表4　妊娠3か月までのfolic acid摂取群と非摂取群の，NTDリスク低減と葉酸摂取に関する認識の比較

	妊娠3か月までのfolic acid摂取状況（n＝1,236）						
	全　体		摂取群		非摂取群		
	n	%	n	%	n	%	P値*1
全体	1,236	100.0	879	71.1	357	28.9	
葉酸とNTDの知識							
葉酸という栄養素							<0.001
知っている	1,217	98.5	875	71.9	342	28.1	
知らない	19	1.5	4	21.1	15	78.9	
葉酸が含まれる食品							<0.001
知っている	976	79.0	752	77.0	224	23.0	
知らない	260	21.0	127	48.8	133	51.2	
葉酸の化学形態による吸収率の違い							0.752
知っている	688	55.7	492	71.5	196	28.5	
知らない	548	44.3	387	70.6	161	29.4	
葉酸とNTDリスクの関連							<0.001
知っている	853	69.0	687	80.5	166	19.5	
知らない	383	31.0	192	50.1	191	49.9	
妊婦に対する葉酸の推奨摂取量							<0.001
知っている	675	54.6	588	87.1	87	12.9	
知らない	561	45.4	291	51.9	270	48.1	
NTDリスク低減の為の葉酸の摂取時期							
開始時期							<0.001
正解	750	60.7	604	80.5	146	19.5	
不正解	486	39.3	275	56.6	211	43.4	
終了時期							0.021
正解	238	19.3	184	77.3	54	22.7	
不正解	998	80.7	695	69.6	303	30.4	
NTDリスク低減の為の葉酸の1日の摂取量							<0.001
正解	156	12.6	117	75.0	39	25.0	
不正解	738	59.7	588	79.7	150	20.3	
まったく分からない	342	27.7	174	50.9	168	49.1	
普段の食事からの自身の葉酸摂取量							<0.001
分かる	122	9.9	109	89.3	13	10.7	
気になるが，分からない	899	72.7	671	74.6	228	25.4	
考えたことがない	215	17.4	99	46.0	116	54.0	
サプリメント利用状況							
妊娠前の葉酸以外のサプリメント利用状況							<0.001
毎日利用していた	235	19.0	212	90.2	23	9.8	
たまに利用していた/利用経験があった	392	31.7	321	81.9	71	18.1	
利用したことがない	609	49.3	346	56.8	263	43.2	
妊娠中の葉酸以外のサプリメント利用状況							<0.001
毎日利用していた	353	28.6	320	90.7	33	9.3	
たまに利用していた/利用経験があった	311	25.2	247	79.4	64	20.6	
利用したことがない	572	46.3	312	54.5	260	45.5	

*1 χ^2検定。

表5　葉酸とNTDリスクの関連について知識のある人における，妊娠3か月までのfolic acid摂取状況と属性の関連

	妊娠3か月までのfolic acid摂取状況（n＝853）							
	全体		摂取群		非摂取群			
	n	%	n	%	n	%	P値[*1]	OR（95%CI）[*2]
全体	853	100.0	687	80.5	166	19.5		
年代							0.165	
20代	301	35.3	236	78.4	65	21.6		1
30代	517	60.6	419	81.0	98	19.0		1.33（0.91–1.93）
40代	35	4.1	32	91.4	3	8.6		3.80（1.08–13.4）
地域							0.127	
北海道	51	6.0	43	84.3	8	15.7		1
東北	37	4.3	29	78.4	8	21.6		0.61（0.20–1.87）
関東	310	36.3	265	85.5	45	14.5		0.92（0.40–2.13）
中部	144	16.9	116	80.6	28	19.4		0.74（0.31–1.80）
近畿	173	20.3	130	75.1	43	24.9		0.51（0.22–1.18）
中国	44	5.2	33	75.0	11	25.0		0.62（0.22–1.77）
四国	21	2.5	15	71.4	6	28.6		0.38（0.11–1.33）
九州	73	8.6	56	76.7	17	23.3		0.58（0.22–1.50）
世帯収入							0.671	
400万未満	229	26.8	181	79.0	48	21.0		1
400～800万未満	366	42.9	294	80.3	72	19.7		0.95（0.61–1.46）
800万以上	70	8.2	60	85.7	10	14.3		0.95（0.43–2.10）
不明/無回答	188	22.0	152	80.9	36	19.1		0.86（0.52–1.43）
妊娠期間							0.286	
初期	237	27.8	184	77.6	53	22.4		1
中期	301	35.3	250	83.1	51	16.9		1.34（0.86–2.10）
末期	315	36.9	253	80.3	62	19.7		1.07（0.70–1.65）
妊娠中の子どもの出生順位							＜0.001	
第1子	482	56.5	419	86.9	63	13.1		1
第2子	291	34.1	215	73.9	76	26.1		0.42（0.29–0.61）
第3子以降	80	9.4	53	66.3	27	33.8		0.27（0.16–0.48）

