Inhibition of Lipogenesis and Stimulation of Lipolysis in 3T3 L1 Cells by a Garcinia Extract

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We studied the influence of a Garcinia extract on the lipogenesis and lipolysis of insulin-differentiated 3T3 L1 cells. After the 2nd week of culture with insulin, the cells exhibited numerous larger intracytoplasmic lipid droplets. This lipogenesis due to insulin was inhibited when the Garcinia extract was administered. When the Garcinia extract was added to the mature adipocytes, the smaller (less than 10μm²) intracytoplasmic lipid droplets selectively disappeared. These data suggest that the Garcinia extract inhibited lipogenesis and stimulated lipolysis.

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INTRODUCTION

A Garcinia extract can be isolated from fruit of the Garcinia genus, and is currently used as a dietary supplement by the health food industry. About 50% of the extract has been identified as (-) hydroxycitrate (HCA), a potent inhibitor of citrate oxaloacetate lyase in cytoplasm. An oral intake of HCA can inhibit fatty acid synthesis and lipogenesis in rat liver. Moreover, HCA-treated rats tended to show a depressed body lipid level.

However, very little is known about the direct effects of HCA on adipocytes. We have previously demonstrated the anti-lipogenic and lipolytic effects of flavonoids on 3T3 L1-preadipocytes. In the present study, a Garcinia extract was tested for its anti-obesity effect on adipocytes by using the same experimental design.

MATERIALS AND METHODS

Chemicals

The Garcinia extract was provided by L.S. Co. (Tokyo, Japan). Dulbecco's modified Eagle's medium (DMEM) and DMEM : Ham F-12 (1 : 1) were purchased from Dainihon Pharmaceutical Co. (Tokyo, Japan). Bovine insulin, calf serum (CS) and fetal calf serum (FCS) were from JRH Biosciences (Lenexa, KS, U.S.A.).

Cell culture

3T3 L1-preadipocytes (American Type Culture Collection, Rockville, MD, U.S.A.) were grown in DMEM containing 10% CS, 100 U/ml of penicillin and 10μg/ml of streptomycin at 37°C in a humidified 5% CO₂ atmosphere as described previously.

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 of insulin. The Garcinia extract (1 mg/ml) was present from the first day of the culture. The extent of differentiation was quantified by staining intracellular oil droplets with Oil Red O after their fixation with 5% formaldehyde.

On day 14 of the culture, the insulin was removed, and 1 mg/ml of the Garcinia extract was added to the medium. We then carefully checked the size of the oil droplets.

Data analyses

Phase-contrast micrographs were recorded on a PC-AT computer through a CCD camera (Ikegami, Tokyo, Japan) and interface board (TARGA+, Truevision, IN, U.S.A.). The area of the droplets was determined by using JAVA software (Jandel Scientific, CA, U.S.A.).

RESULTS AND DISCUSSION

Antilipogenic effect

Figure 1 shows the morphological aspects of cultures treated with insulin and the Garcinia extract after 21 days of treatment. The black droplets found around the nucleus contained Oil Red O stainable...
triglyceride. No oil droplets were observed in the cells in the control medium (not shown). In the presence of insulin, accelerative maturation was observed (Fig. 1, a). The inhibition of cell maturation by the Garcinia extract was obvious when added simultaneously with insulin (Fig. 1, b).

These results demonstrate that the Garcinia extract inhibited the adipose conversion of 3T3 L1-preadipocytes and lipid inclusion. ATP citrate (pro-3S)-lyase is present in human adipose tissue. Together with the foregoing findings, we propose that the Garcinia extract interfered with lipid synthesis in the adipocytes by acting directly and inhibiting cytoplasmic citrate lyase by the same pathway as that described in rat liver.\(^{25-31}\)

**Lipolytic effect**

Figure 2 shows the morphological aspects of cultures treated with the Garcinia extract. Its administration stimulated lipolysis mainly in small droplets. Although the shape of each droplet was unclear, the cells seemed to have lost their cytoplasmic expansion (Fig. 2, b). As a result, the number of

![Fig. 1. Effect of insulin and the Garcinia extract on adipose conversion of 3T3 L1-preadipocytes after 14 days of treatment](image1)

The black droplets contained Oil Red O-stainable triglyceride. Insulin (5 mg/ml) and the Garcinia extract (1 mg/ml) were added at day 0. a: with insulin only; b: with both insulin and the Garcina extract. Bar: 100 μm.

![Fig. 2. Effect of the Garcinia extract on lipolysis in 3T3 L1 cells after 21 days of treatment](image2)

The cells were cultured for 14 days as described in Fig. 1 in the presence of 5 mg/ml of insulin. On day 14 of the culture, the insulin was removed and 1 mg/ml of the Garcinia extract was added to the medium. a: control; b: with the Garcinia extract. Bar: 20 μm.
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Countable droplets decreased. When the droplet area in Fig. 2 is expressed as a histogram, the number of small droplets (<10 μm²) markedly decreased (Fig. 3).

HCA reportedly decreased oleate conversion into cholesterol. Although the exact pathway for the lipolytic effect of HCA is unknown, adipocytes might complement fatty acid enough to maintain their cell membrane by degrading the accumulated lipid.

In summary, the Garcinia extract showed anti-lipogenic and lipolytic effects on adipocytes. HCA might promote more efficient hepatic gluconeogenesis, and effective glucose availability might thus be increased without any calorie intake. Our observations confirm that a Garcinia extract can be a useful dietary supplement for preventing obesity.

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REFERENCES
ガルシニアエキスの3T3細胞における脂肪蓄積阻害および脂肪分解促進作用

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ガルシニアエキスのインシュリンによる3T3-L1細胞の脂肪細胞への分化と、分化後の油滴の分解に対する作用を研究した。3T3-L1細胞をインシュリンで分化させると、2週間で顕著な油滴が細胞質に認められた。この際、ガルシニアエキスを添加しておくと、脂肪細胞への脂肪蓄積が抑制された。油滴が十分に蓄積された後、ガルシニアエキスを加えると、10μm²以下の油滴が選択的に消失した。以上の事実から、ガルシニアエキスは、脂肪の合成を抑制し、脂肪の分解を促進することが明らかとなった。

キーワード：ガルシニアエキス、3T3-L1細胞、脂肪蓄積、脂肪分解、肥満、画像処理。