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Green tea reduces body fat accretion caused by high-fat diet in rats through β-adrenoceptor activation of thermogenesis in brown adipose tissue

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Abstract

The aim of the present study was to investigate body fat-suppressive effects of green tea in rats fed on a high-fat diet and to determine whether the effect is associated with β-adrenoceptor activation of thermogenesis in brown adipose tissue. Feeding a high-fat diet containing water extract of green tea at the concentration of 20g/kg diet prevented the increase in body fat gain caused by high-fat diet without affecting energy intake. Energy expenditure was increased by green tea extract which was associated with an increase in protein content of interscapular brown adipose tissue. The simultaneous administration of the β-adrenoceptor antagonist propranolol(500 mg/kg diet) inhibited the body fat-suppressive effect of green tea extract. Propranolol also prevented the increase in protein content of interscapular brown adipose tissue caused by green tea extract. Digestibility was slightly reduced by green tea extract and this effect was not affected by propranolol. Therefore it appeared that green tea exerts potent body fat-suppressive effects in rats fed on a high-fat diet and the effect was resulted in part from reduction in digestibility and to much greater extent from increase in brown adipose tissue thermogenesis through β-adrenoceptor activation.

Keywords: Green tea; High-fat diet; Body fat; β-adrenoceptor activation; Brown adipose tissue

1. Introduction

Recently considerable interest has arisen concerning the health-promoting potentials of tea [1,2], especially green tea. Green tea is made by steaming or pan-frying to prevent fermentation of the tea leave whereas oolong and black teas undergo fermentation process to some extent [2]. Among a variety of beneficial effects of green tea recently much attention has been focused on body fat-suppressive effects.

It has been shown that green tea and epigallocatechin gallate (EGCG), the major polyphenol of green tea, reduced body weight in experimental animals [3,4] which might be attributed to increased energy expenditure. Green tea increases energy expenditure and fat oxidation [5] through stimulating brown adipose tissue thermogenesis [6]. However, epigallocatechin gallate has been shown to reduce food intake [3]. Therefore, the weight-loss effect of green tea could be due to a reduction in food intake. Systemic investigation looking into body composition and energy balance is needed.

In the present study, effects of green tea on body fat and protein content, food intake, digestibility and energy expenditure in rats fed on a high-fat diet were investigated. In addition, the involvement of β-adrenoceptor activation in the expression of body fat- suppressive effect of green tea was examined.

2. Materials and methods

2.1. Animals and treatment

Male Sprague-Dawley rats weighing between 185 and 190g were housed singly in a temperature-controlled room at 22°C with 12-hr light-dark cycle. They were fed on a semi-synthetic diet for three days prior to the commencement of experiments in order to allow adaptation to the environment and diet. At the beginning of each experiment, animals were divided into three groups according to weight gain during the period of adaptation. At this time, one group

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Table 1
Composition (g/kg) of the experimental diets

Constituent	Normal-fat diet	High-fat diet
Casein	200	200
Maize starch	521	321
Sucrose	100	100
Maize oil	100	100
Lard	-	200
Cellulose	30	30
DL-methionine	2	2
Mineral mix*	35	35
Vitamin mix+	10	10
Choline bitartrate	2	2
Gross energy (kJ/g)	17.8	21.8

* AIN 76 mineral mix containing (g/kg): calcium phosphate dibasic 500, sodium chloride 74, potassium citrate 220, potassium sulfate 52, magnesium oxide 24, manganous carbonate 3.5, ferric citrate 6, zinc carbonate 1.6, cupric carbonate 0.3, potassium iodate 0.01, sodium selenite 0.01, chrominium potassium sulfate 0.55.

+ AIN 76 vitamin mix containing (g/kg): thiamin 0.6, pyridoxine HC1 0.7, niacin 3, calcium pantothenate 1.6, folic acid 0.2, biotin 0.02, vitamin B12 (0.1% trituration in mannitol) 1, dry vitamin A palmitate (500,000 U/g) 0.8, dry vitamin E acetate (500 U/g) 10, vitamin D3 trituration, (400,00 U/g) 0.25, manadione sodium bisulfite complex 0.15.

of animals was killed and their body composition was measured in order to assess initial body energy, with adjustment according to body weight.

Experiment 1 was designed to study effects of green tea on body composition, energy balance and brown adipose tissue activity in rats fed on a high-fat diet. One group of eight animals was given a normal-fat diet and the other two groups also consisted of eight animals were given a high-fat diet. The compositions of the normal- and high-fat diets are shown in Table 1. One of the high-fat groups received high-fat diet containing dry matter of water extract of green tea (green tea extract) at the concentration of 20g/kg diet. Green tea extract was prepared by soaking green tea leaves (Taepyongyang Food, Korea) in hot water (75°C) for five minutes, filtering the solution and then freeze-drying. The macronutrient composition of green tea extract is shown in Table 2.

