Caloric Restriction, the Traditional Okinawan Diet, and Healthy Aging

The Diet of the World's Longest-Lived People and Its Potential Impact on Morbidity and Life Span

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ABSTRACT: **Long-term caloric restriction (CR) is a robust means of reducing age-related diseases and extending life span in multiple species, but the effects in humans are unknown. The low caloric intake, long life expectancy, and the high prevalence of centenarians in Okinawa have been used as an argument to support the CR hypothesis in humans. However, no long-term, epidemiologic analysis has been conducted on traditional dietary patterns, energy balance, and potential CR phenotypes for the specific cohort of Okinawans who are purported to have had a calorically restricted diet. Nor has this cohort's subsequent mortality experience been rigorously studied. Therefore, we investigated six decades of archived population data on the elderly cohort of Okinawans (aged 65-plus) for evidence of CR. Analyses included traditional diet composition, energy intake, energy expenditure, anthropometry, plasma DHEA, mortality from age-related diseases, and current survival patterns. Findings include low caloric intake and negative energy balance at younger ages, little weight gain with age, life-long low BMI, relatively**

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high plasma DHEA levels at older ages, low risk for mortality from age-related diseases, and survival patterns consistent with extended mean and maximum life span. This study lends epidemiologic support for phenotypic benefits of CR in humans and is consistent with the well-known literature on animals with regard to CR phenotypes and healthy aging.

KEYWORDS: **caloric restriction; longevity; Okinawa; DHEA; biomarker; human; healthy aging; life span**

INTRODUCTION

Energy restriction, also known as caloric restriction (CR), is the most robust and reproducible means of reducing age-related diseases and extending life span in short-lived animals, but the effects in humans are unknown.[1–7] Preliminary data from ongoing research with CR on long-lived nonhuman primates (rhesus and squirrel monkeys) also suggest potential health benefits—although it will take some years before final results become available and firmer conclusions can be reached regarding aging and life span.[8,9]

Some epidemiologic and short-term human studies support CR-related health benefits.[9–13] However, a role for CR in human aging is difficult to ascertain since human life span makes long-term investigations impractical and there are no universally accepted biomarkers to measure the rate of human aging.[14] Whether CR (without malnutrition) affects human aging may be among the most significant unanswered questions in modern biogerontology.[9] Yet we have few available human populations or studies that can address this question. Most human populations who have experienced low calorie intake have suffered from high mortality due to infectious diseases and malnutrition.

Studies of human CR volunteers are currently under way with promising early results,[12,15,16] but will be unable to address longevity as an outcome. Only one long-term (>30 years) epidemiologic study has linked CR to human longevity.[17] This 36-year follow-up study reported a weak trend for lower all-cause mortality in healthy never-smoking Japanese–American men whose caloric intake was 15% lower than the cohort average, suggesting that those who maintained a modestly low energy intake (mean kcal/day 1882, range 1705–2061) in mid-life had the lowest late-life mortality risk. There was higher risk for mortality when caloric intake dropped below 50% of the group mean. These data are consistent with previous findings in animals, but much more study is required of healthy human populations with low caloric intake in order to understand the effects of CR on human aging and life span.

Therefore, it is of significant interest that Kagawa[18] reported low caloric intake in the Okinawan population, relative to other Japanese, and hypothesized that this may have been, at least in part, responsible for their healthy longevity. Kagawa[18] reported dietary information from the 1972 Japan National Nutrition survey that suggested Okinawan adults consumed only 83%

of the Japan average caloric intake. This report followed an earlier report by Hokama *et al.*[19] that Okinawan school children consumed only 62% of the calories of other Japanese school children in the early 1960s. Kagawa[18] also presented anthropometric and morbidity data from selected small samples of older Okinawans that were consistent with CR and reported markedly lower risk for age-associated diseases in middle-aged Okinawans (60–64 year olds) than in other Japanese. A later study by Chan *et al.* also reported dietary and phenotypic data in Okinawan septuagenarians and centenarians consistent with CR.[20]

There are several weaknesses with these previous reports. One, to our knowledge no population-based dietary information has been reported in a peer-reviewed journal on Okinawan adults before the 1972 National Nutrition Survey. Since the Japanese lifestyle underwent radical changes from the 1950s,[18] including changes in food choices, caloric intake, and energy expenditure, it is unlikely that the 1972 Japan National Nutrition Survey reflects the traditional CR diet that may be implicated in Okinawan longevity. Two, Okinawans are smaller than other Japanese (and Americans) and likely require fewer calories. Therefore, accounting for their unique energy requirements is necessary before concluding that Okinawans were calorically restricted. For example, Okinawans currently consume 8% fewer calories than other Japanese,[21] yet they now have the largest body mass index (BMI) in Japan.[22] Three, since the Okinawan mortality advantage has all but disappeared except in older cohorts (aged 65-plus),[22,23] it would be informative to have a more detailed, population-based epidemiologic analysis of the traditional diet, energy intake, energy expenditure, phenotype, and the subsequent mortality experience of this older cohort. These data might help answer the question as to whether Okinawans were truly calorically restricted and to what degree, the phenotypic consequences, and the current consequences for age-related mortality and life span. Importantly, an in-depth epidemiologic analysis of this older cohort using longer-term population data might also provide significant new information on the potential human effects of CR.

Fortunately, detailed population surveys of the traditional Okinawan diet and anthropometry were conducted in 1949.[24] Smaller, more limited population surveys were conducted at irregular intervals thereafter by U.S. and Okinawan postwar administrations.[25] These surveys were archived and have not been systematically analyzed. Therefore, we investigated these data in detail and asked three questions: (1) were older Okinawans truly calorically restricted across the life course? (2) is there longer-term anthropometric evidence to support this hypothesis? and (3) is there evidence for slower aging in this cohort?

