Effect of Powdered Green Tea and Its Caffeine Content on Lipogenesis and Lipolysis in 3T3-L1 Cell

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We studied the influence of powdered green tea (PGT) and its caffeine content on the adipose conversion of 3T3-L1 cells by insulin and lipolysis of well-differentiated 3T3-L1 cells. In the 11 days of culture with insulin, the fat cells exhibited more numerous and larger intracytoplasmic lipid droplets, and the activities of glycerophosphate dehydrogenase (GPDH), a marker of adipose conversion, were increased. When PGT and insulin were added simultaneously, the accumulation of lipid droplets and the increase of GPDH were significantly inhibited ($p<0.01$). But the caffeine, which has the same concentration as PGT, accelerated adipose conversion. When PGT or caffeine was exposed to mature adipocytes, smaller-sized intracytoplasmic lipid droplets selectively disappeared. These data suggest that PGT inhibited lipogenesis and stimulated lipolysis.

Key words —— powdered green tea, caffeine, 3T3-L1 cell, lipogenesis, glycerophosphate dehydrogenase, lipolysis

INTRODUCTION

Traditionally-consumed green tea contains about 2.0—3.0% of caffeine[1] which is associated with an increased level of cAMP. In young adipocytes, an increase in β_1-adrenoceptor density has been shown during the differentiation process which is triggered by cAMP.[2] However, catechin inhibits TG accumulation in 3T3-L1 cells.[3] Although these findings suggest an inverse association between the effect of catechin and caffeine on lipogenesis, nothing definite is known in this regard.

In mature adipocytes, the β_3-subtype population coupled to the adenylyl cyclase system reportedly became detectable and played a role in the activation of lipolysis.[2,4] Oolong tea enhanced lipolysis in isolated fat cells, and the active substance was identified as caffeine.[5] However, nothing has been reported about the lipolytic effect of green tea in the literature.

In the present study, powdered green tea (PGT) and caffeine were tested for their antiadipogenic effect and lipolytic effect in 3T3-L1 cells.

MATERIALS AND METHODS

Chemicals —— PGT was provided by Zenken Foods Co. (Tokyo, Japan), was 0.25 μm in size and contained 2.4% caffeine. PGT was dissolved in distilled water, and used after filtration with a 0.45-μm sterilized cellulose acetate filter. Caffeine was purchased from Wako Chemical (Tokyo) and used as an aqueous solution at the same concentration as PGT. Dulbecco's modified Eagle's medium (DMEM) and DMEM : Ham F-12 (1 : 1) were purchased from Dainihon Pharmaceutical Co. (Tokyo). Bovine insulin, calf serum (CS) and fetal calf serum (FCS) were from JRH Biosciences (Lunexa, KS).

Cell Culture —— 3T3-L1 preadipocytes were cultured by the method previously described.[6] Briefly, cells were grown in DMEM containing 10% CS, 100 U/ml penicillin and 10 μg/ml streptomycin, at 37℃ in humidified 5% CO_2. At confluence (day 0), the medium was changed to DMEM : F-12 (1 : 1) containing 10% FCS, and differentiation was induced with 5 mg/ml insulin.

PGT was present from the first day of the culture.

The extent of differentiation was quantified by staining intracellular oil droplets with Oil Red O,[6] as previously described.

Assays —— Cells were removed 21 days after the differentiation process. The activity of GPDH was measured spectrophotometrically in sonicated cell extracts according to an established procedure.[7] One unit of enzyme activity corresponds to the oxidation of 1 nmol of NADH /min per mg of protein. The protein content of the homogenates was measured using a commercially available kit (Coomassie protein assay reagent, Pierce, IL). Bovine serum albumin