[*1] χ^2検定。
[*2] ロジスティック回帰分析によるオッズ比（95%信頼区間）。

取に対する認識と行動について全国規模の調査を実施した。本研究の対象者は社会調査会社のモニターであり，その属性は調査会社のWebサイトに詳細が掲載されている[20]。一般的に，インターネット調査の特徴として，モニターの年齢層に偏りがある点，標本誤差が生じうる可能性がある点，学歴が高い傾向がある点が指摘されている[21,22]。本研究では学歴は尋ねておらず，調査会社のモニターに登録してアンケートに回答したという積極的なユーザーを対象としているなど，対象母集団との関連性が明確ではないという限界がある。一方で，年齢層については2012年人口動態統計[23]における出生児の母親の年齢層とほぼ同等であり偏りはないと考えられる。また，本調査結果における妊婦の葉酸とNTDに関する認知度は質問紙調査による先行研究[17]と同等であったことから，本調査の対象者が特別，知識の高い集団であった可能性は低い。さらに，全国から地域や妊娠期間の偏りのないデータが得られたという利点もある。したがって，本研究結果の基本統計量を国内の妊婦の代表値とみなすことはできないが，関連要因に関する結果は比較的普遍性があるとみなして差支えないであろう。

本研究結果では，85.2%の妊婦が妊娠中に葉酸を意識的に摂取していたものの，妊娠前は37.3%にとどまり，妊娠4か月以降のfolic acidサプリメント摂取者が約5割みられるなど，厚生労働省が推奨している[8]摂取時期である「妊娠の1か月以上前から妊娠3か月まで」よりも意識的な摂取時期が遅すぎることが示された。また，この推奨時期にfolic acidを意識的に摂取していなかった妊婦の特徴とし

て，NTDリスク低減と葉酸に関する知識がないこと，妊娠前および妊娠中を通してサプリメント利用経験がないこと，若年であること，第2子以降を妊娠中であることが認められた。

厚生労働省の通知[8]ではfolic acidの摂取源として「いわゆる栄養補助食品」を挙げている。しかし，市場に出回る葉酸補給を謳った食品の多くはサプリメントであり，folic acid摂取者の摂取源もサプリメントが主流であった。NTDリスク低減のためのfolic acidサプリメントの理想的な利用方法は，必要な時期に必要とされる葉酸摂取量を理解し，自身の天然型葉酸の摂取量を把握したうえで，不足分をサプリメントから補うことと考えられる。しかし，多くの妊婦は，摂取時期が遅く，摂取量の認識もなく，サプリメントを効果的に利用できていない実態が示唆された。一方で，サプリメント利用に否定的な妊婦は葉酸の意識的な摂取自体を行っておらず，葉酸摂取のためにはサプリメントを利用しなければならないと捉えている傾向があることがうかがえた。国内におけるサプリメントを取り巻く環境は整備されているとは言い難く，安全性や品質が様々な製品が存在し[24]，安全な製品の選択が困難という現状がある。したがって，妊娠中もしくは妊娠を考えている女性がサプリメント利用を敬遠することはやむを得ない。そこで，NTD予防のためのfolic acid摂取には，サプリメントではなく，folic acidが添加された加工食品の利用を積極的に推奨することが適切と考える。同時に，様々な通常食品形態のfolic acid添加食品の開発が望まれる。

さらに，必要な時期にfolic acidを摂取しない妊婦を減らすための対応として，経産婦に対する情報提供の強化が挙げられる。NTDの発症は遺伝要因もあるが，その他，多因子による複合的なものである。本研究にて，NTDリスクと葉酸に関する知識がありながらも，必要な時期にfolic acid摂取を行わなかった妊婦は第2子以降の妊娠で多いことが示された。これは，第2子以降ではfolic acidサプリメント利用率が増加する米国の報告と全く逆の傾向である[11]。NTDは第2子以降のみに発症した例もあり[25]，経産婦であってもfolic acid摂取が推奨される旨の情報を積極的に提供して行く必要がある。本研究はインターネット調査であるため，妊婦の情報源はインターネットに偏ると想定されたが，新聞・雑誌や友人・知人・家族からの情報も摂取理由とされており，幅広い情報収集が行われている可能性が示された。しかし，医師・薬剤師等の専門職からの情報を摂取理由に挙げた妊婦は少なかった。必要な時期にfolic acidを摂取していた妊婦では，医師・薬剤師からの情報を摂取理由とした者が多かったため，より早い時期からの専門職による正しい情報提供が必要と考えられる。

