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資　料

葉酸およびそのサプリメント摂取に対する妊婦，管理栄養士・栄養士，管理栄養士・看護師養成校の学生の認識

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【目的】胎児の神経管閉鎖障害（Neural Tube Defect; NTD）リスク低減のため，妊娠可能な女性へ積極的な葉酸摂取が推奨されているが，その内容が正しく理解されているか不明である。そこで本研究は，妊娠期の葉酸およびそのサプリメントの摂取に対する妊婦と，妊婦への情報提供を行う管理栄養士・栄養士，管理栄養士・看護師養成校の学生の認識の実態に関する予備的な調査を実施し，その課題を検討することとした。

【方法】2011年10月～12月に，自治体開催の母親学級の参加者および産院に通う妊婦（東京都，北海道），管理栄養士・栄養士を対象としたサプリメントの講演会の参加者（東京都，神奈川県，広島県），管理栄養士・看護師の養成校の学生（長野県，広島県）を対象とし，無記名自記式のアンケート調査を実施した。

【結果】妊婦104人，管理栄養士・栄養士69人，養成校の学生175人から有効回答を得た。有効回答率はそれぞれ54.5%，51.1%，65.8%であった。妊婦，管理栄養士・栄養士，養成校の学生ともに，90%以上が葉酸という栄養素を知っていたが，胎児のNTD発症リスク低減のために推奨される摂取時期や摂取量についての知識は十分ではなかった。

【結論】本研究は限られた対象者で実施した予備的調査であるが，妊婦および妊婦への情報提供を担う管理栄養士・栄養士や養成校の学生に対して，葉酸摂取とNTDリスク低減に関する，より具体的な情報（適切な摂取時期，摂取量，サプリメントの実態）の提供の必要性が示唆された。

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Ⅰ．緒　言

わが国では，特定成分を濃縮したサプリメント（錠剤・カプセル型の食品）の大半が一般食品として流通しており，安全性の検証が十分にされていない成分が利用されていたり[1,2]，原材料や製品としての品質のばらつきが大きいなど[3]，安全面での問題点が指摘されている。また，食品として流通していながらも形態が錠剤やカプセル状であることから，医薬品と区別せずに利用されている傾向も認められる[4]。

一方で，通常の食事からの十分な栄養素補給が困難な状況では，サプリメントの利用が推奨されている場合があり，その代表例として葉酸が挙げられる。妊娠初期の葉酸強化食品や葉酸サプリメントの摂取が，胎児の神経管閉鎖障害（Neural Tube Defect; NTD）発症リスクの低減と関連することが多くの研究で示されており[5,6]，世界各国で妊娠の可能性のある若年女性に対する葉酸摂取が推奨されている[7,8]。日本においても，2000年に厚生労働省より「神経管閉鎖障害の発症リスク低減のための妊娠可能な年齢の女性等に対する葉酸の摂取に関する情報提供要領」が出され[9]，2002年から母子健康手帳に記載されるなど[10]，妊婦への葉酸摂取に関する情報提供が実施されている。食事中の葉酸の大半はポリグルタミン酸型として存在し，吸収されにくいという特徴がある。一方，サプリメント等に利用されているfolic acidはモノグルタミン酸型で吸収されやすく，化学的にも安定している。そのような理由から，葉酸についてはサプリメントなどの使用も視野に入れた摂取が推奨されている[11]。ちなみに特定保健用食品の疾病リスク低減表示として「二分脊椎などの神経管閉鎖障害を持つ子どもが生まれるリスクの低減」があるが，この表示ができる葉酸の形態はfolic acidとなっている。これらの状況は，必ずしもfolic acidのサプリメントの利用を否定するものではなく，むしろ適切な利用を推奨するものである。しかし，上述のように市場に流通するサプリメントには多様な品質の製品が存在し，消費者はそれらの製品を自己判断で利用していることから，現状ではサプリメントを適切に利用できる環境が整備されているとは言い難い。

こうした中，国内の妊婦によるサプリメント利用の実態調査において，30～40%の妊婦が葉酸サプリメントを

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摂取していることが報告されているが，その摂取時期にはずれがあり，医師や助産師，看護師などの医療従事者による正確な情報提供の必要性が指摘されている[12,13]。管理栄養士・栄養士もコメディカルなスタッフとして，また，食の専門家として妊婦への適切な助言，指導を担う立場であり，正確な知識を有している必要がある。しかし，これらを助言する立場の者のサプリメントや葉酸に関する知識の実態については，葉酸によるNTDリスク低減の認知度が報告されているものの[14,15]，そのための葉酸の適切な摂取時期，摂取量，摂取形態など，具体的知識の程度や製品としてのサプリメントに対する意識に言及した報告は見当たらない。また，指導や助言を受ける側の妊婦についても，そのような視点で調査した研究は見当たらない。

そこで本研究では，葉酸の摂取やサプリメントの利用に対する認識について，当事者である妊婦，ならびに食に関する助言を行う立場にある管理栄養士・栄養士，今後そのような立場となるであろう管理栄養士ならびに看護師養成校の学生を対象に予備的なアンケート調査を実施し，妊娠期の葉酸摂取推奨に関する今後の課題について考察した。

Ⅱ．方　法

1．対象者と調査時期

協力の得られた自治体開催の母親学級（都内3区）の参加者および産院（北海道1病院）に通う妊婦，管理栄養士・栄養士を対象としたサプリメントに関する講演会（東京都，神奈川県，広島県内の計3市）の参加者，管理栄養士・看護師の養成校（長野県，広島県内の計2校）の学生1～4年生を対象とし，2011年10月～12月に無記名自記式のアンケート調査を実施した。対象者数は，妊婦191人，管理栄養士・栄養士135人，養成校の学生266人であった。講演会および自治体開催の母親学級ならびに学校においては，講演または講義の開始前に調査用紙の配布・記入・回収を行った。産院においては診療時に施設を通じて配布，回収を行った。回収は各施設で行ってもらい，取りまとめて郵送で返却するよう依頼した。

倫理的配慮として，質問項目設定の際，特定の個人が識別可能なデータは収集せず，アンケート協力者に対して倫理的な問題となる質問も含まないようにした。また，調査用紙に研究の目的および調査用紙の提出をもって協力の同意を得たものとし，同意しないときは提出する必要がないことを明記した。本研究は，（独）国立健康・栄養研究所研究倫理審査委員会の承認を得て実施した。

2．質問紙内容

質問紙は妊婦用と管理栄養士・栄養士ならびに養成校の学生用の2種類とした。質問紙項目の構成は属性，葉酸および胎児のNTDに対する認識と行動，サプリメント利用に対する認識と行動について関連する文献を参照し[16,17]，以下の質問を設定した。なお，養成校の学生の学年は問わなかった。選択式の設問について，個々の質問内容と回答項目の詳細は表2～5に示した。

1）属　性

全員に年齢，居住地域，妊婦にはさらに妊娠期間と妊娠中の子どもの出生順位を記述式でたずねた。管理栄養士・栄養士は講演会の参加者で，取得資格として「管理栄養士」または「栄養士」のいずれかを選択した者のみを解析対象者とした。

2）葉酸および胎児のNTDに対する認識と行動

葉酸について，名称，栄養素の種類，含まれている食品，化学形態による吸収率の違い，NTDリスク低減効果，摂取が推奨されている開始時期と終了時期，推奨される摂取量について，知っているかどうかを単一回答式（Single Answer; SA）でたずねた。また，妊婦には，妊娠前と妊娠中の葉酸の意識的な摂取の有無（SA），摂取源の形態（複数回答式 Multiple Answer; MA），妊娠中の意識的な摂取期間（SA），摂取理由（MA）を，管理栄養士・栄養士と養成校の学生には，妊婦への推奨経験の有無（SA），推奨した摂取形態（MA），推奨経験がない理由（MA）をたずねた。

3）サプリメントに対する認識と行動

サプリメントと医薬品との違いだと思う点（MA），サプリメントの利用状況（SA），さらに，管理栄養士・栄養士と養成校の学生には妊婦のサプリメント利用に対する考え方（SA）をたずねた。

3．解析方法

全問に不備なく回答した妊婦104人，管理栄養士・栄養士69人，養成校の学生175人（管理栄養士養成校137人，看護師養成校38人）を解析対象者とした。有効回答率はそれぞれ54.5%，51.1%，65.8%であった。集計は各項目について妊婦，管理栄養士・栄養士，養成校の学生ごとに単純集計を行った後，χ^2検定にて対象者間の比較を，McNemar検定にて同一対象者間の比較を行った。統計処理は統計解析ソフトIBM SPSS Statistics 18（日本アイ・ビー・エム株式会社）を用いた。検定は両側検定とし，$p<0.05$を有意とした。

Ⅲ．結　果

1．対象者の特徴

対象者の特徴を表1に示す。妊婦は30代，関東在住，妊娠末期が主であり，管理栄養士・栄養士は40代以上，関東在住，養成校の学生は20代以下，中部または四国在住が主であった。

