# Attitudes of pregnant Japanese women and folic acid intake for the prevention of neural tube defects: A nationwide Internet survey

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**Key words** : pregnancy, women, folic acid, attitudes, dietary supplements, Internet questionnaire

**Objectives** Folic acid intake is recommended for pregnant women because it significantly reduces the risk of neural tube defects (NTD) in the fetus. However, the risk of NTD remains medium in Japan. In this study, the attitudes of pregnant Japanese women and factors related to folic acid intake for the prevention of NTD were evaluated using a nationwide survey.

**Methods** An Internet-based questionnaire was conducted on 2,367 pregnant Japanese women who were registrants of a Japanese social research company in January 2012; 1,236 of these women responded. In the questionnaires, the knowledge regarding the folate intake (i.e., name of folic acid, the risk of NTD, recommended doses, and timing), actual intake of folic acid, demographic factors (i.e., age, geographical area, gestational age, and birth order), and intake of dietary supplements were surveyed.

**Results** Eighty-five percent of respondents consumed folate, which was mostly obtained through dietary folic acid supplements during the first month of pregnancy or after. Factors associated with loss of folic acid intake until 3 months of pregnancy included lack of knowledge, failure to consume dietary supplements, younger age, and multigravida.

**Conclusion** Many pregnant women in Japan consumed folic acid. However, most of them started supplementation after pregnancy recognition, which is too late to reduce the risk of NTD. Alternative strategies to increase the efficacy of folic acid intake, such as recommending folic acid-enriched foods, promoting folic acid fortification efforts, and providing access to practical information, are necessary.

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Toxicology Reports 1 (2014) 787–794

Contents lists available at ScienceDirect

Toxicology Reports

journal homepage: www.elsevier.com/locate/toxrep

# Induction of fatty liver by *Coleus forskohlii* extract through enhancement of de novo triglyceride synthesis in mice

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ARTICLE INFO

*Article history:*
Received 18 July 2014
Received in revised form 15 August 2014
Accepted 8 September 2014

*Keywords:*
*Coleus forskohlii*
Forskolin
Fatty liver
De novo synthesis
PPARγ
Fsp27
Dietary supplement

ABSTRACT

*Coleus forskohlii* extract (CFE), an herbal ingredient, is used for weight-loss products. CFE's alleged efficacy is attributed to forskolin. However, CFE has been shown to induce fatty liver in mice, with components other than forskolin playing a part in this effect. The present study addressed the underlying mechanism of CFE-induced fatty liver by analyzing changes in CFE-treated mice of lipid concentrations and of the levels of mRNAs encoding enzymes and transcription factors known to be related to fatty liver. Mice were fed a diet containing 0, 0.3 and 1% CFE for 2 weeks. CFE at 1% clearly induced fatty liver, as demonstrated by histological examination and confirmed by increases in triglyceride concentrations in liver. However, treated mice did not exhibit elevation in plasma levels of non-esterified fatty acids. Comprehensive analysis of liver mRNA levels revealed accumulation of multiple transcripts, including mRNAs encoding enzymes acetyl-CoA carboxylase and long-chain elongase; transcription factor peroxisome proliferator-activated receptor gamma (PPARγ); and lipid-droplet-associated fat-specific protein 27 (Fsp27). These findings suggest that the de novo synthesis and accumulation of triglyceride in the liver, through the enhanced expression of specific lipogenic mRNAs, is a major underlying mechanism of fatty liver induction by CFE.


## 1. Introduction

Obesity is an ongoing concern in the developed world, because this condition increases the risk of chronic diseases such as diabetes mellitus and cardiovascular disease [1]. To fight obesity, weight-loss dietary supplements often are used without adequate clinical evidence [2]. Among dietary supplements, herbal products are increasingly used [3], and are sometimes perceived as safe because such supplements are "natural". However, recent studies have suggested that herbal products, especially those used for weight loss, can cause adverse events such as serious hepatic failure [4].

*Coleus forskohlii* extract (CFE) has been used for centuries in Ayurvedic medicine to treat various diseases of the cardiovascular, respiratory, gastrointestinal, and central nervous systems [5]. Currently, CFE has received attention as a popular herbal ingredient for weight-loss products, because CFE contains a diterpene compound, forskolin. This compound has been shown to activate adenylate cyclase [6,7] to enhance lipolysis and fat loss in studies performed in cell culture [8,9], rat [10], and human [11,12]. Based on

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http://dx.doi.org/10.1016/j.toxrep.2014.09.013

the activity of this component, CFE is generally standardized at 10% forskolin for use in dietary supplements.

In our previous studies, we observed that CFE induced hepatic cytochrome P450 (CYP) while also inducing fatty liver in mice, although these induction events were not seen with forskolin alone [13,14]. The substance that induced fatty liver and hepatic CYP induction was soluble in ether and ethyl acetate [15]. CYP was induced by CFE at a dose lower than that needed to induce fatty liver, but both phenomena (CYP and fatty liver) seemed to be related. CFE-mediated CYP induction was clearly detected in high-carbohydrate diet [16]. To confirm the safety of CFE as a dietary supplement, we sought to examine the mechanism of action of CFE in the induction of fatty liver.

The development of fatty liver has been attributed to increased release of non-esterified fatty acids from adipose tissue; increased de novo synthesis of fatty acids; decreased beta-oxidation [17]; and decreased export of triglyceride as lipoprotein from liver [18].

In the present study, we examined the possible mechanism of action of CFE on fatty liver in mice by evaluating changes in lipid concentrations in plasma and liver, along with comprehensive profiling of hepatic mRNA expression of genes coding for lipogenic and triglyceride synthesis enzymes, transcription factors, and nuclear receptors.

## 2. Materials and method

### 2.1. Materials

Powdered CFE standardized to contain 10% forskolin was prepared as follows. Dried roots of *C. forskohlii* were obtained from the Bangalore in southern India, crushed, and subjected to extraction with supercritical $CO_2$. The resulting forskolin-rich extract (20–30%) was combined with dextrin to yield a powder containing 10% forskolin. These extraction and preparation steps were outsourced to Tokiwa Phytochemical Co., Ltd. (Chiba, Japan). The final composition of the resulting CFE powder was as follows: water, 5.6%; protein, 0.3%; lipids, 22.7%; ash, 2.2%; and carbohydrates, 69.2%. The HPLC chromatographic profile has been reported elsewhere [19], and the analyzed contents of forskolin and 1,9-dideoxyforskolin, two substances available as standards in the CFE sample, were 10.37% and 1.71%, respectively. CFE was added in the proportions described below to an AIN93G purified diet purchased from Oriental Yeast Co., Ltd. (Tokyo, Japan). All other reagents were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan).

### 2.2. Animal experiment

Male ICR mice, aged 5 weeks (CLEA Japan, Inc., Tokyo, Japan), were housed in polypropylene cages at a constant temperature (22 ± 1 °C) with a 12-h/12-h light-dark cycle. After acclimation for 1 week, mice were allocated into three groups (5 mice per group), and provided with ad libitum access to AIN93G purified diet without CFE (0% CFE; control) or supplemented to 0.3% CFE or 1% CFE. After two weeks on the respective diet, animals were anesthetized with pentobarbital and exsanguinated from inferior vena cava with heparin as an anticoagulant. Livers were immediately removed, weighed, and assessed as follows. For all animals, a portion of the liver was stored in RNAlater (Applied Biosystems, Inc., Foster City, CA, USA) pending processing for mRNA analysis. For a subset of the animals, portions of the livers were fixed in 10% neutral buffered formalin pending processing for histopathological analysis. Other samples were snap frozen on dry ice and stored at −80 °C until analysis. All animal procedures were conducted in accordance with the Japan National Institute of Health and Nutrition Guidelines for the Care and Use of Laboratory Animals, and were approved by ethical committee of the Japan National Institute of Health and Nutrition.

### 2.3. Analysis of mRNA levels

Real time RT-PCR experiments were performed by the method previously described [20]. Briefly, total RNA was extracted using a QuickGene RNA tissue kit SII (Fuji Photo Film Co., Ltd., Tokyo, Japan), and the samples were subjected to real time RT-PCR using the One-Step SYBR RT-PCR kit (Perfect Real Time; Takara Bio Inc., Shiga, Japan) according to the manufacturer's protocol and Mx3000P® (STRATAGENE Co., La Jolla, CA, USA). The results were expressed as copy number ratio of the target mRNA to that of cyclophilin mRNA. The specific primers were synthesized via the Perfect Real Time Primer support system of Takara Bio (http://www.takara-bio.co.jp/prt/intro.htm); primer sequences are shown in Table 1. The mRNAs analyzed in the present study included transcripts encoding glycolytic enzymes Gck, Gapdh, Pklr1 and Pklr2; lipogenesis enzymes Acly, ACC1, ACC2, and Fasn; fatty acid elongation and desaturation enzymes Elovl6 and Scd1; triglyceride synthesis enzymes Gpam, Dgat1, and Dgat2; transcription factor and nuclear receptor proteins ChREBP, Srebp1, Nr1h2, Nr1h3, and PPARα, PPARγ, and PPARδ; and Cidea, Cideb, Cidec/Fsp27 [21].