NTDリスク低減のための葉酸摂取については，2000年の厚生労働省通知[8]から10年以上に渡り啓発が実施されて来たが，本研究結果から，妊婦におけるNTDリスクと葉酸摂取に関する知識は十分ではなく，folic acid摂取時期も適切とは言い難い現状が示されたことから，妊娠前からのfolic acid摂取を周知徹底するには，現状のままの知識の普及と自発的な葉酸摂取行動の推奨だけでは限界があると考えられる。より効果的に妊娠可能な女性のfolic acid摂取を推進するためには，さらなる啓発とともに，folic acidを含む加工食品の利用の推奨や，食品へのfolic acid添加の推進，国内における食材への強制添加の意義についての議論など，次の段階の対策を検討する時期に来ているといえるであろう。

本研究の限界として，インターネット調査を用いた点，年代別回収率を把握できなかった点，妊娠時期の異なる妊婦が混在しており，妊娠初期の記憶の正確性への影響が懸念される点，横断的調査であり因果関係を言及するには限界がある点が挙げられる。しかし，これまでに十分把握されていなかった妊婦の意識的な葉酸摂取の現状とその関連要因を全国規模で明らかにした研究として一定の意義を有し，妊婦に対するより効果的なfolic acid摂取対策を検討するうえで有益な資料となると考える。

V　結　　語

本研究結果より，多くの妊婦が妊娠中に葉酸を意識的に摂取していたものの，その開始時期はNTDリスク低減のためには遅すぎ，NTDリスク低減と葉酸に関する認識不足，サプリメント利用経験がない，若年，第2子以降を妊娠中であることが，必要な時期のfolic acid摂取に負の影響を示すことが明らかとなり，NTDリスク低減のためのfolic acid摂取行動が適切かつ十分に行われていない現状が示された。NTDリスク低減のためのfolic acid摂取を推進するためには，経産婦も対象に含めた正確な情報提供など，さらなる啓発が必要であるとともに，folic acidを添加した加工食品の利用の推奨，食材へのfolic acid添加の推進など，より踏み込んだ対策を検討して行く必要があると考えられる。

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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.

* National Institute of Health and Nutrition

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

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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.
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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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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 $p < 0.05$.

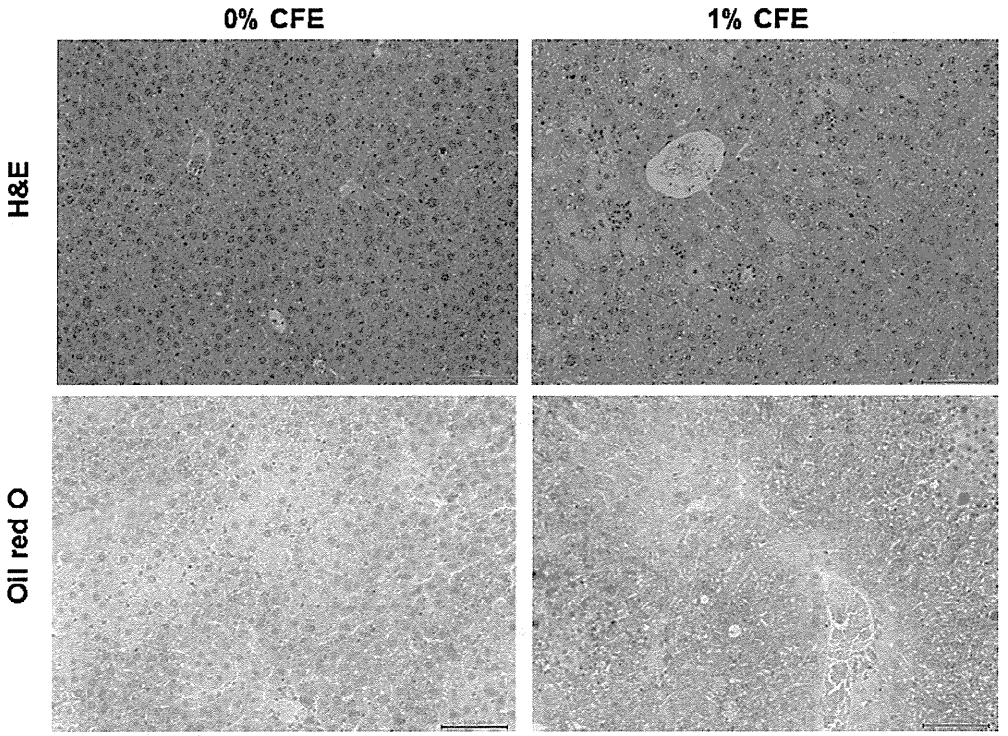

Fig. 1. Representative histopathologic changes in liver sections from mice stained with hematoxylin and eosin (H&E) and Oil red O (magnification 200×). Mice were maintained for 2 weeks on diet supplemented with 0, 0.3, or 1% CFE.