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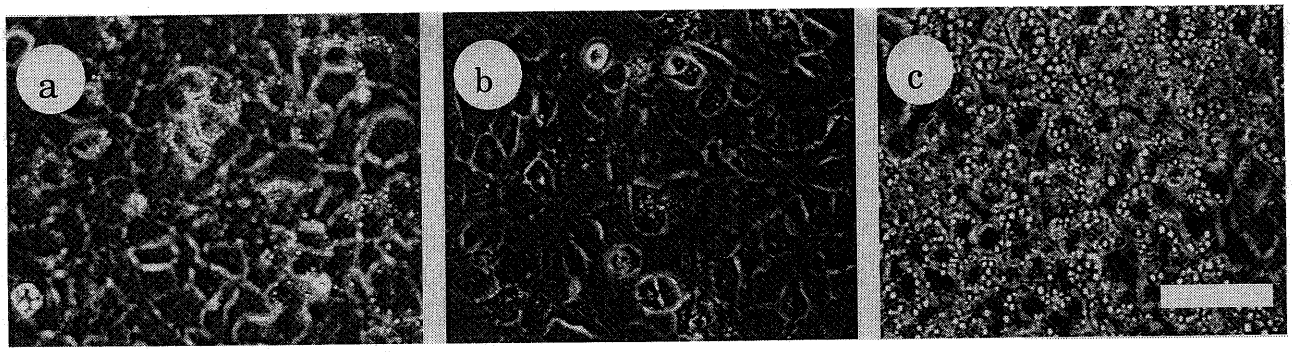

Fig. 1. Effect of PGT and Caffeine on Insulin-Induced Adipose Conversion of 3T3-L1 Preadipocytes after 11 d of Treatment

Insulin (5 mg/ml) and PGT (0.1 mg/ml) or caffeine (2.4 μg/ml) were added at 0 d. a: +insulin; b: +insulin+PGT; c: +insulin+caffeine, Bar=100 μm.

was used as a standard.

Statistics —— Results are expressed as the mean± S.D. One way analysis of variance (ANOVA) and Student's *t*-test were used for statistical comparison.

RESULTS AND DISCUSSION

In the 11 days of culture with insulin, the fat cells exhibited larger intracytoplasmic lipid droplets (Fig. 1). During this period, GPDH activity, which was used as a marker of differentiation, increased from undetectable levels to between 40 and 70 U/mg of cellular protein (Fig. 2). When 0.1 mg/ml of PGT was added, lipogenesis due to insulin was inhibited (Fig. 1) and a significant decrease in GPDH activity was observed (Fig. 2). When an even lower dose of PGT was added (0.05 mg/ml), lipogenesis due to insulin was not inhibited (not presented) and GPDH activity was the same level as that in controls (Fig. 2). At 0.5 mg/ml, the cells were exfoliated from the culture dish within 3—4 d, so no further experiments could be done. Caffeine enhanced insulin-induced differentiation in 3T3-L1 preadipocytes (Figs. 1 and 2).

In adipocytes, cAMP exerts a key role in differentiation processes.[4] Together with the above findings, PGT was suggested to inhibit insulin-induced lipogenesis by prevention of the cAMP-dependent biological process, despite the fact that the caffeine content enhanced adipose conversion. The physiologic mechanism of this finding remains to be elucidated in future studies.

When mature adipocytes were exposed to PGT or caffeine, smaller intracytoplasmic lipid droplets selectively disappeared (Fig. 3). These results suggest that caffeine is the lipolytic substance in PGT, as seen in oolong tea.[5]