2．葉酸の認識

対象者の葉酸に対する認識を表2に示す。妊婦，管理栄養士・栄養士，養成校の学生のいずれも90％以上が「葉酸という栄養素を知っている」と回答し，対象者間で差は認められなかった。「ビタミンである」ことを認識していた者は，妊婦65％，管理栄養士・栄養士82％，養成校の学生83％と妊婦で少なかった（$p<0.01$）。「どのような食品に含まれるか知っている」と回答した者は，妊婦81％，管理栄養士・栄養士68％，養成校の学生57％と妊婦で多かった（$p<0.01$）。葉酸の化学形態により吸収率が異なることについては，約50％の妊婦が「よく分からない」と回答し，管理栄養士・栄養士や養成校の学生よりも多かった（$p<0.05$）。葉酸とNTDリスク低減の関連について認識していたのは妊婦，管理栄養士・栄養士，養成校の学生ともに約50～60％であり，対象者間で差は認められなかった。

NTDリスク低減のために推奨されている葉酸摂取の開始時期が妊娠の1ヶ月くらい前からであることは，妊婦と管理栄養士・栄養士の約70％が知っていたが，養成校の学生では50％であり（$p<0.01$），終了時期が妊娠3ヶ月までであることについて知っていた者は，妊婦48％，管理栄養士・栄養士33％，養成校の学生19％であった（$p<0.01$）。また，その推奨されている摂取量を知っていたのは，妊婦，管理栄養士・栄養士，養成校の学生ともに，約10～20％であり，妊婦で最も多かった（$p<0.05$）。

3．葉酸摂取に対する対応

妊婦の葉酸摂取状況を表3に示す。妊娠前から葉酸を意識的に摂取していたのは22％であり，妊娠中は81％に増加した（$p<0.01$）。摂取源は，「錠剤・カプセルなどのサプリメントから」の選択者が最多であった。摂取時期は「4～5ヶ月くらいまで」が最も多く，葉酸製品の利用理由は「食事だけでは足りないと思ったから」が75％と最多であった。また，71％の妊婦が，自身が食事から摂取している葉酸の量は「気になるが，分からない」と回答した。

管理栄養士・栄養士や養成校の学生の妊婦への葉酸摂取の助言状況を表4に示す。妊婦に葉酸摂取を勧めた経験がある管理栄養士・栄養士は26％，養成校の学生は5％であり（$p<0.01$），そのうちの約90％が野菜などの食事からの摂取を勧めていた。勧めたことがない理由は大半が「機会がないから」であった。

4．サプリメントに対する認識と摂取状況

対象者のサプリメントに対する認識と摂取状況を表5に示す。「サプリメントと医薬品の違いは何だと思いますか？」との問に，妊婦は「有効成分の含有量」の選択者が最も多く，次いで「効果」，「価格」であった。管理栄養士・栄養士では「安全性のエビデンス」が最多で，「有

表1　対象者の特徴

		妊　婦 ($n=104$)		管理栄養士・栄養士 ($n=69$)		養成校の学生 ($n=175$)	
		n	%	n	%	n	%
年齢							
	20代以下	33	31.7	18	26.1	171	97.7
	30代	68	65.4	15	21.7	3	1.7
	40代以上	3	2.9	36	52.2	1	0.6
地域							
	北海道	18	17.3	0	0.0	0	0.0
	関東	85	81.7	52	75.4	1	0.6
	中部	1	1.0	11	15.9	91	52.0
	中国	0	0.0	6	8.7	83	47.4
妊娠期間							
	初期	2	1.9	—	—	—	—
	中期	44	42.3	—	—	—	—
	末期	58	55.8	—	—	—	—
妊娠中の子どもの出生順位	第1子	80	76.9	—	—	—	—
	第2子	24	23.1	—	—	—	—

表2　葉酸に対する認識

質問項目	回答肢	妊婦 ($n=104$)		管理栄養士・栄養士 ($n=69$)		養成校の学生 ($n=175$)		χ^2 検定 p 値
		n	%	n	%	n	%	
葉酸という栄養素を知っていますか？	はい	100	96.2	69	100.0	163	93.1	0.064
	いいえ	4	3.8	0	0.0	12	6.9	
葉酸はどんな栄養素の一つですか？	ビタミン	68	65.4	57	82.6	146	83.4	0.001
	ミネラル	33	31.7	12	17.4	21	12.0	
	たんぱく質	3	2.9	0	0.0	8	4.6	
葉酸はどのような食品に含まれているか知っていますか？	はい	84	80.8	47	68.1	100	57.1	<0.001
	いいえ	20	19.2	22	31.9	75	42.5	
食材に元から含まれる葉酸と，サプリメントなどに添加されている葉酸では，吸収率が異なると思いますか？	思う	50	48.1	40	58.0	96	54.9	0.013
	思わない	3	2.9	9	13.0	19	10.9	
	よく分からない	51	49.0	20	29.0	60	34.3	
葉酸の摂取が，胎児の二分脊椎症，無脳症などの神経管閉鎖障害リスク低減と関連することをご存知ですか？	知っている	51	49.0	41	59.4	95	54.3	0.40
	聞いたことはある	37	35.6	18	26.1	46	26.3	
	知らない	16	15.4	10	14.5	34	19.4	
神経管閉鎖障害リスク低減の為に葉酸を摂った方がよいと推奨されている時期はいつだと思いますか？								
開始時期	1ヶ月くらい前	73	70.2	46	66.7	87	49.7	<0.001
	直後	24	23.1	11	15.9	34	19.4	
	3ヶ月	5	4.8	7	10.1	28	16.0	
	中期（4～7ヶ月）	2	1.9	3	4.3	23	13.1	
	後期（7ヶ月以降）	0	0.0	2	2.9	3	1.7	
終了時期	直後	0	0.0	3	4.3	2	1.1	<0.001
	3ヶ月	50	48.1	23	33.3	33	18.9	
	中期（4～7ヶ月）	18	17.3	8	11.6	27	15.4	
	後期（7ヶ月以降）	13	12.5	11	15.9	57	32.6	
	終了（出産時）	23	22.1	24	34.8	56	32.0	
神経管閉鎖障害リスク低減の為に勧められる葉酸の摂取量を知っていますか？	知っている	25	24.0	12	17.4	20	11.4	0.022
	知らない	79	76.0	57	82.6	155	88.6	

表3　妊婦の葉酸摂取行動

質問項目	回答肢	妊娠前 ($n=104$)		妊娠中 ($n=104$)		McNemar 検定 p 値
		n	%	n	%	
葉酸を意識的に摂っていましたか？	はい	23	22.1	84	80.8	<0.001
	いいえ	81	77.9	20	19.2	
		($n=23$)		($n=84$)		
どのようにして摂っていましたか？†	野菜などの食事から	6	26.1	32	38.1	<0.001
	葉酸が強化された加工食品から	1	4.3	6	7.1	0.063
	錠剤・カプセルなどのサプリメントから	22	95.7	74	88.1	<0.001
	市販薬から	0	0.0	1	1.2	—
どのくらいの期間，意識的に摂っていましたか？	妊娠3ヶ月くらいまで	—	—	16	19.0	—
	妊娠4～5ヶ月くらいまで	—	—	33	39.3	—
	妊娠6～7ヶ月くらいまで	—	—	22	26.2	—
	妊娠8～9ヶ月くらいまで	—	—	13	15.5	—
				($n=80$)		—
葉酸の製品を利用しようと思った理由は何ですか？†	食事だけでは足りないと思ったから	—	—	60	75.0	—
	たくさん摂らなければならないから	—	—	2	2.5	—
	いいと聞いたから	—	—	16	20.0	—
	なんとなく	—	—	2	2.5	—
	その他	—	—	6	7.5	—
				($n=104$)		—
葉酸を食事からどれくらい摂っているか，分かりますか？	栄養計算をしているから分かる	—	—	0	0.0	—
	なんとなく，分かる	—	—	9	8.7	—
	気になるが，分からない	—	—	74	71.2	—
	考えたことがない	—	—	21	20.2	—

† 複数回答項目。%は選択者数の割合を示す。

表4　管理栄養士・栄養士，養成校学生の妊娠中の葉酸摂取に対する対応

質問項目	回答肢	管理栄養士・栄養士 ($n=69$)		養成校の学生 ($n=175$)		χ^2 検定 p 値
		n	%	n	%	
妊婦さんに葉酸摂取を勧めたことがありますか？	はい	18	26.1	9	5.1	<0.001
	いいえ	51	73.9	166	94.9	
		($n=18$)		($n=9$)		
どのような摂取方法を勧めましたか？†	野菜などの食事から	17	94.4	8	88.9	1.0
	葉酸が強化された加工食品から	2	11.1	0	0.0	0.54
	錠剤・カプセルなどのサプリメントから	5	27.8	4	44.4	0.42
	市販薬から	0	0.0	0	0.0	—
		($n=51$)		($n=166$)		
勧めたことがない理由を教えてください†	知らなかったから	5	9.8	29	17.5	0.27
	機会がないから	38	74.5	100	60.2	0.069
	特に必要性を感じないから	3	5.9	2	1.2	0.086
	自信・確信がないから	5	9.8	10	6.0	0.35
	妊娠がわかってからでは遅いと思うから	4	7.8	1	0.6	0.011

