British Journal of Ophthalmology 1995; 79: 202–206

ORIGINAL ARTICLES – Clinical science

Smoking of beedies and cataract: cadmium and vitamin C in the lens and blood

S Ramakrishnan, K N Sulochana, T Selvaraj, A Abdul Rahim, M Lakshmi, K Arunagiri

Abstract

Estimation of cadmium and vitamin C was performed in the blood and lens of smokers in three age groups up to a maximum age of 58, habituated to smoking a minimum of 10 beedies a day for many years, as well as those of non-smokers in the same age groups. Only nuclear cataracts with or without posterior or anterior subcapsular cataract were chosen. It was found that there was a significant accumulation of cadmium in both the blood and the lens of the smokers. Such an accumulation of cadmium might have a role in cataractogenesis in chronic smokers. In a similar experiment, with smokers and non-smokers of two age groups up to a maximum age of 40, both without cataract, increased levels of cadmium were found in the blood of smokers only, though the extent of accumulation was not as high as in chronic smokers of higher age groups. Vitamin C content of lens was on the lower side of normal in both chronic smokers of beedies in the two age groups and non-smokers with nuclear cataract with or without posterior and anterior subcapsular cataract, and there was no significant change brought about by smoking. Vitamin C levels in blood were towards the lower side of the normal in smokers and non-smokers with and without cataract.

(*Br J Ophthalmol* 1995; **79:** 202–206)

Smoking of cigarettes causes cataract at a young age as found by Clayton *et al*,[1] Harding,[2] Harding and Van Heyningen,[3] and Flaye *et al*.[4] West *et al*[5] reported that out of 838 watermen in Maryland, USA free from other probable causes, 229 who smoked, developed nuclear cataract and this was dose dependent. When smoking was stopped for a reasonable length of time, the susceptibility to cataract formation was less. Smoking appears to induce oxidative stress, in that smokers have diminished levels of antioxidants, ascorbate, and carotenoids.[6 7] The constituent aromatic compounds and trace metals in smoke condensates generate long lived reactive oxygen species. These might cause oxidative damage to the lens and produce cataract.[8]

It was assumed that changes in the cadmium and vitamin C levels in the blood might be responsible for cataractogenesis in the smokers for the following reasons.

It is reported that smokers have higher levels of cadmium in blood,[9] milk,[10] and tissues.[11] When a person smokes, his body receives significant amounts of cadmium from the tobacco leaves. This is due to the capacity of the tobacco plants to absorb and concentrate cadmium salts from the earth.[12] The cadmium so received binds to haemoglobin of blood,[13] lungs,[14] and low molecular weight proteins such as metallothioneins[15] of liver and kidneys, and is retained in the body with some free cadmium.[16] While Nath[17] and Racz[18] have reported a progressive accumulation of cadmium in senile cataract as brunescence advances, there are no reports on the cadmium content in the lens tissue of smokers. As smoking causes accumulation of cadmium in blood and tissues, it was thought that cadmium could also accumulate in the lens of smokers with cataract.

It is also reported that there is depletion of vitamin C in the blood and lens of smokers.[6 7 19–22] Jain *et al*[20] have found a depletion of vitamin C in the lens in cases of black cataract but have not given the age of their subjects. As aging is reported to decrease the levels of vitamin C in the lens,[6 7] we fixed the age between 40 and 50. Being an antioxidant and an electron sink, vitamin C quenches the oxygen free radicals produced in the lens.[23] In its deficiency, oxidant challenge may get the upper hand and could cause oxidation of membrane proteins and lipids.

As West *et al*[5] found less susceptibility to cataract among those who stopped smoking, we opined that only biochemical variations and no permanent histological changes in the lens were responsible for the incidence.

Table 1 Subject details

	Number	Age range (years)	% Of total cases
Group I			
Non-smokers with clear lens	25	30–40	16
Group II			
Smokers with clear lens	33	30–40	20
Group III			
Non-smokers with cataract	58	40–58	37
Group IV			
Smokers with cataract	43	40–58	27

Department of Biochemistry, Medical and Vision Research Foundations, Madras 600 006, India
S Ramakrishnan
K N Sulochana
M Lakshmi
K Arunagiri

Stanley Medical College, Madras, India
T Selvaraj
A Abdul Rahim

Correspondence to:
S Ramakrishnan.

