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Physiologic Changes in Humans Subjected to Severe, Selective Calorie Restriction for Two Years in Biosphere 2: Health, Aging, and Toxicological Perspectives

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Biosphere 2 is a closed ecological space of 7-million cubic feet near Tucson, AZ, containing 7 biomes: rain forest, Savannah, ocean, marsh, desert, agricultural station, and habitat for humans and domestic animals. Sealed inside, 4 men and 4 women maintained themselves and the various systems for 2 years. All organic material, all water, and nearly all air was recycled, and virtually all food was grown inside. On the low calorie but nutrient-dense diet available, the men sustained 18% and the women 10% weight loss, mostly within the first 6 to 9 months. The nature of the diet duplicated rodent diets that had been shown to enhance health, lower disease incidence, and retard aging. Using blood specimens frozen at different points during and after the 2 years, determinations were made of a number of biochemical parameters judged to be pertinent based on past studies of rodents and monkeys on similar diets. These included blood lipids, glucose, insulin, glycosylated hemoglobin, renin, and others. The results clearly suggest that humans react to such a nutritional regime similarly to other vertebrates. In addition to these studies, and because this was a tightly closed, isolated environment, the levels of insecticides or pollutants or their derivatives were determined in the sera of 2 crew members. It was found that levels of the lipophilic toxicant DDE and the "total PCB" load increased with the loss of body fat during the first 12–18 months inside Biosphere 2, then decreased.

Key Words: **Biosphere-2; calorie restriction; dietary restriction; primates; humans; DDE; PCB.**

Biosphere 2 is a 3.15-acre, 7-million cubic foot closed ecological space near Tucson, Arizona. At the time of the study presented, it contained 5 wilderness and two domestic biomes (rain forest, savanna, desert, ocean, marsh, agricultural station, living quarters), and a large basement "technosphere" housing computer systems, wind generators, water tanks, water purification and recycling systems, pumps, ovens, threshing and other farm machinery, and additional machinery required for the operation of the total complex. In September 1991, 4 men and 4 women entered Biosphere 2 and the complex was physically sealed (closure) for 2 years. Thermodynamically, it remained open in that sunlight and electric power entered, heat was removed by a sealed water-conduction system, and electronic information was transferred. Except for specific instances employing an air-lock and limited to scientific items, no material entered or left for the 2-year period, except that (for reasons given elsewhere (Walford *et al.*, 1996)) oxygen had to be supplied on two occasions. All organic material was recycled or stored inside, all water and virtually all air was recycled, and about 85% of food was raised inside (the rest coming from pre-entry back-up stores). As much as possible, Biosphere 2 was intended to be a completely closed, self-sustaining ecological system requiring only "adaptive management" by the crew members (Walford *et al.*, 1996).

Before closure, a daily intake in excess of 2500 kcal per person was expected to be supplied entirely by the agricultural system. As it was, however, due to crop problems related to reduced light from an El Nino year and the prevalence of insect pests, caloric intake during the first 6 months averaged only about 1800 kcal/day, rising then to about 2000 kcal for most of the remaining time period. This intake level was low in relation to the large amount of physical labor required of the crew members. All crew members lost significant amounts of weight over the first 6 to 9 months (~18% for the men, 10% for the women), which persisted until nearly the end of the confinement, though was slightly alleviated during the last 2 months when back-up stores could be added to the food intake. On the other hand, the quality of the diverse and largely vegetarian diet, as reflected in essential nutrient content per calorie ("nutrient density"), was superb.

The low-calorie, nutrient-dense character of the diet resembled in principle that which has been shown to retard aging, reduce the incidence of most age-related diseases, and extend life spans in rodents, and other species (Weindruch and Walford, 1988). Animals on such a diet have also shown a substantial number of physiological changes that seem in accord with an enhanced health status. Such a regime had never been studied in humans under carefully monitored conditions.

The present report details the response of selected physiologic variables in the calorie-restricted crew members of Biosphere 2 during the entire 2 years of closure, plus a 30-month follow-up period. The results indicate that their physiological

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responses closely resembled those of rodents and monkeys on such a diet.

MATERIALS AND METHODS

Human subjects and examination protocol. Four men and 4 women made up the crew. Ages at time of closure ranged from 28 to 41, except for one member (RLW) who was 67. All persons were in good health and were nonsmokers. Medical data was collected by one of the present authors (RLW), who served as the medical officer inside Biosphere 2, and was also a crew member. Several months before closure each subject received a complete physical examination, including system review, chest X-ray, electrocardiogram, urinalysis, and fasting laboratory blood analysis, and a medical history was taken, Beginning 2 weeks after closure, every 2 weeks one subject of each sex received a symptom review, physical examination, urinalysis, and fasting blood analysis, all done by RLW inside the enclosure. Thus, each subject was evaluated every 8 weeks.