### 2.4. Other measurements

Liver samples fixed with formalin were embedded in paraffin using standard procedures. The samples were sectioned at 3-μm thicknesses for staining with hematoxylin and eosin (H&E), or sectioned at 10-μm thicknesses for staining with Oil Red O. These morphological analyses were outsourced to Biosafety Research Center, Foods, Drugs and Pesticides (An-Pyo Center), Shizuoka, Japan. Measurement of plasma enzyme activities indicative of hepatic failure was outsourced to SRL Inc., Tokyo, Japan. The analyzed activities were alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP). Hepatic lipids were extracted by the methods of Bligh and Dyer [22]. Concentrations of triglyceride, cholesterol, phospholipid, and non-esterified fatty acid were measured using test kits from Wako Pure Chemical Industries, Ltd., Osaka Japan.

### 2.5. Statistical analyses

The data are presented as the mean and standard error (SE) for the individual groups. Statistical analysis of the data

**Table 1**
Sequences of primers used for real-time RT-PCR analysis (5′–3′).

| Encoded protein | Forward | Reverse |
|---|---|---|
| Cyclophilin | ACGCCACTGTCGCTTTTC | CTGCAAACAGCTCGAAGGA |
| Gck | CTGGATGACAGAGCCAGGAT | CTCTGCCAGGATCTGCTCTAC |
| Gapdh | TGCACCACCAACTGCTTAGC | GGCATGGACTGTGGTCATGAG |
| Pklr1 | ACTTAGCAAAGTCTGCTTTAAGTGG | TGGCACGTCTCAGGTATCC |
| Pklr2 | GTGGAGGCTTCCTTCAAGTG | AGGTCGGTAGCGAGACAGAA |
| Acly | GCCCTGGAAGTGGAGAAGAT | CCGTCCACATTCAGGATAAGA |
| ACC1 | GCGTCGGGTAGATCCAGTT | CTCAGTGGGGCTTAGCTCTG |
| ACC2 | TGAATCTCACGCGCCTACTA | GCCTCTCTTCACCAGATGGA |
| Fasn | GCTGCTGTTGGAAGTCAGC | AGTGTTCGTTCCTCGGAGTG |
| Elovl6 | CAGCAAAGCACCCGAACTA | AGGAGCACAGTGATGTGGTG |
| Scd1 | TTCCCTCCTGCAAGCTCTAC | CAGAGCGCTGGTCATGTAGT |
| Gpam | GGAAGGTGCTGCTATTCCTG | TGGGATACTGGGGTTGAAAA |
| Dgat1 | TCGTGGTATCCTGAATTGGTG | AGGTTCTCTAAAAATAACCTTGCATT |
| Dgat2 | GGCGCTACTTCCGAGACTAC | TGGTCAGCAGGTTGTGTGTC |
| ChREBP | GGCCTGGCTGGAACAGTA | CGAAGGGAATTCAGGACAGT |
| Srebf1 | GGTTTTGAACGACATCGAAGA | CGGGAAGTCACTGTCTTGGT |
| Nr1h2 | GCTCTGCCTACATCGTGGTC | CTCATGGCCCAGCATCTT |
| Nr1h3 | TGTGCGCTCAGCTCTTGT | TGGAGCCCTGGACATTACC |
| Ppara | CTGAGACCCTCGGGGAAC | AAACGTCAGTTCACAGGGAAG |
| Pparg | GAAAGACAACGGACAAATCACC | GGGGGTGATATGTTTGAACTTG |
| Ppard | ATGGGGGACCAGAACACAC | GGAGGAATTCTGGGAGAGGT |
| Cidea | AAACCATGACCGAAGTAGCC | AGGCCAGTTGTGATGACTAAGAC |
| Cideb | CTGCCAGCCTCCAAGAACT | TAGCACTCCACGTAGCAGCA |
| Cidec | GATGGACTACGCCATGAAGTC | GTGCTCACTGCCACATGC |

Protein product abbreviations: Gck, glucokinase; Gapdh, glyceraldehyde-3-phosphate dehydrogenase; Pklr1 and Pklr2, pyruvate kinase liver and red blood cell (Pklr) 1 and 2; Acly, ATP-citrate synthase; ACC1 and ACC2, acetyl-coenzyme A carboxylase 1 and 2; Fasn, fatty acid synthase; Elovl6, long-chain elongase; Scd1, acyl-CoA desaturase 1; Gpam, glycerol-3-phosphate acyltransferase; Dgat1 and Dgat2, diacylglycerol acyltransferase 1 and 2; ChREBP, carbohydrate-responsive element-binding protein; Srebp1, sterol regulatory element binding factor 1; Nr1h2 and Nr1h3, liver X receptor (LXR) beta and alpha; Ppara, Pparg and Ppard, peroxisome proliferator-activated receptors alpha, gamma, and delta; Cidea, Cideb and Cidec/Fsp27, cell death-inducing DNA fragmentation factor 45-like effectors (CIDEs) A, B, and C.

was carried out by one-way ANOVA with post hoc Dunnett's Multiple Comparisons Test where significance was indicated. Differences with $p < 0.05$ were considered to be significant. Statistical analyses were performed using Prism 5.0 (GraphPad Software, Inc., La Jolla, CA, USA).

## 3. Results

Body weight did not change with CFE treatment, but liver weight normalized to body weight (relative liver weight) increased in the 0.3% and 1% CFE groups. Hepatic lipid accumulation was confirmed by staining with Oil Red O. The liver tissue from the 0% CFE mice showed no-remarkable change, but that from the 1% CFE mice exhibited hepatic cellular damage, such as fatty change and necrosis (Fig. 1). Among the liver marker enzymes (AST, ALT, ALP) in plasma, ALP increased in the 1% CFE group; while other enzymes appeared to be elevated by CFE treatment, those changes were not statistically significant due to high standard error (Table 2). Plasma concentration of

**Table 2**
Changes in body weight, liver weight, plasma clinical parameters, and lipid concentration in plasma and liver of mice treated with CFE.

| | CFE-treated | | |
|---|---|---|---|
| | Control (0%) | 0.3% | 1% |
| Final body weight (g) | 36.0 ± 0.93 [1.0] | 34.5 ± 0.46 [0.96] | 33.6 ± 0.65 [0.93] |
| Liver weight (% body weight) | 4.33 ± 0.18 [1.0] | 5.91 ± 0.27 [1.4]* | 8.60 ± 0.40 [2.0]* |
| Plasma clinical parameters | | | |
| AST (IU/L) | 48.0 ± 7.5 [1.0] | 113.4 ± 32.6 [2.4] | 160.0 ± 45.7 [3.3] |
| ALT (IU/L) | 17.0 ± 2.2 [1.0] | 64.2 ± 21.3 [3.8] | 100.6 ± 42.9 [5.9] |
| ALP (IU/L) | 264 ± 35 [1.0] | 380 ± 72 [1.4] | 512 ± 77 [1.9]* |
| Plasma lipids | | | |
| Triglyceride (mg/dL) | 102 ± 19.0 [1.0] | 99 ± 28.2 [1.0] | 208 ± 34.6 [2.0]* |
| Cholesterol (mg/dL) | 208 ± 9.6 [1.0] | 146 ± 24.3 [0.7] | 189 ± 26.9 [0.9] |
| Phospholipid (mg/dL) | 277 ± 14.0 [1.0] | 214 ± 31.6 [0.8] | 268 ± 26.9 [1.0] |
| Non-esterified fatty acid (mequiv./L) | 1.49 ± 0.39 [1.0] | 1.23 ± 0.37 [0.8] | 1.85 ± 0.31 [1.2] |
| Hepatic lipids | | | |
| Triglyceride (mg/g liver) | 14.1 ± 1.2 [1.0] | 32.9 ± 2.7 [2.3]* | 45.2 ± 4.9 [3.2]* |
| Cholesterol (mg/g liver) | 7.1 ± 0.51 [1.0] | 12.1 ± 1.5 [1.7]* | 13.4 ± 1.1 [1.9]* |
| Phospholipid (mg/g liver) | 13.9 ± 0.87 [1.0] | 13.1 ± 1.1 [0.95] | 13.4 ± 1.5 [0.97] |

Male ICR mice were maintained for 2 weeks on a diet supplemented with 0% CFE (control), 0.3% CFE, or 1% CFE. Each value is the mean and SE from 5 mice. Numbers in brackets indicate the ratio compared to the control group.

* Significant difference from the level of control group is indicated by $p < 0.05$.

