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Chlorella (*Chlorella pyrenoidosa*) Supplementation Decreases Dioxin and Increases Immunoglobulin A Concentrations in Breast Milk

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ABSTRACT In addition to meeting nutritional requirements, breast milk plays important roles in biodefense for nursing infants. Dioxins have been detected at high concentrations in breast milk, raising concerns about disorders in nursing infants caused by breast milk containing dioxins in Japan. We analyzed dioxin levels in breast milk and maternal blood samples from 35 pregnant women in Japan. We also measured immunoglobulin (Ig) A concentrations in breast milk and investigated correlations with dioxin concentrations. In addition, 18 of the 35 women took *Chlorella pyrenoidosa* (*Chlorella*) supplements during pregnancy, and the effects on dioxin and IgA concentrations in breast milk were investigated. Toxic equivalents were significantly lower in the breast milk of women taking *Chlorella* tablets than in the Control group ($P = .003$). These results suggest that *Chlorella* supplementation by the mother may reduce transfer of dioxins to the child through breast milk. No significant correlation was identified between dioxin and IgA concentrations in breast milk in the Control group. It is unlikely that normal levels of dioxin exposure via food have a remarkable influence on IgA in breast milk. IgA concentrations in breast milk in the *Chlorella* group were significantly higher than in the Control group ($P = .03$). Increasing IgA levels in breast milk is considered to be effective for reducing the risk of infection in nursing infants. The present results suggest that *Chlorella* supplementation not only reduces dioxin levels in breast milk, but may also have beneficial effects on nursing infants by increasing IgA levels in breast milk.

KEY WORDS: • **Chlorella pyrenoidosa** • ***co-planar polychlorinated biphenyls*** • ***maternal blood*** • ***polychlorinated dibenzo-p-dioxins*** • ***polychlorinated dibenzofurans*** • ***pregnant women*** • ***thyroid hormone***

INTRODUCTION

DIOXINS ARE ENVIRONMENTAL contaminants that exert a variety of harmful effects on humans and animals, including teratogenicity, carcinogenicity, hypofertility, immunosuppression, and thyroid dysfunction.[1–5] Dioxins that have accumulated in the body of the mother can be transferred to the fetus through the placenta during pregnancy, and to the infant via breast milk during nursing.[6,7] Disturbances in brain function and postnatal behavioral dysfunction have been confirmed in experimental animals following transplacental and lactational exposure to environmental contaminants such as dioxins.[8–10] Epidemiological studies have also identified several cases of neurological developmental disorders in children, including hyperactivity and decreased intelligence quotient, following exposure to dioxins or polychlorinated biphenyls (PCBs).[11,12] Increasing incidences of attention deficit hyperactivity disorder, learning disabilities, and autism have been reported in children in Japan and the West in recent years.[13,14] The transplacental and lactational exposure to environmental contaminants such as dioxins are suspected to be one of the causes of attention deficit hyperactivity disorder and learning disabilities in children.

Breast milk is obviously important nutritionally, but also contains a variety of components associated with the immune system of the nursing infant, including immunoglobulin (Ig) A, lactoferrin, and lysozyme. However, since high concentrations of dioxins have been detected in breast milk,[15,16] health disorders in nursing infants caused by breast milk containing dioxins have become a concern, and debate over the pros and cons of breast-feeding has become a social problem in Japan.

Chlorella pyrenoidosa is a unicellular green alga that grows in fresh water. *C. pyrenoidosa* contains much more protein and chlorophyll than other plants, and is also high in vitamins, minerals, dietary fiber, and nucleic acids.[17,18] The proteins contained in *C. pyrenoidosa* include all the essential amino acids necessary for human growth and main-

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tenance of health. *C. pyrenoidosa*, other *Chlorella* species, and *Chlorella* extracts have been reported to exert a variety of effects, including reducing cholesterol,[19] preventing stress-induced ulcers,[20] enhancing resistance to infection,[21] and antineoplastic activity.[22,23] Furthermore, we have previously demonstrated that *C. pyrenoidosa* promotes the fecal excretion of orally ingested dioxins in mice.[24] *C. pyrenoidosa* is currently widely available in many regions of the world as a nutritional supplement and health food.

In the present study, we investigated the maternal transfer of dioxins to children via breast milk and the effects of dioxins on IgA levels in breast milk and thyroid hormone levels in maternal peripheral blood in ordinary pregnant women living in Japan. In addition, in an attempt to reduce transfer of dioxins from mother to child via breast milk, we evaluated the effects of *C. pyrenoidosa* (*Chlorella*) supplements.