† 複数回答項目。%は選択者数の割合を示す。

表5　妊婦，管理栄養士・栄養士，養成校学生のサプリメントに対する認識と行動

質問項目	回答肢	妊　婦 ($n=104$)		管理栄養士・栄養士 ($n=69$)		養成校の学生 ($n=175$)		χ^2 検定 p 値
		n	%	n	%	n	%	
サプリメントと医薬品の違いは何だと思いますか？†	価格	29	27.9	21	30.4	51	29.1	0.91
	有効成分の含有量	49	47.1	38	55.1	72	41.1	0.11
	品質の確保	15	14.4	25	36.2	55	31.4	0.001
	吸収率	16	15.4	12	17.4	35	20.0	0.62
	安全性のエビデンス	17	16.3	47	68.1	66	37.7	<0.001
	吸収性等の試験	5	4.8	15	21.7	12	6.9	<0.001
	副作用の強さ	17	16.3	18	26.1	55	31.4	0.021
	効果	30	28.8	29	42.0	86	49.1	0.004
	その他	10	9.6	2	2.9	5	2.9	0.030
	選択した項目数の平均±標準偏差	1.81 ± 0.94		3.00 ± 1.72		2.50 ± 1.49		
		(妊娠前)	(妊娠中)					
サプリメントを利用していますか？‡	毎日利用している	17 16.3	22 21.2	8	11.6	8	4.6	<0.001§
	たまに利用している	20 19.2	14 13.5	15	21.7	32	18.3	
	過去に利用したことがあった	16 15.4	10 9.6	25	36.2	35	20.0	
	利用したことがない	51 49.0	58 55.8	21	30.4	100	57.1	
妊婦さんがサプリメントを利用することをどう思いますか？	積極的に利用すべき	—	—	8	11.6	6	3.4	<0.001‖
	たまに利用するならよい	—	—	43	62.3	72	41.1	
	あまり利用すべきではない	—	—	16	23.2	80	45.7	
	利用すべきではない	—	—	2	2.9	17	9.7	

† 複数回答項目。%は選択者数の割合を示す。
‡ 妊婦は，葉酸を除くサプリメントの利用状況。
§ 妊婦は，妊娠中のサプリメント利用状況と比較。
‖ 管理栄養士・栄養士と養成校の学生の比較。

効成分の含有量」，「効果」と続いた。養成校の学生では「効果」が最多で，「有効成分の含有量」，「安全性のエビデンス」と続いた。選択した項目数の平均は，妊婦1.8個，管理栄養士・栄養士3.0個，養成校の学生2.5個であった。管理栄養士・栄養士は妊婦よりも，「品質の確保」，「安全性のエビデンス」，「吸収性等の試験」をサプリメントと医薬品の違いとして認識しており，養成校の学生は妊婦よりも「品質の確保」，「安全性のエビデン

ス」,「副作用の強さ」,「効果」をサプリメントと医薬品の違いとして認識していた。

サプリメントを利用した経験がない人は，管理栄養士・栄養士30%，養成校の学生57%であった。妊娠中に葉酸以外のサプリメントを毎日利用している妊婦は21%であった。

妊婦のサプリメント利用について，管理栄養士・栄養士は「たまに利用するならよい」が62%であったが，養成校の学生は「たまに利用するならよい」と「あまり利用すべきではない」がともに約40%であった（$p<0.01$）。

Ⅳ. 考　察

本研究は妊娠期の葉酸およびサプリメント摂取に対する妊婦，管理栄養士・栄養士，管理栄養士・看護師養成校の学生の認識の状況を把握する目的でアンケート調査を実施した。本研究は限定された地域，施設で実施したため，対象者は非常に偏りのある集団であることから，得られた結果を一般化することは難しいという限界がある。特に養成校の学生については，学年により葉酸に関する講義を受ける前と後の学生が混在していると想定される。したがって，この調査結果は養成校の学生全体の認知度を示すデータとは言えないという問題点もある。

葉酸については国内における認知度の低さが指摘されてきたが[15~17]，本研究結果では，90%以上の対象者が葉酸を知っており，その名称に対する認知度は非常に高かった。一方で，摂取が推奨される理由，folic acid としての摂取が推奨されている理由，必要な時期，推奨される摂取量など，具体的な事項の認知度は十分とは言えず，特に，推奨の終了時期と摂取量に関する知識が十分ではないという実態が示された。

日本人の妊婦の葉酸摂取量は妊娠中期以降においても不足していることが報告されている[18,19]。一方，国内外において，妊娠末期の葉酸サプリメントの利用と，胎児の出生時の低体重や[20]出生後の喘息リスクの増加[21]との関連や，葉酸サプリメント利用者による葉酸過剰摂取の指摘[22]もある。したがって，妊婦には葉酸摂取不足の解消と過剰摂取による問題の両者への対応が必要であり，そのためには，妊娠月齢に応じた推奨摂取量や自身の摂取量についての適正な知識が求められる。また，この傾向は妊婦に限らず，管理栄養士・栄養士や養成校の学生においても同様と想定された。この理由として，葉酸による NTD リスク低減に関する情報は比較的新しいものであるため，正確な知識が十分に行き渡っていない可能性が考えられる。それゆえ，妊婦のみならず，管理栄養士・栄養士や養成校の学生に対しても，摂取時期と摂取量に関する重点的な情報提供を行い，妊娠３ヶ月までの妊婦に対し，folic acid としての葉酸を 400 μg/日摂取するように導くとともに，妊娠中期以降の妊婦に対しては，これ以降の葉酸サプリメント摂取には，NTD リスク低減効果は期待できない旨を伝え，通常の食事からのバランスの良い栄養摂取を重視した支援ができる体制を整えることが望まれる。

妊婦に葉酸摂取を推奨した経験のある管理栄養士・栄養士は少数であったが，その大半が「野菜などの食事から」の摂取を勧めたと回答した。一方，妊婦は「錠剤・カプセルから」摂取している者が約70%と最も多く，「野菜などの食事から」摂取したと回答した者は，妊娠前26%，妊娠中38%にとどまった。この結果は，妊婦の間では妊娠中に葉酸を錠剤・カプセル状のサプリメントから摂取することが主流となっており，管理栄養士・栄養士の助言が妊婦の現状に合致していない場合もある可能性を示している。管理栄養士・栄養士自身は，約70%がサプリメント利用経験者であり，妊娠中のサプリメント利用についても「積極的に利用すべき」と「たまに利用するならよい」と回答した者を合わせると70%を超えるなど，サプリメント利用に否定的ではない。本研究結果は，ごく限られた管理栄養士・栄養士の回答であり，助言対象の妊婦の妊娠時期も不明であることから，一般化はできないが，管理栄養士・栄養士が「必要な栄養素は，まず，普段の食事から摂取するように心掛ける」という基本を重視する[14]とともに，サプリメントの安全性のエビデンスや品質が医薬品と異なることに不安を感じ，サプリメント利用に言及しにくい状況が生じている可能性も考えられる。妊娠初期に関しては folic acid としての葉酸摂取が推奨されることから，サプリメントからの摂取についても踏み込み，サプリメント製品の選択方法，利用する時期，利用方法など，具体的な助言や指導を行うことが必要であろう。

妊娠中の葉酸以外のサプリメント利用については，利用者数は妊娠前よりも減少した一方で，毎日利用している常用者は妊娠前よりも増加した。また，妊婦ではサプリメントと医薬品の違いとして安全性や品質に関する項目を選択した者は少なく，全体の選択項目数も少ない結果となった。これは，妊婦はサプリメントを医薬品と同等と誤解している点が多く，医薬品のように，毎日，定期的に摂取するものだと捉えていることを示唆している。葉酸摂取の推奨が，妊娠中の葉酸以外のサプリメントの常用につながることがないよう，妊婦に対し，1）製品の品質や規格が様々で規制のないサプリメントは，その安

全性の面で医薬品とは大きく異なること，2）栄養素摂取の補助として不足した場合に使用すればよいこと，3）その判断のためにも摂取量の概念が重要であること，4）葉酸サプリメントとその他のサプリメントは区別して捉えること，といった事項を伝えることが必要であろう。

葉酸の名称や含まれる食品，効果的な摂取開始時期については，多くの妊婦が「知っている」と答えたものの，実際の積極的な葉酸摂取時期は推奨時期より遅かった。これは，妊婦が情報を入手した時期が，効果的な摂取開始時期を過ぎてしまってからであったことを示唆する。このような問題点は，管理栄養士・栄養士などの専門職が妊娠計画時から継続的な助言をすることにより改善できるであろう。本研究の結果では，管理栄養士・栄養士やその養成校の学生の知識程度は妊婦と大きな違いはなかった。この理由として，今回の調査対象となった管理栄養士・栄養士の大半は実際に妊婦に栄養指導を行う業務に就いていない者であったと想定されるのに対し，妊婦は当事者であるため，葉酸やNTDリスクに関する情報収集への積極性が異なっていたことが考えられる。また，妊婦はインターネットなどによる積極的な情報収集により，知っているつもりになっている可能性も考えられる。

