DOI:10.1111/j.1600-0625.2008.00831.x
www.blackwellpublishing.com/EXD

Letter to the Editor

# Augmentation of sebaceous lipogenesis by an ethanol extract of *Grifola frondosa* (Maitake mushroom) in hamsters *in vivo* and *in vitro*

**Mie Nagao[1,2,3], Takashi Sato[1], Noriko Akimoto[1], Yuya Kato[1,2], Masao Takahashi[2,3] and Akira Ito[1]**

[1]Department of Biochemistry and Molecular Biology, School of Pharmacy, Tokyo University of Pharmacy and Life Sciences, Hachioji, Tokyo, Japan;
[2]Heimat Co Ltd, Chuo-ku, Tokyo, Japan;
[3]Immuno Research Ltd, Auckland, New Zealand
*Correspondence*: Takashi Sato, PhD, Department of Biochemistry and Molecular Biology, School of Pharmacy, Tokyo University of Pharmacy and Life Sciences, 1432-1 Horinouchi, Hachioji, Tokyo 192-0392, Japan, Tel.: +81 42 676 5717, Fax: +81 42 676 5734, e-mail: satotak@ps.toyaku.ac.jp

*Accepted for publication 12 November 2008*

**Abstract:** *Grifola frondosa* (Maitake mushroom) is an edible and medicinal mushroom with versatile effects such as antitumor and immunomodulating actions. Here, we demonstrated that an ethanol extract of *G. frondosa* fruiting body (Maitake extract) augmented intracellular lipid droplet formation and the production of triacylglycerols (TG), a major component of sebum, along with the activation of diacylglycerol acyltransferase, a rate-limiting enzyme of TG synthesis in cultured hamster sebocytes. The topical treatment of Maitake extract on the skin of hamster auricles augmented sebum accumulation in sebaceous glands and ducts. However, in comparison with the Maitake extract, another ethanol extract prepared from *Agaricus blazei* Murill showed less activity in sebaceous lipogenesis in hamsters *in vivo* and *in vitro*. These results provide novel evidence that Maitake extract augments sebaceous lipogenesis in hamsters *in vivo* and *in vitro*. Thus, Maitake extract is likely to be a unique agent leading to the remission of dry skin.

**Key words:** *Grifola frondosa* – sebaceous lipogenesis – sebocytes – xeroderma

*Please cite this paper as:* Augmentation of sebaceous lipogenesis by an ethanol extract of *Grifola frondosa* (Maitake mushroom) in hamsters *in vivo* and *in vitro*. Experimental Dermatology 2009; 18: 730–733.

## Background

Sebaceous gland cells (sebocytes) differentiate to accumulate abundant cytoplasmic lipids, and this results in the secretion of lipids as sebum for the formation of physiological barrier on the skin (1,2). Regarding sebaceous lipogenesis disorders, an excess secretion of sebum has been reported to cause the onset of acne vulgaris and seborrhoea, which are two of the most common skin diseases (2). In contrast, a decrease in sebum secretion has been associated with dry skin (xerosis), the development of which is related to ageing and environmental conditions (3,4). Therefore, pharmacological improvement of sebaceous gland functions is likely to result in the restoration of skin homeostasis (1).

**Abbreviations:** Agaricus extract, an ethanol extract of *Agaricus blazei* Murill; DGAT, diacylglycerol acyltransferase; DMEM/F12, Dulbecco's modified Eagle's medium/Ham's F12; Maitake extract, an ethanol extract of *Grifola frondosa* fruiting body; TG, triacylglycerols.

Edible mushrooms have been reported to be effective for treating disorders including those of the immune system, viral and bacterial infections and cancers (5). *Grifola frondosa* (Maitake mushroom) is a versatile medicinal mushroom with beneficial pharmacological activities (6), and its water-soluble extracts including polysaccharides termed D-fraction have been reported to exhibit antitumorigenesis, immunomodulating, antidiabetes and antiviral activities (6–9). In addition, a clinical study by Kodama et al. (10) has demonstrated that $\beta$-1,6-glucan with $\beta$-1,3 branched chains derived from *G. frondosa*, which has been termed MD-fraction, exerts regression and improvement of symptoms in liver, breast and lung cancer patients. However, the effect of *G. frondosa* on sebaceous lipogenesis remains unclear.