To address question (1), we assessed population food-consumption patterns, energy intake, and energy expenditure for older Okinawans (aged 70s) at two different times—when they were young adults (aged in their 30s) and when they were middle-aged (aged 50s). To answer question (2), we analyzed archived

data on height and weight, and calculated BMI for multiple time points in the Okinawan adult population coincident with whole-population energy balance. To answer question (3), whether delayed aging has occurred in this cohort, we took three approaches. First, since the adrenal steroid dehydroepiandrosterone (DHEA) has been purported to satisfy the main criteria for a candidate biomarker of aging in nonhuman primates,[26] and is of hypothetical utility as a biomarker of the rate of human aging,[27] we measured plasma levels in a sample of older Okinawans (aged 70s). Second, we analyzed late-life survival patterns for potential evidence of extended average and maximum life span. Third, we calculated age-adjusted mortality patterns standardized to the world standard population for age-related diseases. Finally, we compared selected findings to published data from non-CR Americans and Japanese of similar chronological age, in whom differences in diet, energy balance, anthropometry, DHEA, age-related mortality and late-life survival should be robust, if older Okinawans truly experienced long-term CR.

METHODS

Study Materials and Measurements

Population Dietary Intake and Energy Expenditure

Archived dietary data are used to estimate energy balance and potential CR status in Okinawan septuagenarians at younger ages. Okinawan data were derived from the Office of the Civil Administrator of the Ryukyu Islands (Okinawa) for the year 1949, when the current septuagenarians were aged approximately 30 years,[24] and approximately every 5–10 years thereafter by the Okinawa prefectural government.[25] Studies relied on 3-day food records of usual dietary intake to estimate caloric intake in conjunction with dietitian interviews.

For the U.S. population, data collected with comparable methodology for the same birth cohort of Americans (circa 1915–1925 birth cohort) is extremely limited. The closest data set appears to be the NHANES I data set, which used 24-hour dietary recall methods to estimate dietary intake in adult subjects (aged 20–74 years) in 1971–1974.[28] We could find no comparative data set before the 1970s for the U.S. population.

Archived data of anthropometric measurements (height and weight) and demographic data (age, occupation) in the Okinawan population and in the NHANES population were used for estimation of energy expenditure using the Harris–Benedict equation.[29] This equation estimates basal or resting metabolic rate (BMR). An activity factor was then used to calculate additional calories burned according to the following levels of activity:

Sedentary = BMR $\times$ 1.2 (little or no exercise, desk job)

Lightly active = BMR × 1.375 (light exercise/sports 1–3 days/wk)
Moderately active = BMR × 1.55 (moderate exercise/sports 3–5 days/wk)
Very active = BMR × 1.725 (hard exercise/sports 6–7 days/wk or physical job)
Extremely active = BMR × 1.9 (hard daily exercise/sports and physical job).

Energy balance was estimated in the Okinawans and Americans by subtracting estimated energy expenditure from caloric intake measured in the dietary surveys. BMI was also calculated by body weight (kg)/height $(m)^2$ and used as an additional, longer-term marker of energy balance in the Okinawan and U.S. populations.

DHEA Measurement in Study Subjects

All subjects from Okinawa were healthy, community-dwelling men and women aged approximately 75 years ($n = 54$ septuagenarians; 29 males, mean age 74.5 ± 0.7 years and 25 females, mean age 74.7 ± 0.6 years) in 1988, the time of DHEA measurement,[30] and were born into the cohort from which energy balance data were derived. Septuagenarians were selected from subjects who were attending their annual physical exam and were recruited as part of the annual Okinawa Centenarian Study, a population-based study of Okinawans over the age of 100 and selected other elderly controls that begun in 1976.[31]

The reference population for DHEA levels in Americans consisted of healthy, community-dwelling American septuagenarians from the Rancho Bernardo Study ($n = 991$ septuagenarians; 534 men, mean age 68.6 ± 9.0 years; 457 women, mean age 72.1 ± 8.0 years) who had plasma DHEA measured in 1984–1987.[32]

DHEA Measurement Protocol

Similar protocols were used in both the U.S. reference population from the Rancho Bernardo Study and the septuagenarian subjects from the Okinawa Centenarian Study. Specifically, nonfasting venous blood samples were drawn between 8 AM and 4 PM, separated and stored at −20 to −80 °C for up to 8 months. Plasma was assayed for DHEA using a solid-phase 125I RIA (radioimmunoassay) (Okinawa samples: SRL Laboratories, Tokyo, Japan; Rancho Bernardo samples: Rancho Bernardo Study Laboratory, San Diego, CA). The inter- and intra-assay coefficients of variations averaged 5.2% and 10.0% in the Okinawan samples and 6.7% and 6.1% in the Rancho Bernardo samples, respectively.

Statistical Analysis of DHEA Levels

Septuagenarian DHEA levels were compared between the study population in Okinawa and the U.S. reference population in Rancho Bernardo. Two sample unpaired Student *t*-tests with unequal variances were used to compare the Okinawans to the U.S. reference population. A two-tailed *P*-value of 0.05 was considered a statistically significant difference.

