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Effects of Pu-Erh Tea on Lipid Metabolism in Rats[1)]

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The effect of water extracts of Pu-Erh tea (products of Yunnan district, China, preserved for 2 or 20 years) and of green tea (products of Shizuoka prefecture, Japan) on lipid level, tissue weight, lipoprotein lipase (LPL) and adrenalin-induced lipolytic (AIL) activity in rats were examined. Female Wistar rats (8 weeks old) were fed a diet containing 1% cholesterol and given the above tea extracts in drinking water for 8 or 16 weeks *ad libitum*. The levels of plasma cholesterol ester in rats given Pu-Erh tea or green tea were significantly lower than those of control rats after 6—8 weeks, though the difference became smallar after 10 weeks. The triglyceride (TG) level in plasma was also low in rats given Pu-Erh tea for 16 weeks. This effect on TG was not observed in the case of green tea. Among 6 organs or tissues of rats examined, the weight of abdominal adipose tissue was significantly lower in rat fed Pu-Erh tea for 16 weeks. The LPL activity in abdominal adipose tissue tended to be low (though not statistically significant), while the activity of AIL was significantly elevated in rats given Pu-Erh tea for 8 or 16 weeks. A negative correlation was observed between AIL activity and the ratio of adipose tissue/body weight. These data suggest that the successive administration of Pu-Erh tea could stimulate the degradation of TG in adipose tissue and thereby decrease its weight.

Keywords——Chinese tea; cholesterol; triglyceride; lipolytic activity; lipoprotein lipase; adipose tissue

Tea is one of the most popular drinks, and is consumed not only for pleasure but also as a drug with medical effect such as excitation, diuresis or astringency. Caffeine, theophylline and tannins are well-known components in tea which can produce these effects. Caffeine and theophylline are also known to elevate plasma free fatty acid[4,5)] and cholesterol.[6,7)] Numerous controversial studies have been reported on the possible correlation between daily intake of black tea and the incidence of atherosclerosis or heart disease.[8-12)] Some studies with animals have shown that black tea has a significantly lowering effect on plasma cholesterol or triglyceride (TG).[13-15)]

Pu-Erh tea, produced mainly in the Yunnan district of China, is consumed in a fairly large amount among Chinese people also taking a fat-rich diet. The Pu-Erh tea is different from other teas: it is prepared by fermentation, like a black tea, but is usually preserved for a long period. It is believed that the longer the preservation period, the better the quality of Pu-Erh tea. Microorganisms such as *Aspergillus niger* are often found in Pu-Erh tea preparation and hence the tea is musty. These features of Pu-Erh tea differ greatly from those of green tea, which is non-fermented and is preferably taken as fresh as possible.

In this study, we investigated the effects of Pu-Erh tea, preserved for 2 or 20 years, on lipid content in plasma or liver of rats given a 1% cholesterol diet for 8 or 16 weeks. We also measured adrenalin-induced lipolytic (AIL) and lipoprotein lipase (LPL) activities in the

abdominal adipose tissue of rats and compared the effects of Pu-Erh tea with those of green tea.

Experimental

Preparation of the Tea Extracts——Pu-Erh tea was obtained from Jacos Co., Ltd. (Tokyo). Green tea (a product of Shizuoka prefecture) was used within 6 months after harvesting. Tea extract were prepared daily. Ten grams of dry tea leaves were boiled in 500 ml of tap water for 30 min. After cooling, the extracts were centrifuged at $600 \times g$ for 15 min and filtered through gauze.

Animals and Dietary Procedure——Female Wistar rats (8 weeks old) weighing 130—133 g (Clea Japan, Tokyo) were divided into 4 groups: control group (C) given tap water, two Pu-Erh tea groups given Pu-Erh tea of 2 years old (PE-2) or 20 years old (PE-20) and a green tea group (G). They were fed a purified diet containing 1% cholesterol (Nakarai Chemicals, Kyoto); the composition is shown in Table I. The tea extracts (Pu-Erh tea or green tea) were given to the PE-2, PE-20 and G groups and tap water to the C group *ad libitum*. The rats were maintained for 8 or 16 weeks. One more group of rats (PE-20-7) was given Pu-Erh tea from 7 weeks after the start of the experiment. All rats were weighed biweekly and their food intake was recorded every other day.