先行研究において，管理栄養士・栄養士[14]のみならず，医師，薬剤師，看護師などの医療従事者[15]においても葉酸によるNTDリスク低減の認知度は十分とは言えない実態とともに，これら医療従事者からの妊婦への葉酸に関する情報提供の重要性が指摘されている[12]。こうした点を考慮すると，管理栄養士・栄養士や養成校の学生を対象とした，NTDリスク低減と葉酸摂取およびサプリメントの実態に関する正確で具体的な知識，ならびに助言対象者の状況に合わせた柔軟な食生活指導が可能な技術を習得できる，卒前・卒後教育の機会の充実が望まれる。

本研究は，先に述べたように，対象者数が限られ，偏りがある集団を対象としており，また，有効回答率が50％前後と高くなかったことから，一般化は難しいという限界がある。しかし，妊婦および妊婦へ情報を提供する立場である管理栄養士・栄養士，これからそのような立場となり得る学生のいずれにおいても，葉酸に関する知識が十分とは言えない実態が示唆された。本研究結果は，専門職と妊婦の双方が，葉酸およびサプリメントについて適切な理解を得られるような情報提供および教育体制の充実を検討するための一つの資料になると考えられる。

V．結　論

本研究は，葉酸摂取に対する妊婦や，妊婦に情報提供を行う立場である管理栄養士・栄養士，管理栄養士・看護師養成校の学生の認識の現状と課題についてアンケート調査をもとに検討を加えた。妊婦，管理栄養士・栄養士，養成校の学生ともに，葉酸の認知度は高いものの，推奨される摂取時期，摂取量についての知識が十分とは言えず，これらの点について，サプリメントの利用も含めた情報提供および教育の充実が重要と考えられた。ただし，対象者の偏りが大きいため，今後，より質の高い調査の実施が必要である。

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利益相反

利益相反に相当する事項はない。

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Questionnaire Survey on the Understanding of Folic Acid and Dietary Supplementation among Pregnant Women, Dietitians and Students Attending Dietetics or Nursing Training Schools

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ABSTRACT

Objective: Folic acid intake is recommended for fertile women to reduce the risk of neural tube defects in infants. However, the rationale behind this recommendation may not be properly understood. A preliminary study was conducted to examine the current understanding of folic acid and dietary supplementation and associated problems among pregnant women, dietitians, and students attending dietetics or nursing training schools.

Methods: The subjects of this survey were pregnant women who attended pre-natal classes in a local government or maternity hospital (Tokyo and Hokkaido), participants of lectures about dietary supplements for dietitians (Tokyo, Kanagawa, and Hiroshima), and students attending dietetics or nursing training schools (Nagano and Hiroshima) from October to December 2011. The survey was conducted with an anonymous, self-reported questionnaire.

Results: Responses were obtained from 104 pregnant women, 69 dietitians, and 175 students. The effective recovery rates were 54.5%, 51.1%, and 65.8%, respectively. Although more than 90% of the subjects were familiar with the term folic acid, they had inadequate knowledge on the appropriate timing and recommended folic acid intake.

Conclusions: This study was preliminary in nature in that it entailed a small number of participants. However, the data suggest the need to provide detailed information about the association between folic acid intake and neural tube defect risk reduction, such as the appropriate intake timing and amount and the actual situation regarding dietary supplements, for pregnant women, dietitians, and students attending dietetics or nursing training schools.

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Key words: folic acid, pregnant women, dietitian, recognition, cross-sectional survey

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Influence of Dietary Macronutrients on Induction of Hepatic Drug Metabolizing Enzymes by *Coleus forskohlii* Extract in Mice

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Summary From studies in mice, we have reported that *Coleus forskohlii* extract (CFE), a popular herbal weight-loss ingredient, markedly induced hepatic drug metabolizing enzymes, especially cytochrome P450 (CYP), and interacted with co-administered drugs. This study was designed to examine how the induction of drug metabolizing enzymes by CFE was influenced by different levels of macronutrients in the diet. Mice were fed a non-purified diet or semi-purified diet with and without CFE (0.3–0.5%) for 14–18 d, and changes in the ratio of liver weight to body weight, an indicator of hepatic CYP induction, and hepatic drug metabolizing enzymes were analyzed. The ratio of liver weight to body weight, content and activities of CYPs, and activity of glutathione *S*-transferase were higher in a semi-purified standard diet (AIN93G formula) group than in high sucrose (62.9%) and high fat (29.9%) diet groups. Different levels of protein (7%, 20%, and 33%) in the diets did not influence CFE-induced CYP induction or increase the ratio of liver weight to body weight. The effect of CFE on the ratio of liver weight to body weight was higher with a semi-purified diet than with a non-purified diet, and was similar between dietary administration and intragastric gavage when the CFE dose and the diet were the same. There was a positive correlation between CFE-induced CYP induction and the content of starch in the diets, suggesting that dietary starch potentiates CFE-induced CYP induction in mice. The mechanism of enhanced CYP induction remains unclear.

Key Words *Coleus forskohlii*, cytochrome P450, macronutrients, administration route, dietary starch

Coleus forskohlii extract (CFE) is a popular herbal ingredient for commercial weight-loss dietary supplements (*1*). *C. forskohlii* is native to India (*2*), where it has been used for centuries in Ayurvedic medicine to treat various diseases of the cardiovascular, respiratory, gastrointestinal and central nervous systems (*3*). CFE contains the diterpene forskolin as in Fig. 1, which increases cAMP concentrations via the activation of adenylate cyclase, resulting in various therapeutic effects against asthma and idiopathic congestive cardiomyopathy (*4*, *5*). Theoretically, an increase in cAMP induced by forskolin will enhance lipolysis leading to elevated fat degradation and physiological fat utilization, and thus promote fat and weight loss. It has been shown that forskolin increases both cAMP accumulation and lipolysis in fat cells (*6*, *7*), and CFE standardized with forskolin reduces fat accumulation in ovariectomised rats (*8*) and induces favorable effects on body fat in overweight women and obese men (*9*, *10*).

Currently, drug–herb interactions are becoming a source of serious concern in relation to adverse effects, because consumers of herbal supplements often take prescribed drugs concomitantly (*11–13*) and health professionals might be unaware of possible interactions (*14*, *15*). A decrease in efficacy or an increase in the adverse effects of prescribed drugs might interfere with appropriate medical care and have a fatal outcome. Drugs are metabolized by the Phase I and Phase II enzymes; the former is catalyzed by cytochrome P450 (CYP) enzymes, and the latter is catalyzed conjugation enzymes such as glutathione *S*-transferase (GST) and UDP-glucuronosyltransferase (*16*). Interactions between some herbal ingredients, such as St John's wort (*17*) and ginkgo biloba (*18*), have been documented and shown to be mediated by CYPs, but those for other herbal ingredients remain unknown. We previously showed that feeding mice a diet containing CFE (standardized with 10% forskolin) dose- and time-dependently induced hepatic CYPs and GST enzymes (*19*). Significant induction of the hepatic CYP content and CYP2C activity was evident at an intake dose of 0.05%; the CFE dose was 60 mg/kg body weight in mice and corresponded to about 5 mg/kg body weight of a human equivalent dose when calculated using the body surface normalization method (*20*). We also reported the interaction

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of warfarin and CFE in mice in vivo, where CFE attenuated the anticoagulant action of warfarin via induction of hepatic CYPs, especially CYP2C, which is involved in active (*S*)-warfarin metabolism (*21*). Furthermore, we showed that CFE induced CYPs in vivo and directly inhibited CYP2C activity in vitro as well. In both in vivo and in vitro studies, the effect of forskolin, a biologically active marker, was negligible, indicating the contribution of unknown substances in the CFE (*19*, *22*).

Users of weight loss supplements may have an extreme meal with different macronutrient compositions. There are 4 popular weight loss diets: Atkins (very low in carbohydrate), Zone (low in carbohydrate), Ornish (very high in carbohydrate), and LEARN (Life style, Exercise, Attitude, Relationships and Nutrition) (*23*). These differences in dietary macronutrients may influence drug-metabolizing enzymes (*24*). Rats with protein-calorie malnutrition decreased hepatic CYP levels (CYP1A2, 2C11, 2E1 and 3A1/2) (*25*), and rats fed a high-sucrose diet exhibited decreased hepatic content of CYP1A1, CYP3A2 and GST activity (*26*). It has also been shown that a diet deficient in carbohydrate remarkably enhanced liver mixed-function oxidase activity and the metabolism of carbon tetrachloride in rats (*27*). Based on these findings, it is important to determine how dietary macronutrients influence CFE-induced hepatic CYP induction.