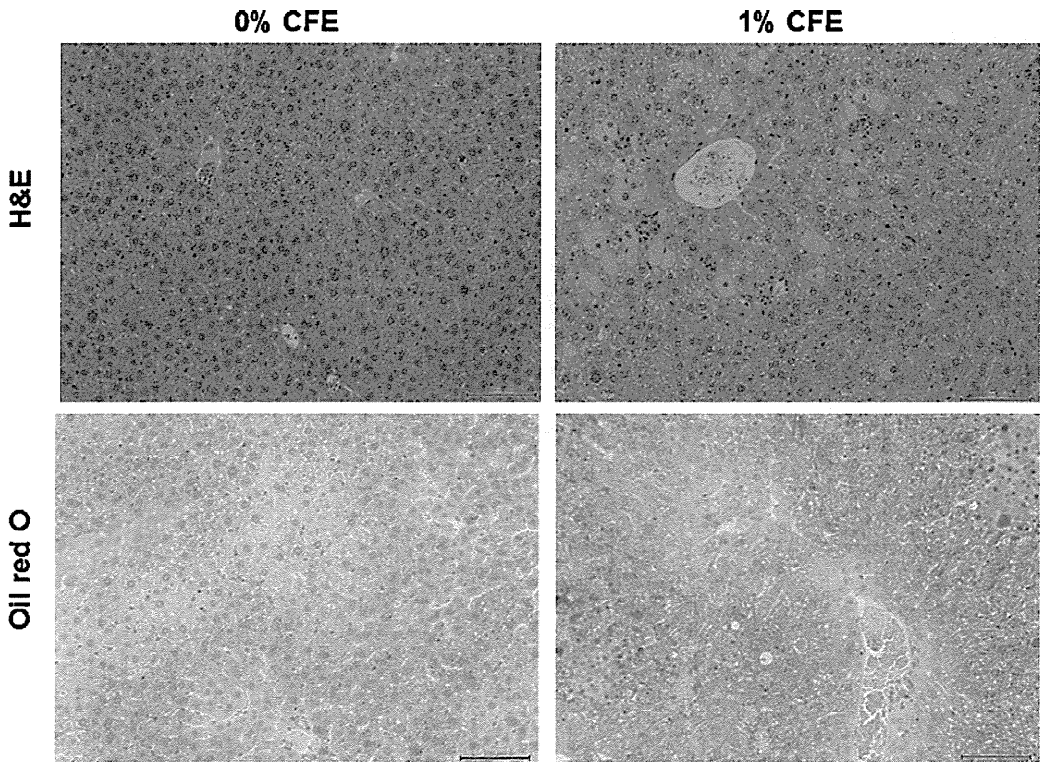


**Fig. 1.** Representative histopathologic changes in liver sections from mice stained with hematoxylin and eosin (H&E) and Oil red O (magnification 200×). Mice were maintained for 2 weeks on diet supplemented with 0, 0.3, or 1% CFE.

triglycerides was increased in the 1% CFE group; plasma levels of cholesterol, phospholipid, and non-esterified fatty acid were not changed by CFE exposure. Liver concentrations of triglyceride and cholesterol were increased in the 0.3% and 1% CFE groups; the increases in liver triglyceride were 2.3- and 3.2-fold in the respective CFE-treated groups compared with the control group.

Expression in the liver of mRNAs encoding lipid synthesis enzymes and transcription factors was analyzed. Dose-related increases were detected in the levels of transcripts encoding various metabolic enzymes, especially those for lipogenesis enzyme ACC2 and fatty acid elongation enzyme Elovl6 (Fig. 2). Also, a clear increase was observed in the levels of the mRNAs encoding transcription factor PPARγ and lipid droplet protein Fsp27 (Fig. 3).

## 4. Discussion

As shown by our results, CFE treatment induced obvious fatty liver even at the lower tested dose of 0.3% in the diet. The possible (non-exclusive) mechanisms of fatty liver induction include: (1) enhanced supply of non-esterified fatty acid from adipose tissue, (2) reduced secretion from liver, and (3) enhanced de novo lipogenesis. In an attempt to distinguish these hypotheses, we examined the possible mechanism of action of fatty liver due to CFE treatment.

An overflow of fatty acid derived from lipolysis has been proposed to be the main cause of the excess accumulation of triglyceride observed in hepatic steatosis [23]. The forskolin in CFE is thought to enhance lipolysis due to activation of adenylate cyclase [8,9]. Therefore, enhanced lipolysis by forskolin is implicated as a source of increased fatty acid, leading to enhanced triglyceride synthesis in the liver. In fact, treatment of mice with 0.5% and 5% CFE for 3 weeks decreased fat tissue weight in our previous study [13]. However, in the present study, non-esterified fatty acid in plasma was not enhanced by 0.3% CFE treatment for 2 weeks, whereas a significant increase in liver triglyceride concentration was detected in this group. This finding suggests that an overflow of fatty acid derived from lipolysis is unlikely to be the mechanism of CFE-induced hepatic steatosis. Triglyceride is exported as lipoprotein from liver into blood. Reduced triglyceride secretion could lead to accumulation of triglyceride in the liver, as observed in the case of $CCl_4$ administration [18]. However, in the present study, treatment with CFE did not yield a decrease in plasma lipid concentration; plasma triglyceride was rather high in 1% CFE group. This fact suggests that the effects of CFE exposure on fatty liver are not mediated by decreases in the secretion of liver triglycerides as in the case of $CCl_4$ administration. Therefore, we speculate that enhanced de novo lipogenesis is involved in CFE-induced fatty liver.

In de novo lipogenesis, ACC and Fasn catalyze the rate-limiting and final steps, respectively [24]. Palmitoyl-CoA is elongated by Elovl6 and Scd1. Enzymes for triglyceride synthesis are transcriptionally regulated by ChREBP, SREBP-1c, and the LXRs (Nr1h2 and Nr1h3) in liver. ChREBP and SREBP-1c induce ACC, Fasn, Elovl6, and Scd1 genes in response to glucose and insulin, respectively. LXRs directly and indirectly activate transcription of the ACC-, Fasn-, and Scd1-encoding loci. In our present comprehensive RT-PCR analysis, expression of mRNAs encoding enzymes

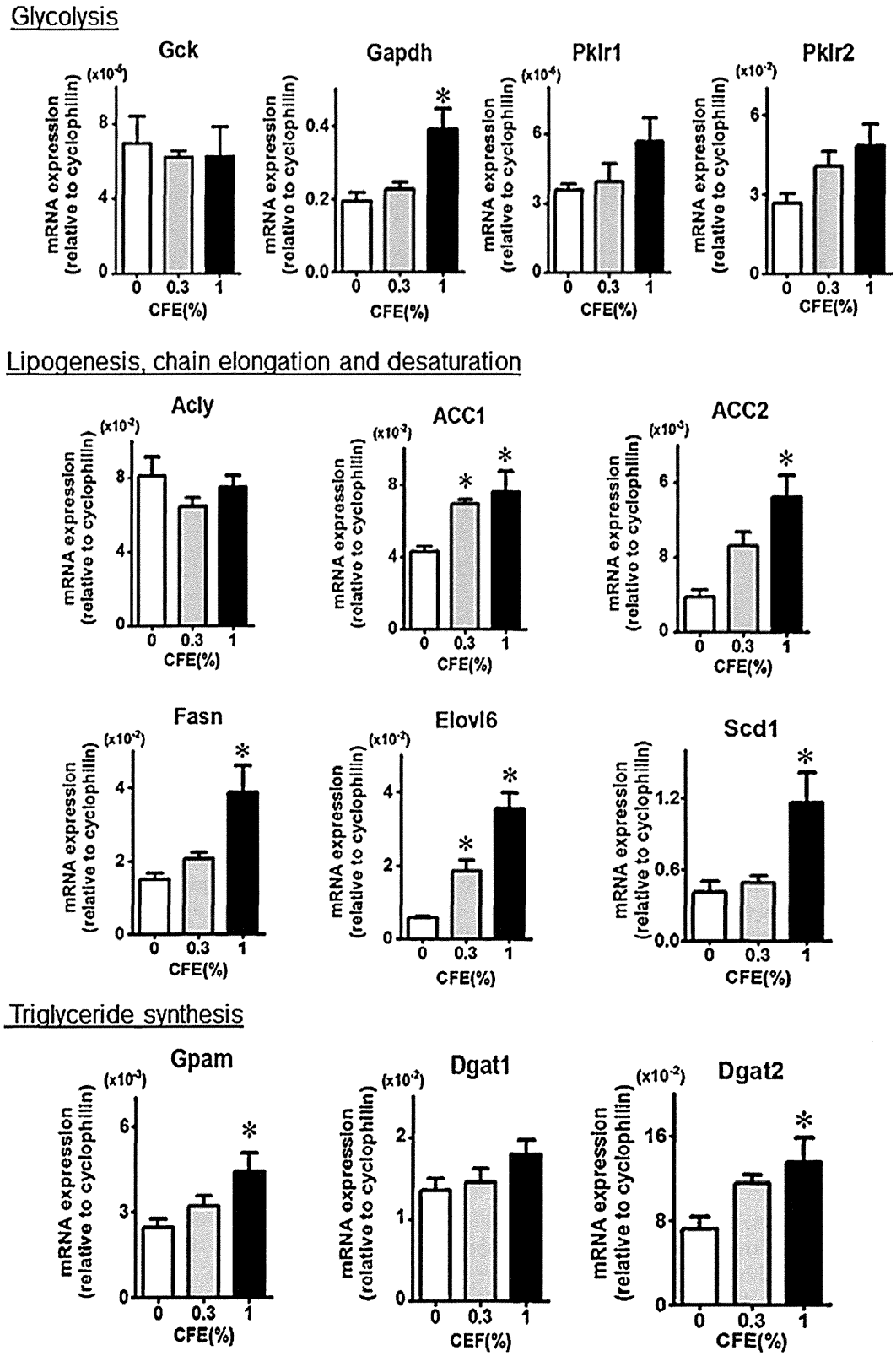


**Fig. 2.** Expression of mRNAs encoding enzymes involved in glycolysis, fatty acid synthesis, chain elongation and desaturation, and triglyceride synthesis. Protein product abbreviations are defined in the main text and Table 1. Each value is the mean and SE from 5 mice. Significant difference from the level of control group (0% CFE) is indicated by $^{*}p < 0.05$.

involved in de novo synthesis of fatty acid and triglyceride were increased, especially those coding for ACC, Fasn, and Elovl6. These changes in mRNA expression could contribute to enhance de novo lipogenesis due to CFE treatment, although mRNAs for the transcription factors themselves did not show significant accumulation.

