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Reduction of leptin secretion by soy isoflavonoids in murine adipocytes *in vitro*

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ABSTRACT

Daidzein and genistein are the main aglycones of soy isoflavonoid, and have many useful activities *in vitro* and *in vivo*. However, equol, a metabolite of daidzein *in vivo*, has attracted attention due to its stronger activity than that of the naturally occurring isoflavonoids. We subjected the soy isoflavonoids, including the naturally occurring (*S*)-equol, to mouse adipocytes, and compared the inhibitory activity on the leptin secretion. Equol, daidzein and genistein inhibited the leptin secretion, whereas *O*-desmethylangolensin had a lower activity. The inhibitory activity of the isoflavones was not affected by the addition of an iNOS inhibitor and an estrogen.

1. Introduction

Recently, an increasing number of obese people have been observed in not only Western countries, but also some Asian countries. It causes not only weight gain, but also many health problems such as diabetes, atherosclerosis and coronary heart disease. The relationships between obesity and these risks are still not established. However, adipocytes, which significantly accumulate in an overweight person, have been suggested as a producer of adipocytokines such as tumor necrosis factor-α, interleukins and adiponectin. Leptin was originally isolated from the obese gene as an endogenous inhibitor of appetite (Zhang et al., 1994), and also secreted by adipocytes. Leptin supplementation has been successfully used to control the body fat mass (Halaas et al., 1997), but the serum leptin level in an obese person is higher than that of normal-weight subjects (Maffei et al., 1995). The controversial results of the leptin concentration in the serum were explained by the leptin resistance (Halaas et al., 1997). Recent studies suggested that the role of leptin is not only dietary control, but also concerns many functions including the health risks (Onuma et al., 2003; Pai et al., 2005). Thus, the reduction of the serum leptin seems to be important for human health, especially for an obese person.

Soy isoflavonoids have become the focus of maintaining human health. Daidzein and genistein have many useful activities (Zhang et al., 1999). However, recent studies suggested the importance of equol, a metabolite of daidzein, because it has a higher activity than that of the parent compound (reviewed by Atkinson et al., 2005). Not all humans produce the useful metabolite because equol is produced by intestinal microflora, which depends on the individual (Fig. 1). Daidzein is also metabolized to *O*-desmethylangolensin (*O*-DMA) in certain amounts in a person (Kelly et al., 1993). Thus, it seems that the soy isoflavonoid activity *in vivo* depends on the individuals due to the metabolic ability. However, the activity of the metabolites have still not been clarified.

Soy supplementations reduce the leptin *in vivo* (Noriega-López et al., 2007; Cederroth et al., 2008), which had been ascribed to the reduced adipocytes. We now examined the effect of soy isoflavonoids, including the metabolite, for the leptin synthesis on the adipocytes *in vitro*.

2. Results

From the reported activity of genistein on the reduction of leptin from rat adipocytes (Szkudelski et al., 2005), we thought that the antioxidative activity would be related to the action. We compared the inhibitory activity of the soy isoflavonoids to that of other strong antioxidants, epigallocatechin gallate (EGCG) and chlorogenic acid. We also applied equol and *O*-DMA, the metabolites of daidzein, to evaluate the effect of the microfloral metabolic activity of daidzein *in vivo*. As shown in Fig. 2a, daidzein and equol effectively reduced the leptin secretion as much as genistein. However, EGCG, chlorogenic acid and *O*-DMA were less effective. Under the experimental conditions, the applied samples

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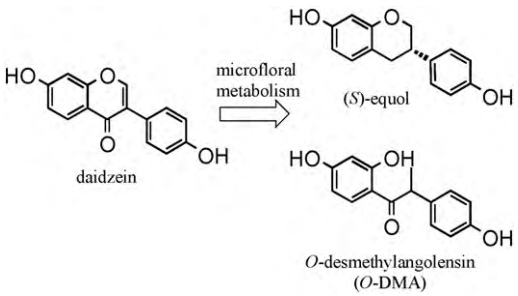

Fig. 1. Microbial metabolism of daidzein.

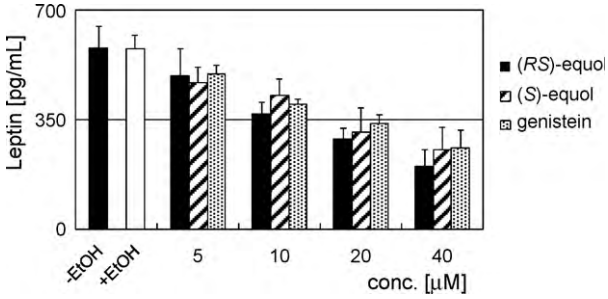

Fig. 3. Comparison of the inhibitory activity of genistein, (RS)- and (S)-equol. The adipocyte cells were treated with samples ranging from 5 to 40 μM. The leptin contents were measured as described in Fig. 2. Data represent the mean ± SD of 3 experiments.

had no cytotoxicity on the cells evaluated by the MTT methods (Fig. 2b). Therefore, the reduced leptin was not derived from the cell damage. Genistein generally has a stronger activity than that of daidzein (Zhang et al., 1999). We compared the dose dependent activity of genistein, (*RS*)-equol and its natural (*S*)-form. These samples dose dependently reduced the leptin secretion of the 3T3-L1 cells. However, there was no remarkable difference among the employed samples (Fig. 3).

