Proc. Natl. Acad. Sci. USA
Vol. 89, pp. 11533–11537, December 1992
Medical Sciences

The calorically restricted low-fat nutrient-dense diet in Biosphere 2 significantly lowers blood glucose, total leukocyte count, cholesterol, and blood pressure in humans

(aging/dietary restriction/risk factor/vegetarian/atherosclerosis)

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Communicated by Robert A. Good, August 25, 1992

ABSTRACT **Biosphere 2 is a 3.15-acre space containing an ecosystem that is energetically open (sunlight, electric power, and heat) but materially closed, with air, water, and organic material being recycled. Since September 1991, eight subjects (four women and four men) have been sealed inside, living on food crops grown within. Their diet, low in calories (average, 1780 kcal/day; 1 kcal = 4.184 kJ), low in fat (10% of calories), and nutrient-dense, conforms to that which in numerous animal experiments has promoted health, retarded aging, and extended maximum life span. We report here medical data on the eight subjects, comparing preclosure data with data through 6 months of closure. Significant changes included: (*i*) weight, 74 to 62 kg (men) and 61 to 54 kg (women); (*ii*) mean systolic/diastolic blood pressure (eight subjects), 109/74 to 89/58 mmHg (1 mmHg = 133 Pa); (*iii*) total serum cholesterol, from 191 ± 11 to 123 ± 9 mg/dl (mean ± SD; 36% mean reduction), and high density lipoprotein, from 62 ± 8 to 38 ± 5 (risk ratio unchanged); (*iv*) triglyceride, 139 to 96 mg/dl (men) and 78 to 114 mg/dl (women); (*v*) fasting glucose, 92 to 74 mg/dl; (*vi*) leukocyte count, 6.7 to 4.7 × 10^9 cells per liter. We conclude that drastic reductions in cholesterol and blood pressure may be instituted in normal individuals in Western countries by application of a carefully chosen diet and that a low-calorie nutrient-dense regime shows physiologic features in humans similar to those in other animal species.**

Biosphere 2 is a 3.15-acre closed space containing an ecosystem that is located near Tucson, Arizona. The large glass and steel structure houses seven biomes: rain forest, savannah, ocean, marsh, desert, an agricultural biome, and a habitat for humans and animals (1). It is serving as a laboratory for ecological investigations (e.g., biogeochemical cycles, food web systems, and the population genetics of speciation). In addition, knowledge from Biosphere 2 should prove useful in the eventual design of extraterrestrial structures capable of sustaining long-term habitation with minimal supply of materials. The enclosure contains >3800 plant and animal species in a state developing toward equilibrium. The system does, however, require "management" by its human occupants to prevent it from evolving to a state possibly biologically stable but not supportive of human life.

On September 26, 1991, four men and four women, the so-called "Biospherians," entered the enclosure via airlock, and the complex was materially sealed ("closure"). Thermodynamically, it remains "open" in that sunlight and electric power enter, and heat is removed. Free transfer of electronic information is also allowed. However, to approximate the isolation of an extraterrestrial location, policy is that (excepting emergency or the rare research need requiring passage of small items) no materials pass in or out for 2 years (until September 26, 1993). Thus all organic material, water, and (virtually all) air must be recycled, and all food is raised inside after closure.

This report concerns the nutritional regime and health status of the Biospherians during the first 6 months of closure. Before closure a daily intake of 2500 kcal per person (1 kcal = 4.184 kJ) had been projected. In the actual event, due to initial crop problems, calorie intake for the first 6 months was limited. Complete food records (kept by R.L.W.) for 21 random days over this period found a mean intake of only 1780 kcal. However, the quality of the diverse and largely vegetarian diet, as reflected in essential nutrient content per calorie (or nutrient density), was excellent. A low-calorie nutrient-dense diet of this nature corresponds to the diet proven in extensive experiments in rodents and other species to retard aging rates, extend maximum life span, and induce characteristic physiologic changes (2).

METHODS AND MATERIALS

Human Subjects and Exam Protocol. Four women and four men (ages 25–67 years) make up the Biospherian crew (Table 1). Ages of males (42 years) and females (36 years) did not differ significantly. All were in good health and nonsmokers. During this study all sustained physical activity judged equivalent to 3–4 h of manual farming daily. All had maintained a similar level of activity for more than a year prior to closure.