Accumulating evidence suggests that nuclear receptor PPARs, which consist of PPARα, PPARγ, and PPARδ, are

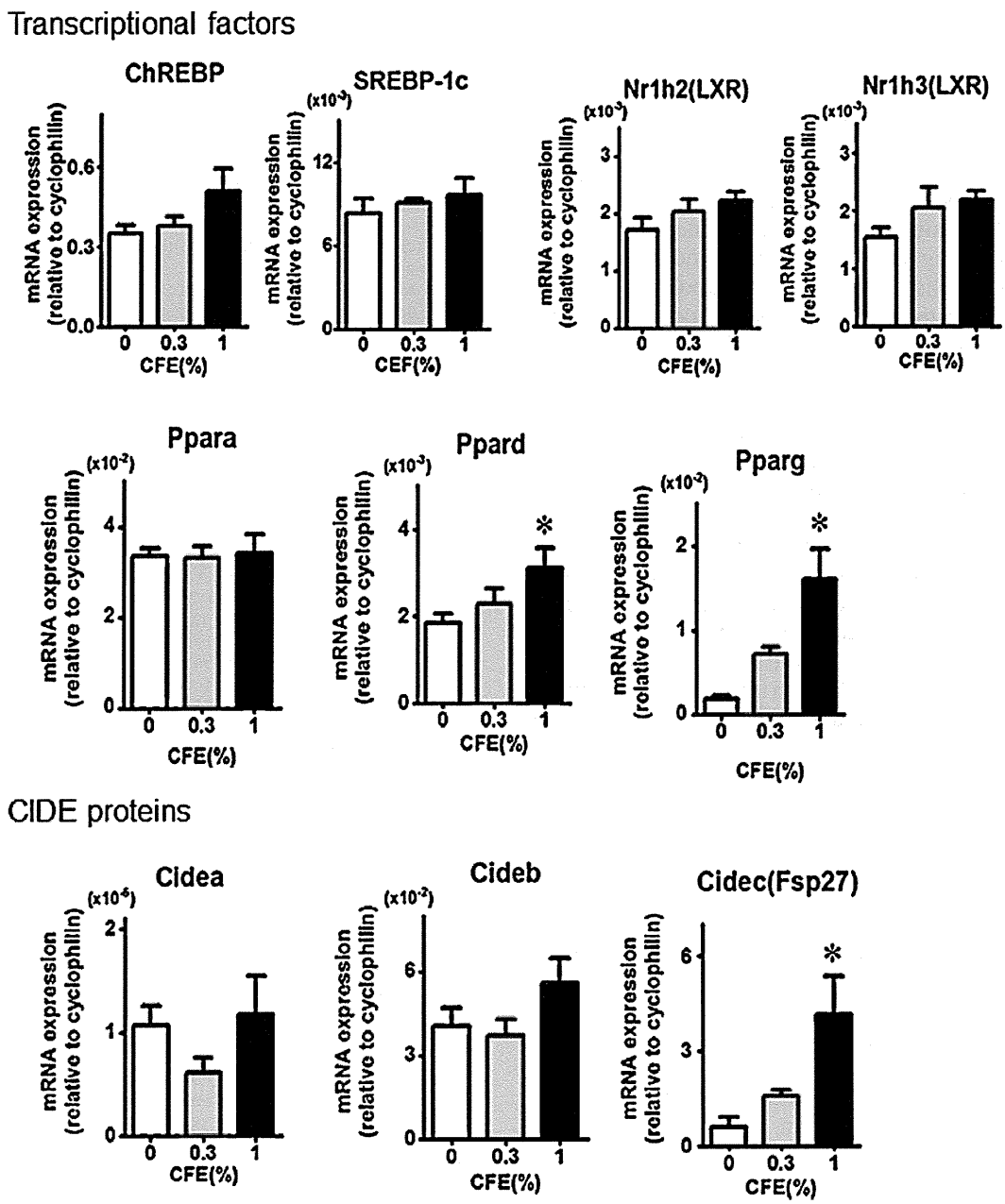


**Fig. 3.** Expression of mRNAs encoding transcription factors and effectors involved in fatty liver. Each values is the mean and SE from 5 mice. Protein product abbreviations are defined in the main text and Table 1. Significant difference from the level of control group (0% CFE) is indicated by $^{*}p < 0.05$.

involved in lipid metabolism [25]. In the present study, expression of the transcript encoding PPARγ showed a clear increase in response to CFE treatment. PPARγ is expressed predominantly in adipose tissue, with low expression in liver [26], although this factor has been shown to play a critical role in hepatic steatosis in obese or diabetic mouse models [27–29]. Among CIDE proteins, which are involved in lipid droplet growth and lipoprotein lipidation [21], Fsp27/Cidec is an adipocyte lipid droplet protein [30]; the Fsp27-encoding gene is directly regulated by PPARγ in hepatic steatosis [28,31]. In the present study, the mRNAs for Fsp27/Cidec and for PPARγ both accumulated following exposure to CFE. On the other hand, the PPARα gene is expressed predominantly in the liver and is a major activator of fatty acid oxidation pathways; elevated PPARα activity leads to decreased lipid levels. PPARδ is ubiquitously expressed in many tissues and has functions similar to those of PPARα. In the present study, expression of the PPARα-encoding mRNA did not exhibit change even at the higher tested dose of 1% CFE. Based on these findings, we conclude that enhanced accumulation of PPARγ- and Fsp27/Cidec-encoding transcripts might be a major contributor to CFE-induced fatty liver. Further detail study will be needed to confirm expression of PPARγ- and Fsp27/Cidec at protein level.

CFE is a natural herbal product, and composition may vary among products. We reported that two sources of CFE standardized with 10% forskolin showed similar increases in relative liver weight and CYP induction [14]. Also, we observed that CFE treatment induced fatty liver and

hepatic CYP induction not only in ICR mice but also in C57BL mice (unpublished observation), suggesting fatty liver is commonly induced by CFE. It will be critical to identify the active substance(s) involved in the CFE induction of fatty liver. Notably, forskolin itself appears not to be involved in CFE induction of fatty liver and CYP [13,14]. In ongoing research, we are seeking to identify the active substance. To date, we have shown that the substance is lipophilic, as demonstrated by solubility in ether and ethyl acetate [15]. Drug metabolism and energy metabolism pathways have been shown to interface through nuclear receptors [32]. The present work suggests that the active substance in CFE affects the expression of the PPARγ-encoding gene; future work will need to examine how the accumulation of PPARγ message is related to CYP induction following CFE treatment. It would be beneficial to seek unidentified substance by the expression of PPARγ in studies in vivo and in vitro.

In conclusion, this study indicated that CFE, a popular weight-loss dietary supplement, induces fatty liver by de novo lipogenesis, a process that may be mediated through enhanced expression of multiple enzymes and transcripts, in particular encoding PPARγ and Fsp27. Previous work suggested that fatty liver induction is the result of an undefined (non-forskolin) component of CFE. Thus, it will be necessary to identify unknown substance by focusing on the expression of PPARγ and Fsp27, and eliminate this activity from the extract to render CFE safe for use as a weight-loss dietary supplement.

## Conflict of interest

The authors declare that there are no conflicts of interest.

## Transparency document

The Transparency document associated with this article can be found in the online version.

## Acknowledgement

This study was financially supported in part by a research grant from the Ministry of Health, Labour and Welfare, Japan (H24-006).