Experiment 2 was planned to investigate involvement of β-adrenoceptor activation in the expression of the body-fat suppressive effect of green tea. Three groups of eight animals were given a high-fat diet but the high-fat diet for two groups contained green tea extract (20g/kg diet) and the high-fat diet for one of these groups also contained the β-adrenoceptor antagonist propranolol (DL-propranolol) at the concentration of 500 mg/kg diet.

Table 2
Macronutrient composition of freeze-dried matter of water extract of green tea

Constituent	g/100g freeze-dried matter
Nitrogen	4.31
Fat	6.78
Sugar	41.02
Dietary fiber	2.57
Ash	22.73
Gross energy (kJ/g)	16.3

Animals were allowed to free access to water and diet throughout the experiment. Daily food intake was recorded precisely, taking into account spillage. Feces was collected everyday. The experimental period was 14 days.

After 14 days on the experimental diets animals were killed, and interscapular brown adipose tissue were removed and stored at −70°C until analysis

2.2. Measurement of body composition

The carcasses were dried at 105°C to constant weight and then thoroughly ground using a grinder. Body protein and fat contents were determined on dried carcasses by the Kjeldahl method and Soxhlet extraction with petroleum ether, respectively.

2.3. Measurement of apparent digestibility

The gross energy contents of diets and feces were determined by ballistic bomb calorimeter and apparent digestibility was calculated from dividing the difference between energy intake and energy in feces by energy intake. Apparent digestibility is expressed as %.

2.4. Measurement of energy expenditure

The energy expenditure was calculated from the difference between metabolizable energy intake and energy gain. Metabolizable energy (kJ/g) content was calculated from the following formula [7]: Metabolizable energy = Gross energy × Apparent digestibility (%/100) − 0.314×N.

The gross energy(kJ/g) and nitrogen(g/100g) contents of diets were determined by ballistic bomb calorimetry and the Kjeldahl method, respectively. Body energy gain over the experimental period was determined by the difference between final and initial body energy contents calculated from body composition using values of 22.7kJ/g protein and 38.6kJ/g fat [8].

2.5. Measurement of protein and DNA contents of brown adipose tissue

The protein content of brown adipose tissue was measured by the method of Lowry et al. [9] using bovine serum albumin as a standard and DNA content by the Burton diphenylamine method modified by Giles and Myers [10].

2.6. Statistical analysis

Results are presented as means with their standard errors. Statistical analysis was performed using the SPSS program.

Data were analyzed by one-way ANOVA. Differences between the groups were established using the least significant difference(LSD) test. Significance was assessed at the $P<0.05$ level.

3. Results

Rats fed on the high-fat diet (30% fat, wt/wt) showed no changes in energy intake when compared with the normal diet group (Fig. 1B). Body weight gain was increased by 6.9% in the high-fat fed group (Fig. 1A) but this change was not statistically significant. However, body fat gain was significantly increased (Fig. 1D) by feeding the high-fat diet with no changes in body protein gain (Fig. 1E). Apparent digestibility was slightly (1.5%) but significantly reduced in the high-fat fed group (Fig. 1C). Therefore the increased body fat gain in high-fat fed rats appeared to be due to decrease in energy expenditure (Fig. 1F).

Addition of green tea extract to the high-fat diet did not alter body weight gain (Fig. 1A) or energy intake (Fig. 1B) but prevented the increase in body fat gain induced by high-fat diet (Fig. 1D) which was associated with restoration of energy expenditure to levels found in the normal-diet group (Fig. 1F). Body protein gain was not affected by the addition of green tea extract (Fig. 1E). A slight (1.5%) but significant reduction in apparent digestibility was observed compared with the high-fat group (Fig. 1C). Therefore, apparent digestibility was 3% lower in rats fed on the high-fat diet with green tea extract than in those fed on the normal diet.

The weight, protein and DNA contents of interscapular brown adipose tissue were not affected by high-fat diet (Table 3). The administration of green tea extract with the high-fat diet had no effects on weight or DNA content of interscapular brown adipose tissue but caused a 26.5% increase in protein content (Table 3).