MATERIALS AND METHODS

Subjects

Subjects in this study comprised 35 healthy pregnant women (age range, 23–40 years; 19 primiparas, 16 multiparas) who were receiving prenatal care at Saiseikai Nara Hospital (Nara, Japan) and who provided written consent to participate in the study. Of these, 18 subjects agreed to take *Chlorella* tablets (*Chlorella* group; 10 primiparas, eight multiparas). These subjects were instructed to take *Chlorella* tablets for approximately 6 months, starting between gestational week 12 and 16, and continuing up to the day of parturition. The dose was 6 g/day of *Chlorella* (30 tablets/day), in 10-tablet portions taken after breakfast, lunch, and dinner. Sun Chlorella A tablets (Sun Chlorella Corp., Kyoto, Japan) containing dried *C. pyrenoidosa* (SUN CHLORELLA strain) powder as the active ingredient were used. *Chlorella* used in this study contained the following components per 100 g: water, 5.3 g; chlorophyll, 2.3 g; protein, 59.7 g; dietary fiber, 9.8 g; fat, 11.2 g; ash, 6.3 g; and carotene, 0.025 g. No restrictions were imposed on the 17 subjects in the Control group (nine primiparas, eight multiparas), with the exception that they were prohibited from taking *Chlorella*.

This study was conducted in accordance with the general principles of the Helsinki Declaration, and all protocols were reviewed and approved by the Institutional Review Board of Saiseikai Nara Hospital.

Analysis of dioxin

Breast milk samples (10 mL per sample, three samples) were collected from all subjects during postpartum days 3–9 and mixed. In addition, maternal peripheral blood samples (20 mL) were taken on the day of parturition, either pre- or postpartum. Given the special circumstances of this study, in that the subjects were pregnant women, maternal blood samples were not drawn under fasting conditions. Samples were collected between August 2001 and October 2002. Samples were immediately frozen after collection, then sent to Obihiro University of Agriculture and Veterinary Medicine, and kept frozen at −20°C until analyzed.

Concentrations of dioxins—six polychlorinated dibenzo-*p*-dioxin (PCDD) congeners, 10 polychlorinated dibenzofuran (PCDF) congeners, and 12 co-planar PCB (co-PCB) congeners (see Table 2)—were measured in each maternal sample. Dioxin analysis was conducted in accordance with the analytical manuals of the Ministry of Health, Labour and Welfare Japan.[25,26] Specifically, maternal blood and breast milk samples were spiked using $^{13}C_{12}$-labeled internal standards (Wellington Laboratories, Guelph, ON, Canada) of PCDDs, PCDFs, and co-PCBs. Dioxin-containing lipid was then separated from maternal blood and breast milk samples by combination with saturated ammonium sulfate, followed by ethanol, after which lipid was extracted using *n*-hexane. The dioxin-containing lipids were applied to a multilayer silica gel column comprising 2% (wt/wt) KOH-silica gel, 44% (wt/wt) H_2SO_4-silica gel, 22% (wt/wt) H_2SO_4-silica gel, and 10% (wt/wt) $AgNO_3$-silica gel, in that order, followed by passage through an alumina (alumina oxide 90; Merck & Co., Inc., Darmstadt, Germany) column for sample clean-up. Analyses of PCDDs and PCDFs were performed using high-resolution gas chromatography-mass spectrometry (GC-MS) (HP-6890 gas chromatograph from Hewlett Packard, Palo Alto, CA; AutoSpec-Ultima mass spectrometer from Micromass, Manchester, UK) equipped with a capillary column (SP-2331, Supelco, Bellefonte, PA; DB-17HT, J&W Scientific, Folsom, CA) employing the electron impact-selected ion monitoring method at 10,000 resolution. Analysis of co-PCBs was performed using GC-MS (HP-6890 and AutoSpec-Ultima) with a DB-5MS fused silica capillary column (J&W Scientific).

Analysis of IgA in breast milk and thyroid hormones in maternal blood

IgA concentrations in breast milk were analyzed by single radial immunodiffusion with a commercial analysis kit (Medical & Biological Laboratories, Nagoya, Japan) using the same samples used to analyze dioxin levels. Specifically, breast milk was centrifuged (9,000 *g*, 10 min) to isolate whey from breast lipids and prepare the samples for analysis. A calibration curve plotted from the concentration of IgA standards and corresponding diameter of the precipitation ring was prepared, and concentrations of IgA in each whey sample were determined. In addition, maternal blood samples were analyzed using enzyme immunoassay for triiodothyronine (T_3), thyroxine (T_4), free T3 (F-T_3), free T4 (F-T_4), and thyroid-stimulating hormone (TSH), and the relationship between concentrations of these hormones and dioxin levels was investigated. Maternal blood samples taken between weeks 35 and 37 of pregnancy were used to measure thyroid hormone concentrations.

