International Journal of Medical Sciences
ISSN 1449-1907 www.medsci.org 2007 4(1):53-58

Research Paper

Does Flavanol Intake Influence Mortality from Nitric Oxide-Dependent Processes? Ischemic Heart Disease, Stroke, Diabetes Mellitus, and Cancer in Panama

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Received: 2006.11.28; Accepted: 2007.01.26; Published: 2007.01.27

Substantial data suggest that flavonoid-rich food could help prevent cardiovascular disease and cancer. Cocoa is the richest source of flavonoids, but current processing reduces the content substantially. The Kuna living in the San Blas drink a flavanol-rich cocoa as their main beverage, contributing more than 900 mg/day and thus probably have the most flavonoid-rich diet of any population. We used diagnosis on death certificates to compare cause-specific death rates from year 2000 to 2004 in mainland and the San Blas islands where only Kuna live. Our hypothesis was that if the high flavanoid intake and consequent nitric oxide system activation were important the result would be a reduction in the frequency of ischemic heart disease, stroke, diabetes mellitus, and cancer – all nitric oxide sensitive processes. There were 77,375 deaths in mainland Panama and 558 deaths in the San Blas. In mainland Panama, as anticipated, cardiovascular disease was the leading cause of death (83.4 ± 0.70 age adjusted deaths/100,000) and cancer was second (68.4 ± 1.6). In contrast, the rate of CVD and cancer among island-dwelling Kuna was much lower (9.2 ± 3.1) and (4.4 ± 4.4) respectively. Similarly deaths due to diabetes mellitus were much more common in the mainland (24.1 ± 0.74) than in the San Blas (6.6 ± 1.94). This comparatively lower risk among Kuna in the San Blas from the most common causes of morbidity and mortality in much of the world, possibly reflects a very high flavanol intake and sustained nitric oxide synthesis activation. However, there are many risk factors and an observational study cannot provide definitive evidence.

Key words: Cocoa, flavanoids, heart disease, diabetes mellitus, cancer, infectious disease

1. Introduction

The effects of plant flavonoids on mammalian cells have been of substantial recent interest, with attention focused on the implications of these agents for cardiovascular disease and for cancer [1]. Some epidemiological studies designed to examine a possible protective effect of flavonoids in cardiovascular disease have reported inverse associations [2-15]. Inconsistencies may be due to measurement error because foods that are flavanol-rich, such as grapes, red wine, or cocoa and chocolate shows remarkable variability in the flavonoid content, largely reflecting processing [16-18].

The Kuna Indians of Panama are known for their low blood pressure levels and little rise in blood pressure with age [19-21]. Our interest began with a search for protective genes, but the protection was environmental rather than genetic [20]. As no environmental factor identified to date will reduce blood pressure levels to 110/70 mm Hg -- the average found in the Kuna, even those over 65 years of age -- identification of potential environmental factors was of considerable interest. We learned that Kuna still residing in their indigenous location in the San Blas islands drank cocoa, all locally grown, as their major source of fluid. The cocoa proved to be flavanol-rich [22]. The flavonoids were shown to cause the activation of nitric oxide synthase in healthy volunteers and patients with atherosclerosis [23, 24].

The very high flavonoid intake taken by virtually all Kuna Indians residing in their indigenous home area, enjoying what is almost certainly the highest flavanoid intake of any community, has created an opportunity to examine the possible role of a sustained high flavonoid intake on cardiovascular disease and cancer -- two major contributors to morbidity and mortality [1]. There are also reasons for suspecting that flavonoids could protect against diabetes mellitus, as they improve sensitivity to insulin [25]. We employed death certificates mandated by the Republic of Panama for all deaths within the Republic. One cannot be buried without a completed death certificate. As only Kuna Indians can own land in the San Blas and all but a very small fraction of the residents are Kuna Indians, this allowed us to compare a population characterized

by a very high sustained flavonoid intake with another population in which flavonoid intake is more variable, on average much lower. Cocoa obtained in the mainland from grocery stores is flavanol-poor [18, 22]. Because there are many risk factors for each of these processes and because death certificates contain little information on risk beyond age and to avoid potentially spurious evidence on risk, we prespecified that a significant outcome from this study would demonstrate a lower frequency of 4 processes in the San Blas -- ischemic heart disease, stroke, diabetes mellitus, and cancer. These complex processes have little in common other than the possibility that flavanols might influence their development and progression [1].