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

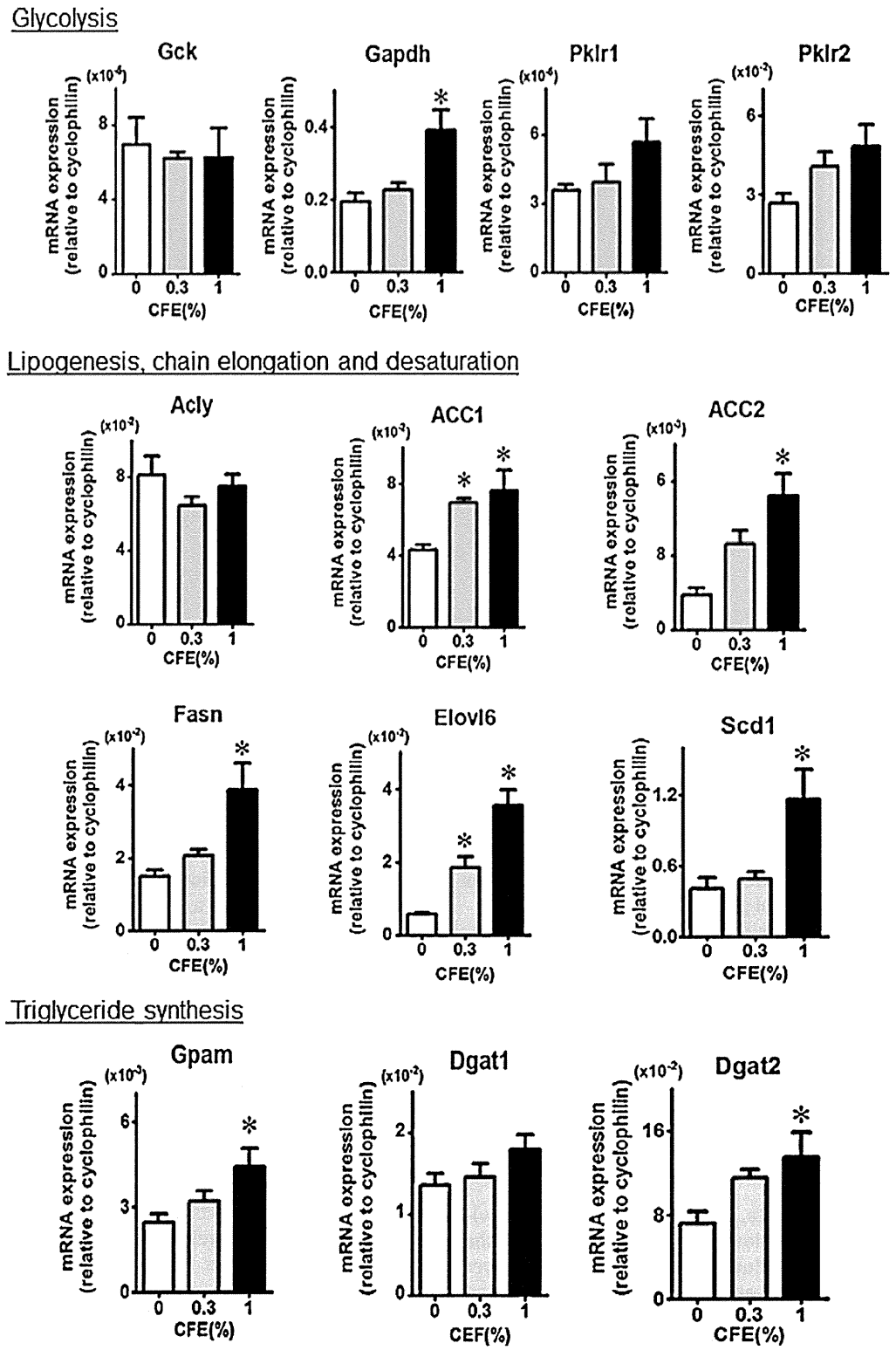

Fig. 2. Expression of mRNAs encoding enzymes involved in glycolysis, fatty acid synthesis, chain elongation and desaturation, and triglyceride synthesis. Protein product abbreviations are defined in the main text and Table 1. Each value is the mean and SE from 5 mice. Significant difference from the 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