Determination of Lipid in Plasma and Liver——For the periodic determination of cholesterol ester (ChE) in plasma, blood (50—100 μl) was taken with heparinized capillaries from the tail artery and the plasma obtained by centrifuging the blood at $1500 \times g$ for 10 min was used. For the determination of lipid in plasma and liver, all rats in each group were fasted for 12 h prior to killing at the end of the experiments and their blood was taken with a heparinized syringe from the abdominal aorta. The plasma was obtained by centrifuging the blood at $1500 \times g$ for 10 min. As for the liver, it was homogenized in 4 vol. of 10 mM Tris buffer (pH 7.4) using a Teflon homogenizer, and the homogenate was centrifuged at $600 \times g$ at 4 °C for 10 min. The supernatant thus obtained was used for lipid analysis. Lipid including ChE, cholesterol (Ch), TG and free fatty acid (FFA) were determined by direct application of the sample (0.5 μl) to a high-performance thin layer chromatography (HPTLC) plate according to the method of Kupke and Sunger.[16] HPTLC plates pre-coated with Silica gel 60 without fluorescent indicater (10 × 20 cm, Merck) were developed in chloroform : methanol : water, 65 : 30 : 5 followed by *n*-hexane : diethyl ether : AcOH = 80 : 20 : 1.5. The fluorescence derived by reacting the spot of lipid with $(NH_4)HCO_3$ was scanned by using a high-speed TLC scanner (model CS-920, Shimadzu Co., Kyoto).

Determination of AIL Activity in Abdominal Adipose Tissues——The rats were killed after 12 h of fasting. The adipose tissues from the abdominal wall were excised and weighed. AIL activity was measured by the method of Hayashi *et al.*[17] with some modifications. Approximately 200 mg of tissue was rinsed in 20 ml of Krebs–Ringer bicarbonate buffer (pH 7.4) containing 10 mg of glucose, bubbled through with a gas mixture of 95% O_2/5% CO_2 at 37 °C for 15 min. The tissues were transferred to a tube with 1.9 ml of the same buffer (pH 7.4) containing 2.5% bovine serum albumin (fatty acid-free, Sigma Chemical Co., St. Louis, USA). One-tenth mililiter of adrenalin (0.2 μg/ml, Dai-ichi Pharmaceutical Co., Tokyo) was then added to the tube and the reaction mixture was incubated at 37 °C for 2 h. The glycerol released in the reaction mixture (medium) was assayed enzymatically with a kit (Boehringer Mannheim Biochemicals, West Germany).

Determination of LPL Activity in Abdominal Adipose Tissues——The extraction and assay of enzyme were done by the procedures of Korn and Quigley[18] and Matsumura *et al.*,[19] respectively. The abdominal adipose tissue was homogenized in a Polytron (model PT10, Kinematica, GmbH, Lausanne, Switzerland) in 5 vol. of cold acetone (−20 °C) and the homogenate was filtered through Toyo No. 2 paper under negative pressure. The tissue residue was washed with 300 ml of acetone followed by 200 ml of diethylether, and dried *in vacuo*. The defatted tissue was stored