Mortality and Survival Comparisons

The late-life survival experience (mortality rates) for Okinawan, Japanese, and American populations was modeled according to the SAS procedure LIFEREG, which fits parametric models to failure-time data.[33] Period life-table data from the U.S. National Center for Health Statistics[34] and the Japan Ministry of Health and Welfare[35] for the calendar year 1995 were used for constructing the survival models. Cause-specific mortality for particular age-related diseases was also calculated for the calendar year 1995 from data obtained from the World Health Statistics Annual[36] and Okinawa Prefectural Government, Division of Statistics[37] and age-standardized to the World Standard Population.[36]

RESULTS

To assess whether there was evidence for CR in elderly Okinawans and if so, for what period of time, we analyzed long-term trends in whole-population caloric intake and energy balance for the years 1949–1998. These data demonstrate that the Okinawan population appeared to be in a relative "energy deficit" consistent with CR until the 1960s, eating approximately 10.9% fewer calories than would normally be recommended for maintenance of body weight, according to the Harris–Benedict equation (FIG. 1A).[29] Consistent with adaptation to a long-term energy deficit, the BMI of adult Okinawans remained stable at a very lean level of approximately 21 kg/m^2 until the 1960s. During the 1960s the Okinawan adult BMI began to rise (FIG. 1A). This was coincident with a shift to consistently positive energy balance.

To further clarify whether these population data indeed support a CR state for Okinawans before 1960, analysis of body weight by age strata was performed for the year 1949, when the Okinawan population appeared to be under CR conditions. Studies in nonhuman primates show that there is consistently lower body weight at all ages and relatively small weight gain beyond adulthood in male CR monkeys in contrast to those with *ad libitum* access to food.[8] Comparisons between the effects of CR on body weight in nonhuman primates (FIG. 1B) and Okinawan men in 1949 (FIG. 1C) show several similarities. There

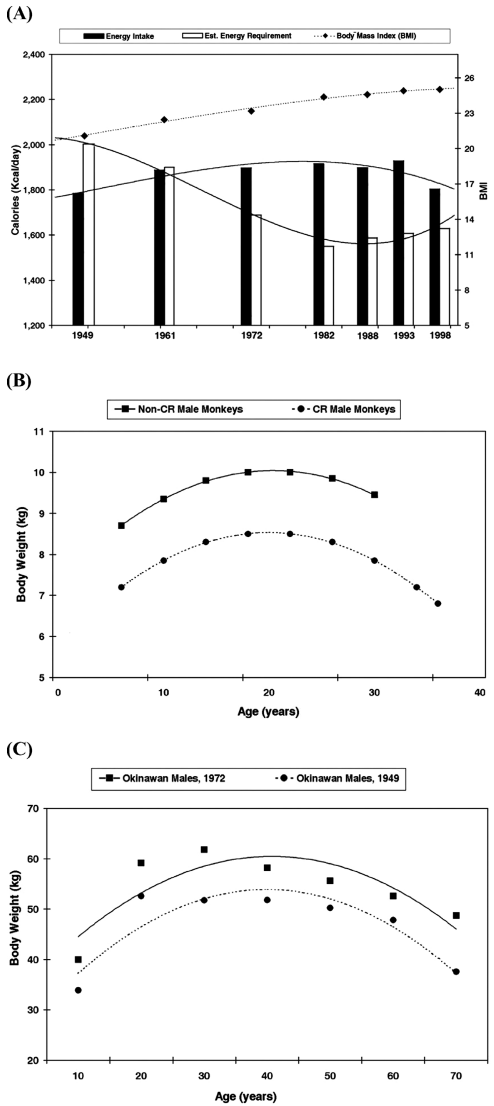

FIGURE 1. Population energy intake, energy expenditure, and anthropometric data for adult Okinawans for the years 1949–1998. (**A**) Data standardized for 50-year-old Okinawans from 1949–1998 show that Okinawans were in negative energy balance (CR) of approximately 11% until the 1960s. As Okinawans transitioned to positive energy balance, BMI began to increase. This was due to a combination of increased energy intake and decreased energy expenditure. BMI peaked at the height of positive energy balance in the mid 1990s. (**B**) Body weight in CR nonhuman primates is markedly lower across all age strata versus non-CR controls.[8] (**C**) Anthropometric data from the year 1949 in Okinawa while under CR conditions demonstrate that body weight is markedly lower across all age strata versus 1972, when energy balance had shifted to non-CR conditions.

was a marked difference in body weight at all ages and relatively small change in body weight across age strata in both groups after peak body weight is achieved at adult ages. In Okinawans during 1949, peak body weight was reached at approximately age 30 years and appeared relatively stable until elderly ages, when it began to decline.

To quantify the degree of CR in the Okinawan population, energy balance calculations are presented in FIGURE 2A. Okinawan whole-population data for the year 1949, when the septuagenarian cohort was aged approximately 30, show an energy intake of 1785 kcal/day and energy requirement estimated at 2003 kcal/day. This energy deficit is quantified in FIGURE 2B as a negative energy balance of −218 kcal/day (10.9%) and is consistent with the very lean adult population BMI of 21.2, thus supporting a CR phenotype. Population data collected in 1972 demonstrate a positive energy balance of 212 calories per day and a corresponding BMI of 23.3, suggesting that the CR phenomenon was largely over for the Okinawan population sometime in the 1960s. For comparative purposes, caloric intake and energy expenditure calculations for the U.S. population using data collected during the years 1971–1974 in the NHANES I study,[28] demonstrate a positive energy balance of 239 calories per day, consistent with a higher population BMI of 25.6 (FIG. 2B).

Since the CR paradigm rests upon "undernutrition" without malnutrition we analyzed whether the traditional Okinawan diet was of sufficient nutritional quality to ensure that widespread nutritional deficiency did not occur and to generate hypotheses as to the impact that other food components might have had on health status (e.g., protein intake, antioxidant vitamins). TABLE 1 presents whole-population dietary intake for the year 1949.[24] Notable is the high intake of vegetables, particularly sweet potatoes and soy, as a principal protein. TABLE 2 presents micronutrient data that suggest that the diet was adequate in most micronutrients. Notable is that intake of the antioxidant vitamins C and E, as well as folate and vitamin B_6 were very high at 289%, 190%, 295% and 221% of recommended intake, respectively, whereas vitamins D, B_2, and B_{12} were quite low at 2%, 45%, and 27% of recommended intake, respectively.