The simultaneous administration of β-adrenoceptor antagonist propranolol with green tea extract in high-fat fed rats resulted in inhibition of changes in body fat gain (Fig. 2D) and energy expenditure (Fig. 2F) caused by green tea extract but the effect on energy expenditure did not reach the statistical significance. The increase in protein content of interscapular brown adipose tissue in response to green tea extract was also prevented by propranolol (Table 4). However, the reduction in apparent digestibility by green tea extract was not affected by propranolol (Fig. 2D).

4. Discussion

In experiments looking into effects on body fat accumulation and energy expenditure by the comparative carcass technique in growing animals, the critical point is how precisely estimate the initial body composition. The final content of body protein and fat can be determined directly by the Kjeldahl method and Soxhlet extraction, respectively. However, The initial body composition has to be estimated according to body weight. In this method, it is assumed that the body composition in relation to body weight is identical to that of rats of similar body weight which is analyzed at the beginning of the experiment. However the ratio of body protein and fat to body weight can be different, being dependent on age. The magnitude of difference increases with aging, especially after ages when fat is predominantly deposited rather than protein. In experiments on body fat accumulation with relatively short period, it is wise to use the sexually mature animal to get a reasonable change in body fat content. In this case a little difference in body weight can result in a relatively considerable difference in the ratio of body protein and fat to body weight. A way to minimize the magnitude of this misestimation is to minimize variations in initial body weight of animals. In the present study, the difference between the largest and smallest initial body weight was within 5g.

Green tea and the principle polyphenol of green tea, epigallocatechin gallate (EGCG) have been reported to suppress body weight and weight of adipose tissue in experimental animals [3,4]. This effect has been thought to be in part due to reduction in food intake. Sayama et al. [4] reported suppression of food intake by green tea in mice. Kao et al. [3] also observed reduction in food intake after the administration of EGCG. However in the present study, green tea prevented the increase in body fat caused by a high-fat diet without affecting energy intake, suggesting that body-fat suppressive effect of green tea is not necessarily related to reduction in food intake. The effect of green tea on food intake seems to be rather complicated. In the study by Sayama et al. [4], the effect of green tea on food intake was dependent on the amount of green tea added, being ineffective at low level. And the food intake-suppressive effect of EGCG, administered intraperitoneally, is diminished when EGCG is administered orally [3]. Recently, we observed a 7%, but statistically not significant, increase in food intake in green tea treated-female rats (our unpublished observations). Further study is required to determine effects of green tea and components of green tea on food intake considering various factors such as sex, dose and the route of administration.

Green tea has been shown to reduce fat and protein digestibility [11,12]. We also observed a reduction in digestibility after the administration of green tea. However, the reduction was only 1.5% which was not big enough to fully explain the decrease in body fat content.

In the present study, the reduction in body fat by green tea was associated with increase in energy expenditure and protein content of brown adipose tissue which is indicative of the capacity of brown adipose tissue thermogenesis [13]. The reduction in body fat and increase in protein content of brown adipose tissue by green tea were inhibited by the simultaneous administration of the β-adrenoceptor antagonist propranolol. The increase in energy expenditure caused