TABLE I. Diet Composition

	g/100 g		g/100 g
Sucrose	63.5	Casein	20.0
Lard	10.0	Vitamin mix.[a]	1.0
Cholic acid	0.5	Mineral mix.[b]	4.0
Cholesterol	1.0		

a) Vitamin mix. (per 100 g): mixture of vitamin A 50000 IU, D_3 10000 IU, $B_1 \cdot HCl$ 120 mg, B_2 400 mg, $B_6 \cdot HCl$ 80 mg, B_{12} 0.05 mg, C 3 g, E acetate 50 mg, K_3 520 mg, *d*-biotin 2 mg, folic acid 20 mg, calcium pantothenate 500 mg, *p*-aminobenzoic acid 500 mg, niacin 600 mg, *myo*-inositol 600 mg, choline chloride 20 g and cellulose powder diluent. *b*) Mineral mix. (per 100 g): mixture of $CaHPO_4 \cdot 2H_2O$ 14.56 g, KH_2PO_4 25.72 g, $NaH_2PO_4 \cdot H_2O$ 9.35 g, NaCl 4.66 g, Ca–lactate 35.09 g, Fe–citrate 3.18 g, $MgSO_4$ 7.17 g, $ZnCO_3$ 0.11 g, $MnSO_4 \cdot 4$—$6H_2O$ 0.12 g, $CuSO_4 \cdot 5H_2O$ 0.03 g and KI 0.01 g. Vitamin mix. and Mineral mix. were purchased from Oriental Yeast Co. (Tokyo).

at −20 °C until use. The tissue (50 mg) was minced finely and incubated in 1 ml of 10 mM NH_4OH solution at 0 °C for 60 min. The extracts containing LPL were centrifuged at 15000 × *g* for 30 min and the pH of the supernatant was adjusted to 8.0 with 1 N acetic acid. The clear supernatant was used for LPL assay. LPL activity was determined by measuring the amount of FFA released during the enzyme reaction. The reaction mixture contained 0.05 ml of 0.3 M Tris–HCl buffer (pH 8.5), 0.05 ml of 20% bovine serum albumin (fatty acid-free), 0.05 ml of substrate solution containing 0.025 ml of 10% Intralipid (10% soybean oil, 1.2% yolk lecithin and 2.5% glycerol, Midori Juji Co., Osaka) which had been preincubated with 0.025 ml of fresh rat serum at 37 °C for 30 min, test solution and water in a final volume of 0.5 ml. The mixture was incubated at 37 °C for 1 h and the FFA released were assayed by the method of Itaya and Ui.[20]

Determination of Protein——Protein was determined by the method of Lowry *et al.*[21] using bovine serum albumin as a reference protein.

Results

Growth and Food Intake

No significant difference was observed in body weight gain or food intake between the groups administered tea (PE-2, PE-20, PE-20-7 and G) and tap water (control group), as shown in Table II.

Organs and Abdominal Adipose Tissue Weight

The weights of the liver, heart, lung and kidney in the tea groups were not significantly different from those of the control group. A slight increase of spleen weight was observed in the PE-20 and G groups at 16 weeks. A significantly lower weight of adipose tissues was noticed in the PE-2, PE-20, and PE-20-7 groups at 16 weeks. The weight ratio of adipose tissue to whole body was significantly lower in all Pu-Erh tea groups at 8 or 16 weeks compared to that of the control group. The effect was not observed in the rats fed green tea for 8 or 16 weeks (Table II).

ChE in Plasma

The fluctuations of the content of ChE in rat plasma are shown in Fig. 1. The ChE level of the control group increased from 75.6 mg/dl to 408.6 mg/dl after 6 weeks and became almost constant (460—480 mg/dl) during 8—14 weeks. The ChE level of the PE-20 group was