In this study in mice, we examined how induction of drug metabolizing enzymes, especially CYPs, was influenced by CFE with regard to route of administration and dietary conditions that differ in macronutrient compositions. In our previous study, CYP induction by CFE was well correlated with an increase in the ratio of liver weight to body weight (*22*). Therefore, we measured CYP content and activities in the liver as well as the ratio of liver weight to body weight as a reliable indicator of CYP induction. The present study in mice had two benefits: one was to clarify dietary conditions that can minimize possible drug–CFE interactions via CYP induction, and the second was to establish experimental diet conditions that can readily be used to seek unknown substances in CFE that induce CYPs in vivo.

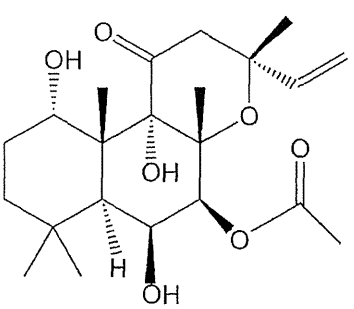

Fig. 1. Chemical structure of forskolin.

MATERIALS AND METHODS

Materials. Powdered CFE standardized with 10% forskolin was prepared as follows. Dried roots of *C. forskohlii* obtained from Bangalore in southern India were crushed and supercritically extracted under CO_2 gas. The forskolin-rich extract (20–30%) was mixed with dextrin to a forskolin concentration of 10%. These processes were performed by Tokiwa Phytochemical Co. Ltd. (Chiba, Japan). CFE comprised: water, 5.6%; protein, 0.3%; lipids, 22.7%; ash, 2.2%; and carbohydrates, 69.2%. For CYP enzyme assays, resorufin, pentoxyresorufin, (*S*)-warfarin, 7-hydroxywarfarin, 7-ethoxycoumarin, testosterone, 6β-hydroxytestosterone, and corticosterone, and glutathione were purchased from Sigma-Aldrich Inc. (St Louis, MO, USA). NADPH was purchased from Oriental Yeast Co., Ltd. (Tokyo, Japan). All other reagents were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan).

Experimental diets. Non-purified commercial rodent diet (CE-2) was supplied by CLEA Japan, Inc. (Tokyo, Japan). The non-purified diet comprised: water, 88 g/kg diet; crude protein, 252 g/kg diet; crude lipids, 44 g/kg diet; total ash, 70 g/kg diet; crude fiber, 44 g/kg diet and soluble non-nitrogenous matter, 502 g/kg diet according to the manufacturer's information. A semi-purified standard diet was prepared based on the composition of the AIN93G formula of Reeves et al. (*28*). Various semi-purified diets with different compositions of macronutrient were prepared as shown in Table 1. The high-starch diet that differed only in the source of the carbohydrate and the high-fat diet were isonitrogenous per kilocalo-

Table 1. Composition of semi-purified experimental diets (g/kg diet).

Ingredient	Standard	High sucrose	High fat	Low protein	High protein
Cornstarch	529.486	0	205.082	641.436	417.536
Sucrose	100	629.486	126	120	80
Casein	200	200	250	70	330
Soybean oil	70	70	292	70	70
Cellulose	50	50	63	50	50
Vitamin mixture (AIN93G)	10	10	13	10	10
Mineral mixture (AIN93G)	35	35	44	35	35
L-Cystine	3	3	3.76	1.05	4.95
Choline hydrogen tartrate	2.5	2.5	3.14	2.5	2.5
Tertiary butylhydroquinone	0.014	0.014	0.018	0.014	0.014

Table 2. Body weight and liver weight of mice fed a non-purified diet or semi-purified standard diet with and without *C. forskohlii* extract (CFE).

Diet	Non-purified		Semi-purified (standard)	
CFE treatment	−	+	−	+
Average daily food intake (g)	5.8±0.21	5.4±0.18 [0.93]	5.0±0.15[a]	4.8±0.11 [0.97][b]
Calculated CFE dose (mg/kg body weight)	0	845±26.0	0	732±17.6[b]
Final body weight (g)	31.4±0.42	30.3±0.30 [0.96]	33.1±0.84[b]	32.9±0.45 [0.99][b]
Liver weight (g)	1.44±0.05	2.08±0.05 [1.4][a]	1.43±0.05[b]	2.63±0.20 [1.8][a,b,c]
(%/body weight)	4.58±0.11	6.87±0.10 [1.5][a]	4.31±0.11[b]	7.99±0.57 [1.9][a,c]

Mice were fed a non-purified diet or semi-purified diet with and without 0.5% *C. forskohlii* extract (CFE) for 2 wk.
Values are expressed as mean and SE ($n=5$). Number in brackets indicates the increase in the ratio for its respective diet group without CFE.
[a] Significant difference from non-purified diet without CFE at $p<0.05$.
[b] Significant difference from non-purified diet with CFE at $p<0.05$.
[c] Significant difference from semi-purified diet without CFE at $p<0.05$.

rie. Low, normal, and high protein diets were prepared as isoenergetic by adjusting the proportion of carbohydrate in the diets. The ingredients for the semi-purified diets were purchased from Oriental Yeast Co., Ltd.

Animal experiments. Male 4-wk-old ICR mice (CLEA Japan, Inc.) were housed at a constant temperature (23±1°C) with a 12-h light-dark cycle in polypropylene cages. After acclimation for 1 wk, the mice were divided into treatment groups (5–6 mice per group) and were administered CFE as follows.

In a comparison of non-purified diet and semi-purified standard diet, CFE was added at a concentration of 0.5% (w/w) to each diet, and given to mice ad libitum for 2 wk. In a comparison of the route of administration, mice were either fed a semi-purified standard diet with 0.5% CFE or given it daily by intragastric gavage of CFE dissolved in 0.5% (w/v) carboxymethylcellulose for 2 wk. In this administration route study, the daily dose of CFE was adjusted to 750 mg/kg body weight. In studies of the effect of macronutrients (i.e., starch, fat and protein), the CFE dose was reduced to 0.3% (w/w) in the semi-purified diets and the treatment term was set at 18 d, because the dietary effects were thought to need a longer period at this CFE dose. In the study, food intake in each group was adjusted to keep a similar intake dose of CFE. At the end of each treatment, mice were anesthetized with pentobarbital and killed. Their livers were removed immediately, weighted, snap frozen with dry ice and stored at −80°C until analysis.

All procedures were in accordance with National Institute of Health and Nutrition Guidelines for the Care and Use of Laboratory Animals and were approved by the Ethical Committee in the same institute.

Analytical methods

Analysis of drug-metabolizing enzymes: The liver was rinsed with 0.9% (w/v) NaCl, homogenized in 50 mmol/L Tris-HCl buffer (pH 7.4) containing 0.25 mol/L sucrose, and separated by centrifugation at 10,000 ×*g* at 4°C for 30 min. The supernatant was centrifuged at 105,000 ×*g* at 4°C for 60 min to prepare microsomal and cytosol fractions. The total CYP content and the activities of various CYP enzymes were determined using the microsomal fraction, and glutathione *S*-transferase (GST) activity was determined using cytosol fraction, as described previously (*29*). The subtypes of CYP enzymes examined and the corresponding CYPs were pentoxyresorufin *O*-dealkylase, CYP2B; (*S*)-warfarin 7-hydroxylase, CYP2C; and testosterone 6β-hydroxylase, CYP3A. Protein concentration was determined using a BCA protein assay kit (Pierce, Rockford, IL, USA).

Statistical analyses. Data are presented as means and standard error (SE) for individual groups and were analyzed statistically using one-way ANOVA (in the non-purified diet versus semi-purified diet and CFE administration route studies) and two-way ANOVA (in the macronutrient studies) with Tukey's multiple comparison test. Differences at $p<0.05$ were considered significant. All statistical analyses were performed using Prism 5.0 (GraphPad Software Inc., La Jolla, CA, USA).

RESULTS

Dietary treatment of CFE with non-purified and purified diets and by different administration routes

Mice were fed a 0.5% CFE in a non-purified commercial rodent diet (CE-2) or semi-purified standard diet for 2 wk. In the semi-purified diet groups, food intake was lower, but body weight was higher compared with the non-purified diet groups (Table 2). This discrepancy might be due to high bioavailability of ingredients in the semi-purified diet compared with crude natural ingredients used in the non-purified diet. In the CFE-treated groups, liver weight in the semi-purified diet group was higher, but the increase in the ratio of liver weight to body weight did not differ between the two CFE groups, which could be caused by the low dose of CFE in the semi-purified diet group. When mice were fed the same semi-purified standard diet, increases in liver weight and

Table 3. Body weight and liver weight of mice fed a semi-purified standard, high-sucrose, or high-fat diet with and without *C. forskohlii* extract (CFE).