All medical data was collected by one of us (R.L.W.), who is the medical officer inside the enclosure and also a crew member. Two to 4 months before closure all subjects received a complete physical examination, medical history, system review, chest x-ray, electrocardiogram, respiratory spirometry, urinalysis, and fasting laboratory blood analysis. Beginning 2 weeks after closure, every 2 weeks one subject of each sex received a symptom review, physical examination, urinalysis, and (overnight fasting) blood analysis, all done by R.L.W. inside the enclosure (3). Thus, each individual was evaluated every 8 weeks. All subjects were weighed before breakfast monthly. Blood pressures (BPs) were taken while seated. To help evaluate the unexpectedly large weight loss by 6 months of closure, body fat was estimated by measuring skinfold thickness of triceps, thigh, and subscapular areas, using a standard formula (4). Body mass index (BMI) was calculated as weight (kg)/[height (m)]^2. Mean BP [or mean arterial pressure (MAP)] was calculated as diastolic BP + 0.33 (systolic BP − diastolic BP).

Nutritional Intake. The diet was essentially vegetarian, with intake of six varieties of fruits (banana, fig, guava, lemon, papaya, and kumquat), five cereal grains (oats, rice,

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Abbreviations: BP, blood pressure; BMI, body mass index; MAP, mean arterial pressure; HDL, high density lipoprotein.

Table 1. Basic data

Subject	Age, years	Weight, kg		BMI, kg/m²		MAP, mmHg		Cholesterol, mg/dl	
		BC	AC	BC	AC	BC	AC	BC	AC
M1	28	94.5	73.6	29.2	22.7	80	70	215/36	125/28
F1	30	59.1	52.3	20.9	18.5	73	62	140/52	92/41
M2	31	67.3	60.5	21.0	18.9	80	70	140/#	104/39
F2	32	52.7	42.8	19.6	17.9	83	70	199/96	144/72
M3	41	67.3	55.5	24.4	20.1	83	50	196/56	107/29
F3	40	75.0	64.5	25.6	22.1	90	73	209/79	122/32
M4	67	68.2	59.1	23.6	20.0	105	83	190/49	125/34
F4	40	55.9	50.9	19.3	17.6	90	70	231/67	168/31

Basic data for the eight Biosphere 2 test subjects and selected physiologic parameters measured before closure (BC) and after 6 months of closure (AC). #, Missing value. Cholesterol levels are given as total serum cholesterol/HDL cholesterol.

sorghum, wheat, and corn), split peas, peanuts, three varieties of beans, 19 vegetables and greens, white and sweet potato, and small quantities of goat milk and yogurt (average, 84 g/day), goat meat, pork, chicken, fish, and eggs (average, 2.5, 6.0, 3.6, 2.0, and 3.0 g/day, respectively). Crops were planned so that as complete a nutritional complement as possible was always available (relative to the recommended daily allowance) despite changing crop cycles. Daily nutritional composition of the diet was determined via computer program (5). Average daily intake for 21 random days was 1780 kcal, range 1672–2143 kcal. Sufficient amounts of all essential nutrients were supplied by the diet, except for vitamins D (the enclosure's glass transmits only a trace of UV radiation), vitamin B_{12} (deficient in the low animal-product diet), and calcium (average, 500 mg/day). The diet contained adequate protein (63 g/day), was high in fiber (52 g/day), and low in cholesterol (36 mg/day) and fat (≈10% of energy intake).

Three meals per day were eaten, with equal portions given to each individual regardless of size or gender. Meals were totally consumed, and no other food (with the exception of fresh herbs for seasoning) was eaten, none being available. Water was taken ad libitum. Individuals also received daily vitamin/mineral supplements consisting of ≈50% of the recommended daily allowance or "safe and adequate" amounts of known essential vitamins and minerals; 100% of the recommended daily allowance of vitamin B_{12}, folic acid, and vitamin D; 400 international units of vitamin E; and 500 mg of ascorbic acid.

Laboratory Examination. This consisted of (*i*) urinalysis by dip stick and microscopic exam, (*ii*) hematologic panel [hemoglobin, hematocrit, erythrocyte and leukocyte counts, and a leukocyte differential count (all performed by R.L.W. using manual techniques)], and (*iii*) standard blood serum chemistry panel [alanine aminotransferase, alkaline phosphatase, amylase, aspartate aminotransferase, γ-glutamyltransferase, lactate dehydrogenase, Ca^{2+}, Mg^{2+}, phosphorus, total cholesterol, high density lipoprotein (HDL) cholesterol, triglycerides, glucose, total protein, blood urea nitrogen, uric acid, creatinine, total bilirubin, Na^+, K^+, Cl^-, and CO_2 (all performed by R.L.W. on a Kodak Ektachem DT system inside the enclosure, with control specimens being run each time).