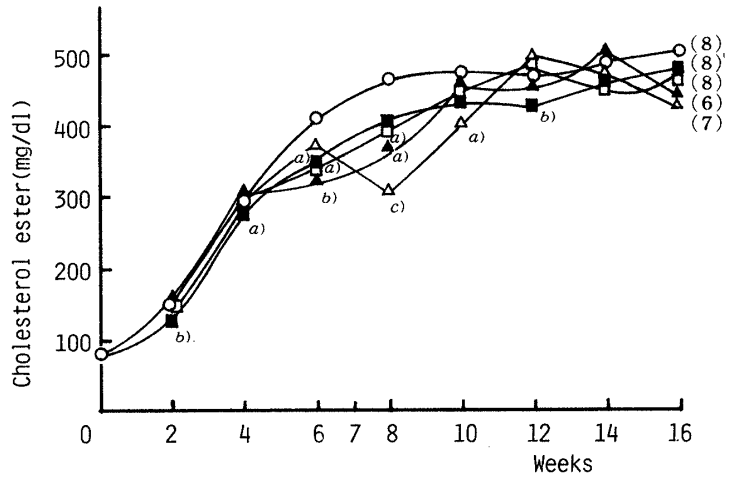

Fig. 1. Fluctuation of the Contents of Cholesterol Ester in Rat Plasma

Tea extracts (Pu-Erh tea 2 years old □, 20 years old ■, △, green tea ▲) or tap water (control ○) were given *ad libitum* to rats fed diet containing 1% cholesterol for 16 weeks. In one group of rats, the extract of Pu-Erh tea (20 years old) was given from 7 weeks after the start of feeding 1% cholesterol diet (△). Figures in parentheses indicate the number of rats (female) used. Cholesterol ester was determined by the HPTLC method with blood samples (50—100 μl) taken from the aorta. Significantly different from the control group: *a*) $p<0.05$; *b*) $p<0.01$; *c*) $p<0.001$.

TABLE II. Effects of Pu-Erh Tea on Food Intake and Body and Tissue Weights in Rats

Group	8 weeks feeding			16 weeks feeding				
Determination	Control (6)[a]	Pu-Erh tea (6) (20 yrs old)	Green tea (6)	Control (8)	Pu-Erh tea (8) (2 yrs old)	Pu-Erh tea (8) (20 yrs old)	Pu-Erh tea (7) (20 yrs old)[b]	Green tea (6)
Food intake, g/d	—[c]	—	—	21.4±2.2[d]	23.2±3.6	22.8±3.4	22.4±2.5	22.3±3.5
Body wt. initial, g	128.7±4.3	129.3±3.6	130.0±4.0	132.8±4.7	129.5±6.3	132.0±4.7	129.7±5.0	131.5±4.5
final, g	227.0±13.8	227.2±10.6	232.8±13.3	251.0±12.6	258.5±16.5	261.0±16.1	255.7±13.4	263.3±10.9
Liver wt. g	10.53±1.00	10.50±1.51	10.78±1.78	15.09±1.72	13.62±2.14	14.42±1.46	13.14±2.22	15.70±2.03
Heart wt. g	0.68±0.01	0.73±0.06	0.72±0.04	0.81±0.10	0.83±0.09	0.79±0.05	0.80±0.10	0.79±0.07
Lung wt. g	1.19±0.16	1.13±0.04	1.41±0.21	1.32±0.25	1.47±0.11	1.49±0.23	1.45±0.11	1.39±0.22
Kidney wt. g	1.36±0.09	1.42±0.12	1.48±0.14	1.65±0.19	1.75±0.25	1.65±0.08	1.61±0.18	1.80±0.10
Spleen wt. g	0.68±0.06	0.64±0.08	0.69±0.08	0.73±0.07	0.83±0.17	0.82±0.09[e]	0.81±0.17	0.87±0.06[g]
Adipose wt. g	9.92±3.15	7.18±1.25	7.62±3.13	8.72±1.44	5.32±1.15[h]	5.50±2.78[f]	6.87±1.01[f]	7.90±1.41
Adipose/body wt. %	4.32±1.17	3.15±0.49[e]	3.28±1.34	3.46±0.56	2.07±0.41[h]	2.12±1.09[g]	2.72±0.43[f]	2.94±0.47