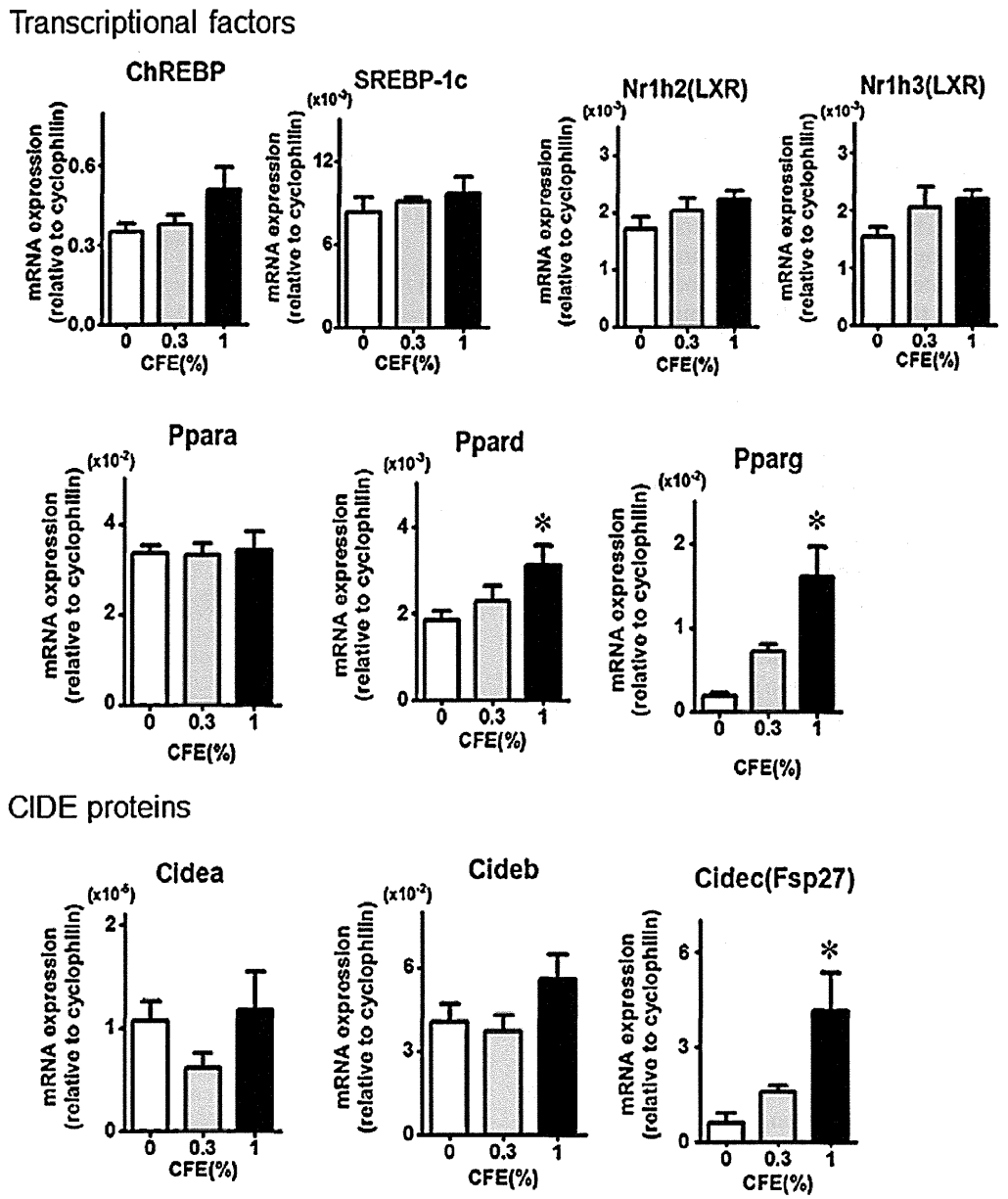

Fig. 3. Expression of mRNAs encoding transcription factors and effectors involved in fatty liver. Each values is the mean and SE from 5 mice. Protein product abbreviations are defined in the main text and Table 1. Significant difference from the level of control group (0% CFE) is indicated by $^{*}p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

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.

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Article

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

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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 χ^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 χ^2 test. Multivariable analysis was also done using the logistic regression analysis adjusted for sex and age. *P* values less than 0.05 in χ^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 χ^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 χ^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%).