Data Analysis. Baseline preclosure data were designated "period 0" data. Beginning 2 weeks after closure, the three successive 8-week time periods during which each subject was evaluated once were designated periods 1, 2, and 3. Data were analyzed using both raw scores at each time period and percent change scores relative to period 0, for all dependent variables except age (note: all percent change scores are the means of individual percent change scores and may in exceptional cases be quite different from the percent change in mean scores). Multiple regression on the independent variables "gender" and "time period" was followed by Duncan's multiple range test. For variables that did not show a significant effect of gender, only the main effect of time period was examined with Duncan's test; for variables showing a significant effect of gender, the gender–time-period interaction was evaluated.

RESULTS

Table 1 shows individual subject data preclosure and at 6 months for weight, BMI, MAP, and cholesterol. Figs. 1–5 give BPs and laboratory results at four time periods: preclosure (period 0) and the three subsequent 8-week periods; each subject was evaluated once per period. There were no changes in average body temperatures over the 6 months, and no noteworthy changes in sleep patterns or in the subjective sense of health or well-being among the Biospherians.

Male subjects' mean weight decreased (74 ± 7 to 62 ± 4 kg) over 6 months (mean 16% decrease in BMI), and females decreased (61 ± 5 to 54 ± 4 kg, mean 11% decrease in BMI). Weight losses became highly significant ($P < 0.01$) for both sexes by period 2 (≈3.3 months). Men lost weight and BMI faster than women (partly due to the lower caloric intake per kg for men). The gender difference in BMI change relative to preclosure became significant ($P < 0.01$) by 6 months. Percent body fat was measured at the 6-month interval. Men (range, 7–12%; mean, 8.5%) differed significantly ($P < 0.01$) from women (range, 15–24%; mean, 18%). At 6 months, both sexes were at a body fat composition at the lower bounds of normal (4) but reported no ill effects. In particular, the women experienced none of the menstrual irregularities reported to accompany low body fat composition (6).

Mean systolic BP decreased (109 ± 4 to 89 ± 3 mmHg) over 6 months (1 mmHg = 133 Pa), a mean decrease of 18%. Mean diastolic BP decreased (74 ± 3 to 58 ± 3 mmHg, a 21% decrease), and mean MAP decreased (86 ± 3 to 67 ± 3

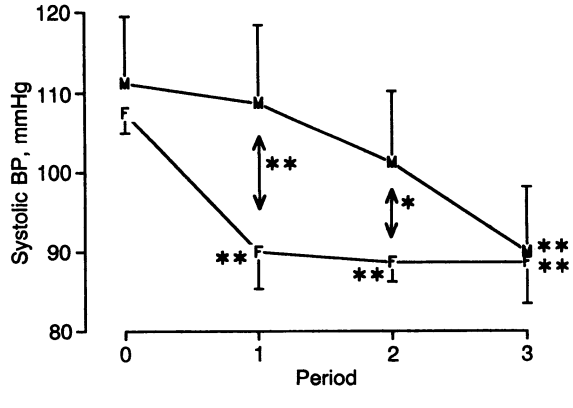

FIG. 1. Mean systolic BP for males (M, four subjects) and females (F, four subjects) preclosure (period 0) and during the following 6 months. *, $P < 0.05$; **, $P < 0.01$; symbols near data points, significance of difference compared to period 0; double arrows with * or **, significance comparing data from males and females.

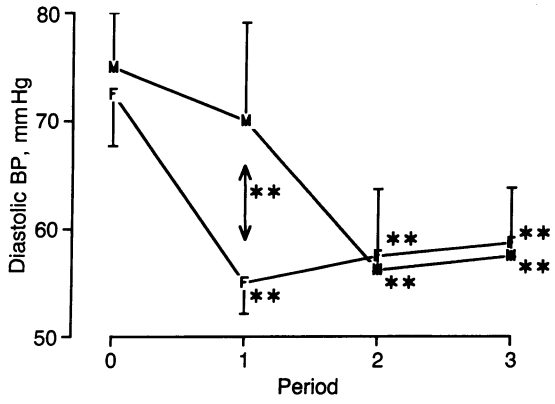

FIG. 2. Mean diastolic BP for males (M) and females (F) preclosure (period 0) and during the following 6 months. **, $P < 0.01$; symbols near data points, significance of difference compared to period 0; double arrow **, significance ($P < 0.01$) comparing data for males and females at period 1.