TABLE 3 presents physical examination data from the same subjects from whom dietary information was collected in 1949. Notable is the relatively high prevalence of cheilosis (dry, cracked lips and mouth) at 10.7% of the population. This is consistent with the low consumption of vitamin B_2 (riboflavin) reported in TABLE 2. Notable as well is the relatively high prevalence of delayed menstruation and deficient lactation, consistent with low caloric intake and/or low body fat levels in women.[38,39]

If indeed a CR phenomenon occurred in elderly Okinawans at younger ages, it is theoretically possible that there might be biomarker evidence of CR-linked delayed physiological aging, as suggested by animal data.[8] Therefore, we measured DHEA in 54 Okinawan septuagenarians, who would have undergone CR until at least middle age, according to the previous population data. As

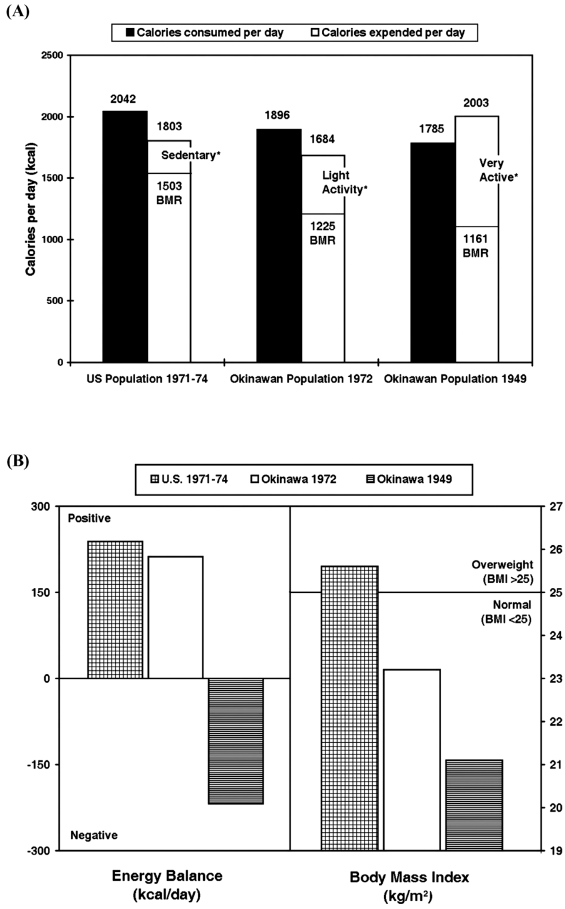

FIGURE 2. Daily energy balance and BMI in Okinawans and Americans (kcal/day). (**A**) Caloric expenditure for various levels of activity is based on reported occupation and activity levels from NHANES I (U.S. National Center for Health Statistics 1978), the Office of the Civil Administrator of the Ryukyu Islands (1949), the U.S. Department of the Office of the Civil Administrator of the Ryukyu Islands (1949), and the National Nutrition Survey, Japan Ministry of Health, Labor and Welfare (1972). BMR = basal metabolic rate (based on sex, height, body weight at age 50, Harris–Benedict equation).[29] Energy balance shifted from negative to positive in Okinawa from 1949 to 1972, supporting an early-life CR phenotype for older Okinawans. By the 1970s, population data from the U.S. and Okinawa standardized to 50 year olds show that both Americans and Okinawans were in positive energy balance. (**B**) Population data from the U.S. and Okinawa show that Okinawans were in negative energy balance of approximately −218 kcal/day in 1949. Both Okinawans and Americans were in positive energy balance in the 1970s. Americans had a positive energy balance of approximately 239 kcal/day in the 1970s, while Okinawans were in positive energy balance of approximately 212 kcal/day. This supports an energy balance shift of approximately 400 kcal/day for Okinawans during these years.

TABLE 1. Traditional dietary intake of Okinawans and other Japanese circa 1950

	Okinawa, 1949[a]	Japan, 1950[b]
Total calories	1785[c]	2068
Total weight (grams)	1262	1057
Caloric density (calories/gram)	1.4	2.0
Total protein in grams (% total calories)	39 (9)	68 (13)
Total carbohydrate in grams (% total calories)	382 (85)	409 (79)
Total fat in grams (% total calories)	12 (6)	18 (8)
Saturated fatty acid	3.7	4.7
Monounsaturated fatty acid	3.6	5.3
Polyunsaturated fatty acid	4.8	8.0
Total fiber (grams)	23	23
Food group	**Weight in grams (% total calories)**	
Grains		
Rice	154 (12)	328 (54)
Wheat, barley, and other grains	38 (7)	153 (24)
Nuts, seeds	<1 (<1)	<1 (<1)
Sugars	3 (<1)	8 (1)
Oils	3 (2)	3 (1)
Legumes (e.g., soy and other beans)	71 (6)	55 (3)
Fish	15 (1)	62 (4)
Meat (including poultry)	3 (<1)	11 (<1)
Eggs	1 (<1)	7 (<1)
Dairy	<1 (<1)	8 (<1)
Vegetables		
Sweet potatoes	849 (69)	66 (3)
Other potatoes	2 (<1)	47 (2)
Other vegetables	114 (3)	188 (1)
Fruit[d]	<1 (<1)	44 (1)
Seaweed	1 (<1)	3 (<1)
Pickled vegetables	0 (0)	42 (<1)
Foods: flavors & alcohol	7 (<1)	31 (2)

[a]Data derived from analysis of U.S. National Archives, archived food records, 1949 and based on survey of 2279 persons.