Accepted for publication
5 September 1994

Table 2 Cadmium levels in lens (smokers v non-smokers both with cataract)

Age (years)	Category	N	Mean (SEM) (µg/g) lens tissue	Mean (SEM) (µg/g) lens proteins	p Value
40–50	Non-smokers with cataract (group III)	5	0·2896 (0·072)	1·176 (0·178)	
	Smokers with cataract (group IV)	3	0·6010 (0·0812)	2·15 (0·398)	
	Difference		0·3114	0·974	<0·05
	t Values		2·76	2·61	
51–55	Non-smokers with cataract (group III)	5	0·1350 (0·023)	0·688 (0·98)	
	Smokers with cataract (group IV)	6	1·4615 (0·509)	6·267 (2·568)	
	Difference		1·3265	5·579	<0·05
	t Values		2·36	2·41	
56–58	Non-smokers with cataract (group III)	22	0·2274 (0·23)	0·9391 (0·77)	
	Smokers with cataract (group IV)	22	1·1134 (0·227)	5·3020 (1·202)	
	Difference		0·8860	4·3629	<0·01
	t Values		3·89	3·25	

Materials and methods

A total of 159 cases divided into four groups were studied as given in Table 1. They all belonged to the lower middle class and were treated in Stanley Medical College Hospital, Madras.

Owing to difficulty in getting specific cases of nuclear cataract alone and in those under the age of 50 as initially stipulated, two modifications had to be made in the original criteria. The maximum age was raised to 58 and patients with nuclear cataract with and without posterior or anterior subcapsular cataract were included. Likewise, it was difficult to get controls and smokers with clear lens over the age of 40. Hence the lower age limit was reduced to 30 for normals and smokers without cataract. Groups I and II were divided into two age groups each, 30–35 and 36–40, while groups III and IV were divided into three age groups each, 40–50, 51–55, and 56–58 for cadmium and two each, 40–54 and 55–60 for vitamin C studies.

Beedies contain only powdered tobacco leaves wrapped up in a dry leaf while cigarettes contain the same wrapped up in paper. The quality of tobacco used in cigarettes is different. The weight of a whole beedi (random sample) was found to be 0·3509 g while that of a cigarette 0·8924 g. The tobacco contents weighed 0·223 g in the beedi and 0·782 g in the cigarette. Tobacco used in the beedies contains 6·5 to 8·2% alkaloid nicotine, while that used in cigarettes has 2·7 to 3·7%.[24] The other constituents are nicotimine, nicoteine, nicotellines, nicotianine, resins, fats and sulphates, nitrates, chlorides, phosphates, malates and citrates of potassium, ammonium, calcium, etc, and oxalic acid.[25] Beedies are commonly smoked by lower middle class people in India.

A double blind method was followed and the authors did not know of the affiliation of any subject to any of the prescribed groups beforehand.

The inclusion and exclusion criteria adopted were as follows:

(A) Inclusion criteria: studies were confined to

(i) only males. This is because it is reported that females are less susceptible to cadmium accumulation than males who smoke a larger number of cigarettes and hold more smoke in their lungs for a longer time
(ii) those treated in Stanley Medical Hospital, Madras, India
(iii) those up to a maximum age of 58 years; higher age was avoided as aging itself could decrease vitamin C in lens and cause accumulation of cadmium
(iv) those habituated to smoking a minimum of 10 beedies a day
(v) those who had no break in smoking for more than 1 month during the past year, and
(vi) those who had only nuclear cataract with or without anterior or posterior subcapsular cataract.

(B) Exclusion criteria. The following were excluded:

(i) those who had cortical cataract
(ii) those who had diabetes mellitus
(iii) hypertension
(iv) glaucoma
(v) alcoholics, and
(vi) those on steroid treatment.

The exclusion criteria were assessed by studying the case history of the patients, blood sugar investigation for diabetes mellitus, and examination by a physician and an ophthalmologist.

Blood taken by venepuncture and the intact cataractous lenses operated out were used immediately. All the patients were examined by slit-lamp and then categorised into groups.