## Questions addressed

The aim of this study was to address whether or not *G. frondosa* exhibits a biological activity to modulate


 

sebaceous lipogenesis, which may be associated with the treatment of sebaceous disorders such as acne or xerosis.

## Experimental design

Ethanol extracts of Maitake (Maitake extract) and Agaricus (Agaricus extract) were prepared from dried powders of *G. frondosa (*Maitake mushroom*)* fruiting body (Hokuto Co, Nagano, Japan) and *Agaricus blazei* Murill myceria (Kyowa Hakko Kogyo Co, Tokyo, Japan) (Appendix S1, Preparation of ethanol extracts of *Grifola frondosa* (Maitake mushroom) fruiting body and *Agaricus blazei* Murill myceria). Hamster sebocytes (11) and auricle skin were treated with Maitake extract (100–400 $\mu$g/ml), Agaricus extract (100–400 $\mu$g/ml), or vehicle solution at the respective concentrations (Appendix S1, Cell culture and treatments and *In-vivo* treatment of Maitake extract). Lipid droplets in hamster sebocytes and sebum in the sebaceous glands and ducts of frozen auricle tissues were stained with oil red O (Sigma Chemical, St. Louis, MO, USA) (11,12), and then the intensity of oil red O was quantified by an image analysis system, Lumina Vision (Ver. 2.2.2; Mitani, Fukui, Japan) (Appendix S1, Oil red O staining) (13). Lipid composition of the extracts of the intracellular lipids, and diacylglycerol acyltransferase (DGAT) activity in hamster sebocytes were analysed as previously described (11,14) (see Supporting Information).

## Results

*In-vitro* image analysis of the oil red O stained cells showed that the intracellular levels of sebum was dose-dependently augmented by Maitake extract (Fig. 1a). The augmentation of sebum production by Maitake extract (400 $\mu$g/ml) was greater than that by insulin (10 nM). The increased sebum was found to mostly consist of triacylglycerols (TG), a major component of sebum in both humans and hamsters (11), while there was a slight but significant increase in the level of free fatty acids and no change in cholesterol, cholesterol ester and wax ester in the Maitake extract-treated cells (Fig. 1b). Furthermore, the augmented TG production resulted from an increase in the activity of DGAT, a rate-limiting enzyme of TG synthesis (15), in the hamster sebocytes (Fig. 1b, inserted panel). On the other hand, Agaricus extract could be seen to augment intracellular lipid droplet formation, but this augmentation was significantly less than that of the Maitake extract at all respective concentrations (100–400 $\mu$g/ml) (Fig. 1a and Fig. S1). We also confirmed no augmentation of sebum production in hamster sebocytes treated with the same volume of ethanol at the respective concentrations in the control treatment (data not shown). These results indicate that the Maitake extract facilitates sebum production via the stimulation of mainly *de novo* TG synthesis in hamster sebocytes, and further that the sebaceous lipogenic activity is predominant when treated with the Maitake extract rather than with the Agaricus extract.