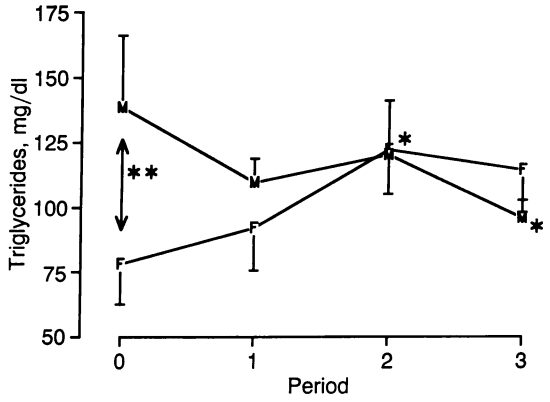

FIG. 4. Mean fasting serum triglyceride levels for males (M) and females (F) preclosure (period 0) and during the following 6 months. * at period 2, significant ($P < 0.05$) difference in females relative to period 0; * at period 3, difference ($P < 0.05$) in males relative to period 0; double arrow **, difference ($P < 0.01$) comparing male and female preclosure values.

mmHg, a mean 20% decrease). All BPs after closure differed significantly from preclosure values ($P < 0.05$ for period 1, $P < 0.01$ for periods 2 and 3). Males and females had equivalent BPs preclosure and at 6 months, but at intermediate times males had higher systolic BPs and (at period 1) also higher diastolic BPs (Figs. 1 and 2).

There was no significant gender difference in serum cholesterol or HDL at any period, but all total cholesterol levels after closure were lower ($P < 0.01$) than preclosure levels (Fig. 3). Mean total cholesterol decreased (191 ± 11 to 123 ± 9 mg/dl; for cholesterol, 1 mg/dl = 0.0259 mM) over 6 months (mean decrease, 35%). Mean HDL cholesterol fell from 62 ± 8 mg/dl (data for seven subjects only; one sample was accidentally destroyed) to 38 ± 5 (seven-subject mean decrease of 37%). The ratio of total cholesterol to HDL cholesterol ("risk ratio") did not change significantly (preclosure, 3.45 ± 0.48; 6 months, 3.49 ± 0.41). Triglyceride levels were significantly different in the sexes preclosure, but subsequently converged (Fig. 4). Over 6 months, triglycerides decreased in men (139 ± 28 to 96 ± 7 mg/dl; mean decrease, 21%) and increased in women (78 ± 15 to 114 ± 16 mg/dl; mean increase, 53%).

There was no gender difference in fasting serum glucose at any time period, but mean glucoses (eight subjects) were significantly ($P < 0.01$) lower at all times after closure. Mean fasting glucose fell from 92 ± 4 to 74 ± 2 mg/dl (mean decrease, 18%) over 6 months.

There was no gender difference for total leukocyte count, absolute neutrophil count, or absolute lymphocyte counts during the study (Fig. 5). However, the mean eight-subject total leukocyte count was significantly lower ($P < 0.01$) at all periods after closure vs. preclosure, falling from 6.66 ± 0.28 to 4.71 ± 0.22 × 10^9 cells per liter of blood over 6 months (mean decrease, 28%). Neutrophil counts fell (3.69 ± 0.22 to 2.04 ± 0.22 × 10^9 cells per liter of blood; mean decrease, 43%; $P < 0.01$), and lymphocyte counts rose (2.15 ± 0.28 to 2.48 ± 0.11 × 10^9 cells per liter of blood; mean increase, 29%; $P < 0.01$).

Blood urea nitrogen concentration decreased ($P < 0.01$) from 15.8 ± 1.7 mg/dl at preclosure to 9.3 ± 0.5 mg/dl at 6 months (data available on six of eight subjects only and not shown). All other laboratory measurements showed no significant alterations between preclosure and after closure periods and are not here reported. Outside control subjects eating an ad libitum standard U.S. diet and taking nutritional supplements identical to those taken by the Biospherians showed none of the changes reported here.

