective tissue. IBAT samples were weighed and homogenized in an ice-cold medium containing 250 mM sucrose and 5 mM TES (pH 7.2). The mitochondria of IBAT were isolated by differential centrifugation and then the mitochondria protein content was measured (11). GDP binding and oxygen consumption in IBAT mitochondria was determined as described elsewhere (12). IBAT samples from two mice were combined for one measurement of GDP binding and oxygen consumption. Statistical analysis was done using Student's *t*-test.

As shown in Fig. 1, the mean change in T_{BAT} relative to T_{core} ($T_{\text{BAT}} - T_{\text{core}}$) after the intraperitoneal injection of 40 mg/kg caffeine was slightly higher than that after the injection of saline (T_{BAT} elevated from $37.2 \pm 0.1^\circ\text{C}$ to $37.6 \pm 0.1^\circ\text{C}$, while T_{core} elevated from $37.2 \pm 0.1^\circ\text{C}$ to $37.4 \pm 0.1^\circ\text{C}$). After the injection of 60 mg/kg caffeine, T_{BAT} elevated from $37.1 \pm 0.1^\circ\text{C}$ to $38.1 \pm 0.2^\circ\text{C}$, but T_{core} elevated only from $37.1 \pm 0.1^\circ\text{C}$ to $37.5 \pm 0.2^\circ\text{C}$. The mean change in $T_{\text{BAT}} - T_{\text{core}}$ after the injection of 60 mg/kg caffeine was, therefore, significantly higher ($p < 0.05$) from 4 to 60 min after the injection. The injection of 80 mg/kg caffeine, however, elevated both T_{BAT} and T_{core} by more than 3.0°C . The optimal dose of intraperitoneal injection of caffeine for activation of BAT function in mice is 60 mg/kg. Wellman and Marmon (9) also showed that the injection of 40 mg/kg caffeine (i.p.) exhibited a significant increase in IBAT temperature at 5 min after the injection in rats. The difference in optimal dose that stimulates BAT thermogenesis between our study and theirs (9) may be due to the difference of species.

No significant differences in IBAT weight and mitochondrial protein content in IBAT were observed among control and caffeine (40 and 60 mg/kg) groups. GDP binding and oxygen consumption in IBAT mitochondria in 60 mg/kg caffeine-injected group were significantly increased compared with those in the control group, but not significantly in 40 mg/kg caffeine-injected group (Table 1). The present results indicate that intraperitoneal injection of caffeine activates BAT

Fig. 1. Effect of intraperitoneal injection of caffeine and saline on changes in BAT temperature relative to core temperature ($T_{BAT}-T_{core}$). ●, caffeine (60 mg/kg) ($n=5$); ○, caffeine (40 mg/kg) ($n=5$); △, saline ($n=5$); * $p<0.05$ (vs. saline); Values are mean $\pm$ SE for 5 mice.

Table 1. Effect of caffeine (40 and 60 mg/kg) on IBAT weight, IBAT mitochondrial protein content, GDP binding, and oxygen consumption in IBAT mitochondria, and RMR.

		Control	Caffeine (40 mg/kg)	Caffeine (60 mg/kg)
IBAT weight (mg)	($n=4$)	104.7 ± 0.8	98.7 ± 1.0	101.1 ± 0.9
Mitochondrial protein content in IBAT (mg)	($n=4$)	1.05 ± 0.14	1.10 ± 0.08	1.10 ± 0.11
GDP binding (pmol/mg · mitochondrial protein)	($n=4$)	143.9 ± 5.9	152.3 ± 7.1	$169.2 \pm 4.2^*$
Oxygen consumption (nmol/min · mg · mitochondrial protein)	($n=4$)	22.6 ± 0.6	24.5 ± 2.0	$31.3 \pm 1.3^{**}$
RMR (ml/min · BW ^{0.75})	($n=6$)	21.0 ± 1.1	23.1 ± 1.0	$30.8 \pm 1.6^*$

Values are mean $\pm$ SE. BW, body weight (kg). Significant difference between control and caffeine: * $p<0.05$; ** $p<0.01$.

thermogenesis. Because BAT thermogenesis is controlled primarily by SNS activity (7), the rapid increase in BAT temperature after 60 mg/kg caffeine injection might have resulted from the activation of SNS activity. Caffeine inhibits the action of cyclic AMP (cAMP) phosphodiesterase (13), thus increasing cellular cAMP, which would lead to prolongation of the effects of sympathetic stimulation. It is known that caffeine promotes the release of norepinephrine and especially epinephrine from adrenal medulla (3, 4), which might have also stimulated BAT thermogenesis. In addition, caffeine may contribute to the activation of BAT thermogenesis, in part, by mobilizing fuel substrates *via* activation of lipolysis in white adipose tissue (14). A detailed study of the mechanism by which BAT function is potentiated by caffeine should be done by measuring norepinephrine (NE) turnover, a reliable indicator of SNS activity (15), or using adrenodemedullated mice.

Various experimental and genetic obese models have been proven to have abnormalities of BAT function (16, 17), which contribute partly to their obesity (18). A number of novel sympathetic stimulants that are specific for BAT were currently used for mitigation of obesity in rodents (19). Caffeine is, however, effective as a thermogenic anti-obese agent only at high doses (20). In practice, caffeine has not been used alone but used in combination with sympathomimetics such as ephedrine or phenylpropanolamine, to mitigate obesity in animal models (9, 21). The thermogenic effect of caffeine may be, therefore, synergistic with sympathomimetic agents.

It is well established that caffeine increases metabolic rate in man (1, 2). In this study, administration of 60 mg/kg caffeine significantly elevated RMR, which was compatible with the results of BAT temperature and BAT thermogenesis. Therefore, the effect of caffeine on RMR reflects an action of this drug on BAT thermogenesis. It is demonstrated in human studies that 250 mg of caffeine caused a 14% increase in RMR (2), and that single-dose oral administration of 100 mg of caffeine (as in a cup of coffee) increased RMR by 3–4% (22). It is also possible in humans that increased RMR by caffeine may be attributed, in part, to activation of BAT thermogenesis, although the energy relevance of BAT in human adults is less than in rodents (23).

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