Clinical laboratory examination. These evaluations consisted of urinalysis performed by dip stick and microscopic examination; CBC and differential count, and the standard blood chemistries available with a Kodak Ektachem DT dry reagent chemistry system (Walford *et al*, 1996). In addition, aliquots of serum and plasma were obtained at intervals from all crew members, frozen, and stored at –70°C inside Biosphere 2. Specimens were also obtained at four time periods up to 30 months following the exit of the crew from Biosphere 2 and return to a normal *ad libitum* diet. These frozen specimens were later analyzed at the UCLA Clinical Laboratories for a number of variables, using results of calorie restriction studies in rodents as a general comparison guide. For determination of glycated hemoglobin, frozen packed red blood cells were thawed, reconstituted to 2× volume with saline, and subjected to analysis. This method gives a somewhat higher reading than is found with freshly drawn blood, but comparisons over time remain valid.

Toxicological analyses. All determinations were done at ACCU-CHEM Laboratories, Richardson, Texas (Laseter *et al*, 1983).

Nutritional intake. The diet inside Biosphere 2 was essentially vegetarian. Fruits were chiefly bananas and papaya; grains were chiefly wheat, rice, and sorghum. Peanuts, split peas, and several types of beans were raised, as well as 24 varieties of vegetables and greens. The animal facility provided small quantities of goat milk, goat meat, pork, chicken, fish, and eggs. Crops were planted so that as complete a nutritional complement as possible was always available in terms of the recommended daily allowances, despite changing crop cycles. Nutritional composition of the diet was determined on representative days via a computer program (Walford, 1996).

Three meals per day were eaten by the crew members, with equal portions given to each individual regardless of size or gender. While this may seem surprising, the crew felt that attempts to apportion food, labor, and other items according to body weight, sex, age, subjective sense of hunger, or other considerations would be hopelessly complex. Meals were always totally consumed, and no other food was eaten, none being available. Water was taken *ad libitum*. Crew members also received daily vitamin/mineral supplements consisting of ~50 to 100% of the recommended daily allowance or "safe and adequate" amounts of known essential vitamins and minerals.

Statistical analysis. Nonparametric exploratory data analysis was implemented on the time series data requiring fewer distributional assumptions than a more classical approach. This procedure was used due to the relatively few data points (i.e., only eight subjects) and inherent biological variability. A "meta"-approach method included tools applied in computational intensive statistics such as bootstrapping and a nonparametric additive model that employed the Fisher Chi-square test (Effron and Tibshirani, 1991; Hedges and Olkin, 1985; Huber, 1981). The experimental design involved using each subject as their own control and estimating the distributional range of values "inside" and "outside" Biosphere 2 (note: values "outside" included 1-month pre-entry but excluded the "transitional" period of 1-month post-exit). Percent differences were transformed in an additive model and tested against separate null hypotheses with a Chi-square distribution for significance. Independent random range distribution estimations using bootstrapping methods reconfirmed the results of the composite meta-approach.

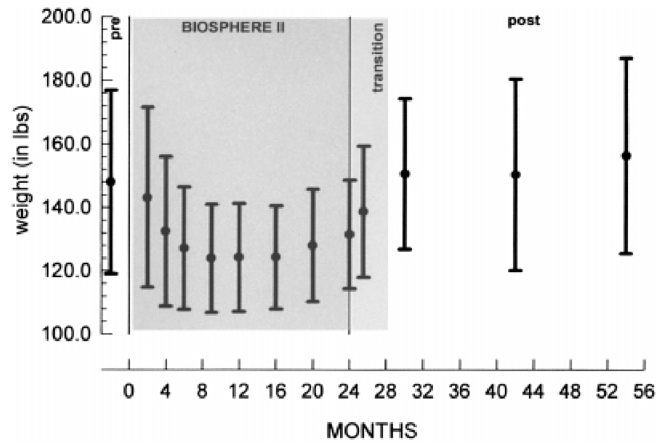

FIG. 1. Average weights ± SD of crew members before entry, during residence in, and after exit from Biosphere 2.

RESULTS

Except for minor ailments described elsewhere (Walford *et al*, 1996), the health of the crew remained excellent throughout the 2 years. Electrocardiographs, chest X-rays, respiratory spirometry, and urinalyses were normal. Quantitative data for body temperature inside Biosphere 2 are not available as the available clinical thermometers were not calibrated for values below 96°F and measurements were sometimes below this level. Pre- and post-exit average body temperature of all crew members were in the normal range of about 98.6°F; inside Biosphere 2 they were often in the 96 to 97°F range, and sometimes below 96°F.