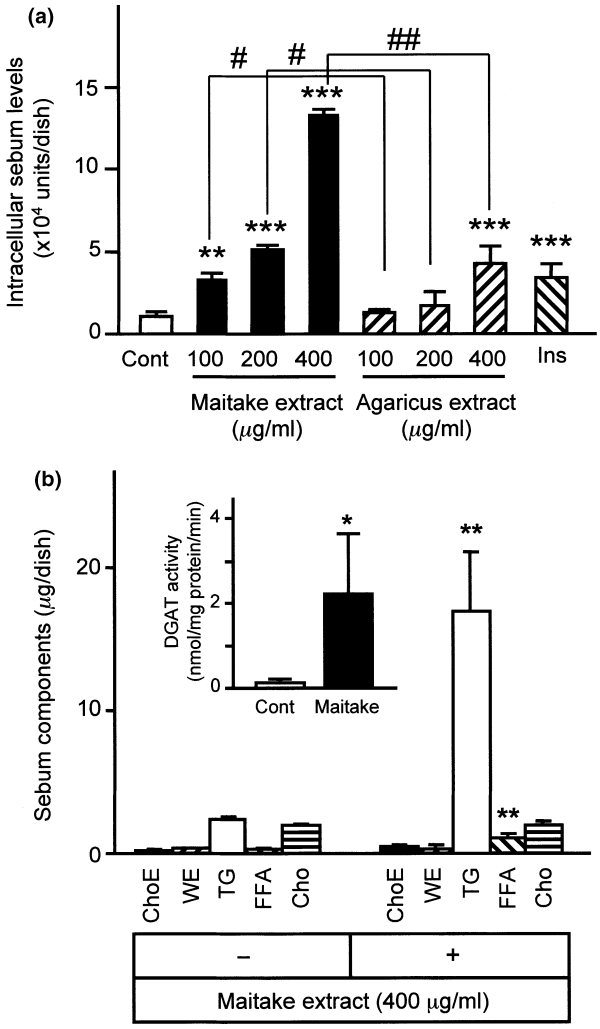


**Figure 1.** Augmentation of intracellular sebum accumulation and characterization of sebum composition in Maitake and Agaricus extracts-treated hamster sebocytes. (a) Hamster sebocytes at the third passage were treated every 2 days over a period of 6 days with Maitake extract (100–400 $\mu$g/ml), Agaricus extract (100–400 $\mu$g/ml), or insulin (Ins) (10 nM), and then the levels of oil red O stained sebum in the cells were quantified by image analysis as described in the Experimental design. (b) The levels of sebum components in the cell lysate in Maitake extract (400 $\mu$g/ml)-treated hamster sebocytes were quantified by automatic thin-layer chromatography as described in the Experimental design. An inserted panel indicates an increase in DGAT activity in the Maitake extract (400 $\mu$g/ml)-treated cells. Data are shown as mean ± standard deviation of three individual dishes. *, ** and ***, significantly different from untreated cells (Cont) ($P < 0.05$, 0.01 and 0.001, respectively). # and ##, significantly different from Maitake extract-treated cells at each concentration ($P < 0.05$ and 0.01, respectively). ChoE, cholesterol esters; WE, wax esters; TG, triacylglycerols; FFA, free fatty acids; and Cho, cholesterol.

 

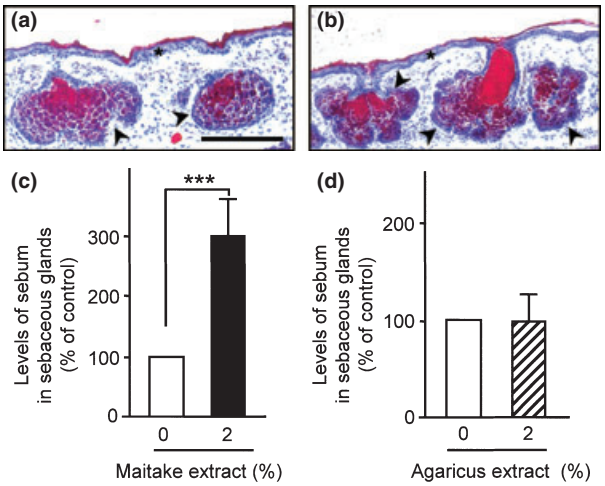


**Figure 2.** Effects of the Maitake extract, but not that of Agaricus, on sebum accumulation in sebaceous glands in the skin of hamster auricles. Left auricles in hamsters were treated every day for 2 weeks with 50 $\mu$l of Maitake extract (1 and 2%) or Agaricus extract (1 and 2%) in 95% ethanol/5% glycerol. As the control, the right auricle skin of the same hamsters was similarly treated with a vehicle solution. The fixed tissues were stained with oil red O, and then typical data in the case of the 2% Maitake-extract treatment are shown. (a, b) Vehicle- and Maitake extract-treated skin, respectively. Arrowheads and asterisks indicate sebaceous glands and epidermis, respectively. Bar 300 $\mu$m. (c, d) The relative amounts of sebum accumulated in sebaceous glands were quantified from three individual areas of the skin treated with Maitake extract (c) or Agaricus extract (d), and then were expressed by taking vehicle-treated hamsters as 100%. Data are shown as mean ± standard deviation of three independent areas. ***Significantly different from the vehicle-treated hamsters (white column) ($P < 0.001$).