Data analysis

Mean values and standard deviations (SDs) were calculated for the concentrations of each dioxin congener and for

total PCDDs, total PCDFs, total co-PCBs, and total dioxin concentrations (PCDDs + PCDFs + co-PCBs) in breast milk and maternal blood samples. Toxic equivalents (TEQ) were calculated using World Health Organization toxicity equivalency factors.[27] In these calculations, measured values of congeners with concentrations below the detection limit were regarded as 0. Spearman's rank correlation test was used to investigate correlations between dioxin concentrations and IgA levels in breast milk and thyroid hormone levels in maternal blood, and to investigate correlations between dioxin levels in breast milk and maternal blood, within each group. To evaluate the effects of taking *Chlorella*, the *Chlorella* group ($n = 18$) and Control group ($n = 17$) were compared for concentrations of each dioxin congener and for total PCDDs, total PCDFs, total co-PCBs, total dioxin concentrations (PCDDs + PCDFs + co-PCBs), and TEQ in breast milk and maternal blood. The Mann-Whitney U test was used to compare differences in mean values for each group. Values of $P < .05$ were considered statistically significant. All statistical procedures were performed using SPSS version 11.0 for Windows statistical software (SPSS, Chicago, IL).

RESULTS

Subject characteristics

Characteristics of mothers and their infants are shown in Table 1. No differences between the *Chlorella* and Control groups were observed with regard to results of normal clinical examinations or interviews conducted at Saiseikai Nara Hospital during pregnancy (data not shown). The stools of subjects in the *Chlorella* group were seen to display green discoloration, but this was attributed to the excretion of chlorophyll contained in *C. pyrenoidosa*. No other adverse reactions were observed.

Dioxins in breast milk and maternal blood

The principal congeners found in breast milk and maternal blood were 1,2,3,7,8-PentaCDD, 1,2,3,6,7,8-HexaCDD, 2,3,4,7,8-PentaCDF, 3,3′,4,4′,5-PentaCB (IUPAC number 126), and 2,3,3′,4,4′,5-HexaCB (IUPAC number 156). These five congeners represented approximately 80% of the TEQ (Table 2). In the Control group, PCDD, PCDF, non-*ortho*-co-PCBs, and mono-*ortho*-co-PCB profiles in breast milk and maternal blood were very similar (Fig. 1). A similar profile for dioxins was shown in the *Chlorella* group (Fig. 1). In the Control group, a significant correlation was observed between TEQ (pg of TEQ/g of whole sample) in breast milk and TEQ (pg of TEQ/g of whole sample) in maternal blood ($r = .676$, $P < .01$; Fig. 2). Likewise, a significant correlation was also observed between TEQ in breast milk and TEQ in maternal blood in the *Chlorella* group ($r = .759$, $P < .01$; Fig. 2).

Relationship between IgA and dioxin concentrations in breast milk

The mean (±SD) IgA level in breast milk of the Control group was 105.9 ± 77.7 mg/dL (Table 3). In the Control group, no significant correlation was observed between IgA concentrations and TEQ in breast milk (Fig. 3). On the other hand, a significant correlation was observed between IgA concentrations and TEQ in breast milk in the *Chlorella* group ($r = .502$, $P = .005$; Fig. 3).

Relationship between thyroid hormone and dioxin levels in maternal blood

No major deviations from the normal range were observed in T_3, F-T_3, T_4, F-T_4, or TSH levels in either the Control group or the *Chlorella* group (Table 3). F-T_3 levels in maternal blood of women between 35 and 37 weeks of pregnancy were below normal in almost all subjects, but no deviations from the normal range for F-T_4 levels were observed in any subject. TSH levels deviated from the normal range in a small number of subjects (data not shown). In the Control group, no significant correlation was observed between TEQ in maternal blood and F-T_4, F-T_3, or TSH concentrations in maternal blood (Fig. 4).

Effects of Chlorella *supplementation*

Mean TEQ was significantly lower in breast milk of the *Chlorella* group (0.554 ± 0.216 pg of TEQ/g of whole sam-

TABLE 1. SUBJECT CHARACTERISTICS

	Control group (n = *17*)			Chlorella *group* (n = *18*)			
Characteristic	*Mean*	*SD*	*%*	*Mean*	*SD*	*%*	P[a]
Maternal							
Age (years)	28.6	4.1	—	30.4	3.3	—	.303
Weight before pregnancy (kg)	54.0	10.0	—	53.2	8.6	—	.708
Parity (% primipara)	—	—	52.9	—	—	55.6	1.000
Newborn							
Male (%)	—	—	29.4	—	—	50.0	.305
Weight (g)	2,861.4	334.3	—	3,125.1	342.5	—	.022

[a]Mann-Whitney U test or Fisher's exact test.