[b]Japan National Nutrition Survey, 1950.

[c]Total daily caloric intake was originally reported as 1785 kcal/day in 1949. This was estimated to be 17% less than government-recommended daily intake. Differences in assumptions regarding particular foods, cooking methods, and choice of nutritional analysis programs result in a range of 1605 to 2012 kcal/day.

[d]Papaya and tomatoes were classified as vegetables.

predicted by the CR hypothesis, the mean value of DHEA for male septuagenarians in Okinawa was significantly higher at 2.59 ng/mL (95% CI: 2.24–2.94) versus 2.00 ng/mL (95% CI: 1.91–2.10) in non-CR American men ($P < 0.001$) (FIG. 3). Larger differences were seen in similarly aged women from the two populations with levels of 3.03 ng/mL (95% CI: 2.48–3.58) found in Okinawans versus 1.13 ng/mL (95% CI: 1.06–1.20) in Americans ($P < 0.001$) (FIG. 3).

TABLE 2. Micronutrient sufficiency of the traditional Okinawan diet and Japanese diet

	Okinawa, 1949[b]		Japan, 1950[c]	
Micronutrient	Amount	% RDAJ[a]	Amount	% RDAJ[a]
Vitamin A (RE)	602	110	337	62
Vitamin D (mcg)[d]	0.4	2	7.9	31
Vitamin E (mg)	16.6	190	6.3	72
Vitamin K (mcg)	87.6	160	65.8	120
Vitamin B_1: thiamin (mg)	1.4	137	1.1	113
Vitamin B_2: riboflavin (mg)	0.5	45	0.5	47
Niacin (mg)	13.2	93	18.1	127
Vitamin B_6: pyridoxine (mg)	3.0	221	1.6	118
Folate (mcg)	557.4	295	267.2	141
Vitamin B_{12}: cobalamin (mcg)[e]	0.6	27	4.0	176
Vitamin C (mg)	273.4	289	94.9	100
Calcium (mg)[f]	505.3	82	325.5	53
Iron (mg)	11.6	109	11.0	103
Phosphorus (mg)	864.1	115	1191.2	159
Magnesium (mg)	396.1	151	327.4	125
Potassium (mg)	5199.6	272	2712.3	142
Zinc (mg)	6.2	62	10.6	107
Sodium (mg)	1133.0	113	2450.8	245

[a]Micronutrient requirements are from 6th Recommended Dietary Allowances for the Japanese (RDAJ), 1996.

[b]Calculated from U.S. National Archive, archived food records, 1949.

[c]Calculated from the Japan National Nutrition Survey and the Statistics Record of the Ministry of Agriculture, Forestry and Fisheries, Government of Japan, 1950.

[d]Okinawa is located at a subtropical (26.4°) latitude and subjects would likely have manufactured enough *in vivo* vitamin D from sunlight to meet RDAJ.[70]

[e]Periodic festivals (approximately monthly) in which pork and other meats were consumed are not accounted for in this analysis.

[f]Okinawan drinking water is high in calcium and other minerals, which are not accounted for in this analysis.

In addition, if delayed aging occurred in Okinawans, there may be a rightward shift in the survival curve as seen in animal studies,[3] with increases in both average life span (defined here as age at death for 50th percentile of population) and maximum life span (defined here as age at death of 99th percentile of survival). FIGURE 4 displays survival curves for Okinawan, Japanese, and U.S. populations for the year 1995. These data show increases in both average and maximum life span in the Okinawan population compared to Japanese and American populations, consistent with CR. Average life span and maximum life span in the Okinawan, Japanese, and U.S. populations was 83.8 and 104.9 years, 82.3 and 101.1 years, and 78.9 and 101.3 years, respectively. These data are based on a conservative survival model (LIFEREG Procedure) and differences are even greater in maximum life span between Okinawans and Japanese and/or Americans using a Weibull survival model (data not shown).

TABLE 3. Nutritional deficiency symptoms in Okinawa, 1949

	Urban	Rural	All Okinawa
Number of persons examined	797	1029	1826
Persons with no symptoms (%)	74.3	81.6	80.5
Persons with one or more symptoms (%)	25.7	18.4	19.5
Anemia	3.1	1.1	1.4
Hyperkeratosis	0.4	0.7	0.7
Xerophthalmia	–	0.7	0.6
Cheilosis	14.1	10.1	10.7
Glossitis	2.0	0.7	0.9
Loss of knee jerk	5.5	2.7	3.1
Edema	0.5	1.6	1.5
Chronic diarrhea	0.9	0.9	0.9
Bradycardia	0.8	1.8	1.6
Delayed menarche[a]	4.7	10.2	9.4
Deficient lactation[a]	5.6	20.0	17.8

[a] In women only.

Finally, since CR induces profound reductions in risk for chronic disease and increases the age at onset of chronic diseases,[2] we summarize age-adjusted mortality data for specific age-related diseases in FIGURE 5. Coronary heart disease, and forms of cancer, such as lymphoma, and cancer of the prostate, breast, and colon are remarkably low in age-matched Okinawans versus other Japanese and Americans. Since the forces of mortality act most strongly at older ages, these mortality differences reflect mainly the mortality experience of the older Okinawan cohort, which appears to have been subjected to mild CR.