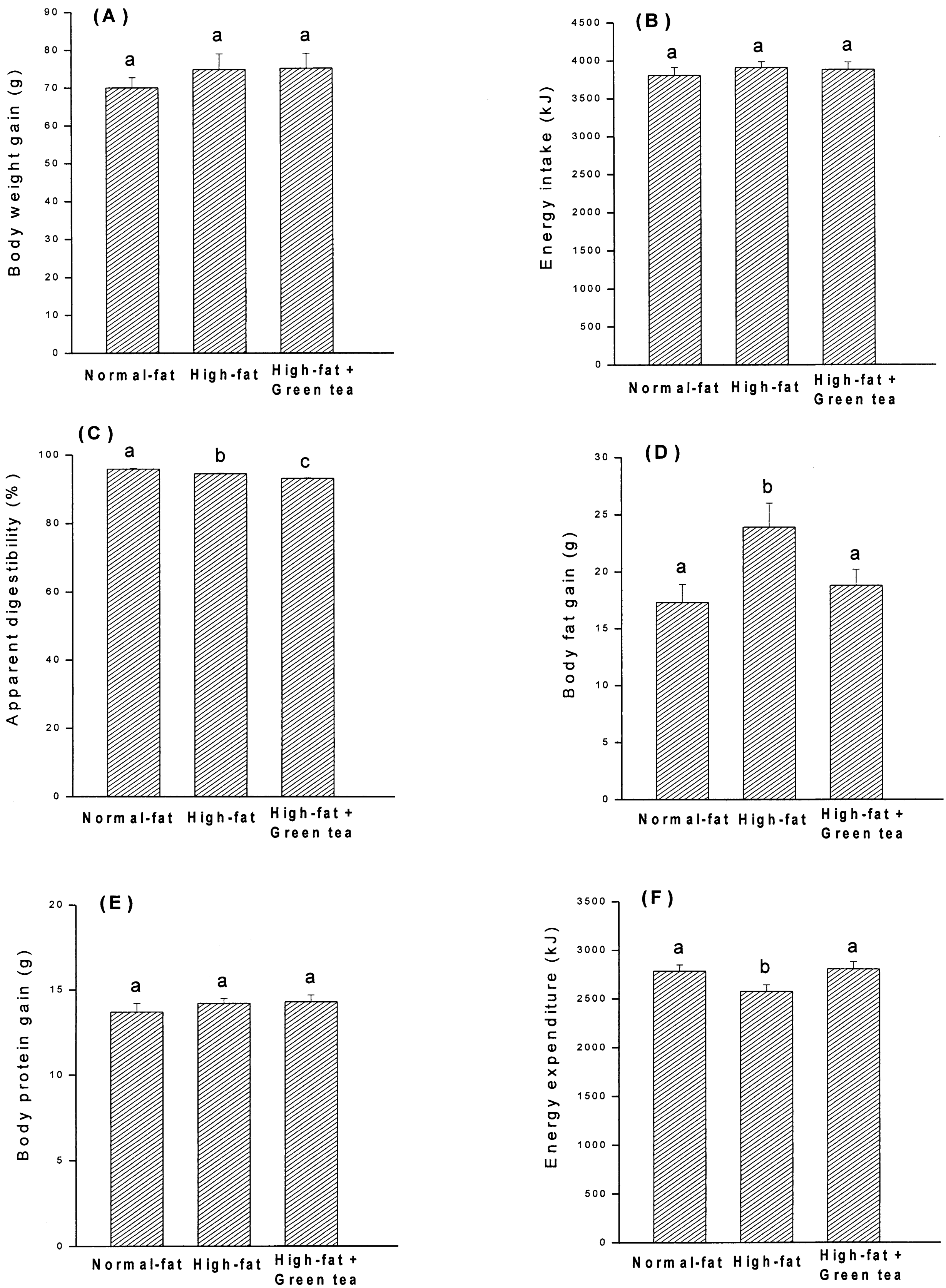

Fig. 1. Effects of green tea extract on body weight gain(A), energy intake(B), apparent digestibility(C), body fat(D) and protein gain(E), and energy expenditure(F) in rats fed on a high-fat diet over 14 days. Values are means for eight rats, with their standard errors indicated by vertical bars. Bars not sharing a common letter were significantly different, $P<0.05$.