A volume of 2 ml of whole blood was digested with 3 ml of concentrated nitric acid:perchloric acid (5:1) by heating to a paste. The residue was made up to 2 ml with five times glass distilled water and a drop of concentrated nitric acid was added to prevent adsorption of cadmium on the wall of the container. Half the lens material was dried between the folds of filter paper, weighed accurately, digested, and made up as in the case of blood. The cadmium nitrate extract was analysed in an atomic absorption spectrophotometer using cadmium standards received from Sigma Chemical Company, St Louis, USA. A volume of 3 ml of nitric acid:perchloric acid mixture was also digested and treated similarly for blank values. Results were expressed as µg/dl of blood, µg/g of wet lens, and µg/g of lens proteins. Cadmium levels in beedies and cigarettes were also estimated in the same way.

After using 2 ml of whole blood for cadmium, the remaining blood was centrifuged and plasma separated. A volume of 1 ml of plasma was mixed with equal volume of 10% trichloroacetic acid. This mixture was titrated against 0·2 ml of 2,6-dichlorophenol indophenol solution.[26]

After using half the lens for cadmium estimation, the remaining half was dried between the folds of filter paper, weighed and treated with 2 ml of 10% trichloroacetic acid. The precipitated lens proteins were centrifuged, removed, dissolved in 0·25 N sodium hydroxide and estimated by the method of Lowry *et al*[27] using bovine serum albumin from Sigma as standard. The trichloroacetic acid extract was titrated against 0·2 ml of 2,6-dichlorophenol indophenol[26] and vitamin C was calculated per gram of lens tissue and of

Table 3 Cadmium levels in blood (smokers v non-smokers both with cataract)

Age (years)	Category	N	Mean (μg/dl)	SD	SEM	t test	p Value
40–50	Non-smokers with cataract (group III)	5	0·362	0·220	0·098		
	Smokers with cataract (group IV)	3	0·3067	0·219	0·127		
	Difference		0·0553			0·35	NS
51–55	Non-smokers with cataract (group III)	6	0·2750	0·132	0·054		
	Smokers with cataract (group IV)	6	1·5733	1·191	0·486		
	Difference		1·2983			2·65	<0·05
56–58	Non-smokers with cataract (group III)	26	0·2637	0·192	0·038		
	Smokers with cataract (group IV)	23	1·4452	1·166	0·243		
	Difference		1·1815			5·10	<0·01

lens proteins. All the values were subjected to statistical analysis by Student's *t* test.

Results and discussion

It was found that there was significantly high accumulation of cadmium in the lenses of smokers with cataract in all three age groups, 40–50, 51–55, and 56–58 (Table 2) compared with that of non-smokers in the same age groups with cataract. There was a twofold increase in lens cadmium in the 40–50 year age group while the increase was five to 10 times in the higher age groups, perhaps, because of chronic smoking.

With cadmium in the lens, the results are better expressed in terms of proteins to avoid any complications due to hormonal disturbances, fatty infiltration, or hydration which could alter the tissue weight. There was a sixfold increase in blood cadmium in smokers with cataract belonging to the two age groups, 51 to 55 and 56–58, compared with that of non-smokers with cataract (Table 3). The results for blood cadmium and lens cadmium are statistically significant in respect of differences between non-smokers and smokers. The absence of any significant increase of cadmium in the blood of smokers in the 40–50 year age groups in group IV (Table 3) might be due to their lower age, and fewer years of smoking. The number of subjects in the group was also comparatively less than the other age groups in groups III and IV owing to difficulty in getting patients between 40 and 50 with nuclear cataract. The maximum age of the subjects in groups III and IV was 58 and these subjects had been smoking beedies for many years at the rate of more than 10 beedies a day without a break even for a month. The studies clearly show that there is accumulation of the heavy metal ion of cadmium in the lens of chronic smokers which might have a role in cataractogenesis. The accumulation of cadmium in group IV is not due to age as, in the non-smokers of the same age group with cataract, there was much less cadmium in their lens and blood (Tables 2 and 3). No doubt cadmium has been reported to be present in the lens in senile cataract[28] and its concentration is directly proportional to the development of brunescence, but this is due to old age. The levels reported are 0·209 μg/g for immature, 0·317 for mature, and 0·505 for hypermature cataractous lenses[28] but much less than the values of 1·11 to 1·46 found by us (Table 2). Normal lens has no cadmium.[17] It is reported that there is an eightfold increase of cadmium in the lungs of rats exposed to cigarette smoke.[29]

In groups I and II also – that is, subjects without cataract in age groups 30–35 and 36–40, there is accumulation of cadmium in the blood of smokers (Table 4) confirming the finding of previous workers in the field. The difference in our values is statistically significant. The values reported in the literature are different – for instance, 0·5 μg/dl,[14 30] 0·31 to 1·2 μg/l,[13] 0·3–7 μg/l[13] for non-smokers, and 0·6 to 3·9 μg/l for smokers.[13] In view of ecological and environmental conditions, differences in the cadmium content of cigarettes and beedies, number smoked and, above all, absence of a homeostatic mechanism for cadmium in the living system, such variations are bound to occur and comparison of results has to be made in the studies in the particular centre.