Diet	Standard		High sucrose		High fat	
CFE treatment	−	+	−	+	−	+
Average daily food intake (g)	4.8±0.08	4.7±0.07 [0.98]	4.8±0.08	4.7±0.10 [0.99]	3.6±0.04	3.6±0.10 [0.99]
Calculated CFE dose (mg/kg body weight)	0	380±10.5	0	364±4.6	0	375±5.8
Final body weight (g)	39.0±1.1	38.2±1.1 [0.98]	40.1±0.57	39.9±0.90 [0.99]	39.2±0.35	39.6±1.1 [1.0]
Liver weight (g)	1.51±0.07	3.21±0.22 [2.1][a]	1.55±0.04	2.71±0.25 [1.8][a]	1.42±0.04	2.22±0.15 [1.6][a,b]
(%/body weight)	3.87±0.084	8.41±0.51 [2.2][a]	3.86±0.11	6.75±0.50 [1.7][a, b]	3.61±0.093	5.61±0.30 [1.6][a,b]

Mice were fed a semi-purified standard (10% sucrose and 7% soybean oil), high-sucrose (62.9% sucrose) or high-fat diet (29.9% soybean oil) with and without 0.3% *C. forskohlii* extract (CFE) for 18 d. The detailed composition of the experimental diets is shown in Table 1.
Values are expressed as mean and SE (n=6). Number in brackets indicates the increase in the ratio for its respective diet group without CFE.
[a] Significant difference from its respective diet without CFE at $p<0.05$.
[b] Significant difference from standard diet with CFE at $p<0.05$.

Table 4. Body weight and liver weight of mice fed a semi-purified standard, low-protein, or high-protein diet with and without *C. forskohlii* extract (CFE).

Diet	Standard		Low-protein		High-protein	
CFE treatment	−	+	−	+	−	+
Average daily food intake (g)	4.3±0.090	4.3±0.15 [1.0]	4.4±0.082	4.5±0.16 [1.0]	4.4±0.099	4.5±0.14 [1.0]
Calculated CFE dose (mg/kg body weight)	0	330±7.9	0	364±11.8	0	344±12.8
Final body weight (g)	40.6±1.3	39.1±1.3 [0.96]	37.5±0.89	36.3±1.1 [0.97]	41.1±1.2	38.9±1.2 [0.95]
Liver weight (g)	1.61±0.03	2.94±0.24 [1.8][a]	1.43±0.03[b]	2.47±0.22 [1.7][a]	1.50±0.052	2.57±0.19 [1.7][a]
(%/body weight)	3.96±0.10	7.48±0.50 [1.9][a]	3.82±0.092	6.76±0.45 [1.8][a]	3.66±0.098	6.63±0.49 [1.8][a]

Mice were fed a semi-purified standard (20% casein), low-protein (7% casein) or high-protein (33% casein) diet with and without 0.3% *C. forskohlii* extract (CFE) for 18 d. The detailed composition of the experimental diets is shown in Table 1.
Values are expressed as mean and SE (n=6).
Number in brackets indicates the increase in the ratio for its respective diet without CFE.
[a] Significant difference from its respective diet without CFE at $p<0.05$.
[b] Significant difference from standard diet without CFE at $p<0.05$.

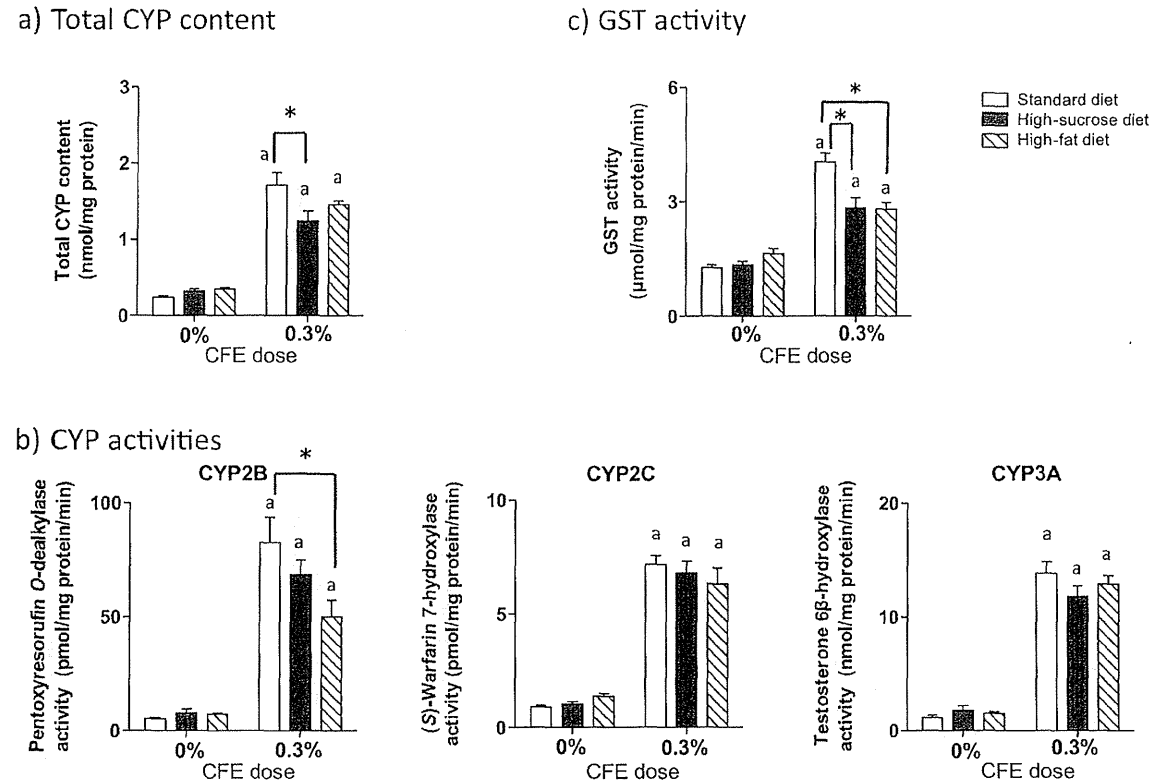

Fig. 2. Hepatic drug-metabolizing enzymes in mice fed a semi-purified standard, high-sucrose, or high-fat diet with and without 0.3% *C. forskohlii* extract (CFE). Mice were fed a semi-purified standard (10% sucrose and 7% soybean oil), high-sucrose (62.9% sucrose) or high-fat diet (29.9% soybean oil) with and without 0.3% *C. forskohlii* extract (CFE) for 18 d. The detailed composition of the experimental diets is shown in Table 1. Values are expressed as mean and SE (n=6). [a] Significant difference from the respective diet without CFE at $p<0.05$. * Significant difference between two groups at $p<0.05$.

the ratio of liver weight to body weight by the CFE treatment were similar between feeding with 0.5% CFE diet and intragastric gavage at a single dose of CFE 750 mg/kg body weight/d, a dose equivalent to that given by the 0.5% CFE diet. The ratio of liver to body weight was 4.07±0.12% in the control group, 6.89±0.25% in the CFE treatment by intragastric gavage group, and 7.36±0.40% in CFE treatment by diet group.

Effect of macronutrients in the diet on induction of hepatic drug-metabolizing enzymes by CFE

Mice were fed semi-purified diets with different macronutrient compositions with and without 0.3% CFE for 18 d. In mice fed a high-sucrose (62.9% sucrose), a high-fat (29.9% soybean oil), or a semi-purified standard diet (10% sucrose and 7% soybean oil), final body weight did not differ among the groups (Table 3). In addition, liver weight and hepatic drug-metabolizing enzymes did not differ among the groups without CFE treatment (Fig. 2). Calculated intakes of CFE in the CFE-treated groups were similar because daily food intake did not differ. In the CFE-treated groups, the ratio of liver to body weight was higher in the standard diet groups than in the high-sucrose and high-fat diet groups. Similar phenomena were observed for the total CYP content and the activities of CYP2B and GST (Fig. 2). These findings indicate that dietary effects on drug metabolizing enzymes became clearer with the treatment with CFE.

In mice fed a low-protein (7% casein), a high-protein (33% casein), or a standard diet (20% casein), the ratio of liver weight to body weight was higher in the CFE-treated groups, but the values did not differ among the three CFE-treated groups (Table 4). The influence of dietary protein on the activities of CYP3A and GST was detected in low-protein and high-protein diets, but overall changes were inconsistent (Fig. 3).