2. Materials and methods

Death certificates for the years 2000 to 2004 were available for 77,375 in mainland Panama and 548 individuals in the San Blas. A single cause of death was listed by either the attending physician or by a local health authority. Contributing causes of death were not listed, nor was ethnicity listed. All of the data were submitted to the Panamanian Ministry of Health in Panama City and were available for analysis. The code of the specific cause of death was based on the detailed list of the International Class of Occasional Statistics of Diseases and Problems Related Health Problems (CID 9 and 10).

The age distribution in the San Blas and in the Panama mainland was obtained from the most recent census information obtained in the year 2004.

Frequency of deaths per hundred thousand population were tabulated for various causes of death, both with and without age-adjustment and relative risk and 95 percent confidence interval were calculated. We used the Student's t-test and Chi Square to compare the frequency of cause-specific deaths in mainland Panama and the San Blas. Chi square was used to assess differences in age distribution across the two regions. We considered a p value of less than 0.05 as statistically significant.

3. Results

In mainland Panama, the most common cause of death was diseases of the circulation with a frequency of 119/100,000/year (Table 1). This was made up almost equally of ischemic heart disease with a frequency of 43.94/100,000/year and stroke with a frequency of 41.34/100,000/year. The frequency was somewhat greater in males, 132/100,000/year, than in females, 106/100,000/year, and as expected showed a striking increase with age, reaching a peak of 1,193/100,000/year in those over 60 years of age. Cancer followed with a frequency of 74.71/100,000/year. The frequency in males (77.6/100,000/yr) exceeded only slightly the frequency in females (71.8/100,000/yr). Again, rates peaked among those over 60 years of age, at 620/100,000/yr. Diabetes mellitus was sixth in frequency with an average of 24.4 deaths/100,000 with a frequency significantly higher in women than in men. The frequency peaked at 237.6/100,000/yr in those over 60 years of age.

TABLE 1. Death rate for Panama per 100,000: effect of gender and age

				AGES						
DIAGNOSIS	TOTAL	MALE	FEMALE	**0-10**	**10-20**	**20-25**	**25-35**	**35-50**	**50-60**	**60+**
Total	424.76	486.80	361.57	203.51	50.61	109.94	131.72	205.68	485.15	3126.11
CV Disease	119.06	131.99	105.89	2.25	2.82	5.44	6.31	32.15	119.00	1193.65
Cancers	74.71	77.58	71.78	5.40	6.64	7.62	10.71	36.86	135.23	620.18
Accidents	43.53	71.52	15.02	16.80	23.89	63.13	59.65	49.48	42.44	53.01
Respiratory Diseases	40.19	44.16	36.14	21.90	1.99	4.72	3.63	10.94	30.37	351.05
Infectious	31.62	39.48	23.61	24.00	2.32	13.42	31.16	40.56	39.94	108.24
Diabetes	24.40	19.93	28.95	0.30	0.00	0.36	0.57	7.74	33.70	237.62
GI Disorders	17.65	21.55	13.68	2.10	1.16	2.54	2.29	8.92	29.54	146.80
Urinary Sys Diseases	13.87	16.30	11.39	1.80	1.33	0.73	3.06	5.39	18.31	120.85
Unclassified Symptoms	12.33	13.99	10.63	8.85	0.50	2.90	3.44	2.02	11.23	97.86
Brain Disorders Excl Strokes	12.17	13.87	10.44	7.80	3.82	2.18	3.63	4.21	13.73	84.52
Perinatal	10.94	12.74	9.10	52.04	0.00	0.00	0.00	0.00	0.00	0.00
Deformities	10.02	11.43	8.59	45.59	1.49	0.36	0.57	0.17	0.00	0.00
Malnutrition	9.80	9.68	9.93	14.70	2.82	3.63	3.25	3.03	7.07	49.67
Subcu & Connec-Tissue Disorders	3.63	2.56	4.71	0.00	0.66	1.45	1.72	2.69	4.58	26.32
Childbirth Problems	0.85	0.00	1.72	0.00	0.50	1.45	1.72	1.51	0.00	0.74