TABLE 2. TEQ (PG OF TEQ/G OF WHOLE SAMPLE) AND CONCENTRATIONS (PG/G OF WHOLE SAMPLE) OF PCDDS, PCDFS, AND CO-PCBS IN MATERNAL BLOOD AND BREAST MILK IN CONTROL AND *CHLORELLA* GROUPS

		Control group (n = 17)		Chlorella *group* (n = 18)	
	WHO-TEF	*Blood*	*Milk*	*Blood*	*Milk*
PCDDs					
2,3,7,8-TetraCDD	1	0.007 (0.007)	0.045 (0.021)	0.005 (0.004)	0.024 (0.011)*
1,2,3,7,8-PentaCDD	1	0.034 (0.017)	0.193 (0.079)	0.030 (0.011)	0.119 (0.044)*
1,2,3,6,7,8-HexaCDD	0.1	0.143 (0.086)	1.004 (0.480)	0.118 (0.047)	0.590 (0.214)*
1,2,3,7,8,9-HexaCDD	0.1	0.022 (0.017)	0.147 (0.094)	0.017 (0.015)	0.092 (0.065)
1,2,3,4,6,7,8-HeptaCDD	0.01	0.084 (0.040)	0.309 (0.196)	0.228 (0.171)	0.253 (0.166)
OctaCDD	0.0001	16.18 (1.054)	17.471 (.319)	14.778 (0.943)*	17.222 (2.798)
PCDFs					
2,3,7,8-TetraCDF	0.1	0.007 (0.006)	0.027 (0.017)	<LOD	0.013 (0.013)*
1,2,3,7,8-PentaCDF	0.05	0.005 (0.005)	0.028 (0.017)	<LOD	0.008 (0.010)*
2,3,4,7,8-PentaCDF	0.5	0.051 (0.030)	0.348 (0.141)	0.046 (0.021)	0.202 (0.074)*
1,2,3,4,7,8-HexaCDF	0.1	0.022 (0.014)	0.107 (0.049)	0.015 (0.011)	0.065 (0.036)*
1,2,3,6,8,9-HexaCDF	0.1	0.024 (0.014)	0.110 (0.048)	0.020 (0.013)	0.70 (0.035)*
1,2,3,7,8,9-HexaCDF	0.1	<LOD	<LOD	<LOD	<LOD
2,3,4,6,7,8-HexaCDF	0.1	<LOD	0.062 (0.034)	<LOD	0.041 (0.035)
1,2,3,4,6,7,8-HeptaCDF	0.01	0.013 (0.006)	0.045 (0.019)	<LOD	0.041 (0.027)
1,2,3,4,7,8,9-HeptaCDF	0.01	<LOD	<LOD	<LOD	<LOD
OctaCDF	0.0001	<LOD	<LOD	<LOD	<LOD
Non-*ortho*-Co-PCBs					
3,4,4',5-TetraCB (#81)	0.0001	0.042 (0.025)	0.191 (0.093)	0.051 (0.030)	<LOD*
3,3',4,4'-TetraCB (#77)	0.0001	0.359 (0.109)	0.838 (0.239)	0.264 (0.091)*	0.483 (0.210)*
3,3',4,4',5-PentaCB (#126)	0.1	0.279 (0.258)	2.149 (1.232)	0.298 (0.188)	1.231 (0.664)*
3,3',4,4',5,5'-HexaCB (#169)	0.01	0.182 (0.108)	1.370 (0.474)	0.182 (0.081)	0.933 (0.351)*
Mono-*ortho*-Co-PCBs					
2',3,4,4',5-PentaCB (#123)	0.0001	0.0949 (0.749)	6.624 (3.948)	0.838 (0.513)	3.528 (2.042)*
2,3',4,4',5-PentaCB (#118)	0.0001	51.412 (43.748)	388.824 (232.107)	40.222 (16.658)	201.611 (120.422)*
2,3,3',4,4'-PentaCB (#105)	0.0001	12.688 (11.408)	93.53 (60.196)	10.522 (4.585)	53.500 (35.357)*
2,3,4,4',5-PentaCB (#114)	0.0005	3.205 (2.212)	28.353 (16.681)	2.367 (1.144)	12.978 (5.719)*
2,3',4,4',5,5'-HexaCB (#167)	0.00001	5.841 (4.327)	42.882 (25.124)	4.500 (1.935)	22.778 (10.282)*
2,3,3',4,4',5-HexaCB (#156)	0.0005	17.629 (10.572)	150.471 (89.824)	14.389 (6.626)	82.566 (37.722)*
2,3,3',4,4',5'-HexaCB (#157)	0.0005	4.529 (2.738)	36.177 (20.467)	3.744 (1.675)	19.856 (8.731)*
2,3,3',4,4',5,5'-HeptaCB (#189)	0.0001	1.678 (1.054)	11.159 (4.548)	1.379 (0.541)	7.706 (3.749)*
Total PCDDs		16.407 (1.152)	19.168 (3.759)	15.030 (0.998)*	18.301 (3.098)
Total PCDFs		0.126 (0.076)	0.731 (0.295)	0.102 (0.060)	0.455 (0.210)*
Total Co-PCBs		98.794 (75.233)	762.390 (423.150)	78.756 (29.964)	407.159 (201.023)*
PCDDs/PCDFs/Co-PCBs		115.327 (76.037)	782.288 (425.806)	98.888 (30.125)	425.915 (203.026)*
TEQ		0.141 (0.090)	0.951 (0.423)	0.126 (0.048)	0.554 (0.216)*