## References

[1] S. Low, M.C. Chin, M. Deurenberg-Yap, Review on epidemic of obesity, Ann. Acad. Med. Singapore 38 (2009) 57–59.
[2] A.M. Egras, W.R. Hamilton, T.L. Lenz, M.S. Monaghan, An evidence-based review of fat modifying supplemental weight loss products, J. Obes. (2011) 2011.
[3] M.E. Gershwin, A.T. Borchers, C.L. Keen, S. Hendler, F. Hagie, M.R. Greenwood, Public safety and dietary supplementation, Ann. N. Y. Acad. Sci. 1190 (2010) 104–117.
[4] R.K. Yellapu, V. Mittal, P. Grewal, M. Fiel, T. Schiano, Acute liver failure caused by 'fat burners' and dietary supplements: a case report and literature review, Can. J. Gastroenterol. 25 (2011) 157–160.
[5] H.P. Ammon, A.B. Muller, Forskolin: from an ayurvedic remedy to a modern agent, Planta Med. 51 (1985) 473–477.
[6] H. Metzger, E. Lindner, The positive inotropic-acting forskolin, a potent adenylate cyclase activator, Arzneimittelforschung 31 (1981) 1248–1250.
[7] K.B. Seamon, W. Padgett, J.W. Daly, Forskolin: unique diterpene activator of adenylate cyclase in membranes and in intact cells, Proc. Natl. Acad. Sci. U. S. A. 78 (1981) 3363–3367.
[8] D.O. Allen, B. Ahmed, K. Naseer, Relationships between cyclic AMP levels and lipolysis in fat cells after isoproterenol and forskolin stimulation, J. Pharmacol. Exp. Ther. 238 (1986) 659–664.
[9] H. Okuda, C. Morimoto, T. Tsujita, Relationship between cyclic AMP production and lipolysis induced by forskolin in rat fat cells, J. Lipid Res. 33 (1992) 225–231.
[10] L.K. Han, C. Morimoto, R.H. Yu, H. Okuda, Effects of Coleus forskohlii on fat storage in ovariectomized rats, Yakugaku Zasshi 125 (2005) 449–453.
[11] M.P. Godard, B.A. Johnson, S.R. Richmond, Body composition and hormonal adaptations associated with forskolin consumption in overweight and obese men, Obes. Res. 13 (2005) 1335–1343.
[12] S. Henderson, B. Magu, C. Rasmussen, S. Lancaster, C. Kerksick, P. Smith, C. Melton, P. Cowan, M. Greenwood, C. Earnest, A. Almada, P. Milnor, T. Magrans, R. Bowden, S. Ounpraseuth, A. Thomas, R.B. Kreider, Effects of Coleus forskohlii supplementation on body composition and hematological profiles in mildly overweight women, J. Int. Soc. Sports Nutr. 2 (2005) 54–62.
[13] N. Virgona, Y. Taki, S. Yamada, K. Umegaki, Dietary Coleus forskohlii extract generates dose-related hepatotoxicity in mice, J. Appl. Toxicol. 33 (2013) 924–932.
[14] N. Virgona, K. Yokotani, Y. Yamazaki, F. Shimura, T. Chiba, Y. Taki, S. Yamada, K. Shinozuka, M. Murata, K. Umegaki, Coleus forskohlii extract induces hepatic cytochrome P450 enzymes in mice, Food Chem. Toxicol. 50 (2012) 750–755.
[15] K. Yokotani, T. Chiba, Y. Sato, Y. Kubota, Y. Watanabe, M. Murata, K. Umegaki, Estimation of components which induce mice cytochrome P450 in Coleus forskohlii extract, Pharmacometrics 82 (2012) 67–73.
[16] K. Yokotani, T. Chiba, Y. Sato, T. Nakanishi, M. Murata, K. Umegaki, Influence of dietary macronutrients on induction of hepatic drug metabolizing enzymes by Coleus forskohlii extract in mice, J. Nutr. Sci. Vitaminol. (Tokyo) 59 (2013) 37–44.
[17] C. Postic, J. Girard, The role of the lipogenic pathway in the development of hepatic steatosis, Diabetes Metab. 34 (2008) 643–648.
[18] X. Pan, F.N. Hussain, J. Iqbal, M.H. Feuerman, M.M. Hussain, Inhibiting proteasomal degradation of microsomal triglyceride transfer protein prevents CCl4-induced steatosis, J. Biol. Chem. 282 (2007) 17078–17089.
[19] K. Yokotani, T. Chiba, Y. Sato, Y. Taki, S. Yamada, K. Shinozuka, M. Murata, K. Umegaki, Hepatic cytochrome P450 mediates interaction between warfarin and Coleus forskohlii extract in vivo and in vitro, J. Pharm. Pharmacol. 64 (2012) 1793–1801.
[20] Y. Taki, Y. Yamazaki, F. Shimura, S. Yamada, K. Umegaki, Time-dependent induction of hepatic cytochrome P450 enzyme activity and mRNA expression by bilobalide in rats, J. Pharmacol. Sci. 109 (2009) 459–462.
[21] L. Xu, L. Zhou, P. Li, CIDE proteins and lipid metabolism, Arterioscler. Thromb. Vasc. Biol. 32 (2012) 1094–1098.
[22] E.G. Bligh, W.J. Dyer, A rapid method of total lipid extraction and purification, Can. J. Biochem. Physiol. 37 (1959) 911–917.
[23] P. Ferre, F. Foufelle, Hepatic steatosis: a role for de novo lipogenesis and the transcription factor SREBP-1c, Diabetes Obes. Metab. 12 (Suppl. 2) (2010) 83–92.
[24] Y. Kawano, D.E. Cohen, Mechanisms of hepatic triglyceride accumulation in non-alcoholic fatty liver disease, J. Gastroenterol. 48 (2013) 434–441.
[25] B. Grygiel-Gorniak, Peroxisome proliferator-activated receptors and their ligands: nutritional and clinical implications – a review, Nutr. J. 13 (2014) 17.
[26] M. Ahmadian, J.M. Suh, N. Hah, C. Liddle, A.R. Atkins, M. Downes, R.M. Evans, PPARgamma signaling and metabolism: the good, the bad and the future, Nat. Med. 19 (2013) 557–566.
[27] O. Gavrilova, M. Haluzik, K. Matsusue, J.J. Cutson, L. Johnson, K.R. Dietz, C.J. Nicol, C. Vinson, F.J. Gonzalez, M.L. Reitman, Liver peroxisome proliferator-activated receptor gamma contributes to hepatic steatosis, triglyceride clearance, and regulation of body fat mass, J. Biol. Chem. 278 (2003) 34268–34276.
[28] K. Matsusue, T. Kusakabe, T. Noguchi, S. Takiguchi, T. Suzuki, S. Yamano, F.J. Gonzalez, Hepatic steatosis in leptin-deficient mice is promoted by the PPARgamma target gene Fsp27, Cell Metab. 7 (2008) 302–311.
[29] S.E. Schadinger, N.L. Bucher, B.M. Schreiber, S.R. Farmer, PPARgamma2 regulates lipogenesis and lipid accumulation in

steatotic hepatocytes, Am. J. Physiol. Endocrinol. Metab. 288 (2005) E1195–E1205.
[30] V. Puri, S. Konda, S. Ranjit, M. Aouadi, A. Chawla, M. Chouinard, A. Chakladar, M.P. Czech, Fat-specific protein 27, a novel lipid droplet protein that enhances triglyceride storage, J. Biol. Chem. 282 (2007) 34213–34218.
[31] K. Matsusue, A physiological role for fat specific protein 27/cell death-inducing DFF45-like effector C in adipose and liver, Biol. Pharm. Bull. 33 (2010) 346–350.
[32] J. Gao, W. Xie, Pregnane X receptor and constitutive androstane receptor at the crossroads of drug metabolism and energy metabolism, Drug Metab. Dispos. 38 (2010) 2091–2095.

*Nutrients* **2014**, *6*, 5392-5404; doi:10.3390/nu6125392

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*nutrients*

**ISSN 2072-6643**

www.mdpi.com/journal/nutrients

*Article*

# Inappropriate Usage of Dietary Supplements in Patients by Miscommunication with Physicians in Japan

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*Received: 11 August 2014; in revised form: 8 September 2014 / Accepted: 17 October 2014 / Published: 26 November 2014*

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**Abstract:** Recently, people have used dietary supplements not only for nutritional supplementation, but also for treatment of their diseases. However, use of dietary supplements to treat diseases, especially with medications, may cause health problems in patients. In this study, we investigated use of dietary supplements in patients in Japan. This survey was conducted from January to December 2012, and was completed by 2732 people, including 599 admitted patients, 1154 ambulatory patients, and 979 healthy subjects who attended a seminar about dietary supplements. At the time of the questionnaire, 20.4% of admitted patients, 39.1% of ambulatory patients, and 30.7% of healthy subjects were using dietary supplements, which including vitamin/mineral supplements, herbal extracts, its ingredients, or food for specified health uses. The primary purpose for use in all groups was health maintenance, whereas 3.7% of healthy subjects, 10.0% of ambulatory patients, and 13.2% of admitted patients used dietary supplements to treat diseases. In addition, 17.7% of admitted patients and 36.8% of ambulatory patients were using dietary supplements concomitantly with their medications. However, among both admitted patients and ambulatory patients, almost 70% did not mention dietary supplement use to their physicians. Overall, 3.3% of all subjects realized adverse effects associated with dietary supplements. Communication between patients and physicians is important to avoid health problems associated with the use of dietary supplements.

**Keywords:** dietary supplements; patients; treatment of diseases; adverse effects

## 1. Introduction

With the rapid increase in the senior population in Japan, chronic diseases associated with aging, such as diabetes mellitus, cardiovascular disease, hypertension, osteoporosis, and cancer have become a widely recognized social issue. Against this background, an increase in health consciousness prompts people to use dietary supplements to maintain health and prevent diseases. Most people use vitamin or mineral supplements, whereas other herbal extracts (e.g., blueberry, coleus forskohlii, ginkgo, or green tea) and ingredients (e.g., collagen, catechins, fish oil, glucosamine, hyaluronic acid, and isoflavones) are also popular in Japan. People tend to believe that dietary supplements are as safe as food and as beneficial as medicine.

The beneficial effects of food and its nutrients and other ingredients have been recognized for a long time. Previously, people obtained nutrients and other ingredients only as foods, such as vegetables, fruits, fish, meat, tea, and other items. Over time, manufacturers learned to extract and condense some of the specific nutrients or ingredients in food and offer them as dietary supplements in the form of tablets, capsules, or powders. The concentrated ingredients in dietary supplements carry not only the benefits but also the risk of toxicity, interaction with drugs, and adverse reactions compared with the ingredients in whole foods [1]. However, manufacturers tend to emphasize key characteristics of their products and promote sales using attractive claims. In addition, in some cases, manufacturers claim that medicines are more likely than dietary supplements to cause side effects, because medicines are synthetic compounds, whereas dietary supplements are made from natural substances and thus safe and suitable for everybody. Currently, there is insufficient evidence that dietary supplements improve disease in humans, and if patients turn to dietary supplements instead of medicines, health problems might occur. Indeed, adverse effects caused by dietary supplements, especially hepatotoxicity associated herbal supplement use, are reported worldwide [2–4].