Death rates for the 15 most common causes of death in Panama. The effects of gender and age are shown. The leading causes of death are cardiovascular disease, cancer, and accidents as in much of the world.

In the San Blas, the pattern differed strikingly (Table 2). The most common cause of death in the San Blas was infections with a frequency of 80.5/100,000/yr. The frequency of death due to infections was substantially greater in men

(130.1/100,000/yr) than in women (61/100,000/yr). The age distribution showed two peaks. An early peak in those under 10 years of age was dominated by diarrheal illness in the very young. A secondary peak occurred in those over 60 years of age. The common infections, in addition to diarrhea were tuberculosis, malaria, influenza, and HIV.

In the conditions of interest (Table 2), heart disease showed a frequency of only 8.3/100,000/yr and stroke was even lower, with a frequency of 3.2/100,000/yr. Cancer and diabetes were equally low with a frequency of 3.7/100,000/yr (Table 2).

Four conditions were predetermined before the analysis to be specific targets for effectiveness of flavanols, heart disease, stroke, cancer, and diabetes mellitus. The year-by-year findings in Panama and in the San Blas for those four conditions are shown in Table 3. The stability year-by-year for each diagnosis in Panama was evident. In the case of cancer, the frequency over the five years ranged from 64.2 to 72.2/100,000/yr with a mean of 68.4±1.60. In San Blas during the same interval four of five years showed no cancers, and one year showed a peak of 22/100,000/yr with a mean of 4.4±4.4 in San Blas: the difference in frequency is highly significant ($p<0.001$). A similar pattern exists for ischemic heart disease and stroke, each relatively stable in Panama and showing more variation in San Blas: The frequency in San Blas for both was significantly lower than in mainland Panama ($p<0.001$). In the case of diabetes (Table 3) the same consistent difference was found, but the difference was smaller: The frequency in San Blas was 39 percent of that in Panama. The data are presented by age for these 4 conditions for the most recent year in which data are available, 2004 (Table 4).

TABLE 2. Death rate for San Blas per 100,000: By Age and Gender

				AGES						
DIAGNOSIS	TOTAL	MALE	FEMALE	**0-10**	**10-20**	**20-25**	**25-35**	**35-50**	**50-60**	**60+**
Total Regional	192.82	232.06	158.84	174.42	25.25	88.85	120.30	151.71	139.83	928.25
Infections	80.53	103.14	60.96	62.53	6.31	44.42	75.19	90.38	76.27	336.18
Respiratory Disease	48.78	55.54	42.93	78.98	6.31	12.69	11.28	12.91	19.07	215.76
GI Disease	11.04	12.89	9.44	1.65	2.10	0.00	11.28	9.68	6.36	75.26
Malnutrition	10.12	11.90	8.59	4.94	0.00	0.00	11.28	9.68	25.42	45.16
Brain Disorders, excl Strokes	9.66	12.89	6.87	4.94	8.42	6.35	3.76	3.23	6.36	50.18
CV Disease	8.28	9.92	6.87	8.23	0.00	6.35	0.00	3.23	0.00	55.19
Soft Tissue Disorders	6.90	4.96	8.59	0.00	0.00	0.00	0.00	3.23	6.36	65.23
Diabetes Mellitus	3.68	5.95	1.72	0.00	0.00	0.00	0.00	6.46	0.00	30.11
Accidents	3.68	5.95	1.72	1.65	0.00	12.69	7.52	6.46	0.00	5.02
Cancers	3.68	2.98	4.29	0.00	2.10	0.00	0.00	6.46	0.00	25.09
Stroke	3.22	3.97	2.58	0.00	0.00	6.35	6.35	0.00	0.00	30.11
Perinatal Diseases	3.22	1.98	4.29	11.52	0.00	0.00	0.00	0.00	0.00	0.00