We could not find anyone over 40 with a clear lens. As smoking was relatively for a much shorter duration, though there was accumulation of cadmium in group II, it was not as high as in group IV. The relatively higher levels of cadmium in different age groups in group IV might be due to chronic smoking – that is, these subjects of maximum age 58 years were smoking for more than 20 years compared with smokers aged 30–40 in group II. In respect of non-smokers in group III, the high value in age groups 40–50, 51–55, and 56–58 compared with group I (30–40 years) could be due to exposure to cadmium in the environment[31] and possibly other factors.

Cadmium may hasten cataractogenesis by various mechanisms. Normally it is bound to lungs and low molecular weight proteins, the metallothioneins in the kidneys, liver, and eye lens and also haemoglobin in blood. There is also free cadmium.[16] It is likely that as the concentration of cadmium increases in blood and tissues, the haemoglobin, as result of bound cadmium, might lose its full power of delivery of oxygen to tissues including ocular structures. This would mean decreased ATP production and availability of energy. Cadmium could also bind and intercalate with lens proteins and denature them. It is known that heavy metal ions of mercury, lead, etc, can precipitate blood and body proteins. Again, cadmium is known to compete with copper[32] and zinc[33] in the body and could affect the copper and zinc homeostasis of blood and copper containing protein such as ceruloplasmin and enzymes such as superoxide dismutase and cytochrome a_3, the respiratory enzyme. Superoxide dismutase activity can be

Table 4 Cadmium levels in blood (smokers v non-smokers both without cataract)

Age (years)	Category	N	Mean (μg/dl)	SD	SEM	t test	p Value
30–35	Non-smokers without cataract (group I)	14	0·069	0·008	0·002		
	Smokers without cataract (group II)	11	0·0815	0·021	0·006		
	Difference		0·0125			1·98	<0·05
36–40	Non-smokers without cataract (group I)	7	0·0654	0·006	0·002		
	Smokers without cataract (group II)	8	0·1121	0·024	0·006		
	Difference		0·0467			1·80	<0·05

Table 5 Vitamin C levels in blood (smokers v non-smokers both with cataract)

Age (years)	Category	N	Mean (μg/l)	SD	SEM	t test	p Value
40–54	Non-smokers with cataract (group III)	6	13·51	2·668	1·089		
	Smokers with cataract (group IV)	6	14·05	3·491	1·425		
	Difference		0·54			0·3	NS
55–60	Non-smokers with cataract (group III)	26	14·86	3·578	0·702		
	Smokers with cataract (group IV)	25	13·66	2·921	0·584		
	Difference		1·28			1·31	NS

Table 6 Vitamin C levels in blood (smokers v non-smokers both without cataract)

Age (years)	Category	N	Mean (μg/l)	SD	SEM	t test	p Value
30–35	Non-smokers without cataract (group I)	14	16·12	3·496	0·934		
	Smokers without cataract (group II)	20	15·88	3·483	0·779		
	Difference		0·24			0·2	NS
36–40	Non-smokers without cataract (group I)	7	15·28	3·551	1·342		
	Smokers without cataract (group II)	12	13·5	3·606	1·041		
	Difference		1·78			1·04	NS

Table 7 Vitamin C levels in lens (smokers v non-smokers both with cataract)

Age (years)	Category	N	Mean (SEM) (μg/g) lens tissue	Mean (SEM) (μg/g) lens proteins	p Value
40–54	Non-smokers with cataract (group III)	5	0·0956 (0·025)	0·5418 (0·188)	NS
	Smokers with cataract (group IV)	4	0·1964 (0·016)	0·7041 (0·064)	
	Difference		0·1008	0·1623	NS
	t Values		0·85	0·88	
55–60	Non-smokers with cataract (group III)	26	0·1178 (0·014)	0·5602 (0·042)	
	Smokers with cataract (group IV)	25	0·1357 (0·016)	0·8539 (0·274)	
	Difference		0·0179	0·2937	NS
	t Values		0·84	1·08	

affected[32] through competition with zinc also. Cadmium decreases the bioavailability of selenium[33] and this may affect the biosynthesis of glutathione peroxidase. Deficiency of vitamin E is also reported.[33] By a decrease in antioxidants such as ceruloplasmin, superoxide dismutase, and glutathione peroxidase, the defence against oxidative damage could be weakened by the accumulation of cadmium.