DISCUSSION

The older cohort of Okinawans (aged 65-plus) is remarkable in many ways. Of particular interest is that they possess among the highest functional capacity and the longest survival in Japan, the country with world's longest-lived population. Life expectancy at birth for the year 2000 was 86.0 years for Okinawan women and 77.6 years for Okinawan men, respectively. Life expectancy for the septuagenarian cohort (life expectancy at age 65) is the highest in Japan, and possibly the world, at 24.1 years for females and 18.5 years for males, respectively.[40] This compares to 22.5 years and 17.6 years for the same birth cohort in mainland Japan and 19.3 years and 16.2 years for the corresponding U.S. birth cohort of females and males, respectively.[41]

The question addressed by this article is whether or not CR is linked to this phenomenon. Since this is an epidemiologic study, reporting mainly population data, the results must be viewed as hypothesis-generating and not conclusive. The question of whether CR or some other unknown factor, such as genetics,

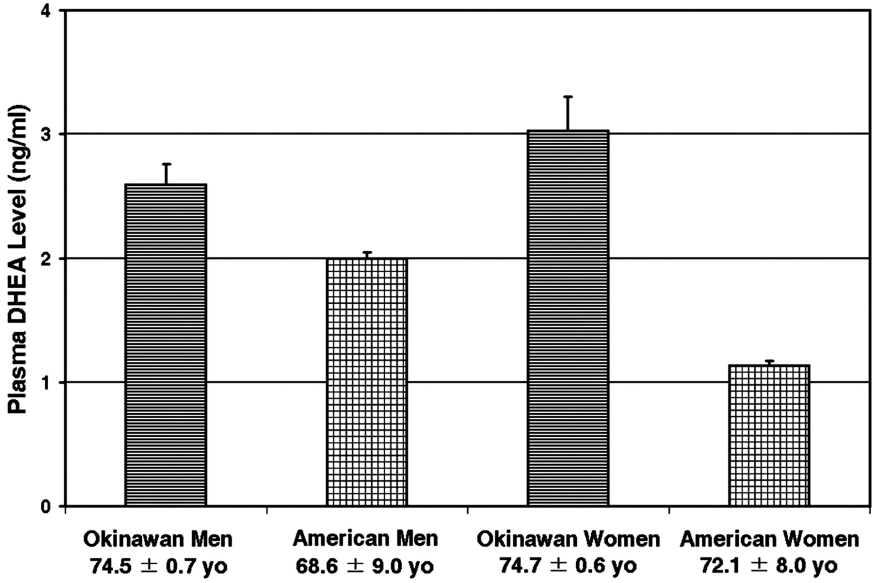

FIGURE 3. Plasma DHEA levels (ng/mL) in elderly Okinawans and Americans. DHEA was significantly higher in older Okinawan men ($P < 0.001$) and Okinawan women ($P < 0.001$) versus older American men and women, respectively. yo = years old.

is responsible for the healthy longevity of the Okinawans are hypotheses that require further testing. Nevertheless, this study is supportive of at least a partial role for CR in Okinawan longevity and is significant for several reasons.

One, this is the first in-depth epidemiologic study of long-term CR in the Okinawan population, which has been cited as a potential human example of the effects of CR on aging and age-related disease.[18] Two, this study is the only study to report population-based dietary and phenotypic data over the adult life span for the older cohort of Okinawans. Previous studies reported on small selected samples of Okinawans, which are more subject to bias, and reported data at a single point in time.[18,20] Three, this study supports and extends earlier observations from the 1970s regarding the potential impact of CR on Okinawan health and longevity.[18] Okinawans indeed appear to be one of the few populations in the world that may have experienced mild long-term CR without significant malnutrition, and this may be linked to their exceptionally healthy survival. That this phenomenon appears to have occurred in their natural environment also makes them a population of special interest.

Four, there is a dearth of research on factors that may affect the rate of human aging and controversy on how exactly to measure this. While there are no universally accepted biomarkers of aging,[14] this study adds human data that are consistent with the literature in animals with regard to the effects of CR on DHEA levels.[8,27] Furthermore, this is the first study that has shown extended

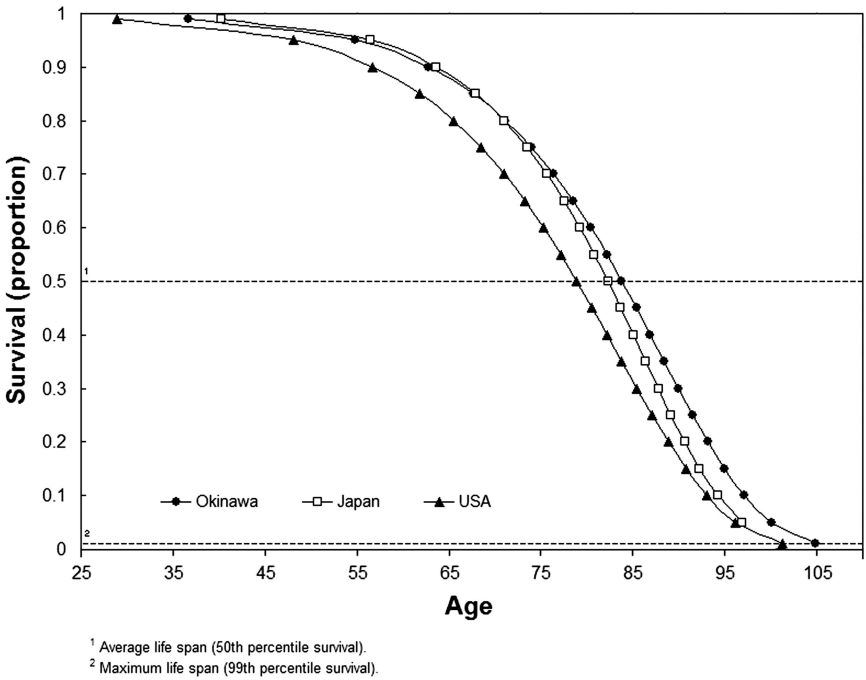

FIGURE 4. Survival model for Okinawans, Japanese, and American populations. Data show extended life span (average, maximum) for Okinawans (83.8 years, 104.9 years) compared to other Japanese (82.3 years, 101.1 years), and Americans (78.9 years, 101.3 years).