Death rate for the San Blas Island Chain per 100,000. A similar presentation to Table 1, but focused on the San Blas Islands off the Caribbean Coast. Note that cardiovascular disease, cancer, and diabetes are less than 10% of their frequency in the mainland.

TABLE 3. Age Adjusted Death rate/100,000 for selected causes in Panama

	YEAR	2000	2001	2002	2003	2004				
							Mean	SD	SEM	**n**
IHD*										
Panama		42.1	45.5	42.8	44.6	44.7	43.94	1.43	0.64	5
San Blas		8.4	2.8	16.8	8.3	7.0	8.7	6.75	3.02	5
CANCER										
Panama		67.9	64.2	65.7	71.8	72.7	68.36	3.58	1.60	5
San Blas		0.0	0.0	0.0	22.0	0.0	4.41	9.86	4.41	5
STROKE										
Panama		40.2	40.4	40.0	42.3	43.8	41.34	1.65	0.74	5
San Blas		0.0	0.0	0.0	2.8	0.0	0.55	1.23	0.55	5
DIABETES										
Panama		21.8	23.2	26.0	25.2	24.4	24.12	1.66	0.74	5
San Blas		5.6	8.3	11.1	2.8	5.0	6.6	4.33	1.94	5

*ISH=Ischemic Heart Disease

Age adjusted death rates per 100,000 over 5 years for the 4 conditions specified in the hypothesis that a very high dietary intake of flavanoids might predict. Note the remarkable year-to-year consistency in Panama and the very much lower frequency each year in the San Blas.

TABLE 4. Age Adjusted death rate in Panama and Kuna Yala for 2004

Disease	Total/100,000	25-34 Years	35-44 Years	45-54 Years	55-64 Years	65-74 Years	75 + Years
IHD							
Panama	44.7	2.5	7.6	19.6	70.8	204.6	1339
Kuna Yala	7	0	0	0	0	1	6
STROKE							
Panama	43.8	0	8.8	27.4	96.2	253.4	1143.1
Kuna Yala	0	0	0	0	0	0	0
CANCER							
Panama	72.7	10.7	29.9	81.9	187.1	503.3	1220
Kuna Yala	0	0	0	0	0	0	0
DIABETES							
Panama	24.4	0	0	0	64.1	172.4	586.8
Kuna Yala	0	0	1	0	0	2	2

Age-adjusted death rate in Panama and Kuna Yala for one year, 2004, for the 4 conditions specified in the hypothesis. Note that in Panama each diagnosis shows an age-dependent frequency, and that this does not appear in the San Blas.

Four conditions were predetermined before the analysis to be specific targets for effectiveness of flavanols, heart disease, stroke, cancer, and diabetes mellitus. The year-by-year findings in Panama and in the San Blas for those four conditions are shown in Table 3. The stability year-by-year for each diagnosis in Panama was evident. In the case of cancer, the frequency over the five years ranged from 64.2 to 72.2/100,000/yr with a mean of 68.4±1.60. In San Blas during the same interval four of five years showed no cancers, and one year showed a peak of 22/100,000/yr with a mean of 4.4±4.4 in San Blas: the difference in frequency is highly significant ($p<0.001$). A similar pattern exists for ischemic heart disease and stroke, each relatively stable in Panama and showing more variation in San Blas: The frequency in San Blas for both was significantly lower than in mainland Panama ($p<0.001$). In the case of diabetes (Table 3) the same consistent difference was found, but the difference was smaller: The frequency in San Blas was 39 percent of that in Panama. The data are presented by age for these 4 conditions for the most recent year in which data are available, 2004 (Table 4).