Data are mean (SD) values. Mean value below limit of detection (LOD) was as follows: TetraCDD/CDF, PentaCDD/CDF, HexaCDD/CDF, HeptaCDD/CDF, OctaCDD/CDF, and Co-PCB were 0.005 pg/g, 0.005 pg/g, 0.01 pg/g, 0.02 pg/g, and 0.01 pg/g, respectively. WHO-TEF, World Health Organization toxicity equivalency factors.

*$P < .05$.

Numbers in parentheses are IUPAC numbers.

ple) than in the Control group (0.951 ± 0.423 pg of TEQ/g of whole sample) (Table 2). Mean breast milk IgA concentrations were significantly different at 154.8 ± 74.9 mg/dL and 105.9 ± 77.7 mg/dL in the *Chlorella* and Control groups, respectively (Table 3).

DISCUSSION

This study investigated the maternal transfer of dioxins to children via breast milk in ordinary mothers living in Japan. In addition, we investigated the possibility that maternal transfer of dioxins to children via breast milk could be reduced by supplementing the mothers with *Chlorella*. To evaluate the toxicity of dioxins to mothers and children, relationships between maternal dioxin levels and both IgA in breast milk and thyroid hormones in maternal blood were investigated.

Dioxin intake by infants through nursing was calculated from TEQ in the breast milk of subjects. Dioxin intake by nursing infants was estimated at approximately 118 pg of

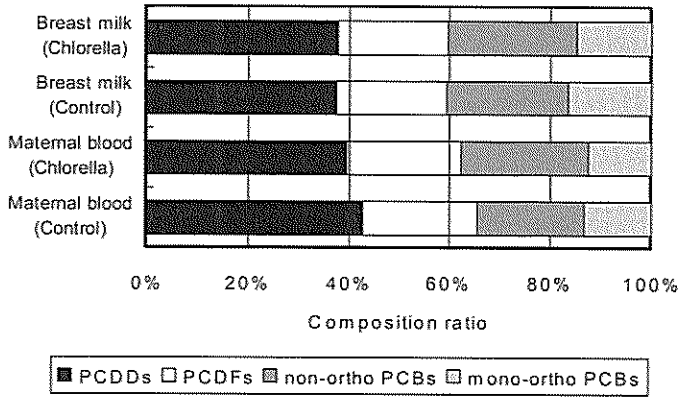

FIG. 1. Composition ratios of TEQ for PCDDs, PCDFs, non-*ortho*-co-PCBs, and mono-*ortho*-co-PCBs in breast milk and maternal blood in the Control and *Chlorella* groups.

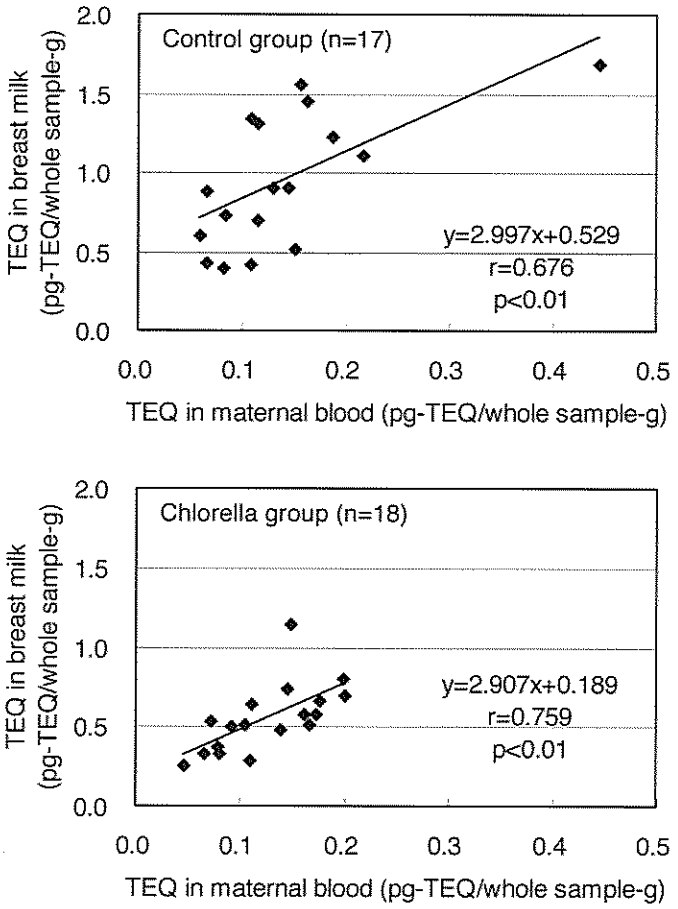

FIG. 2. Correlation between TEQ in maternal blood and breast milk in a subset of the Control and *Chlorella* groups.