Vitamin C

Tables 5 and 6 show levels of vitamin C in the blood of smokers and non-smokers. There is no significant difference between the four groups though the average value of vitamin C was not at the peak of normal – that is, 20 mg/l reported in the literature.[34]

Vitamin C levels in lens tissue and lens proteins are given in Table 7. In this case also there is no variation between groups III and IV though the values are on the lower side of the normal – that is, 0·14 mg/g lens tissue as against an upper normal of 0·35 mg/g lens tissue. Similar results, however, are obtained in the lens of non-smokers with cataract. Smoking beedies did not cause any significant decrease of vitamin C either in the blood or lens in our studies. Perhaps smoking a good number of beedies for a prolonged time might have brought about a significant decrease in vitamin C as reported for cigarettes by earlier workers. Also, what is applicable to cigarette smoking need not be applicable to beedi smoking, as for instance, cadmium is present to only one third of the level in beedies compared with cigarettes (0·1 μg/100 mg in cigarettes and 0·03 μg/100 mg in beedies).[2 35] There are different theories to explain cataract formation in cigarette smokers. It could be caused by the reactive oxygen species (ROS) and oxidant effect,[8] thiocyanate,[2] or naphthalene.[2]

We conclude that one of the major causes of nuclear cataract, as reported by West *et al*,[5] could be biochemical as a result of accumulation of cadmium from smoking in the lens. Cadmium may hasten cataractogenesis directly by interaction with lens proteins and indirectly by its competition with copper, zinc, and selenium and causing a decrease of antioxidants. On cessation of smoking, cadmium accumulation may stop and the individuals become less susceptible to cataractogenesis.[5]