average *and* maximum life span in a human population that is potentially due to CR, as indicated by a rightward shift of the survival curve for Okinawans. While mortality due to age-related disease was much lower in the Okinawans and helped lead to a higher average life span, the apparent increase in maximum life span is suggestive of slower aging in the Okinawans. Finally, this study population has among the longest life expectancy in the world, and includes what may be the world's highest prevalence of exceptionally aged individuals, such as centenarians, and therefore is an important population for the study of environmental and genetic factors that may predict or lead to exceptional survival.[42]

There are several strengths to this study. One, this is among the most comprehensive studies of dietary and phenotypic data related to CR in Okinawa to date and the first to report population-based energy balance and phenotypic data in older Okinawan adults before the 1970s, when they were young adults. This is important for establishing a link between CR and longevity in Okinawa since it appears that only older Okinawans have a survival advantage compared to other Japanese.[22] Two, a sample of fairly well-defined subjects from the older cohort of Okinawans was available for biomarker

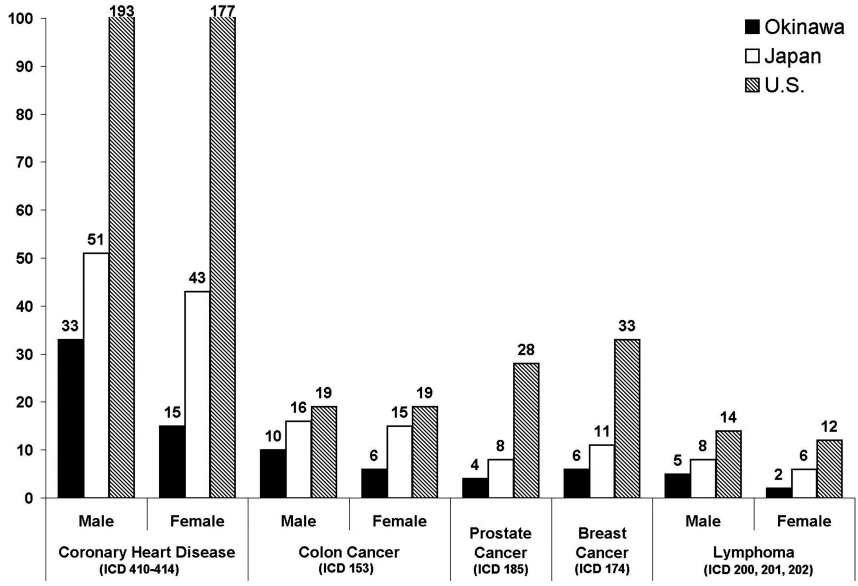

FIGURE 5. Mortality from age-associated diseases in Okinawans versus Americans. Numbers represent age-adjusted mortality rate in deaths per hundred thousand persons per year for 1995. Coding was according to ICD-9 codes; populations were age-adjusted to World Standard Population. These data show markedly lower mortality risk from age-related diseases in Okinawans versus other Japanese and Americans.

(DHEA) study, and both the Okinawan and American samples were selected from specific populations of healthy septuagenarians in geographically defined communities living in their natural environments. Three, while exact dietary data are not known for these individuals, population data are otherwise known for their birth cohorts, collected with good dietary methodology. While these are only crude measurements of energy balance, the population data support an energy deficit in Okinawans (but not Americans) that would require physiological adaptations consistent with a CR phenotype.

Four, anthropometric data, such as BMI, are consistent with a large difference in energy balance between the study populations and support a CR status for Okinawan septuagenarians at younger ages. The study cohort to which these Okinawans belong was relatively thin and appeared to have been calorically restricted at least until middle age. Support for a CR phenotype for members of this older cohort can also be derived from later-life measurements of their BMI, which was 23.5 for males and 24.1 for females when aged approximately 75 years.[20] When corrected for potential loss of height with aging[43] the true BMI for these subjects may be closer to 22.4 for males and 22.0 for females. This low BMI, compared with an average BMI of 21.2 in 1949 at approximately age 30, suggests minimal weight gain with age that is also consistent with CR.

There were several limitations to this study, mostly centered on the comparisons with the U.S. study population. However, this study is mainly concerned with the cumulative evidence that CR occurred in the older Okinawan population and whether there is evidence that CR-linked outcomes may have occurred, including phenotypic outcomes, lower mortality from age-associated diseases, and extended survival.

Limitations include the fact that population data are used to estimate whether this older generation of Okinawans were calorically restricted and these data are subject to measurement error. Clearly it is not possible to maintain an energy deficit in the long term and still be healthy. Nevertheless, the low BMI from the Okinawan population until the 1960s is consistent with a long-term adaptive response to limited energy availability and periodic energy deficits. These energy deficits are supported by historical reports of periodic crop failures that occurred in Okinawa in the early 20th century and a long history of marginal food supply.[44] A CR status appears to have remained until at least middle age for Okinawan septuagenarians since dietary and physical activity patterns remained relatively consistent in Okinawa until the 1960s (FIG. 1A). After the 1960s standards of living improved, physical activity declined, and Westernization (increased fat, meat and bread) and Japanization (increased polished Japanese or "Japonica" white rice) of the diet occurred, with large shifts in energy balance.

Another weakness of this study centers on interpretation of the DHEA levels. Since the populations were of different ethnicity and the Okinawans come from an island population with restricted gene flow, there may be genetic or other unique features that account, at least in part, for the differences in DHEA levels and health status of the populations.[45] However, we could find no published data on DHEA in other Japanese populations, and the only prior reported comparisons of Japanese and Americans involved dehyroepiandrosterone sulfate (DHEAS). These data demonstrate that Japanese men actually have lower DHEAS levels than American men when younger.[46] Therefore, persistently higher life-long DHEA levels in Okinawans compared with Americans is unlikely to account for the older-age differences in DHEA we found in this study. More likely is a slower age-related decline in DHEA in the Okinawans, consistent with CR.

