HOKKAIDO UNIVERSITY

Title	Study on Factors Related to the Effects of Edible Mushroom-Derived Sphingomyelin Synthase Inhibitors on Obesity
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Description	この博士論文の全文は利用可能日以降に公開されます。利用可能日以前の閲覧方法は https://www.lib.hokudai.ac.jp/dissertations/copy-guides/ をご参照ください。
Degree Grantor	北海道大学
Degree Name	博士(生命科学)
Dissertation Number	乙第7235号
Issue Date	2026-03-25
DOI	https://doi.org/10.14943/doctoral.r7235
Doc URL	https://hdl.handle.net/2115/99851
Type	doctoral thesis
File Information	Enkhmaa_Enkhbat_summary.pdf, 全文の要約

Hokkaido University Collection of Scholarly and Academic Papers : HUSCAP

Summary of Doctoral Dissertation

Degree requested: Doctor of Life Science Applicant's name Enkhmaa Enkhbat

Title of Doctoral Dissertation

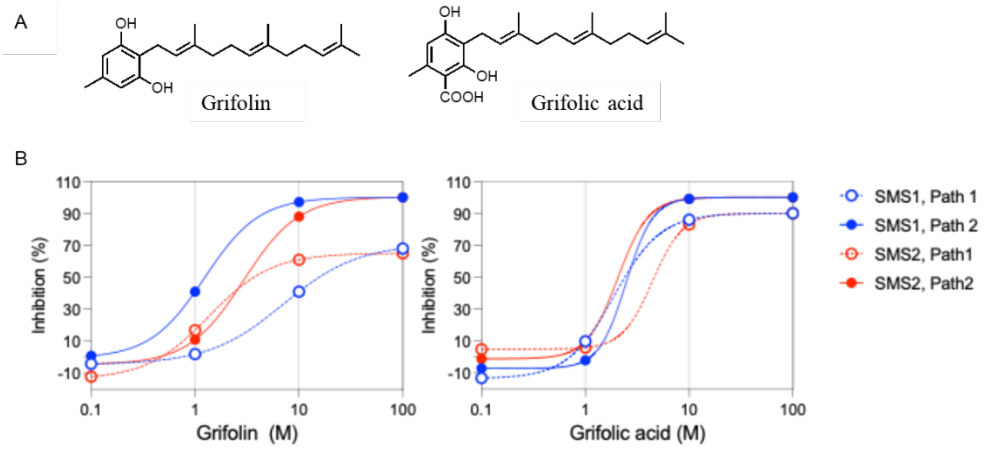
Study on Factors Related to the Effects of Edible Mushroom–Derived Sphingomyelin Synthase Inhibitors on Obesity
(食用キノコ由来スフィンゴミエリン合成酵素阻害剤の肥満等に及ぼす影響要因に関する研究)

Recently, obesity and vitamin D deficiency have been increasingly recognized as two major and interrelated public health concerns that contribute to the development of metabolic syndrome, insulin resistance, and fatty liver disease. Their coexistence reflects a disruption of lipid metabolism and endocrine regulation, yet the biochemical mechanisms linking these two physiological processes remain insufficiently understood.

It has been argued that imbalances in sphingolipid metabolism, particularly the altered conversion between ceramide and sphingomyelin, play a central role in these disorders. The enzyme sphingomyelin synthase (SMS), which catalyzes the formation of sphingomyelin and diacylglycerol from ceramide and phosphatidylcholine, functions as a crucial metabolic regulator. Abnormally elevated SMS activity promotes lipid accumulation and inflammation in hepatocytes. Although synthetic inhibitors of SMS have been developed for experimental use, the discovery of naturally occurring, food-derived inhibitors with physiological compatibility and nutritional safety remains an unexplored research frontier.

In this study, we screened 212 mushroom species and 650 plant materials collected across Japan to identify natural sphingomyelin synthase (SMS) inhibitors and evaluate their biological effects on obesity and vitamin D homeostasis. A high-performance liquid chromatography (HPLC)–based fluorescence assay was employed to measure sphingomyelin synthase (SMS) activity in vitro, and the extract of *Albatrellus confluens* exhibited the strongest inhibitory potential among all tested samples.

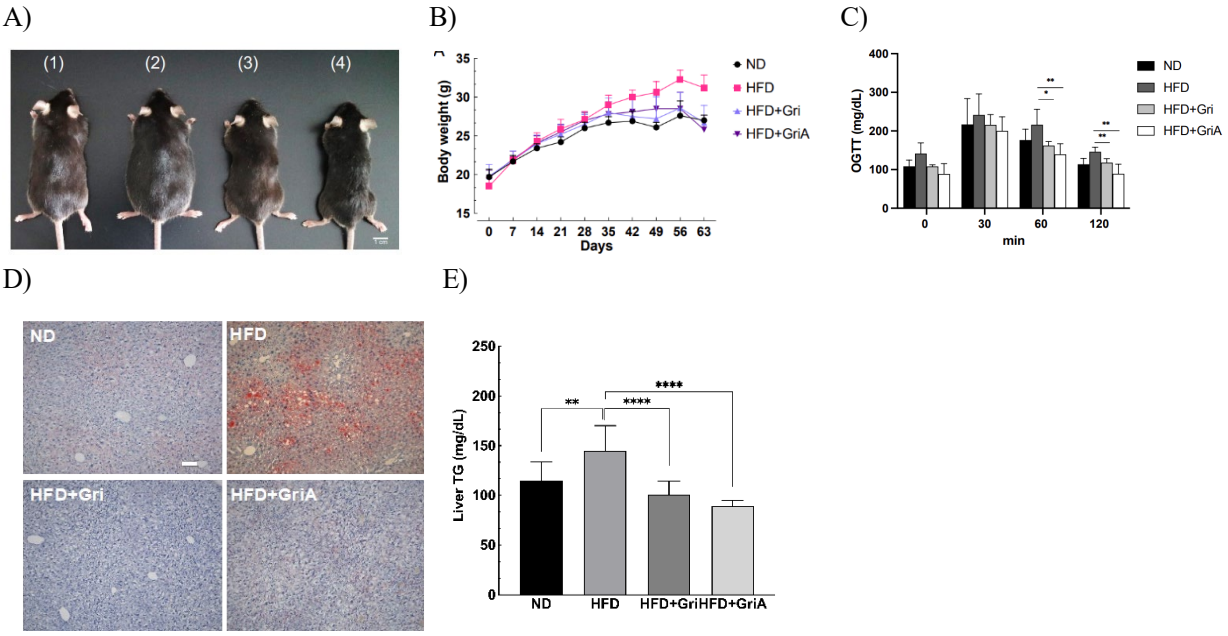
Subsequent chromatographic purification and spectral analyses using NMR and high-resolution mass spectrometry led to the isolation and structural identification of two phenolic compounds, grifolin and grifolic acid. Both compounds exhibited potent and selective inhibition toward SMS1 and SMS2 isoforms, with half-maximal inhibitory concentrations (IC_{50}) ranging from 0.6 to 18 μM, and effectively reduced lipid-droplet formation in cultured HepG2 cells without any cytotoxic effect.