TABLE 5. Age distribution in San Blas and mainland Panama

	PANAMA	SAN BLAS
AGE (Years)	MALE FEMALE	MALE FEMALE
<55	94.1% 93.9%	88% 86.1%
55-64	3.1% 3.0%	5.8% 6.7%
65-74	1.8% 1.9%	3.9% 4.7%
75+	1.0% 1.2%	2.3% 2.5%

$X^2 = 11.04$. df = 1. $P<0.001$

Age-distribution in the San Blas and mainland Panama from the census of 2004. Note that there are more individuals over the age of 55 years among the Kuna in the San Blas than mainland Panama, and the frequency is about double during each decade.

Death from trauma was also more frequent in the mainland than in the indigenous island location, largely reflecting the influence of motor vehicle accidents. In the San Blas, there are neither roads nor automobiles, and death from this source was zero. An increase in risk in the San Blas was associated with perinatal medical problems, infections, and all other causes.

The average age of the Kuna in the islands was very much influenced by the mortality in the very young, primarily due to diarrhea. As an index of aging, we examined the age distribution on the mainland and in the islands (Table 5). About 12 percent of both males and females in the San Blas were 55 years of age or older, about double the 6 percent over that age in the Panama mainland (Chi Square = 11.04; $p<0.001$).

4. Discussion

In Panama, as in much of the western world, diseases of the circulation are the most common cause of death, and cancer is second. The difference in risk of death due to these two classes of illness, cancer and heart disease, between San Blas and mainland Panama was very large. Our original hypothesis was that this would be the case if flavanol-rich cocoa and its influence on nitric oxide synthesis had implications for disease pathogenesis, as has been suggested [26]. We could explain this extraordinary difference in the risk of death by proposing either a better prognosis or a lower incidence of these illnesses in San Blas. A reduced incidence is the most likely explanation, but both a better prognosis and a reduced incidence could have a similar explanation.

The San Blas is the oldest indigenous district in the Republic of Panama, having a relatively homogeneous population both biologically and ethnically. It exhibits one of the highest levels of poverty and economic and social exclusion in Panama. The district has two hospitals, six health centers, and eleven health posts, which do not guarantee access to all levels of service. About 40,000 inhabitants are spread over forty-nine communities that are widely dispersed along the entire Caribbean Coast of Panama. The profile of health of the Kuna residing in the San Blas revealed substantial premature death due to infections: Infectious and parasitic diseases made a contribution more than 4-fold higher than in the rest of the country. The principle reasons were diarrhea, tuberculosis, AIDS, influenza, and malaria.

One major limitation of our findings involves how the diagnosis on the death certificates was established. Medical diagnosis follows a well-defined sequence in which suspicion is raised from findings obtained from a medical history and physical examination, followed up with specific tests designed to establish the diagnosis. In the case of cancer, for example, examination of tissue obtained at biopsy is required for diagnosis. In the case of heart disease, the diagnosis will be established through some combination of medical history and physical findings supplemented by the results of tests, including x-ray, angiography, ultrasound examination, the electrocardiogram, and occasionally a biopsy. The availability of the laboratory examination probably differs in the San Blas and the mainland, but the difference is relative, not absolute. In the hospital in Ailigandi, for example, an electrocardiogram machine has been available for more than fifteen years, and the physicians who staff the hospital have worked in Panama City and know how to diagnose myocardial infarction. During those fifteen years, there have been no cases of myocardial infarction diagnosed on Ailigandi (personal communication). Differences in the ability to establish a diagnosis may have played a larger role in the frequency of cancer diagnosis than in the frequency with which heart disease was diagnosed, although thousands of Pap smears and biopsies are performed annually in the San Blas. In 4 of 5 years, no cases of cervical cancer were diagnosed.