Tea extracts were given *ad libitum* to rats fed 1% cholesterol diet for 8 or 16 weeks. *a*) Figures in parentheses indicate the number of rats (female) used. *b*) Tea extracts were given from 7 weeks after the start of feeding 1% cholesterol diet. *c*) Food intake in the 8-week feeding study was not recorded. *d*) Mean±S.D. Significantly different from the control: *e*) $p<0.05$; *f*) $p<0.02$; *g*) $p<0.01$; *h*) $p<0.001$.

significantly lower than that of the control group at 2 weeks. The levels of the other tea groups (PE-2 and G) were also significantly lower at 6—8 weeks. There was, however, no statistically significant change in any of the tea groups after 14 weeks. In the PE-20-7 group, the ChE level

TABLE III. Effects of Pu-Erh Tea on Plasma and Liver Lipid of Rats

Group / Determination	Control (8)[a]	Pu-Erh tea (8) (2 yrs old)	Pu-Erh tea (8) (20 yrs old)	Pu-Erh tea (7) (20 yrs old)[b]	Green tea (6)
Plasma					
Cholesterol ester, mg/dl	500.86±168.50[c]	454.82±126.76	478.14±151.29	411.00±109.47	443.85±115.87
Cholesterol, mg/dl	116.57±40.73	102.88±34.24	107.71±36.77	88.07±27.49	98.15±25.50
Triglyceride, mg/dl	137.14±48.05	62.14±18.27[e]	68.50±24.72[e]	73.29±41.61[d]	128.63±66.68
Free fatty acid, meq/dl	1.45±0.36	1.28±0.20	1.11±0.17	1.13±0.26	1.43±0.49
Liver					
Total cholesterol, mg/g	35.41±5.01	35.04±6.31	39.60±5.12	33.12±8.49	34.64±6.40
Triglyceride, mg/g	18.64±5.30	15.51±5.28	16.36±5.55	15.45±3.70	20.79±5.09
Free fatty acid, mg/g	1.31±0.43	1.28±0.46	1.37±0.45	2.06±1.18	1.64±0.91

Tea extracts were given *ad libitum* to rats fed 1% cholesterol diet for 16 weeks. *a*) Figures in parentheses indicate the number of rats (female) used. *b*) Tea extracts were given from 7 weeks after the start of feeding 1% cholesterol diet. *c*) Mean±S.D. Significantly different from control rats: *d*) $p<0.05$; *e*) $p<0.01$.

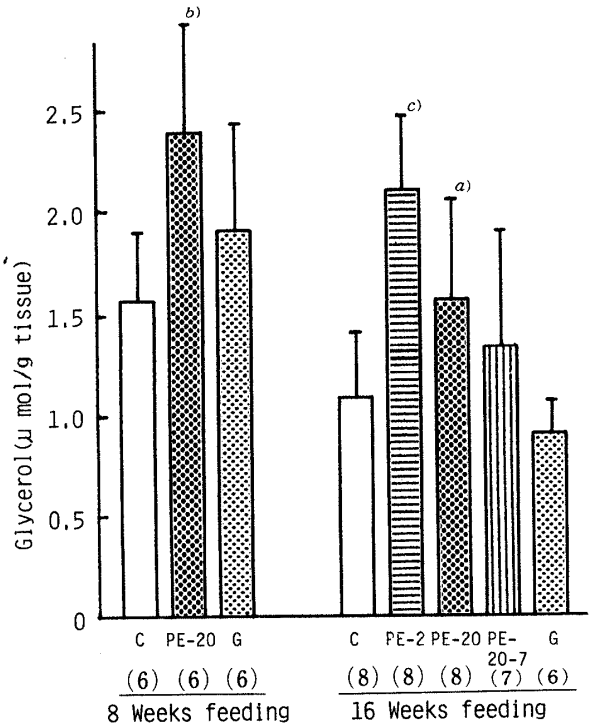

Fig. 2. Effects of Pu-Erh Tea on Adrenalin-Induced Lipolytic Activity in Rat Adipose Tissue

Tea extracts (PE-2, Pu-Erh tea 2 years old; PE-20 and PE-20-7, Pu-Erh tea 20 years old; G, green tea) or tap water (C, control) were given *ad libitum* to rats fed diet containing 1% cholesterol for 8 or 16 weeks. Tea extract (PE-20-7) was given from 7 weeks after the start of feeding 1% cholesterol diet. Figures in parentheses indicate the number of rats (female) used. Significantly different from the control group: *a*) $p<0.05$; *b*) $p<0.02$; *c*) $p<0.01$. The vertical bars represent the S.D.