DISCUSSION

The special nature of the low-calorie low-fat high-complex-carbohydrate low-animal-product Biosphere 2 diet, taken

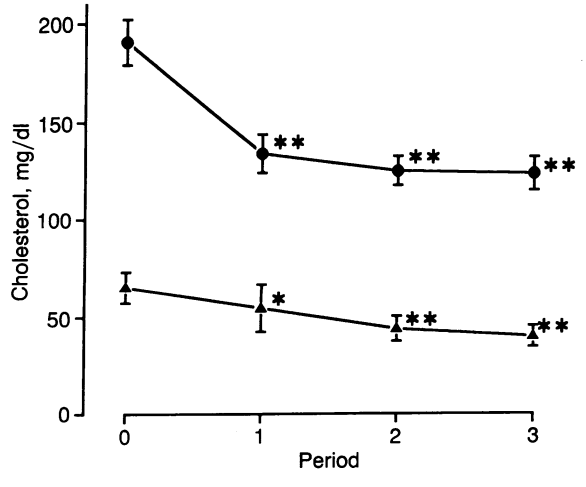

FIG. 3. Mean fasting serum total cholesterol (●) and HDL cholesterol (▲) preclosure (period 0) and during the following 6 months (all values are for eight subjects except the HDL value at period 0, representing seven subjects). *, Difference ($P < 0.05$) compared to period 0; **, $P < 0.01$.

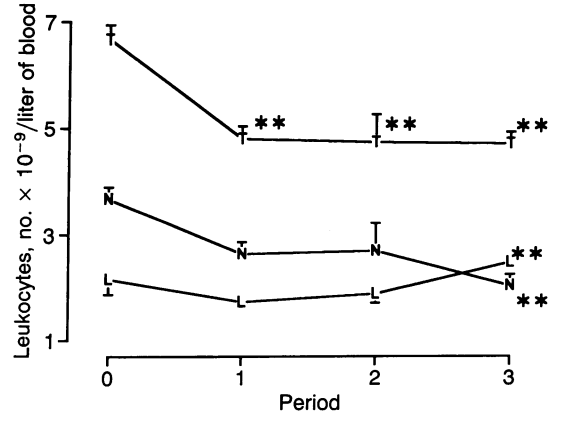

FIG. 5. Mean leukocyte counts (eight subjects) preclosure (period 0) and during the following 6 months. T, mean total leukocyte count; N, mean absolute neutrophil count; L, mean absolute lymphocyte count; **, difference ($P < 0.01$) compared to period 0.

along with the accompanying striking physiologic changes in subjects consuming it, may have both specific and general implications regarding human health.

Low-calorie nutrient-dense diets have been found to retard aging and prolong maximum life span in all species so far tested, ranging from unicellular organisms to rodents (2). In rodents such diets prevent or delay cancer and many age-related diseases (7) and also ameliorate the age-related decline in immune response (8). It is known that calorie restriction alone, independent of diet composition (so long as the diet is not deficient in essential nutrients), leads to most of the above effects, including prolongation of life span (2).

Does the antiaging effect of a low-calorie nutrient-dense diet apply to primates? Population data on humans does not allow evaluation of such a diet, since restricted human populations tend to be nutrient deficient. Although estimates of restriction effects on human life span would require multigenerational efforts, shorter studies of physiologic effects might yield insight as to whether humans and other animal species might respond similarly to a nutrient-dense calorie-restricted diet. The present report is a closely monitored long-term study of the effect of such a diet upon human physiologic parameters that uses healthy nonobese humans.

In addition to weight loss, findings from this study that are common to restriction experiments with other species include the development of borderline low leukocyte counts, low fasting blood sugars, and low blood cholesterol values. Total leukocyte count decreases have been noted in experimental strict vegetarianism in humans (9), but serum glucose decreases in vegetarianism are typically much less than in the present study (10, 11) and probably here represent a restriction effect. Marked serum glucose decreases and decreases in both lymphocytes and neutrophils are among the hallmarks of the alterations induced in rodents by antiaging low-calorie regimes (2, 12, 13) and in fact serve as important evidence for two current theories of aging (14, 15). Studies of long-term caloric restriction in nonhuman primates are also now under way, although for technical reasons the degree of restriction is less than has been employed in rodents, and one group has reported a lack of classical effects (16, 17). Nevertheless, the University of Wisconsin restricted primate colony (18) has begun to show low fasting blood sugars and a trend toward low absolute lymphocyte counts (R. Weindruch, personal communication).