Leptin secretion was affected by many compounds including the endogenous materials. Treatment with iNOS inhibitors increased the leptin secretion reduced by IFN-γ-LPS (Unno et al., 2006). We then added N^{ω}-nitro-L-arginine methyl ester (L-NAME), an iNOS inhibitor, to the cells treated with isoflavonoids to elucidate the effect of NO. Under our conditions, the iNOS inhibitor itself had no apparent effect on the leptin secretion from the adipocytes (Fig. 4a). The L-NAME also failed to recover the decreased leptin secretion caused by the isoflavonoids.

Leptin is increased by sex steroid hormones (Machinal et al., 1999), and soy isoflavonoids have a weak estrogenic activity (Zhang et al., 1999; Muthyala et al., 2004). It could be possible that the soy isoflavonoids acted as estrogen receptor antagonists in the adipocytes. We then applied estradiol to adipocytes for the evaluation of the effect on the estrogen receptors. However, 17β-estradiol had no apparent effect on the leptin secretion with or without the soy isoflavonoids (Fig. 4b).

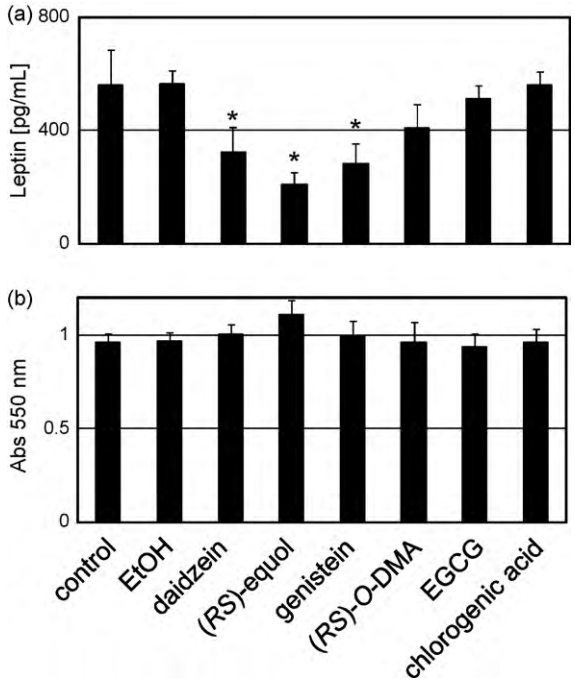

Fig. 2. Inhibitory effect of polyphenols on leptin secretion from adipocytes. The adipocytes differentiated from 3T3-L1 cells were treated with 40 μM samples and cultured for 2 days. (a) The mediums were collected and the leptin contents were measured using a commercially available ELISA kit. (b) The cell viability was evaluated by the MTT method. Data represent the mean ± SD of 3 experiments. $^{*}p < 0.05$ vs. Vehicle (EtOH).

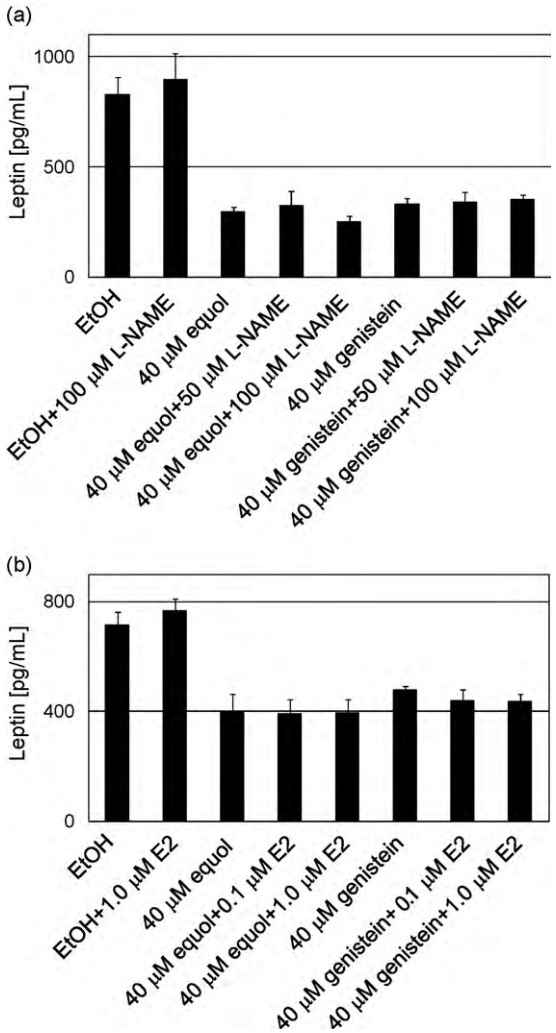

Fig. 4. Effect of an iNOS inhibitor and an estrogen on the soy isoflavonoids-reduced leptin secretion. The adipocyte cells were treated with 40 μM (*RS*)-equol or genistein, with or without (a) L-NAME and (b) 17β-estradiol (E2). The leptin contents were measured as described in Fig. 2. Data represent the mean ± SD of 3 experiments.