*In-vivo* image analysis of the oil red O stained tissues revealed that 2% Maitake extract augmented 2.8-fold sebum accumulation in sebaceous glands and ducts ($P < 0.001$) (Fig. 2a–c). A similar augmentation of sebum accumulation was observed in 1% Maitake extract-treated skin in hamsters (1.6-fold, data not shown). In contrast, there was no change in the amount of sebum in sebaceous glands and ducts in the Agaricus extract-treated hamsters (Fig. 2d). Therefore, these results indicate that topical application of Maitake extract exhibits *in vivo* lipogenic activity in hamster sebaceous glands.

## Conclusions

As natural dietary agents such as flavonoids have been reported to suppress sebaceous lipogenesis in hamsters and humans *in vivo* and *in vitro* (16–19), to the best of our knowledge, there is no natural agent to stimulate sebum production in sebaceous glands. In this study, we demonstrated for the first time that an ethanol extract of Maitake mushroom fruiting body exhibits a stimulatory effect on sebum production through DGAT-dependent augmentation of TG synthesis in hamster sebaceous glands *in vivo* and *in vitro*. Therefore, Maitake extract is likely to be a natural promoter for sebum production that may be useful for the maintenance of skin barrier function.

Other medicinal extracts from *A. blazei* and *Agaricus bisporus* have been reported to exhibit anti-inflammatory, antioxidant, antitumor and immunoenhancing actions (20,21), which are common properties among edible and medicinal mushrooms including *G. frondosa* (Maitake) (6–8). In this study, however, we found that the stimulatory effect of Agaricus extract on sebaceous lipogenesis was significantly weaker or negligible in comparison with that of Maitake extract both *in vitro* and *in vivo*. Therefore, our results strongly suggest that the inducible effect of sebum production is predominant when treated with the Maitake extract rather than with the Agaricus extract.

It has been reported that a water-soluble fraction of Maitake mushroom, termed MD-fraction, effectively diminishes cancer progression in liver, breast and lung tumors (10). Our finding that another Maitake extract prepared similarly with a 60% ethanol solution did not exert lipogenesis stimulation in hamster sebocytes (data not shown). In light of this result and a previous report of Gu and Belury (22), we feel that the pharmacological effects of Maitake extract may be because of a hydrophobic component(s) rather than a hydrophilic one(s). Further experiments are needed to identify the crucial component(s) of Maitake extract for the activation of sebaceous lipogenesis.

In conclusion, these results provide novel evidence that Maitake extract is a natural product for the augmentation of sebaceous lipogenesis. Moreover, Maitake extract may be useful for the improvement of dry skin and xeroderma associated with an ageing-dependent decrease of sebum production (23). We look forward to further investigation of this aspect of Maitake extract in a clinical setting.

## Acknowledgements

This work was supported in part by a grant to private universities provided by The Promotion and Mutual Aid Corporation for Private Schools of Japan, and by Research Fund of Mitsukoshi Health and Welfare Foundation 2008. The authors wish to thank Mr T. Ogura and Ms S. Tashiro for their technical assistance.

## Supporting Information

Additional Supporting Information may be found in the online version of this article:

**Appendix S1**. Cell culture, treatments, and oil red O staining.

**Figure S1**. Effect of Maitake mushroom and Agaricus extracts on lipid droplet formation in hamster sebocytes. Hamster sebocytes at the third passage were treated every 2 days over a period of 6 days with or without Maitake (400 $\mu$g/ml) or Agaricus extracts (400 $\mu$g/ml). Cells were fixed and then stained with oil red O as described in the Experimental design section. a, untreated cells; b, Maitake extract (400 $\mu$g/ml)-treated cells; and c, Agaricus extract (400 $\mu$g/ml)-treated cells. Bar 50 $\mu$m.


 