In support of this, Nafziger *et al.*[47] reported DHEA levels from a study of community-dwelling white Americans that were not markedly different from the values for the comparison group in this study (Rancho Bernardo cohort). Plasma levels were reported as 1.6 ng/mL for septuagenarians of both sexes—lower than Okinawans of similar age. In addition, in a separate study of plasma estrogen levels in these same subjects, similar differences were observed between Okinawan and U.S. populations, with significantly higher levels of estrogen seen in Okinawans at older ages.[48] Estrogen, as a downstream byproduct of DHEA, usually reflects DHEA's plasma level (i.e., high DHEA means relatively high estrogen).

Finally, other factors might be responsible for the mortality advantages and phenotypic differences between Okinawans, other Japanese, and Americans. These include differences in dietary macronutrients, such as differences in protein intake or amino acid composition of the diet, which has been linked to longevity in rodents[49,50] and fruit flies[51] and to stroke risk in animals and humans,[52,53] lower glycemic index carbohydrates in the Okinawans, which may lower cardiovascular and cancer risk,[54] higher consumption of flavonoids (e.g., soy foods and Okinawan sweet potatoes),[55] which may stimulate sirtuin proteins and have been implicated as CR mimetics,[56] higher intake of antioxidant-rich vegetables, lower intake of sodium and higher K/Na ratio (both implicated in lower blood pressure),[57] or multiple other dietary differences.

Chronic low caloric intake and chronic low protein and/or low intake of particular amino acids (e.g., methionine) may also be chronic low-intensity stressors and may have contributed to a phenomenon known as "hormesis," where low levels of otherwise damaging agents have positive, potentially life-extending effects.[6,58]

Other factors, such as genetic differences[41,59,60] and psychosocial factors,[61] are important survival factors that have not been accounted for in this study. In particular, recent work suggests that Okinawans may possess familial survival advantages that account, at least in part, for their remarkable longevity.[42] On the other hand, fuller expression of the potential health and longevity benefits of CR in Okinawans would have been limited by the lack of a good public health infrastructure until the 1960s, and resultant high death rates due to tuberculosis and other infectious diseases.[62] Furthermore, of the nutritional factors, only CR has been found in animal studies capable of consistently increasing both average *and* maximum life span, indicated by a rightward shift of the survival curve.[63]

Thus, the septuagenarian cohort of Okinawans appears to have experienced CR of approximately 11% with respect to their estimated energy requirements, until middle age. Older cohorts in Okinawa may have experienced CR for even longer or at greater levels, consistent with their increased survival advantage compared with that of other Japanese, although estimates of the degree of CR should be viewed with caution since they were obtained from energy balance estimates for the whole Okinawan population and were not validated by precise metabolic methods, such as indirect calorimetry or doubly labeled water.

The CR status of the older Okinawans appears to be due, in part, to a high level of occupational energy demand from their main vocation as farmers. This was coupled to low caloric intake from an energy-poor but nutrient-dense diet rich in Okinawan sweet potatoes, other vegetables, legumes, and other foods low in energy density (FIG. 1).[24] The Okinawan sweet potato, with a caloric density of 1.0 kcal/gram, has been the main carbohydrate of the Okinawan diet from the 1600s until approximately 1960, accounting for more than 50% of calories.[64] The higher caloric density of the traditional Japanese diet is partly

due to their higher consumption (75% of calories) of high caloric density Japonica (white) rice, with a caloric density of 1.5 kcal/gram.

The CR phenotype appears to be disappearing in Okinawa except among older birth cohorts (aged 65-plus years). Calorie intake has been increasing and activity levels decreasing since the 1960s with concomitant population-wide increases in BMI, although this trend appears to have stabilized after reversion of Okinawa from U.S. to Japanese governance in the 1970s (FIG. 1A). Loss of the CR phenotype among younger Okinawans and increased weight gain in this cohort is also associated with higher mortality from obesity-linked diseases. This can be inferred from a mortality crossover, which has followed a BMI crossover in Okinawans versus other Japanese.[22] Concomitantly, there has been a significant slowing of life expectancy gains over the last two decades. This may, as is hypothesized in Americans,[65] have an unfavorable impact upon the health and longevity of subsequent generations.

Early-life energy deficits experienced by older Okinawans would have required compensatory responses in terms of more efficient use of energy[66] since long-term energy deficits are unsustainable and inconsistent with health. These adaptations may have included less body fat, lower blood sugar and insulin levels, hormetic responses, and multiple changes in gene expression, among other adaptations potentially linked to longer life span.[3–6,8,67–69] Animal studies suggest that CR at any stage of the life cycle (early or later life) may potentially result in mortality advantages versus non-CR controls.[4]

In conclusion, we observed low calorie intake coupled with high physical activity levels that appear to have contributed to a CR phenotype in older Okinawans. This phenotype includes a life-long low BMI, relatively high plasma levels of DHEA at older ages, reduced mortality from age-associated diseases, and extended average and maximum survival. While these conclusions are tentative in nature, an adaptive response to early and mid-life energy restriction in the older cohort of Okinawans may be implicated in their low morbidity and exceptionally long survival. This is consistent with the well-known animal literature that supports a beneficial effect of CR on BMI, age-related biomarkers, morbidity/mortality, and life span. More studies using the CR paradigm (energy restriction without malnutrition) are required to validate the effects of low energy intake and/or energy balance in human populations.

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