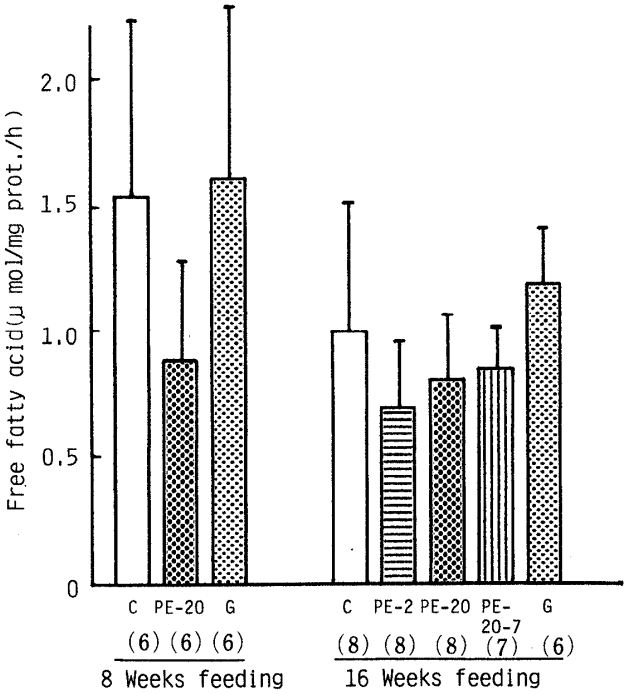

Fig. 3. Effects of Pu-Erh Tea on Lipoprotein Lipase in Rat Adipose Tissue

Tea extracts (PE-2, Pu-Erh tea 2 years old; PE-20 and PE-20-7, Pu-Erh tea 20 years old; G, green tea) or tap water (C, control) were given *ad libitum* to rats fed diet containing 1% cholesterol for 8 or 16 weeks. Tea extract (PE-20-7) was given from 7 weeks after the start of feeding 1% cholesterol diet. Figures in parentheses indicate the number of rats (female) used. The vertical bars represent the S.D.

decreased sharply for a short period of time after the start of tea administration.

Lipid Contents in Plasma and Liver

TG levels of plasma in the PE-2 and PE-20 groups were significantly lowered. TG levels in the liver of Pu-Erh tea groups were also low, though the effect was not statistically significant (Table III). The levels of Ch and FFA were not changed in the Pu-Erh tea and green tea groups compared with those of the control group after 16 weeks.

AIL Activity

The lipolytic activities in abdominal adipose tissue are shown in Fig. 2. The AIL activity in all Pu-Erh tea groups (PE-2 and PE-20) was significantly higher than that of the control group regardless of the administration period. The activities of the PE-20 and PE-2 groups were approximately 1.5 and 1.9 times higher than that of the control group at 8 and 16 weeks, respectively, while that of G group showed no significant change. A negative correlation was observed between the activities and the ratio of adipose tissue to body weight at 8 or 16 weeks ($r=-0.71$, $p<0.01$ and $r=-0.80$, $p<0.01$ for 8 and 16 weeks, respectively).

LPL Activity

The activity in abdominal adipose tissue is shown in Fig. 3. LPL activity tended to be low in all Pu-Erh tea groups (PE-2, PE-20 and PE-20-7) at 8 or 16 weeks though the effect was not statistically significant.