TEQ/kg of body weight/day, assuming a mean breast milk intake of 120 mL/kg of body weight/day.[28] This value is considerably more than 1.63 pg of TEQ/kg of body weight/day, the daily intake of dioxins from food calculated in a study conducted by the Ministry of Health, Labour and Welfare Japan in 2001,[29] and is more than 20-fold greater than the World Health Organization recommended tolerable daily intake of dioxins of 1–4 pg of TEQ/kg of body weight/day.[30] Although nursing occurs for only a limited period of time, more detailed research is needed to reveal the consequences by high intakes of dioxin via breast milk. The linear regression equation introduced from the results of this study expressing the relationship between TEQ of breast milk and TEQ of maternal blood should prove useful for predicting dioxin concentrations in breast milk based on concentrations in maternal blood.

In this study, IgA levels in breast milk and thyroid hormone levels in maternal blood were used as indicators for examining the health effects of dioxins on pregnant women and their children. Following the Yu-Cheng incident in Taiwan in 1979, suppressed antibody production, including decreased serum levels of IgA and IgM, was reported in patients who had ingested dioxin-contaminated cooking oil.[31] However, no observable effects of dioxins were seen in the present study on IgA concentrations in breast milk. This may be because dioxins have essentially no effect on IgA levels in breast milk at the normal level of dioxin exposure via

TABLE 3. CONCENTRATION OF IGA IN BREAST MILK AND CONCENTRATION OF THYROID-RELATED HORMONES IN MATERNAL BLOOD IN CONTROL AND *CHLORELLA* GROUPS

	Control group (n = *17*)		Chlorella *group* (n = *18*)	
	Milk	*Blood*	*Milk*	*Blood*
IgA (mg/dL)	105.9 (77.7)	—	154.8 (74.9)*	—
T_3 (ng/mL)	—	1.87 (0.23)	—	1.77 (0.39)
F-T_3 (pg/mL)	—	2.24 (0.18)	—	2.34 (0.27)
T_4 (μg/dL)	—	11.71 (1.65)	—	11.80 (1.56)
F-T_4 (ng/dL)	—	1.01 (0.13)	—	1.01 (0.11)
TSH (μU/mL)	—	1.68 (1.09)	—	1.50 (1.06)

Data are mean (SD) values.
*$P < .05$.

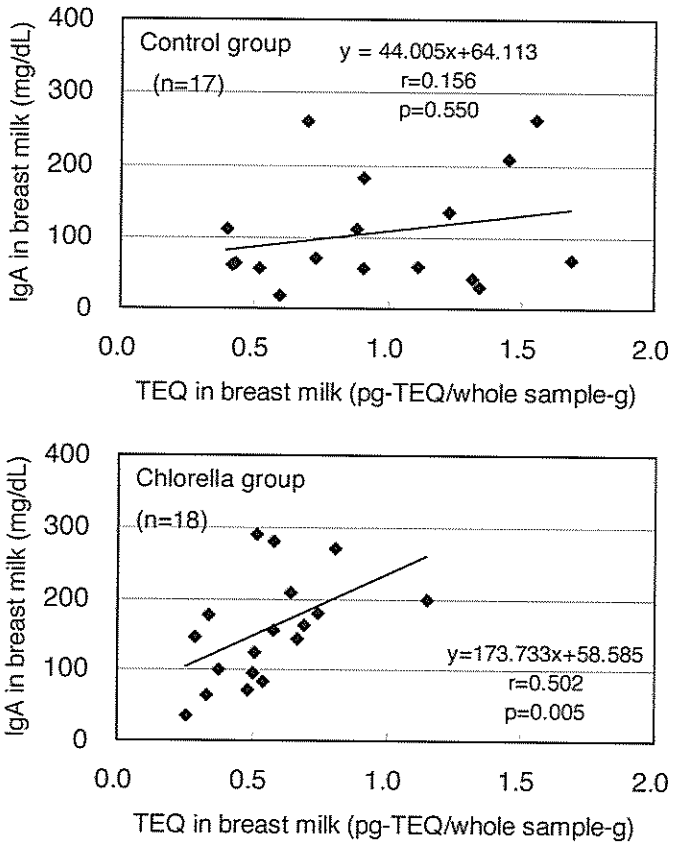

FIG. 3. Correlation between TEQ and concentration of IgA in breast milk in each participant of the Control and *Chlorella* groups.

food. Thyroid dysfunction has been reported to be a toxic effect of dioxins.[4,5] As depressed thyroid function in pregnant women is reportedly linked to mental retardation in children,[32] our study also investigated the effects of dioxins on thyroid hormone levels in maternal blood. However, no significant correlation between thyroid hormone concentrations and dioxin concentrations in maternal blood was observed.