Regulation of dietary supplement in Japan is more complicated compared to other countries such as the USA or European countries. In 1991, the Ministry of Health, Labour and Welfare set up the Food for Specified Health Uses to provide people with accurate health information about foods. The current Japanese system for regulation of health foods is called Food with Health Claims and is made up of two categories: (1) "Food with Nutrient Function Claims" and (2) "Food for Specified Health Uses". Most of "Food for Specified Health Uses" products are the form of regular food, such as tea, beverage, yogurt, and flakes. On the other hand, except for "Food with Health Claims", laws for dietary supplements are not defined in Japan. This means that most dietary supplements on the market are considered the same as other foods, even if they are in the form of capsules or tablets [5,6].

Consumers tend to have only a vague understanding that dietary supplements are different from medicines, and some consumers use dietary supplements as medicines to treat specific diseases in Japan. Several reasons contribute to this inappropriate use of dietary supplements. First, there is no clear, official definition of dietary supplements in Japan. Because of this, many dietary supplements claim to treat specific diseases, especially cancer, even though such claims are illegal in Japan. Secondly, dietary

supplements available as capsules or tablets look like medicines and thus are often thought to be as effective as medicines. Thirdly, consumers do not understand the properties of dietary supplements. Physicians are concerned about the use of dietary supplements by their patients, because of the possibility of dietary supplement–drug interactions [7]. In particular, dietary supplements may interact with some medicines as well as affect anaesthesia and bleeding during surgery [8].

Dietary supplements are helpful to complement nutrition in not only healthy subjects but also patients. However, if patients use dietary supplements to treat diseases without consulting physicians, it may cause health problems. This study used a self-administered questionnaire to clarify awareness and use of dietary supplements among patients in Japan.

## 2. Methods

### *2.1. Subjects*

Subjects included 2732 people, who either attended health food seminars (Iwate, Ibaragi, Fukushima, Tokyo, Kanagawa, Shizuoka, Gifu, Wakayama, Fukui, Okayama, and Ehime), visited pharmacies (Tokyo, Shizuoka, Okayama), or were admitted to hospitals (Iwate, Tokyo, Saitama, Chiba, Shizuoka, Aichi, Okayama, Fukuoka, Nagasaki, Miyazaki, Saga, Kumamoto, Kagoshima) from January to December 2012. To clarify the recognition and use of dietary supplements among patients, we asked all subjects about their medical status and divided into three categories, admitted patients, ambulatory patients, and healthy subjects. Healthy subjects were defined as people who were not hospitalized or making regular visits to the hospital. This study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human subjects/patients were approved by the Research Ethics Committee of the National Institute of Health and Nutrition and each participating institute.

### *2.2. Definition of Dietary Supplements*

Dietary supplements are well defined in the USA and European countries, because they are regulated by law, but they are not regulated in Japan. Dietary supplements were usually recognized in the form of capsules, tablets, powders, or liquid. However, some dairy or soybean products are also produced as dietary supplements, even if they are in the form of regular foods. Thus, we did not define a specific form for dietary supplements in this survey. Dietary supplements were defined as foods, other than vegetables and fruits that subjects thought would have beneficial effect on their health.

### *2.3. Questionnaire*

The questionnaire included demographic characteristics (sex and age), information on use of supplements, awareness of dietary supplements (safety, price, effectiveness, substitute for medicines, and co-administration with medicines), purpose (maintenance of health, nutritional support, prevention of disease, treatment of disease, beauty, no specific purpose), number of dietary supplements used, realization of beneficial and adverse effects, and type and their situation of medications. In addition, the questionnaire asked whether subjects informed their physicians of their use of dietary supplements.

### *2.4. Statistical Analysis*

Differences in demographic characteristics or supplement use among admitted patients, ambulatory patients, and healthy subjects were tested using the $\chi^2$ test or Kruskal-wallis test with Bonferroni correction. Univariate analysis for the association of supplement use with various variables in the patients and healthy subjects was done using the $\chi^2$ test. Multivariable analysis was also done using the logistic regression analysis adjusted for sex and age. *P* values less than 0.05 in $\chi^2$ test and 0.0167 in Kruskal-wallis test were considered significant. A statistical analysis was performed using SPSS 18.0J for Windows (IBM Co., Armonk, New York, NY, USA).

## 3. Results

### *3.1. Characteristics*

Characteristics of all subjects ($n = 2732$) are shown in Table 1. More than half of subjects were female, and ages ranged from younger than 20 years to older than 80 years. Among all subjects, 21.9% were admitted patients, 42.2% were ambulatory patients, and 35.8% were healthy subjects.

**Table 1.** Characteristics of each group.

| | Healthy Subjects | Ambulatory Patients | Admitted Patients | Total | *p*-value |
|---|---|---|---|---|---|
| **Number of Subjects (%)** | 979 (35.8) | 1154 (42.2) | 599 (21.9) | 2732 (100.0) | |
| **Sex, *n* (%)** | | | | | <0.001 |
| Male | 251 (25.6) | 342 (29.6) | 335 (55.9) | 928 (34.0) | |
| Female | 728 (74.4) | 812 (70.4) | 264 (44.1) | 1804 (66.0) | |
| **Age, *n* (%)** | | | | | <0.001 |
| Under 20's | 62 (6.3) | 6 (0.5) | 9 (1.5) | 77 (2.8) | |
| 20's | 183 (18.7) | 49 (4.2) | 42 (7.0) | 274 (10.0) | |
| 30's | 133 (13.6) | 98 (8.5) | 50 (8.3) | 281 (10.3) | |
| 40's | 163 (16.6) | 110 (9.5) | 69 (11.5) | 342 (12.5) | |
| 50's | 140 (14.3) | 148 (12.8) | 95 (15.9) | 383 (14.0) | |
| 60's | 177 (18.1) | 336 (29.1) | 160 (26.7) | 673 (24.6) | |
| 70's | 107 (10.9) | 318 (27.6) | 134 (22.4) | 559 (20.5) | |
| Over 80's | 14 (1.4) | 89 (7.7) | 40 (6.7) | 143 (5.2) | |
| **Dietary Supplement Use, *n* (%)** | | | | | <0.001 |
| Present | 301(30.7) | 451 (39.1) | 122 (20.4) | 874 (32.0) | |
| Past | 298 (30.4) | 355 (30.8) | 209 (34.9) | 862 (31.6) | |
| Never | 125 (12.8) | 74 (6.4) | 64 (10.7) | 263 (9.6) | |
| Never but Future | 255 (26.0) | 274 (23.7) | 204 (34.1) | 733 (26.8) | |

*p*-values were calculated $\chi^2$ test.

### *3.2. Use of Dietary Supplements*

Among all subjects, 32.0% were currently using dietary supplements and 31.6% had used dietary supplements in the past (Table 1). Past use of dietary supplements in healthy subjects (30.4%) was

similar to present use (30.7%). In contrast, use increased over time in ambulatory patients (from 30.8% to 39.1%) and decreased over time in admitted patients (from 34.9% to 20.4%). However, 20.4% of admitted patients still used dietary supplements. In this survey, we did not ask what kind of dietary supplements they used, but previous reports [9] and some internet surveys showed that various type of dietary supplements including not only vitamins/minerals, but also herbal extracts and its ingredients, were used in Japan.

### *3.3. Awareness of Dietary Supplements*

Awareness of dietary supplements is shown in Table 2. In terms of safety, more than 40% of subjects thought that dietary supplements were safe (strongly agree or agree). There were no differences between ambulatory patients and healthy subjects. However, more admitted patients thought that dietary supplements were safe (strongly agree: 24.9%) compared with ambulatory patients (14.1%) and healthy subjects (12.3%). In terms of price, more than 80% of subjects thought that dietary supplements were expensive (strongly agree: 51.1%; agree: 31.3%). However, fewer admitted patients thought that dietary supplements were expensive (strongly agree: 45.9%; agree: 25.6%). In terms of effectiveness, about 40% of subjects thought that dietary supplements were effective (strongly agree: 5.6%; agree: 33.2%). There were no significant differences among three groups. However, admitted patients likely thought that dietary supplements were effective (strongly agree: 9.6%) compared with ambulatory patients (5.4%) and healthy subjects (3.5%).