Increases in a macronutrient in a diet are synonymous with a decrease in other macronutrients. We adjusted the total amount of macronutrients with starch, which was 0% to 53% in the experimental diets as in Table 1. As shown in Figs. 2 and 3, the CYP induction seemed to be high in the semi-purified standard diet, which is high in starch content. To confirm the contribution of dietary starch to CYP induction in association with and without CFE treatment, the relationship between CYP content and dietary starch levels were examined using the data in Figs. 2 and 3. There was a significant positive correlation between total CYP content in liver and starch levels in the diet (Fig. 4). The phenomenon was clearer in the CFE-treated groups; the correlation coefficient was 0.44 in the control groups and 0.69 in the CFE-treated groups. Similar positive correlation was observed between GST activity and dietary starch levels;

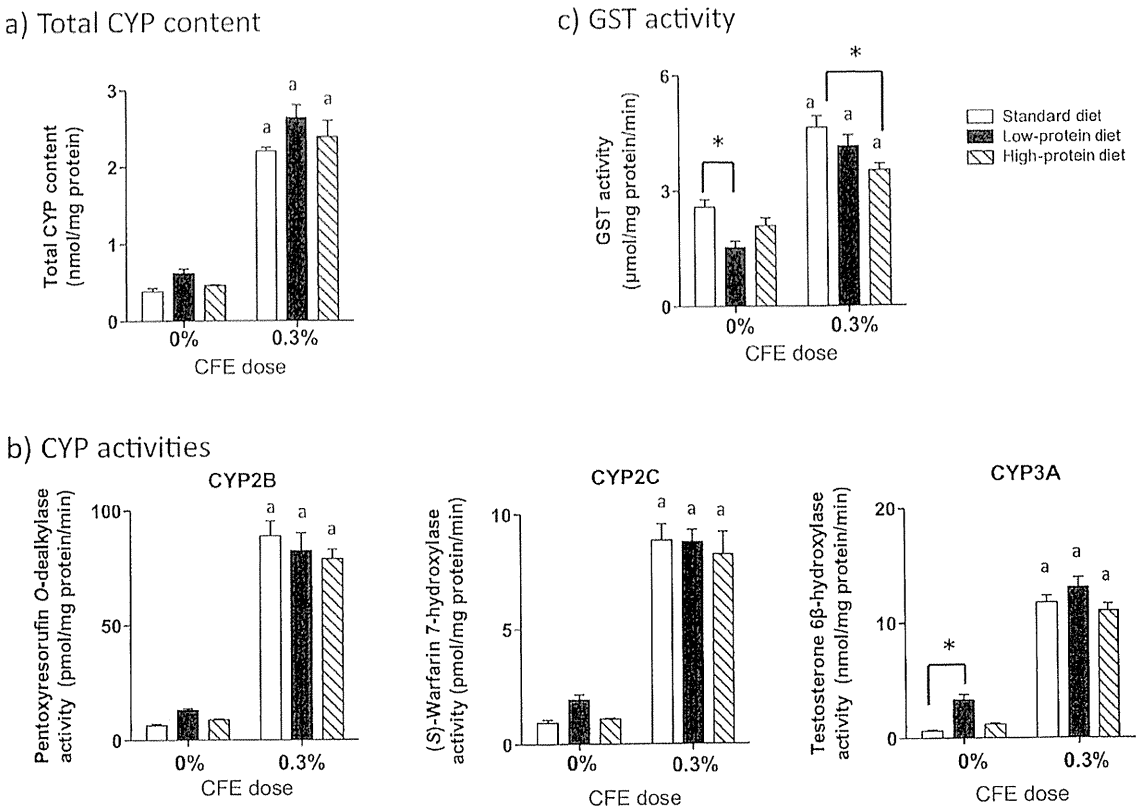

Fig. 3. Hepatic drug-metabolizing enzymes in mice fed a semi-purified standard, low-protein or high-protein diet with and without 0.3% *C. forskohlii* extract (CFE). Mice were fed a semi-purified standard (20% casein), low-protein (7% casein) or high-protein (33% casein) diet with and without 0.3% *C. forskohlii* extract (CFE) for 18 d. The detailed composition of the experimental diets is shown in Table 1. Values are expressed as mean and SE ($n=6$). [a] Significant difference from the respective diet without CFE at $p<0.05$. * Significant difference between the two groups at $p<0.05$.

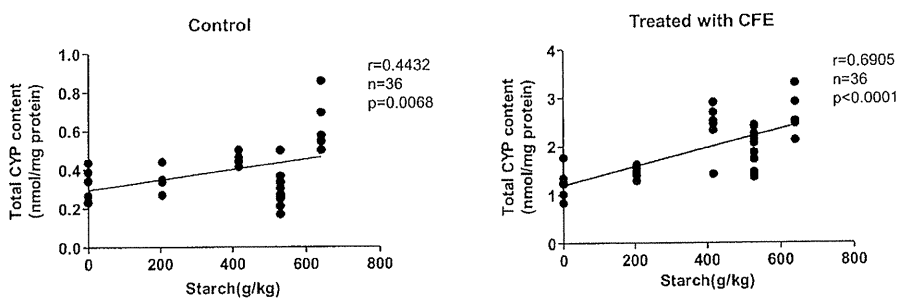

Fig. 4. Correlation between starch in the diet and hepatic CYP content in mice treated with and without *C. forskohlii* extract (CFE). Data were obtained from Figs. 2 and 3.

the correlation coefficient was 0.24 ($p=0.154$) in the control groups and 0.69 ($p<0.0001$) in the CFE-treated groups.

DISCUSSION

In the present study, we examined how dietary macronutrients influence CFE-induced hepatic drug-metabolizing enzymes, especially CYPs, in mice, and whether there is a difference in CYP induction by CFE between dietary treatment and intragastric gavage. The total content and activities of hepatic CYPs may fluctuate depending on liver sample storage, microsome preparation, and the measurement condition of CYPs. In contrast, the measurement of liver weight was simple and the increased ratio of liver weight to body weight corresponded well to the induction of CYP by CFE (*22*); the correlation coefficient was 0.85 ($n=35$, $p<0.001$). When the relationship between hepatic CYP content and liver weight to body weight was reanalyzed using the data from the CFE dose-response study (*17*), a significant positive correlation was also detected ($r=0.78$, $n=26$, $p<0.001$). Thus, we used the increase in the ratio

of liver weight to body weight as a simple and reliable indicator of CYP induction following CFE treatment. As a result, the induction of CYP, which was estimated by the increased ratio of liver weight to body weight, was similar between CFE administration by diet and by intragastric gavage, while it was higher in the semi-purified standard diet compared with the high-fat, high-protein, and low-protein diets. Analysis of CYP content and activities showed a similar trend. It was determined that the level of hepatic CYP and GST in CFE-treated groups was positively correlated with the level of starch in the semi-purified diet. In addition, it is worth noting that the high-starch diet in the present study was the standard diet generally used as the AIN93G formula.

The influence of dietary macronutrients on CYP activity has been shown in previous studies in extreme dietary conditions (*25–27*). Lee et al. (*25*) showed that hepatic CYP (CYP1A2, 2C11, 2E1 and 3A1/2) activities were decreased in the rats with protein-calorie malnutrition (feeding of 5% casein diet for 4 wk). Peters et al. (*26*) reported that the activities of hepatic CYP1A1 and CYP3A2 were decreased in rats fed a high-sucrose diet (60% of total calories) compared with a control diet (0% sucrose). Nakajima et al. (*27*) reported that the activity of hepatic mixed function oxygenase was increased in rats fed a low-sucrose diet for 3 wk, resulting in the toxicity of carbon tetrachloride. In contrast to those studies, we selected rather mild changes in the dietary macronutrient compositions in the present study, and found that content of starch in the diet correlated with the increase in drug-metabolizing enzymes, especially in the CFE-treated groups. The increases in a macronutrient in a diet were synonymous with the decrease in other macronutrients, and change in each ingredient may independently affect the drug-metabolizing enzymes. Accordingly, it will be hard to understand the dietary effect on the drug-metabolizing enzymes. Nevertheless, to the best of our knowledge, there are no reports showing the relation between CYP induction and dietary starch. Thus, this will be a first report that shows an enhanced induction of CYPs by dietary starch, and not by a diet with an extreme level of macronutrients, but by the semi-purified standard diet.

A non-purified diet is composed of natural crude ingredients that may contain substances inducing drug-metabolizing enzymes. In the present study, we observed the induction of CYPs in the semi-purified diet that was composed of isolated ingredients such as sucrose, starch and casein. Therefore, it is unlikely that unknown substance inducing CYPs was present in the ingredients such as starch. At present there is no explanation why a high starch level potentiates the induction of hepatic CYPs by CFE. We speculated that the nature of the induction of drug-metabolizing enzymes by CFE is related to such a mechanism. As shown in our previous studies (*19, 21*), CFE induced various drug-metabolizing enzymes such as CYP2B, CYP2C, CYP3A and GST, suggesting that the activation of transcription of drug-metabolizing enzymes is involved. Ding and Staudinger clearly showed that constituents of CFE, namely forskolin and 1,9-dideoxyforskoiln, induced CYP3A gene expression through the pregnane X receptor (PXR) in cultured hepatocytes (*30*). Activation of nuclear receptors PXR and constitutive androstane receptor (CAR) has been shown to regulate drug-metabolizing enzymes as well as glucose and lipid metabolism (*31*). CFE used in the present study also induced hepatic steatosis in mice fed the semi-purified standard diet, although the effective dose was 10 times higher than the dose that induced CYPs (*32*). These facts suggest that changes in dietary starch level affect the induction of drug-metabolizing enzymes. CFE is composed of various substances; however, forskolin was not involved in CYP activation or hepatic steatosis (*22, 32*), indicating the contribution of unidentified substances. In a study of solvent fractionation of CFE, we found that the unidentified substances involved in CYP induction were mainly distributed in the diethyl ether-fraction (*22*). Further detailed studies are needed to clarify the mechanism of action of CYP induction and steatosis associated with CFE treatment and to identify the active substances other than forskolin in CFE. The results of the present dietary study will be helpful in guiding the in vivo studies necessary to identify these active substances.