Approximately 90% of human intake of dioxins is through food.[33,34] We investigated whether dioxins in breast milk reflected the content of dioxins ingested by mothers through food by comparing data on dioxins in breast milk in this study to data on dioxins from dietary sources in Japanese from a survey conducted by the Ministry of Health, Labour and Welfare, Japan in 2001.[29] The results revealed significant correlations between concentrations of dioxin congeners for both dioxins in breast milk and dietary intake of dioxins ($r = .878$, $P < .01$; Fig. 5). Huisman *et al.*[35] investigated the relationship between dioxins in breast milk and dietary intake of dioxins during pregnancy in 418 pregnant women, and reported a weak correlation between dioxin concentrations in breast milk and dioxin intake during pregnancy. It appears that dioxins in breast milk are influenced by the dietary intake of dioxins by the mother.

We investigated the potential for reducing the maternal transfer of dioxins to the child through breast milk by dietary supplementation of *C. pyrenoidosa* during pregnancy. In this study, the TEQ in breast milk of pregnant women who took *Chlorella* tablets during pregnancy was approximately 40% lower than in controls. Pluim *et al.*[36] investigated the effects of a 5-day low-dioxin diet in nursing mothers during week 4 postpartum, measuring dioxin concentrations in breast milk both before and after the diet. Although they reported no change in dioxin concentrations during this short-term study, they suggested that a diet low in dioxin levels continued over the long term could potentially

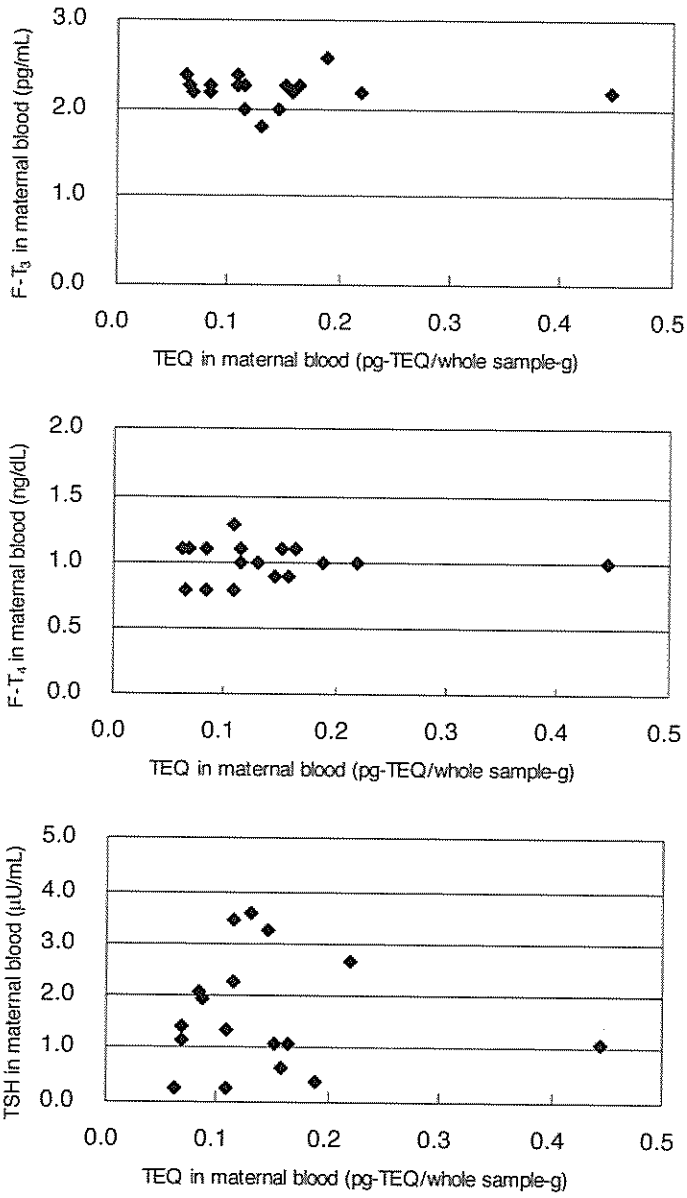

FIG. 4. Correlation between TEQ and concentration of F-T_3, F-T_4, and TSH in maternal blood in a subset of the Control group ($n = 17$).