**Table 2.** Awareness of dietary supplements.

| | Strongly Agree | Agree | Neither Agree nor Disagree | Disagree | Strongly Disagree | *p*-value |
|---|---|---|---|---|---|---|
| **Safe (%)** | | | | | | <0.001 |
| All subjects | 15.7 | 29.5 | 30.8 | 17.2 | 6.8 | |
| Healthy subjects | 12.3 | 30.9 | 31.3 | 18.6 | 6.9 | |
| Ambulatory patients | 14.1 | 28.8 | 31.6 | 18.8 | 6.8 | |
| Admitted patients **,†† | 24.9 | 28.3 | 28.5 | 11.9 | 6.4 | |
| **Expensive (%)** | | | | | | <0.001 |
| All subjects | 51.1 | 31.3 | 12.2 | 3.4 | 2.1 | |
| Healthy subjects | 49.0 | 35.4 | 11.5 | 2.1 | 2.0 | |
| Ambulatory patients * | 55.5 | 30.5 | 8.4 | 3.8 | 1.7 | |
| Admitted patients **,†† | 45.9 | 25.6 | 20.6 | 5.0 | 2.9 | |
| **Effective (%)** | | | | | | 0.232 |
| All subjects | 5.6 | 33.2 | 28.6 | 23.2 | 9.4 | |
| Healthy subjects | 3.5 | 34.9 | 30.0 | 22.5 | 9.1 | |
| Ambulatory patients | 5.4 | 33.3 | 26.2 | 25.3 | 9.8 | |
| Admitted patients | 9.6 | 29.8 | 30.7 | 20.6 | 9.2 | |
| **Substitute for medicines (%)** | | | | | | <0.001 |
| All subjects | 2.0 | 8.1 | 14.2 | 28.3 | 47.4 | |
| Healthy subjects | 1.2 | 6.4 | 13.8 | 27.4 | 51.3 | |
| Ambulatory patients | 1.6 | 8.7 | 11.8 | 29.7 | 48.2 | |
| Admitted patients **,†† | 4.3 | 9.8 | 19.3 | 27.3 | 39.2 | |

**Table 2.** *Cont.*

| | | | | | | |
|---|---|---|---|---|---|---|
| **No problem in co-administration with medicines (%)** | | | | | | <0.001 |
| All subjects | 11.9 | 19.4 | 22.4 | 18.8 | 27.4 | |
| Healthy subjects | 7.4 | 18.1 | 22.0 | 22.3 | 30.1 | |
| Ambulatory patients ** | 12.4 | 20.9 | 21.3 | 17.7 | 27.7 | |
| Admitted patients **,†† | 18.6 | 18.8 | 25.5 | 14.8 | 22.4 | |

Missing values were excluded; $p$-values were calculated Kruskal-Wallis test; * $p < 0.0167$, ** $p < 0.0033$ *vs.* health subjects, and †† $p < 0.0033$ *vs.* ambulatory subjects by Bonferroni post hoc test.

Overall, most subjects did not believe that dietary supplements were a substitute for medicines, with no differences between ambulatory patients and healthy subjects. In contrast, more admitted patients thought that dietary supplements could be substituted for medicines (strongly agree: 4.3%) compared to ambulatory patients (1.6%) and healthy subjects (1.2%). There was a significant difference among groups in terms of concomitant use of supplements with medicines. Admitted patients (strongly agree: 18.6%; strongly disagree: 22.4%) and ambulatory patients (strongly agree: 12.4%; strongly disagree: 27.7%) were more likely to think that concomitant use of supplements and medicines was safe compared with healthy subjects (strongly agree: 7.4%; strongly disagree: 30.1%).

Current user of dietary supplements thought that dietary supplements were effective (strongly agree: 5.8% healthy subjects, 7.2% ambulatory patients, and 17.7% admitted patients), substitute for medicines (strongly agree: 1.7% healthy subjects, 2.6% ambulatory patients, and 6.1% admitted patients), and no problem in co-administration with medicines (strongly agree: 10.0% healthy subjects, 17.3% ambulatory patients, and 29.3% admitted patients). All of these numbers are higher than those in Table 2. In addition, ambulatory and admitted patients using dietary supplements tended to think that dietary supplements were safe (strongly agree: 12.8% healthy subjects, 16.1% ambulatory patients, and 31.9% admitted patients). However, price (strongly agree: 43.2% healthy subjects, 52.4% ambulatory patients, and 45.3% admitted patients) did not affect dietary supplement use in all groups.

### *3.4. Purpose of Using Dietary Supplements*

Purpose of using dietary supplements is shown in Table 3. Among all subjects, maintenance of health and nutritional support were the primary and secondary reasons, respectively, for using dietary supplements; there were no significant differences among groups. More ambulatory patients (33.3%) used dietary supplements to prevent disease compared with admitted patients (23.1%) and healthy subjects (24.9%). Only 3.7% of healthy subjects used dietary supplements to treat their diseases, whereas, 10.0% of ambulatory patients and 13.2% of admitted patients used dietary supplement for this purpose. Use of supplements for beauty purposes was lowest in admitted patients (5.8%) and highest in healthy subjects (23.6%). There was no significant difference among groups; however, 4.4% of ambulatory patients and 4.1% of admitted patients used dietary supplements without any specific purpose.

**Table 3.** Purpose of using dietary supplements.

| | Yes | No | *p*-value | Odds Ratio | 95% CI |
|---|---|---|---|---|---|
| **Maintenance of health (%)** | | | 0.250 | | |
| All subjects | 70.6 | 29.4 | | | |
| Healthy subjects | 73.8 | 26.2 | | 1 | |
| Ambulatory patients | 68.2 | 31.8 | | 0.68 | 0.48–0.96 |
| Admitted patients | 71.9 | 28.1 | | 0.84 | 0.51–1.39 |
| **Nutritional support (%)** | | | 0.161 | | |
| All subjects | 36.7 | 63.3 | | | |
| Healthy subjects | 40.5 | 59.5 | | 1 | |
| Ambulatory patients | 33.8 | 66.2 | | 0.86 | 0.63–1.19 |
| Admitted patients | 38.0 | 62.0 | | 0.95 | 0.60–1.51 |
| **Prevention of disease (%)** | | | 0.014 | | |
| All subjects | 29.0 | 71.0 | | | |
| Healthy subjects | 24.9 | 75.1 | | 1 | |
| Ambulatory patients | 33.3 | 66.7 | | 1.30 | 0.92–1.84 |
| Admitted patients | 23.1 | 76.9 | | 0.79 | 0.47–1.32 |
| **Treatment of disease (%)** | | | 0.001 | | |
| All subjects | 8.3 | 91.7 | | | |
| Healthy subjects | 3.7 | 96.3 | | 1 | |
| Ambulatory patients | 10.0 | 90.0 | | 2.87 | 1.42–5.78 |
| Admitted patients | 13.2 | 86.8 | | 4.03 | 1.75–9.28 |
| **For beauty (%)** | | | <0.001 | | |
| All subjects | 15.3 | 84.7 | | 1 | |
| Healthy subjects | 23.6 | 76.4 | | 0.61 | 0.40–0.93 |
| Ambulatory patients | 12.2 | 87.8 | | 0.29 | 0.13–0.69 |
| Admitted patients | 5.8 | 94.2 | | | |
| **Without any specific purpose (%)** | | | 0.911 | | |
| All subjects | 4.6 | 95.4 | | | |
| Healthy subjects | 5.0 | 95.0 | | 1 | |
| Ambulatory patients | 4.4 | 95.6 | | 0.76 | 0.37–1.56 |
| Admitted patients | 4.1 | 95.9 | | 0.69 | 0.24–2.04 |

$n$ = 872; Subjects answered dietary supplement use "present"; $p$-values were calculated $\chi^2$ test; Odds Ratio were calculated logistic regression analysis adjusted for sex and age.

### *3.5. Experience with Beneficial or Adverse Effects from Using Dietary Supplements*

Among subjects who used dietary supplements in the past and present, only 23.8% experienced beneficial effects from dietary supplements. However, more admitted patients (28.4%) felt beneficial effects compared to ambulatory patients (25.1%) or healthy subjects (22.1%). On the other hand, 3.3% of subjects experienced adverse effects from using dietary supplements. The most common adverse effects were diarrhea and constipation (28.3%), fatigue (18.3%), allergic reactions (16.7%), abdominal pain (15.0%), vomiting (11.7%), and headache (6.7%).

### *3.6. Concomitant Use of Dietary Supplements and Medicines*

Most ambulatory patients (91.3%) and admitted patients (81.8%) took medicines; in contrast, only 10.6% of healthy subject took medicines (Table 4). Furthermore, 36.8% of ambulatory patients, 17.7% of admitted patients, and 4.3% of healthy subjects took dietary supplements and medicines concomitantly.

**Table 4.** Concomitant use of dietary supplements and medicines.

| | **Medicines** *n* **(%)** | **Dietary Supplements** *n* **(%)** | **Parallel Use** *n* **(%)** |
|---|---|---|---|
| Healthy Subjects ($n = 979$) | 104 (10.6) | 301 (30.7) | 42 (4.3) |
| Ambulatory Patients ($n = 1154$) | 1054 (91.3) | 451 (39.1) | 425 (36.8) |
| Admitted Patients ($n = 599$) | 490 (81.8) | 122 (20.4) | 106 (17.7) |

Table 5 shows the number of subjects taking concomitant dietary supplements and medicines. The most common pattern was one kind of dietary supplement and one kind of medicine ($n = 44$). However, six subjects took more than five dietary supplements and more than five medicines concomitantly.

**Table 5.** Number of dietary supplements and medicines used concomitantly.

| **Number of** | **Number of Medicines** | | | | |
|---|---|---|---|---|---|
| **Dietary Supplements** | 1 | 2 | 3 | 4 | 5≤ |
| 1 | 44 | 43 | 27 | 28 | 37 |
| 2 | 43 | 40 | 33 | 14 | 32 |
| 3 | 16 | 17 | 13 | 8 | 13 |
| 4 | 4 | 4 | 3 | 2 | 4 |
| 5≤ | 10 | 5 | 3 | 3 | 6 |

$n = 452$; Missing values were excluded.