Currently, several weight-loss diets are widely used, including Atkins, Zone, Ornish and LEARN (*23*). The lowest carbohydrate diet was shown to be more effective for weight loss at 12 mo in premenopausal overweight and obese women (*23*). If we applied the present data, intake of the lowest carbohydrate diet and CFE-containing weight loss supplement would be less vulnerable toward the induction of hepatic CYPs. On the other hand, intake of a high-starch diet and a supplement with CFE may induce CYPs, thereby potentially causing adverse events though drug–herb interactions. This may be substantiated by adverse event reports from careful examination of CFE supplement users in practice.

In conclusion, we showed that CYP induction by CFE was potentiated in mice fed a high-starch diet, corresponding to a semi-purified standard diet with the AIN93G formula, compared with low- or high-protein, and high-fat diets. The route of CFE administration, with the diet or by intragastric gavage, did not influence the induction of CYPs as long as the CFE dose and feeding diet were the same. These findings will be helpful in searches for unknown substances involved in hepatic CYP induction and steatosis and in finding a way to minimize CFE–drug interactions caused by the intake of dietary supplements with CFE.

Acknowledgment

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Short Communication

Prevalence of inappropriate dietary supplement use among pregnant women in Japan

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We investigated the characteristics of dietary supplements and their use by 1,076 Japanese pregnant women, the majority of whom were in mid- to late pregnancy. The subjects completed a self-reported survey on their sociodemographic characteristics, supplement use, and attitudes towards diet. The overall prevalence of supplement use did not change before and after pregnancy (75%); however, daily use increased by approximately two-fold with pregnancy (20.2% versus 37.2%). After the onset of pregnancy, supplements containing folic acid were taken for fetal health. Daily users were more likely to be older, have a greater awareness of the risk of neural tube defects (NTD), view supplement use as acceptable, have less diet anxiety, and have more advisers regarding diet. Respondents used supplements containing folic acid alone or with other ingredients. Folic acid intake is recommended to reduce the risk of NTD. However, supplement use began after pregnancy recognition, suggesting a lack of knowledge on the appropriate timing of folic acid use. Information about supplements was obtained mostly from newspapers, magazines, flyers, and stores. These results indicate that more accurate information regarding the optimal timing of folic acid intake and the safety of dietary supplements must be disseminated.

Key Words: dietary supplements, pregnancy, folic acid, information, Japan

INTRODUCTION

The use of dietary supplements is increasing worldwide, including among pregnant women. It has been shown that 72% to 86% of pregnant women in the United States and 81% to 94% of those in European countries use dietary supplements.[1-4] Nutritional status during pregnancy plays an important role in the well-being of both mother and fetus; therefore, pregnant women tend to use dietary supplements for fear of nutritional deficiencies. Among nutrients, folic acid has received a great deal of attention. It is well known that periconceptional use of folic acid supplements reduces the risk of neural tube defects (NTD) in infants.[5,6] Because of this beneficial effect, health authorities in numerous countries recommend periconceptional folic acid intake in fertile women.[5,7] In pregnant women not at risk of malnutrition, the necessity of nutrients supplied via dietary supplements is unclear, with the exception of folic acid. A variety of dietary supplements are available in the marketplace; some contain folic acid alone, while others contain additional vitamins and minerals or herbal ingredients. Some studies have pointed out that the safety of dietary supplements containing herbal ingredients is not conclusive for pregnancy, and evidence-based data are lacking.[8,9] Furthermore, unlike the United States, there is no clear definition on what constitutes a dietary supplement in Japan, allowing the existence of a variety of dietary supplements in the marketplace. The Japanese government does not regulate the quality, safety and efficacy of most of these products. With the increased availability of dietary supplements in the marketplace and heightened awareness of folic acid requirements during pregnancy, dietary supplement use in pregnant women is expected to increase in Japan. However, few reports have examined this topic. Thus, in this study we conducted a questionnaire survey to examine the current status of dietary supplement use, and their characteristics, by pregnant women.

METHODS

A cross-sectional survey was conducted from January through November 2010. We used an anonymous, self-reporting survey. The questionnaire was distributed at maternity hospitals and public health centers. The forms were mailed to the party conducting the survey and were returned by mail after completion.

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Subjects

We identified 3,650 pregnant women in 18 prefectures in Japan. Responses were obtained from 1,076 pregnant women (effective recovery rate: 29.5%), with a mean age of 32.2±4.4 years and a mean gestational age of 25.7±6.8 weeks.

Completion of the survey was considered informed consent. This study was conducted with the approval of the Research Ethics Committee of the National Institute of Health and Nutrition of Japan.

Questionnaire

The questionnaire topics were as follows.

Background characteristics: age, gestational age, number of people in the household, education level, smoking status.

Dietary supplement use before and during pregnancy: supplement use was defined according to four categories ("daily use", "occasional use", "past use", and "have never used"). For each user, questions were asked about ingredients in the supplements and the purpose of their use. Additionally, the pregnant supplement users were asked for the prenatal label, information source, supplier, observations/notes at purchase, important points when purchasing, consulted person(s), and precautions for use. Additionally, folic acid supplement users were asked what other ingredients were contained in the product.

With regard to attitudes towards diet, in order to assess the respondents' level of knowledge on food, questions were asked about their awareness of the standard dietary intake, the dietary balance guide and NTD risk with folic acid intake. The standard dietary intake refers to "Dietary Reference Intakes for Japanese (2010)",[7] which was formulated by the Ministry of Health, Labour, and Welfare of Japan. The dietary balance guide[10] was prepared by the Ministry of Health, Labour, and Welfare and the Ministry of Agriculture, Forestry, and Fisheries of Japan. Furthermore, questions were asked regarding supplement use during pregnancy, anxiety regarding diet during pregnancy, adviser(s) for diet and supplement use, and the number of items of concern regarding diet selected from a list.

Statistical analysis

The distribution of sociodemographic and other characteristics was presented as percentages, and comparisons of these characteristics between daily supplement users during pregnancy and non-daily users were performed using the chi-square test and t-test for categorical and continuous variables, respectively. Prevalence odds ratios (OR) and the corresponding 95% confidence intervals (95% CI) from logistic regressions were used to determine the factors associated with daily supplement use by pregnant women while adjusting for all the other variables included in the model. Variables that were not significantly multicollinear were included in the final regression model after being identified by a multicollinearity test using Spearman's rank correlation. The awareness of dietary reference intake was excluded because of its significant multicollinearity. Adjustments were made for the following variables: age, gestational age, geographical area, education level, smoking status, supplement use during pregnancy, awareness of NTD risk, awareness of the dietary balance guide, diet anxiety during pregnancy, number of advisers, and the number of items of concern regarding diet.

Table 1. Dietary supplement use before and during pregnancy

	Before pregnancy n (%)	During pregnancy n (%)
Total	1,076 (100)	1,076 (100)
Dietary supplement use		
Have used	806 (74.9)	810 (75.3)
Daily	217 (20.2)	400 (37.2)
Occasional	373 (34.7)	245 (22.8)
Past	216 (20.1)	165 (15.3)
Have never used	270 (25.1)	266 (24.7)
Alterations due to pregnancy		
Frequency of use		
Increased		374 (34.8)
Decreased		253 (23.5)
No change		449 (41.7)
Number of use		
Increased		262 (24.3)
Decreased		352 (32.7)
No change		461 (42.9)

Missing values were excluded.

Participants using supplements during pregnancy were divided into two groups: users of only folic acid supplements ("Only folic acid") and users of supplements containing additional ingredients or only non-folic acid ingredients ("Not only folic acid"), and comparisons were made based on the status of their use. Inter-group comparisons were performed using the chi-sqaure test and t-test for categorical and continuous variables, respectively.

Data were analyzed using PASW Statistics 18.0 for Windows, and the level of significance was set at $p<0.05$.

RESULTS

Supplement use before and during pregnancy

Of the total respondents, 74.9% (n = 806) had used supplements before pregnancy, and 75.3% (n = 810) used supplements during pregnancy (Table 1). Daily users increased from 20.2% (n = 217) before pregnancy to 37.2% (n = 400) after pregnancy. Alterations in the frequency and number of supplements used due to pregnancy varied among individuals.

Characteristics of daily users during pregnancy

In the multivariable analysis, the five characteristics associated with daily supplement use during pregnancy included: higher age (OR = 1.06, 95% CI = 1.02-1.09), approval of supplement use during pregnancy (65.6%, OR = 21.0, 95% CI = 4.93-89.8), higher awareness of NTD risk (70.2%, OR = 2.81, 95% CI = 1.79-4.40), lack of diet anxiety (the other OR = 0.5-0.57), and greater number of advisers (OR = 1.18, 95% CI = 1.01-1.38) (Table 2). There were no statistical differences in daily supplement use during pregnancy among variables such as gestational age, geographical area, education level, and smoking habits.