Discussion

Plasma ChE levels of rats fed 1% cholesterol diet with Pu-Erh tea or green tea for 6—8 weeks were significantly lower than those of the control group. This result agrees with the finding of Akinyanju and Yudkin,[13] who observed that the administration of black tea for 7 weeks lowered ChE levels in rat plasma.

The level of ChE in the PE-20 group was significantly lower than that of the control group and it was remarkably (though transiently) lowered in the PE-20-7 group, in which Pu-Erh tea was administered 7 weeks after the start of the experiment. These results indicate that the tea not only acts in a preventive manner against the accumulation of ChE in plasma but also reduces ChE, at least for a short period of time. This reduction of the ChE level might be maintained longer if the tea consumption of rats were to be appropriately regulated.

Further, the plasma TG levels of rats administered Pu-Erh tea for 16 weeks were 45—50% lower than that of the control group.

It may thus be concluded that Pu-Erh tea would be a suitable beverage in hyperlipidemia, though the mechanism of the above effects remains uncertain. It is known that caffeine or theophylline lowers the level of plasma TG,[7] but elevates the level of Ch or FFA.[4-7] Green tea (in this study) or coffee[13,15] showed no significant effect *in vivo*, though they contain caffeine or theophylline. Akinyanju and Yudkin[13] suggested in their comparative study of ordinary and decaffeinated coffee that the effect of tea on lipid metabolism is due to some factor(s) other than caffeine. The main differences between green tea and Pu-Erh or black tea are in the processes of their preparation; the former is prepared by heating the leaves for about 30—60 s to inactivate enzymes such as oxidase, *etc.*, while the latter is produced by fermentation, in which the components of the leaves are oxidized by the enzymes and various active substances may be formed. Table II shows that the weight of abdominal adipose tissue body weight was lowered in all Pu-Erh tea-administered rats. AIL activity was significantly higher in all Pu-Erh tea groups except PE-20-7 (Fig. 2), and a negative correlation was observed between the AIL activity and the ratio of adipose tissue/body weight. LPL activity, which primarily affects the uptake of lipid into adipose tissues, tended to

decrease, though not significantly. Changes of these lipolytic enzyme activities may be associated with a lower adipose tissue weight in Pu-Erh tea rats comparing to that in control rats. AIL activity is known to be elevated by caffeine.[22,23] The contents of caffeine (analyzed by spectrophotometric determination)[24] were 61, 52 mg/100 ml in Pu-Erh tea extracts of 2 and 20 years old, respectively, and 66 mg/100 ml in green tea extracts used in this study. However, the AIL activities of the above 3 kinds of tea extracts (measured by adding the extracts to the assay system *in vitro*) were 132, 245 and 166% of the control value, respectively, and the green tea extracts had about the same effect as the Pu-Erh tea extracts (2 years old). These results are not in agreement with the results obtained *in vivo* (see Fig. 2) and may indicate that the elevation of adrenalin-induced lipolysis *in vivo* by Pu-Erh tea is not simply attributable to caffeine. Tannin is another biologically active principle in tea and is known to inhibit the absorption of cholesterol from the intestine.[25] Moreover, certain hydrolyzable tannins have an antioxidative effect[26,27] and are known to enhance or inhibit lipolysis of fat cells *in vitro*.[28] Various polyphenols or aldehydes released from tea tannin in the process of fermentation may affect lipid metabolism. However, the effects of tannin on the activities of LPL and AIL have not been investigated.

Pu-Erh tea which has been preserved for a longer time generally has slight musty odor and is considered to be more valuable than younger preparations. The effects of Pu-Erh tea mentioned above may be ascribable to cyclic AMP, for example, which is known to be produced as a metabolic product of *Aspergillus* strains and which accelerates lipid metabolism.[29] However, under our experimental conditions, the effects of 20-years-old Pu-Erh tea seemed to be similar to that of 2-years-old tea.

Further study is needed to identify the active principle of Pu-Erh tea and to elucidate the mechanism of its effects on lipid metabolism in rats.

References and Notes

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