### *3.7. Declaration of Dietary Supplements Use to Primary Care Doctors*

In patients who took dietary supplements and medicines concomitantly, only 30.2% of admitted patients and 28.0% of ambulatory patients declared their use of dietary supplements to their attending physicians. In other words, almost 70% of patients used dietary supplements on their own, without consulting physicians. Table 6 shows reasons for no declaring dietary supplements use to physicians in each ambulatory and admitted patient.

**Table 6.** Reasons for not discussing dietary supplement use with physicians.

| **Reasons** | **Ambulatory (*n*)** | **Admitted (*n*)** |
|---|---|---|
| Dietary supplements that they use does not relate to their treatment | 25 | 2 |
| Doctors might deny dietary supplements use | 19 | 2 |
| Doctors never ask about dietary supplements use | 14 | 5 |
| Dietary supplements are just food | 16 | 2 |
| No need to say | 7 | 6 |
| There are any influences to medication (self-judgment) | 8 | 1 |

**Table 6.** *Cont.*

| | | |
|---|---|---|
| There are not any opportunities to tell | 5 | 1 |
| Doctors do not have any knowledge about dietary supplements | 3 | 1 |
| There are not any problems in using dietary supplements | 3 | 0 |
| Use dietary supplements only as needed | 3 | 0 |
| Other | 12 | 4 |

$n = 112$ in ambulatory patients and $n = 24$ in admitted patients; Subjects answered this question.

## 4. Discussion

In this study, we clarified that not only ambulatory patients but also admitted patients used dietary supplements, and they used it for treatment their diseases. These patients also took medicines concurrently without consulting physicians.

In the United States, 48.8% of people used dietary supplements from 2007 to 2010 [10]. Previous reports show that use of dietary supplements in Japan has increased over time from 10.9% in 2001 [11], 11.0% in males and 16.4% in females in 2003 [12], and 45.8% in older adults in 2008 [9], even if factors such as sex, age, socioeconomic status, and health-related characteristics are known to affect use of dietary supplements [10,13–16]. In addition, recognition also affects dietary supplement use. Dietary supplements were not regulated in Japan. Most dietary supplements are the form of capsules, tablets, powders, or liquid, and some are the form of regular foods in Japan. In this situation, some people take dietary supplements without consideration for the risk of them. In these days, as dietary supplement use increases, associated health problems also increase. Health problems associated with dietary supplement use have two causes. One is use of low quality or illegal products that contain drug ingredients [17,18]. To avoid health problems caused by these products, the Japanese government constantly surveys and checks these products on websites and retail stores. Another is inappropriate use of dietary supplements, including excessive intake and concomitant use of various dietary supplements and/or medicines. In particular, inappropriate use of dietary supplements in patients may be associated with severe health problems. To avoid health problems caused by inappropriate use, communication between patients and physicians are important.

It is recognized that infants, children, pregnant women, the elderly, and patients are susceptible to dietary supplements. It is important to identify dietary supplement use in these high-risk groups and to stop inappropriate use. Inappropriate use of dietary supplements by Japanese children [19] and pregnant women [20] has been defined. In Japan, most children have a good nutritional state and thus do not require dietary supplements. On the other hand, folic acid supplements are recommended for pregnant women because it is difficult to obtain adequate amounts of folic acid from food [21]. However, we confirmed that pregnant women could not avail of dietary supplements appropriately [20]. Aside from children and pregnant women, many older people in Japan appear to use dietary supplements; some of them use dietary supplements for treatment of diseases [22]. In this study, we investigated the awareness and use of dietary supplements among Japanese subjects.

Our results showed that 36.8% of ambulatory patients and 17.7% of admitted patients took dietary supplements with their medicines and thought that this practice was safe. However, many reports indicate that dietary supplements interact with medicines. The most well-known example is St. John's

wort (*Hypericum perforatum* L.). St. John's wort contains hyperforin, which increases the expression of cytochrome P450 (CYP), especially CYP3A4, and affects drug metabolism in the liver [23]. Other herbs (e.g., black cohosh, coleus forskohlii, echinacea, garlic, ginkgo, ginseng, green tea, kava, and milk thistle) [24–28] and ingredients (e.g., catechins [29], curcuminoids [30], isoflavones [31], quercetin [32], polyphenols [33], and resveratrol [34]) also affect drug metabolizing enzymes.

To avoid interactions between prescription medications and dietary supplements, physicians need to know whether their patients use dietary supplements or not. However, as shown in this survey, most patients do not discuss these supplements with their physicians, which is consistent with previous reports [35]. One reason for this lack of discussion is that most physicians do not ask about dietary supplement use, probably because the consultation time for each patient is limited. In addition, 5 admitted patients answered "Doctors never ask about dietary supplements use" (Table 6), it means that some of physicians did not care whether their patients used dietary supplements or not. It might be caused by insufficient recognition of dietary supplements. At the same time, most patients do not think that dietary supplements will affect their medication. Thus, both patients and physicians do not fully recognize the risk of interactions between dietary supplements and medications [36]. It is also reported that both of patients and physicians are poorly understood the regulation of dietary supplement in the USA [37], even though dietary supplements are regulated by the U.S. Food and Drug Administration (FDA) under Dietary Supplement Health and Education Act. As dietary supplements are not as safe as they believe [38], education for both physicians and patients is important in order to avoid health problems associated with dietary supplements.

Consistent with a previous internet survey in Japan, 3.3% of all subjects experienced adverse effects by using dietary supplements, even if most cases were not severe. In this survey, we did not ask which type of product was used. Thus we could not determine any relationship between dietary supplements and adverse effects. However, many subjects used several dietary supplements and medicines concurrently. Even if we asked which type of product was used, it would be impossible to determine the cause of health problems. To avoid unexpected health problems caused by dietary supplements, patients should not use dietary supplements for disease treatment or concurrently with medicines without consulting by physicians.

There are some limitations in this study. The number of admitted patients was lower than the number of ambulatory patients or healthy subjects, because cooperation with primary doctors was essential to conduct this survey in admitted patients. In addition, we did not ask type, periods, and frequency of dietary supplements use or medications. So, we could not evaluate the exact risk of concomitant use of dietary supplements and medicines in this study. Further investigations are needed.

## 5. Conclusions

We clarified that most patients used dietary supplements without consulting physicians, and some of them experienced adverse effects from using dietary supplements. To avoid health problems, it is important that physicians ask patients about dietary supplement use and those patients should inform their physicians about these supplements if physicians do not ask.

## Acknowledgments

This work was supported in part by Health and Labour Sciences Research Grants (Research on Food Safety). The authors thank the pharmacies, hospitals, and autonomous communities that cooperated with this study.

## Author Contributions

Tsuyoshi Chiba formulating the research question, designing the study, carrying it out, analyzing the data, and writing the article; Yoko Sato carrying it out and analyzing the data; Tomoko Nakanishi, Kaori Yokotani, and Sachina Suzuki carrying it out; Keizo Umegaki designing the study and writing the article.

## Conflicts of Interest

The authors declare no conflict of interest.

## References

1. American Dietetic Association. Position of the american dietetic association: Fortification and nutritional supplements. *J. Am. Diet. Assoc.* **2005**, *105*, 1300–1311.
2. Teschke, R.; Wolff, A.; Frenzel, C.; Schulze, J.; Eickhoff, A. Herbal hepatotoxicity: A tabular compilation of reported cases. *Liver Int.* **2012**, *32*, 1543–1556.
3. Bjelakovic, G.; Gluud, L.L.; Nikolova, D.; Bjelakovic, M.; Nagorni, A.; Gluud, C. Antioxidant supplements for liver diseases. *Cochrane Database Syst. Rev.* **2011**, *3*, doi:10.1002/14651858.CD007749.pub2.
4. Pilkington, K.; Boshnakova, A. Complementary medicine and safety: A systematic investigation of design and reporting of systematic reviews. *Complement. Ther. Med.* **2012**, *20*, 73–82.
5. Yamada, K.; Sato-Mito, N.; Nagata, J.; Umegaki, K. Health claim evidence requirements in Japan. *J. Nutr.* **2008**, *138*, 1192–1198.
6. Ohama, H.; Ikeda, H.; Moriyama, H. Health foods and foods with health claims in Japan. *Toxicology* **2006**, *221*, 95–111.
7. Sadovsky, R.; Collins, N.; Tighe, A.P.; Brunton, S.A.; Safeer, R. Patient use of dietary supplements: A clinician's perspective. *Curr. Med. Res. Opin.* **2008**, *24*, 1209–1216.
8. Messina, B.A. Herbal supplements: Facts and myths-talking to your patients about herbal supplements. *J. Perianesth Nurs.* **2006**, *21*, 268–281.
9. Hirayama, F.; Lee, A.H.; Binns, C.W.; Watanabe, F.; Ogawa, T. Dietary supplementation by older adults in Japan. *Asia Pac. J. Clin. Nutr.* **2008**, *17*, 280–284.
10. Bailey, R.L.; Gahche, J.J.; Miller, P.E.; Thomas, P.R.; Dwyer, J.T. Why US adults use dietary supplements. *JAMA Intern. Med.* **2013**, *173*, 355–361.
11. Ishihara, J.; Sobue, T.; Yamamoto, S.; Sasaki, S.; Akabane, M.; Tsugane, S. Validity and reproducibility of a self-administered questionnaire to determine dietary supplement users among Japanese. *Eur. J. Clin. Nutr.* **2001**, *55*, 360–365.