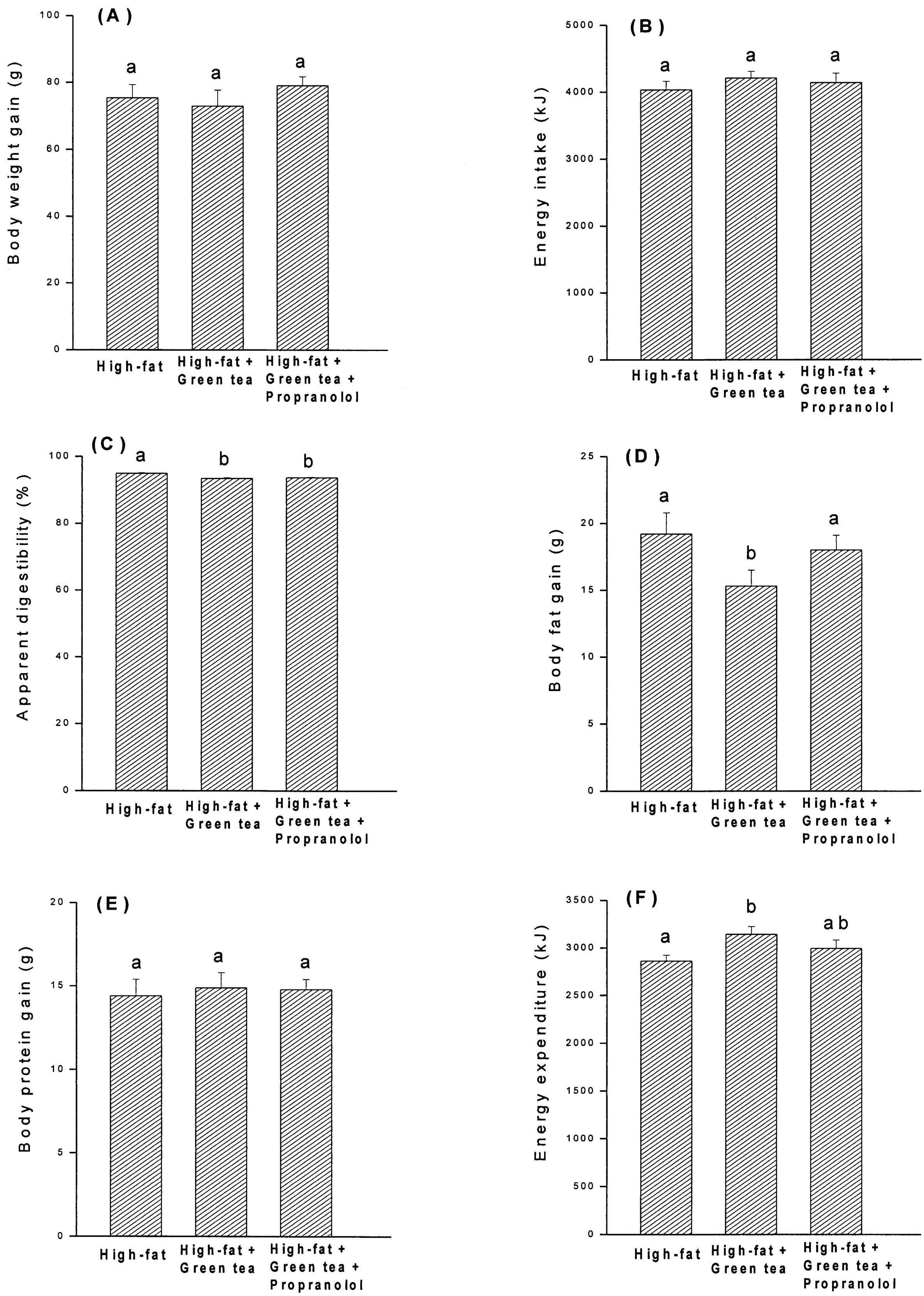

Fig. 2. Effects of propranolol (500mg/kg diet) on responses to the administration of green tea extract in rats fed on a high-fat diet over 14 days. Values are means for eight rats, with their standard errors indicated by vertical bars. Bars not sharing a common letter were significantly different, $P<0.05$. A=body weight gain; B=energy intake; C=apparent digestibility; D=body fat gain; E=body protein gain; F=energy expenditure.

Table 3
Effects of green tea extract on weight, protein and DNA content of interscapular brown adipose tissue (IBAT) in rats fed on a high-fat diet over 14 days

	Normal diet	High-fat diet	High-fat diet + Green tea
Weight (g)	0.243 ± 0.029[a]	0.229 ± 0.031[a]	0.246 ± 0.035[a]
Protein content (mg)	35.2 ± 1.4[a]	32.1 ± 2.2[a]	40.6 ± 2.8[b]
DNA content (μg)	415 ± 28[a]	397 ± 35[a]	418 ± 30[a]

Values are means for eight rats, with their standard errors. Values within a row not sharing a common superscript letter were significantly different, $P < 0.05$.

Table 4
Effects of propranolol (500mg/kg diet) on responses to the administration of green tea extract in rats fed on high-fat diet over 14 days

	High-fat	High-fat + Green tea	High-fat + Green tea + Proprandolol
Weight (g)	0.256 ± 0.041[a]	0.274 ± 0.030[a]	0.239 ± 0.045[a]
Protein content (mg)	38.1 ± 2.0[a]	49.4 ± 1.6[b]	37.7 ± 2.6[a]
DNA content (μg)	431 ± 34[a]	455 ± 37[a]	419 ± 42[a]

Values are means for eight rats, with their standard errors. Values within a row not sharing a common superscript letter were significantly different. $P < 0.05$.

by green tea was also blocked by propranolol. Though this effect did not reach statistical significance it is reasonable to regard as an evidence that body fat-suppressive effects of green tea is to a great extent resulted from increase in energy expenditure mainly through stimulation of β-adrenoceptor activation in brown adipose tissue. Dulloo et al. [5] also reported increases in 24-hr energy expenditure and fat oxidation in response to the administration of green tea in humans and the same research group subsequently showed an increase in O_2 uptake rate of brown adipose tissue in vitro by the addition of ethanol extract of green tea, indicating stimulation of brown adipose tissue thermogenesis [6].

In summary, green tea inhibited the increase in body fat content caused by feeding a high-fat diet without affecting energy intake and this body fat-suppressive effect of green tea was resulted in part from reduction in digestibility and to much greater extent from increase in brown adipose tissue thermogenesis through β-adrenoceptor activation.

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