A) Chemical structures of grifolin and grifolic acid; B) Dose–dependent inhibition of SMS1 and SMS2

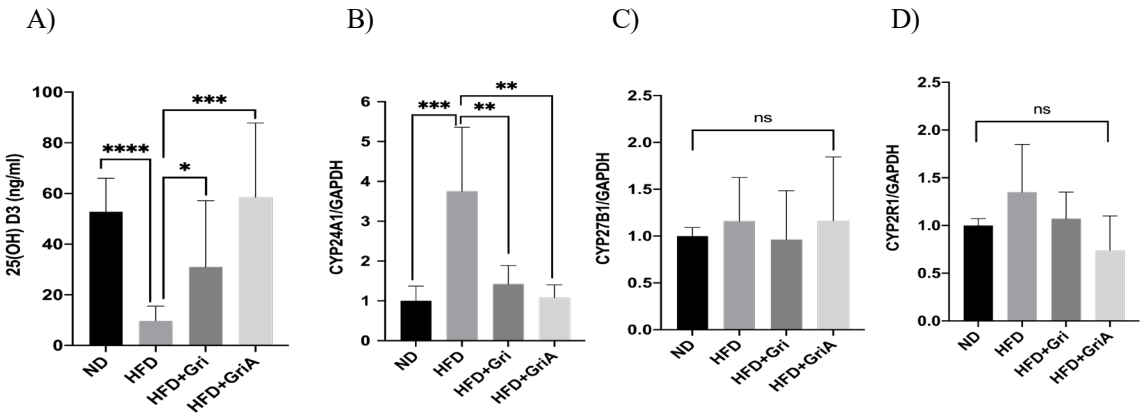
To evaluate their physiological efficacy, male C57BL/6J mice were fed a high-fat diet (HFD) supplemented with 0.1 % grifolin or grifolic acid for nine weeks. The treatment groups exhibited 14 – 17 % lower body-weight gain,

improved glucose tolerance, and markedly reduced hepatic lipid accumulation compared with untreated controls. Lipidomic analysis confirmed significant decreases in hepatic sphingomyelin and diacylglycerol levels, consistent with suppression of SMS activity and re-establishment of a balanced ceramide/sphingomyelin ratio.



A) Representative images of mice; (B) Weekly body weight gain; C) Oral glucose tolerance test; D) Representative Oil Red O–stained liver sections; E) Hepatic triglyceride levels (mg/dL);

Moreover, serum 25-hydroxyvitamin D_3 concentrations were preserved in treated mice, accompanied by reduced expression of renal CYP24A1 and unchanged expression of CYP27B1 and CYP2R1, suggesting inhibition of vitamin D catabolism and maintenance of vitamin D metabolic stability. At the molecular level, biochemical assays and gene-expression profiling demonstrated normalization of hepatic lipid homeostasis and up-regulation of genes associated with lipid catabolism, transport, and storage regulation. Together, these findings indicate that SMS inhibition by mushroom-derived metabolites can simultaneously influence sphingolipid signaling and vitamin D metabolic pathways, leading to broad improvements in lipid and hormonal balance.



A) Serum 25(OH)D_3 concentrations; B) Renal CYP24A1 expression; C) Hepatic CYP27B1 expression; (D) Hepatic CYP2R1 expression;

Structural analysis of the grifolin framework revealed an amphipathic configuration with both hydrophilic and hydrophobic domains, resembling sphingosine. This structural similarity suggests that the compounds may interact with sphingomyelin synthase in a substrate-mimicry manner, contributing to their inhibitory effect. Importantly, the observed metabolic benefits occurred independently of caloric restriction, implying that the anti-

obesity effects arise primarily from biochemical regulation rather than reduced energy intake.

In conclusion, this study demonstrates that grifolin and grifolic acid act as dual-substrate SMS inhibitors with beneficial effects on obesity, glucose tolerance, hepatic lipid accumulation, and vitamin D metabolism in a DIO mouse model. These findings highlight the potential of mushroom-derived natural products as functional food ingredients or nutraceuticals for the prevention and management of obesity and related metabolic disorders. By targeting both lipid signaling and vitamin D pathways, grifolin and grifolic acid represent a unique class of multifunctional bioactives that warrant further investigation in translational and clinical settings.