1 Clayton RM, Cuthbert J, Seth J, Phillips CI, Bartholmew RS, McK Rad J. Epidemiological and other studies in the assessment of factors contributing to cataractogenesis. *Ciba Fdn Symp* 1984; **106:** 25–47.
2 Harding JJ. *Cataract, biochemistry, epidemiology and pharmacology.* London: Chapman & Hall, 1991: 122.
3 Harding JJ, Van Heyningen R. Beer, cigarette and military work as risk factors for cataract. *Dev Ophthalmol* 1989; **17:** 13–6.
4 Flaye De, Sullivan KN, Cullinan TR, Silver JH, Whitelocke RAF. Cataract and cigarette smoking: the city eye study. *Eye* 1989; **3:** 379–84.
5 West S, Munoz B, Edward AE, Taylor HR. Cigarette smoking and risk of nuclear cataract. *Arch Ophthalmol* 1989; **107:** 1166–9.
6 Taylor A. Role of nutrient in delaying cataract. *Ann NY Acad Sci* 1992; **669:** 111–24.
7 Taylor A, Jacques PF, Dorey KC. Oxidation and aging. *Impact on Vision,* 1993.
8 Balasubramanian D, Shalini VK, Luthra M, Rao SHN, Basti S, *et al.* Molecular epidemiology of the smoke cataract connection. Oxidant stress mechanism. *Invest Ophthalmol Vis Sci* 1993; abstract 2925.
9 Iyyengar GV. *Milestones in biological trace element research. The science of the total environment.* Amsterdam: Elsevier, 1991; **100:** 1–15.
10 Debeka RW, Karpinski KF, McKenzie AD, Bajdik CD. Survey of lead, cadmium and fluoride in human milk and correlation of levels with environmental and food factors. *Food Chem Toxicol* 1986; **24:** 913–21.
11 Loh HS, Wilson CWM. Cigarette smoking and cadmium. *Lancet* 1972; ii: 491–3.
12 Chiba Momoko. Effects of smoking on human health from the aspect of trace elements. International conference on alcoholism and trace elements, Trivandrum, India, 1992.
13 Iyyengar GV, Woittiez J. Trace elements in human clinical specimens; evaluation of literature data to identify reference value. *Clin Chem* 1988; **34:** 474–81.
14 World Health Organisation. Diseases caused by cadmium and its toxic compounds. Early detection of occupational disease. *WHO Technical Series, Geneva* 1986; **8:** 48–52.
15 Savita P, Nath R. Studies on trace metals and metal binding protein in human senile cataractous lens. In: Gupta K, ed. *Ocular pharmacology, recent advances.* 1991: 49–56.
16 Bhattacharyya MH, Whelton BFD, Stern PH, Peterson DP. Cadmium accelerates bone loss in ovariectomized mice and fetal rat limb bones in culture. *Proc Natl Acad Sci USA* 1988; **85:** 8761–5.
17 Nath R. *Studies on the intralenticular distribution of Cu, Zn, Cd, and Se, in normal human senile cataractous lens and experimental rabbit model and role in catactogenesis.* Annual report on the ICMR project.
18 Racz P, Erdophelyi A. Cadmium, lead and copper; concentration in normal and senile cataractous human lens. *Ophthalmic Res* 1988; **20:** 10–3.
19 Wilczek M, Zygulska Machowa H. Zawartose Witamine CW. Roznych New Typack Zaem. *J Klin Oczne* 1968; **38:** 477–80.
20 Jain IS, Ram Sharma A, Gupta A, Nath R. Black cataract. A result of decreased ascorbic acid in chronic smokers. *Afro Asian J Ophthalmol* 1990; **9:** 91–2.
21 Berger J, Shepard D, Morrow F, Taylor A. Relationship between dietary intake and tissue levels of reduced and total vitamin C in the non scorbutic guinea pig. *J Nutr* 1989; **119:** 734–40.
22 Pelletier O. Smokers have decreased vitamin C. *Am J Clin Nutr* 1968; **21:** 1259–61.
23 Ramakrishnan S. *Essentials of biochemistry and ocular biochemistry.* Annamalainagar, Chidambaram, India: Annamalai University publication, 1993: 53.
24 Narishmarao CV, Chakraborty MK. *Cultivation and utilisation of medicinal plants.* Jammu, Kashmir: Regional Research Laboratory, 1986: 673.
25 Nadkarni AK. *Indian Materia Medica.* Vol I. Bombay, India: Popular Prakashan, 1976: 850, 851.

26 Harris LJ, Ray SN. Determination of ascorbic acid by the method using titration with 2,6, dichlorophenol indophenol. *Lancet* 1935; i: 462.
27 Lowry OH, Rosenbrough NH, Farr AL, Randall RN. Protein measurement with Folin's phenol reagent. *J Biol Chem* 1951; **193:** 265–73.
28 Savita P, Nath R. In: Gupta SK, ed. *Ocular pharmacology, recent advances.* New Delhi: Papyrus Publishing House, 1991: 53.
29 Gairola CG. Cadmium enriched cigarette smoke induced cytological and biochemical alterations in rat lungs. *J Toxicol Environ Health* 1989; **3:** 317–29.
30 *Encylopaedia of occupational health and safety. Cadmium and compounds.* Geneva: ILO, 1986: 357.
31 Lauwerys R, Amery A, Bernard A, Bruaux P, Buchet JP, Claeys F, *et al.* Health effects of environmental exposure to cadmium – objectives design and organization of the cadmium study; a cross-sectional morbidity study carried out in Belgium from 1985–1989. *Environ Health Perspect* 1990; **87:** 283–9.
32 Marklund SL. Superoxide dismutase in human tissue cells and extracellular fluids, clinical implication. *Free radicals aging and degenerative disease.* New York: Alan R Liss, 1986: 509.
33 Preston AM. Cigarette smoking – nutritional implications. *Prog Food Nutr Sci* 1991; **15:** 183–217.
34 Varley H, Gowenlock AH, Bell M. In: *Practical clinical biochemistry.* Vol 2. London: Heinemann, 1976: 255.
35 Kuhnert BR, Kunnert PM, Lazebnik N, Erhard P. The effect of maternal smoking on the relationship between maternal and fetal zinc status and infant birth weight. *J Am Coll Nutr* 1988; **7:** 309–16.