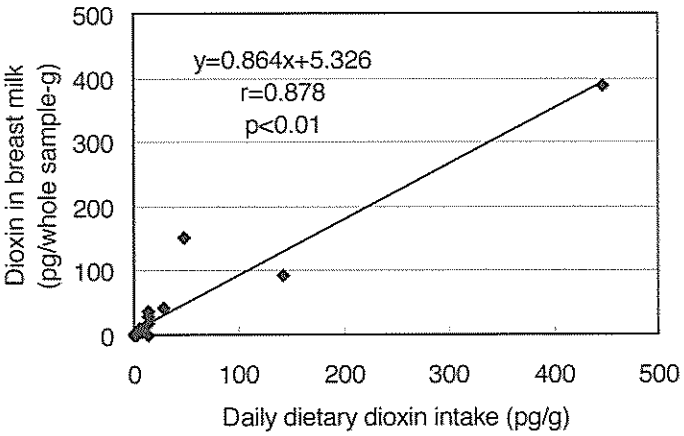

FIG. 5. Correlation between 28 dioxin congeners in breast milk in the Control group and dietary dioxin intake. Data relating to dietary intake of dioxins in Japanese quoted from the "National survey of daily intake (total diet study) of dioxins from food in Japan" (2001) conducted by the Ministry of Health, Labour and Welfare Japan.[29]

reduce dioxin levels in adipose tissue and breast milk. Beck *et al.*[37] reported that the breast milk of vegetarians contained low PCDD/PCDF concentrations. This suggests the possibility that dioxin levels in breast milk can be reduced through a diet low in dioxins, and through inhibiting the absorption and accelerating the excretion of dioxins ingested through food. The primary mechanism by which *C. pyrenoidosa* promotes excretion of dioxins is presumably through the formation of complexes between dioxins and the chlorophyll in *C. pyrenoidosa*.[38] Dioxins are then absorbed by the dietary fiber also contained in *C. pyrenoidosa*,[39,40] thereby inhibiting absorption of dioxins from the intestinal tract and reabsorption of dioxins through the enterohepatic circulation. However, no significant difference in TEQ in maternal blood was observed between groups. *Chlorella* supplements reportedly reduced serum cholesterol levels in hyperlipidemic patients,[19] suggesting the need to consider the effects of *C. pyrenoidosa* on maternal lipid metabolism, including the transfer of lipids containing dioxins to the mammary glands. However, the physiological functions of *C. pyrenoidosa* and the associated mechanisms are not yet thoroughly understood, and represent an important topic for future investigation.

The present study found no effects of dioxins on IgA concentrations in breast milk, although IgA levels in breast milk were significantly increased in the *Chlorella* group compared to the Control group. Increased concentrations of IgA were seen in breast milk from the *Chlorella* group, while dioxin concentrations were reduced. However, the lack of a correlation between IgA and dioxin concentrations in breast milk suggests that this was caused not by decreased dioxin levels, but rather as the effect of *Chlorella* on the immune system of pregnant women. IgA in breast milk is known to have specificity against antigens such as bacteria, viruses, and harmful substances in the digestive tract of the nursing infant. In addition, IgA also provides nonspecific immunity, forming complexes with invasive factors and with orally ingested food antigens by high agglutinating activity, preventing invasion into the intestinal mucosal cells.[41] Consequently, from a qualitative perspective, the significance of IgA as a breast milk component lies not in specific antibody activity, but in the total quantity of IgA present. IgA-producing B cells primed by antigens in gut-associated lymphoid tissue such as Peyer's patches transfer to mammary glands via the enteromammary pathway, and then produce IgA. Kitamura *et al.*[42] conducted a study in which pigs were given feed containing casein phosphopeptide from day 45 of gestation until weaning, and investigated immunoglobulin levels in milk. IgA levels in the casein phosphopeptide-fed group were significantly increased on the day of parturition and 10 days postpartum. Kitamura and Otani[43] also reported that IgA production in the gastrointestinal tract is promoted by oral intake of casein phosphopeptide in humans. Some reports have noted that dietary fiber and vegetable proteins such as soy protein enhance IgA production in mesenteric lymphocytes.[44,45] Components contained in *C. pyrenoidosa* such as peptides or dietary fiber presumably stimulate IgA-producing B cells in gut-associated lymphoid tissue and increase IgA in breast milk through this mechanism via the enteromammary pathway. Increasing IgA in the breast milk through maternal *Chlorella* supplementation may be effective for reducing the risk of infectious disease in nursing children.

The latent risks of environmental contaminants such as dioxins may well be manifest as symptoms such as attention deficit hyperactivity disorder and learning disabilities in children, who are more sensitive than adults to chemical substances and toxins. However, to thoroughly ascertain any causal relationships, we must await the results of detailed long-term studies. At present, parallel measures should also be pursued to reduce, however slightly, any health risks posed by environmental contaminants such as dioxins to fetuses and nursing infants. Reducing maternal dioxin levels and the transfer of dioxins to fetuses and nursing infants represent one of the most realistic ways to accomplish this goal. Our results suggest that *Chlorella* supplementation by the mother may effectively reduce transfer of dioxins to children through breast milk. Our results also indicate that maternal *Chlorella* supplementation may have beneficial effects on nursing infants by increasing IgA levels in breast milk.

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