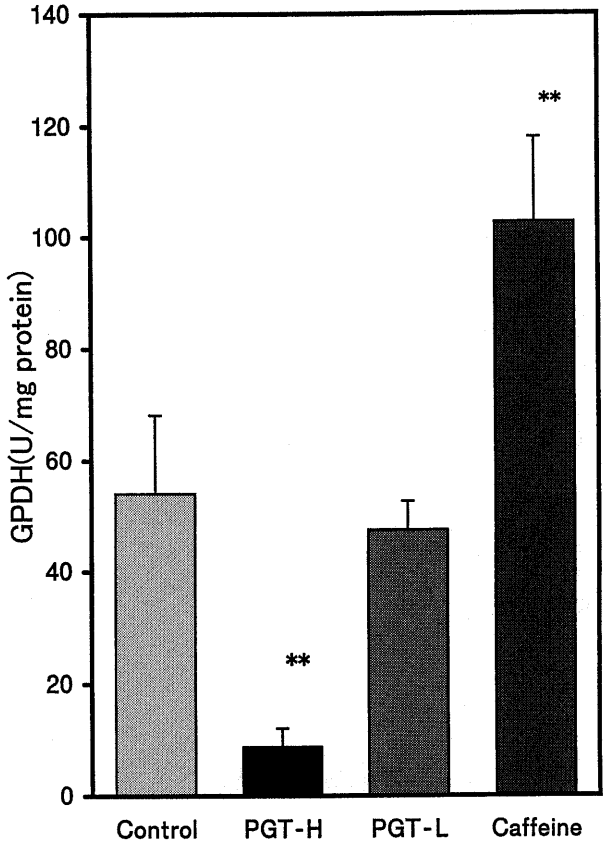

Fig. 2. Effect of PGT and Caffeine on Insulin-Induced GPDH Activity in 3T3-L1 Cells

Insulin (5 mg/ml) and PGT (PGT-H: 0.1 mg/ml; PGT-L: 0.05 mg/ml) or caffeine (2.4 μg/ml) were added at 0 d. Results are presented as the mean±S.D. of four experiments in duplicate; $^{**}p<0.01$.

In summary, this *in vitro* study shows that the antiadipogenic effect of PTG is mediated *via* the mechanism which inhibits the adipogenic effect of caffeine, and that the lipolytic effect is *via* an increased level of cAMP by caffeine. In addition to other benefical effects, PGT can also

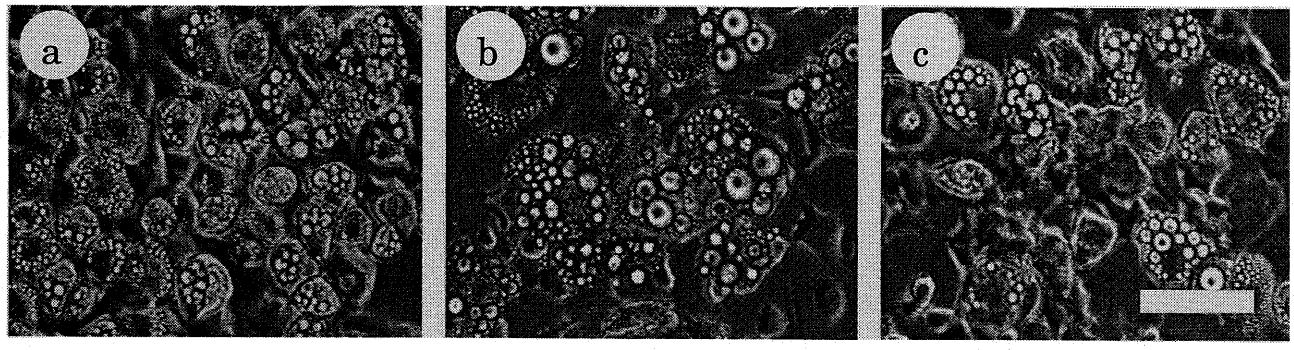

Fig. 3. Effect of PGT and Caffeine on Lipolysis in 3T3-L1 Cells after 4 d of Treatment

The cells were cultured for 10 d as described in Fig. 1, in the presence of 5 mg/ml insulin. On the 10th d of culture, insulin was removed and 0.1 mg/ml PGT or 2.4 μg/ml caffeine was added to the medium. a: control; b: +PGT; c: +caffeine, bar=100 μm.

be useful in preventing obesity and maintaining optimal health.

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REFERENCES

1) Resources Council, Science and Technology Agency, Japan, "Standard Tables of Food Composition in Fourth Revised Edition," Ministry of Finance Printing Breau, Tokyo, 1982.
2) Hadri K.E., Fève B., Pairault J., *Eur. J. Pharmacol.*, **297**, 107—119 (1996).
3) Watanabe J., Kawabata J., Niki R., *Biosci. Biotechnol. Biochem.*, **62**, 532—534 (1998).
4) Sugihara H., Yonemitsu N., Miyabara S., Toda S., *J. Lipid Res.*, **28**, 1038—1045 (1987).
5) Han L.K., Takaku T., Kimura Y., Okuda H., *Int. J. Obes. Relat. Metab. Disord.*, **23**, 98—105 (1999).
6) Hasegawa N., *J. Home Econ. Jpn.*, **49**, 889—892 (1998).
7) Wise L. S., Green H., *J. Biol. Chem.*, **254**, 273—275 (1979).