Age of the communities at risk is important. As both diseases of the circulation and cancer increase in frequency with age, death at an earlier age from infection could have influenced the outcome. In fact, the communities living in San Blas are not younger: The percentage of Kuna who are over the age of 55 years is substantially and statistically higher than in mainland communities. Adjustment for age served to enhance the difference in the frequency with which disease of the circulation or cancer led to death in the San Blas versus the mainland.

Although compatible with the possibility that a diet extraordinarily rich in flavonoids contributed to the low frequency of circulatory disease and cancer, it is obvious that other possibilities exist. These include especially different patterns of diagnosis discussed above. As another alternative to flavanoid intake to account for the difference in disease frequency, the possibility that differences in other risk factors contributed cannot be ignored. Unfortunately, the death certificates contained none of this information beyond age. As the difference was so very large, and the explanation is so important, follow-up population-based studies will be important to examine these risk factors prospectively. Until such data are available, the results of this study should be interpreted with great caution.

Other lifestyle variables may play a role in health outcomes by region. Stress is commonly suspected to be lower on the islands than on the mainland (commonly by people who have actually never visited the islands). We have assessed stress in the Panamanian mainland and islands, and there is a difference favoring the islands, but it is small [30]. Another possible difference is diet. We and others have examined the diet in the San Blas and Kuna living in the Panamanian mainland carefully and although several differences are noted, the most remarkable is cocoa intake [14]. However, we have no information on diet and the frequency of other risk factors in non-Kuna living in the Panamanian mainland. Obtaining this information should have a high priority. There is very probably less exposure to pollutants in the water and in the air in the San Blas compared to the mainland, especially for those inhabitants that live in the city. Tobacco use by Kuna is limited both in the indigenous island site and the mainland.

Other variables may influence age-specific death rates by region. One common practice among the Kuna involves working on the mainland on a job for a salary for many years, followed by retirement to the San Blas indigenous island life where life is less stressful, and less expensive. To the extent that occurred, and if twenty or thirty years of life on the Panamanian mainland do not lead to changes that cannot be reversed by return to the island, differences between the two locations may have been underestimated.

If indeed they do protect, how might the flavonoids provide protection? Our hypothesis was that in the case of vascular disease the responsible pathway could involve stimulation of nitric oxide synthesis and thereby reversal of endothelial dysfunction [23, 24]. Alternative possibilities, also possibly related to nitric oxide, include improvements in insulin sensitivity [25], improvements in platelet function [15], inhibition of LDL oxidation [28], and an increase in plasma HDL cholesterol concentration [29]. The influence of nitric oxide on insulin sensitivity would also provide an explanation for the difference in the frequency of diabetes mellitus [25] in the San Blas and the mainland [1]. In addition, a role in carcinogenesis may be hypothesized through nitric oxide cell-to-cell communication [1].

Death certificates provide core information, especially important at the national level [31, 32]. The findings can be misleading. For example, if someone dies with both cancer and diabetes, the cancer is far more likely to be recorded [33]. This problem should not have influenced our findings, as heart attack, stroke, and cancer are likely to have been chosen from among a range of possible diagnoses, and there is no reason why the pattern should differ on the mainland and in the San Blas.

Despite the other possible explanations for the large differences in deaths from cardiovascular disease and cancer with an array of possible confounding factors, it is improbable that these could abolish the cardiovascular and cancer protective effect observed among the San Blas Kuna as compared to the mainland. Although the findings are compatible with effect of the flavanol-rich cocoa on health, clearly a large number of alternative possibilities exist involving diet, physical activity, stress and genetic factors. An observational study of this kind cannot prove causality. Indeed, only

a randomized, controlled clinical trial in which all of these factors can be controlled will lead to a definitive conclusion. All of these issues can be addressed, but that will take time and substantial resources.

Acknowledgments

This study was funded in part by the Instituto Commemorativo Gorgas de Estudios de la Salud and the M&M/Mars Company. None of these funding sources participated in the collection of the data or its analysis.

Conflicts of interest

The authors have declared that no conflict of interest exists.

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