Ghrelin is a 28-amino acid peptide hormone with orexigenic effect and has gained interest in the field of obesity treatment. It is mainly synthesized in the stomach. Ghrelin circulates in two forms: octanoylated ghrelin with an octanoyl moiety at the serine-3 residue, and des-acyl ghrelin without any acyl-modification. Octanoylated ghrelin stimulates appetite, but des-acyl ghrelin does not. Ghrelin O-acyltransferase (GOAT) and prohormone convertase (PC) are involved in the processing of proghrelin to octanoylated ghrelin. GOAT catalyzes proghrelin octanoylation, PCs including furin, PC1/3, or PC2, cleave C-terminal amino acids of proghrelin. Inhibition of octanoylated ghrelin production and secretion has been considered effective for obesity treatment.

Our laboratory previously developed a cell-based assay system using the ghrelin-expressing cell line, AGS-GHRL8, established by transfecting the human ghrelin gene into AGS human gastric carcinoma cells. In this study, phytochemicals having inhibitory effects on octanoylated ghrelin production and secretion were explored using AGS-GHRL8 cells, and their effects were examined in vivo. Furthermore, the mechanisms underlying the inhibition of octanoylated ghrelin production and secretion were examined.

1. Exploration of compounds having inhibitory effects on octanoylated ghrelin production and secretion

The effects of six triterpenes (asiatic acid, betulinic acid, corosolic acid, glycyrrhetinic acid, oleanolic acid, and ursolic acid), caffeic acid, chlorogenic acid, citric acid, and epigallocatechin gallate (EGCG) on octanoylated ghrelin levels were examined. Triterpenes, except for betulinic acid, and EGCG decreased octanoylated ghrelin levels. Epigallocatechin and gallic acid, hydrolysates of EGCG, had no effect on octanoylated ghrelin levels in AGS-GHRL8 cells, suggesting that EGCG itself is the active molecule involved in lowering octanoylated ghrelin levels.

2. Inhibitory effect of phytochemicals in vivo

The effects of oleanolic acid and EGCG on octanoylated ghrelin levels were evaluated in mice. Oral administration of oleanolic acid did not influence octanoylated ghrelin levels and body weight gain in high-fat and high-glucose diet-fed mice. In standard diet-fed mice, oleanolic acid significantly reduced plasma octanoylated ghrelin levels and body weight gain. Oral administration of TEAVIGO®, a green tea extract containing >94% EGCG, significantly reduced plasma octanoylated ghrelin levels. Oleanolic acid and EGCG have been reported to
show anti-obesity effects in mice. The results of this study suggest that the decrease in octanoylated ghrelin levels may be involved in the anti-obesity effects of oleanolic acid and EGCG.

3. Mechanism underlying the inhibition of octanoylated ghrelin production and secretion by phytochemicals

The involvement of decreased expression of related genes in the reduction of octanoylated ghrelin levels by triterpenes and EGCG was investigated. Five triterpenes which reduced octanoylated ghrelin levels in AGS-GHRL8 cells, and EGCG did not decrease GOAT and furin mRNA expression levels, implying that gene expression is not related to the inhibitory effects in AGS-GHRL8 cells. Oleanolic acid had no effect on the mRNA expression of ghrelin, GOAT, furin, PC1/3, or PC2 in mice stomach. In the stomach of TEAVIGO®-treated mice, the mRNA expression of ghrelin and PC1/3 was significantly downregulated, suggesting the involvement in the reduction of plasma octanoylated ghrelin levels in TEAVIGO®-treated mice.

Octanoyl coenzyme A (CoA) is a substrate for GOAT in the octanoylation of a serine-3 residue in ghrelin. Octanoyl CoA is formed by dehydrocondensation between a carboxyl group in octanoic acid and a CoA thiol group. It is thus possible that a triterpene having a carboxyl group may competitively inhibit the binding of octanoic acid to CoA. Therefore, the relationship between the presence of carboxyl group in triterpenes and the inhibitory effect on octanoylated ghrelin levels was investigated using β-amyrin and uvaol, which have similar structures to those of oleanolic acid and ursolic acid, except that the carboxyl group is substituted with methyl and hydroxymethyl groups, respectively. Oleanolic acid but not β-amyrin suppressed octanoylated ghrelin levels in AGS-GHRL8 cells. Ursolic acid significantly lowered octanoylated ghrelin levels, whereas uvaol had a slight suppressive effect. These findings indicate that the carboxyl group in triterpenes might be important for the suppressive effect on octanoylated ghrelin production and secretion.

In conclusion, triterpenes and EGCG have an inhibitory effect on octanoylated ghrelin production and secretion. The carboxyl group in triterpenes may be involved in the inhibitory effect. Downregulation of ghrelin and PC1/3 mRNA expression may be related to the suppression of octanoylated ghrelin levels in mice by EGCG. The data presented here suggest that triterpenes and EGCG could be developed as a novel prevention strategy for obesity that acts via the suppression of octanoylated ghrelin production and secretion.