## References


1 Zouboulis C C, Baron J M, Böhm M *et al.* Frontiers in sebaceous gland biology and pathology. Exp Dermatol 2008: **17**: 542–551.
2 Zouboulis C C, Schagen S, Alestas T. The sebocyte culture: a model to study the pathophysiology of the sebaceous gland in sebostasis, seborrhea and acne. Arch Dermatol Res 2008: **300**: 397–413.
3 Fenske N A, Lober C W. Structural and functional changes of normal aging skin. J Am Acad Dermatol 1986: **15**: 571–585.
4 Zouboulis C C, Boschnakow A. Chronological ageing and photoageing of the human sebaceous gland. Clin Exp Dermatol 2001: **26**: 600–607.
5 Chang R. Functional properties of edible mushrooms. Nutr Rev 1996: **54**: S91–S93.
6 Mayell M. Maitake extracts and their therapeutic potential. Altern Med Rev 2001: **6**: 48–60.
7 Inoue A, Kodama N, Nanba H. Effect of maitake (*Grifola frondosa*) D-fraction on the control of the T lymph node Th-1/Th-2 proportion. Biol Pharm Bull 2002: **25**: 536–540.
8 Manohar V, Talpur N A, Echard B W, Lieberman S, Preuss H G. Effects of a water-soluble extract of maitake mushroom on circulating glucose/insulin concentrations in KK mice. Diabetes Obes Metab 2002: **4**: 43–48.
9 Preuss H G, Echard B, Bagchi D *et al.* Enhanced insulin–hypoglycemic activity in rats consuming a specific glycoprotein extracted from maitake mushroom. Mol Cell Biochem 2007: **306**: 105–113.
10 Kodama N, Komuta K, Nanba H. Can maitake MD-fraction aid cancer patients? Altern Med Rev 2002: **7**: 236–239.
11 Sato T, Imai N, Akimoto N, Sakiguchi T, Kitamura K, Ito A. Epidermal growth factor and 1$\alpha$,25-dihydroxyvitamin $D_3$ suppress lipogenesis in hamster sebaceous gland cells *in vitro*. J Invest Dermatol 2001: **117**: 965–970.
12 Akimoto N, Sato T, Iwata C *et al.* Expression of perilipin A on the surface of lipid droplets increases along with the differentiation of hamster sebocytes *in vivo* and *in vitro*. J Invest Dermatol 2005: **124**: 1127–1133.
13 Kuwahara M, Sugimoto M, Tsuji S *et al.* Cytosolic calcium changes in a process of platelet adhesion and cohesion on a von Willebrand factor-coated surface under flow conditions. Blood 1999: **94**: 1149–1155.
14 Iwata C, Akimoto N, Sato T *et al.* Augmentation of lipogenesis by 15-deoxy-$\Delta^{12,14}$-prostaglandin $J_2$ in hamster sebaceous glands: identification of cytochrome P-450-mediated 15-deoxy-$\Delta^{12,14}$-prostaglandin $J_2$ production. J Invest Dermatol 2005: **125**: 865–872.
15 Turkish A, Sturley S L. Regulation of triglyceride metabolism. I. Eukaryotic neutral lipid synthesis: ''many ways to skin ACAT or a DGAT''. Am J Physiol Gastrointest Liver Physiol 2007: **292**: G953–G957.
16 Seki T, Morohashi M. Effect of some alkaloids, flavonoids and triterpenoids, contents of Japanese–Chinese traditional herbal medicines, on the lipogenesis of sebaceous glands. Skin Pharmacol 1993: **6**: 56–60.
17 Chen W, Yang C C, Sheu H M *et al.* Expression of peroxisome proliferator-activated receptor and CCAAT/enhancer binding protein transcription factors in cultured human sebocytes. J Invest Dermatol 2003: **121**: 441–447.
18 Sato T, Takahashi A, Kojima M *et al.* A citrus polymethoxy flavonoid, nobiletin inhibits sebum production and sebocyte proliferation, and augments sebum excretion in hamsters. J Invest Dermatol 2007: **127**: 2740–2748.
19 Sato T, Ito A. Beneficial actions of citrus polymethoxy flavonoids in treating acne. Clin Dermatol Retinoids Other Treat 2008: **24**: 22–26.
20 Kimura Y, Kido T, Takaku T *et al.* Isolation of an anti-angiogenic substance from *Agaricus blazei* Murill: its antitumor and antimetastatic actions. Cancer Sci 2004: **95**: 758–764.
21 Yuminamochi E, Koike T, Takeda K *et al.* Interleukin-12- and interferon-$\gamma$-mediated natural killer cell activation by *Agaricus blazei* Murill. Immunology 2007: **121**: 197–206.
22 Gu Y H, Belury M A. Selective induction of apoptosis in murine skin carcinoma cells (CH72) by an ethanol extract of Lentinula edodes. Cancer Lett 2005: **220**: 21–28.
23 Makrantonaki E, Zouboulis C C. Molecular mechanisms of skin aging: state of the art. Ann N Y Acad Sci 2007: **1119**: 40–50.


 