Other alterations in physiology seen in the present study relate as clearly to susceptibility to age-related disease as to aging retardation. Foremost among these is the striking and fairly rapid drop in blood cholesterol (average, 35% decrease) seen even in individuals beginning with low cholesterol levels (Table 1). At 6 months, all eight individuals displayed total blood cholesterol levels < 140 mg/dl. No other dietary or drug regime known to us has been shown to exert a comparable effect. The extremely low levels of cholesterol and saturated fat in the Biosphere 2 diet doubtless play a role, but there is also the possibility that, even in nonobese persons, calorie restriction functions additively with fat restriction to decrease total serum cholesterol.

The dietary regimen most resembling that of Biosphere 2 is possibly the 26-day Pritikin Longevity Center "residential" program, which has reported as much as 25% blood cholesterol reduction (10) in healthy humans. The Pritikin diet is largely vegetarian, high in fiber, and derives 10% energy from fat but is eaten ad libitum (10). Another diet qualitatively resembling that of Biosphere 2 is the "vegan" diet that eschews animal products entirely. A modified vegan diet lowered serum cholesterol by 13% in normal subjects (11). Typically in vegan and Pritikin diets the total cholesterol/HDL ratio ("risk ratio") remains fairly constant, as in the present study (10, 19, 20).

Departures of average serum lipid concentrations far from those values "normal" for industrialized countries have been found in certain isolated low-technology populations. The largely vegan Tarahumaras of Mexico consume 9–12% of calories as fat and display mean total adult cholesterol levels of 137 mg/dl, with HDL cholesterol at 36 mg/dl (21). The Yanomamos of Brazil on a similar diet show adult cholesterol levels of 133 mg/dl, with an HDL level of 37 mg/dl (22). Because these data are epidemiologic, it is difficult to separate dietary from other variables; however, such societies are in many ways dietarily similar to Biospherians in not having free access to vegetable oils and nut products, which often increase fat and calories in Western vegan diets (23, 24). One group of Western near-vegan "macrobiotic" diet enthusiasts has been reported with adult cholesterol levels in the range of 125 mg/dl (25).

There is now a consensus that elevated blood cholesterol constitutes a major etiologic factor in atherosclerosis and that lowering high cholesterol decreases cardiac risk (26–28). Of note is the study by Ornish *et al.* (20) in which subjects randomized to a stringent vegetarian diet (10% fat-derived calories) achieved a 26% cholesterol drop relative to an already fat-restricted diet and angiographic regression of coronary atherosclerosis (20). Controversy exists as to the optimal decrease in cholesterol levels for regression of atheromatous disease and also to what degree HDL exerts an independent protective role, since HDL is often also reduced by very low fat diets. Western studies have little mortality data for cholesterol levels <150 mg/dl, although in Framingham risk reduction continues (for men, but not women) down to at least 180 mg/dl (29, 30). In the largest most complete prospective epidemiologic study known to us, the "China Health Project," 6500 Chinese were examined with regard to lifestyle, physiologic parameters, and mortality (31). This population eats a plant-based diet in which 15% of calories are fat-derived (slightly more than in Biosphere 2) and has a mean total plasma cholesterol level of 127 mg/dl (similar to the present study) with a range of 94–162 mg/dl. Over this very low range there was no correlation between total cholesterol and cardiac mortality in the China Health Project, suggesting that near-maximal cardiac risk reduction is achieved at somewhat higher cholesterol levels than 125 mg/dl. Moreover, there was no correlation between HDL cholesterol levels and cardiac mortality, suggesting that moderately reduced HDL levels do not contribute much to cardiac risk if total cholesterol levels are very, very low. HDL values in the China Project (mean, 44 mg/dl; range, 31–59 mg/dl) are similar to those reached by the Biospherians.

In summary: we present evidence that a low-calorie nutrient-dense diet produces in primates—in this case, humans—physiologic changes similar to those seen in calorie-restricted rodents. In addition, such a diet may bring specific health benefits. Although there is increasing evidence of health benefits to adults from maintaining low serum cholesterol levels (31), natural populations with average very low blood lipid concentrations have usually been found in places relatively exotic to Western standards. Thus the possibility always exists that in these populations, large departures from typical industrialized country ranges of blood lipids, BPs, etc., are due to different genetics or aspects of lifestyle other than diet. The present study provides evidence that, on the contrary, radical and possibly beneficial changes in physiologic risk factors can be produced in normal affluent individuals in Western countries quickly and reproducibly by dietary manipulation.

We thank the Biosphere 2 crew for their participation. This work was supported by Space Biospheres Ventures, Veteran's Adminis-

tration Research Funds, National Science Foundation Grant NS-20660, and Public Health Service Grants AG-00424 and AG-00121.

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