Figure 1 shows average weights of the crew before, during, and after their stay in Biosphere 2. The weights of the men decreased by approximately 18%, and of the women by 10% during calorie restriction inside Biosphere 2. The lost weight was completely restored by 6 months after exit and return to an *ad libitum* diet.

Figure 2 shows systolic and diastolic blood pressure values of the crew before, during, and after their stay in Biosphere 2. There were significant decreases in both measurements for both sexes while in the study, reverting to pre-entry levels after return to an *ad libitum* diet. Figure 2 also shows renin levels of the crew inside Biosphere 2 at the 22-month time point, compared to renin levels after exiting from Biosphere 2. Again, a significant decrease was found.

Figure 3 gives values for glucose, insulin, and glycated hemoglobin inside Biosphere 2, and at several time-points after exiting Biosphere 2. All were significantly decreased inside Biosphere 2.

Figure 4 shows levels of DDE in two crew members before

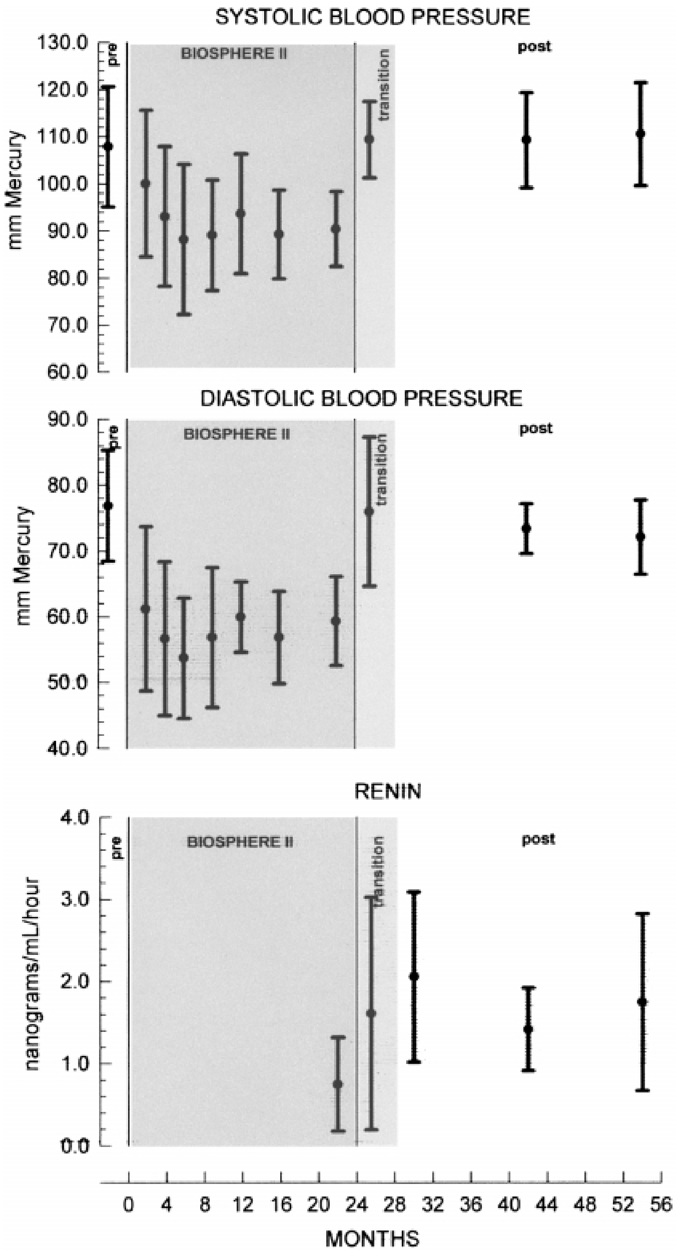

FIG. 2. Systolic and diastolic blood pressure ± SD of crew members before entry, during residence in, and after exit from Biosphere 2. Renin values ± SD during residence in, and after exit from Biosphere 2.

entry, during residence in, and after exit from Biosphere 2. The levels increased during the first 12 to 18 months, then declined, and increased slightly after exit and re-exposure to the outside environment. Figure 4 also shows levels of "total PCBs" in the same 2 crew members. Again, levels tended to be higher inside Biosphere 2 than in the outside environment.

DISCUSSION

Because the crew, when entering Biosphere 2, had not been expected to undergo calorie restriction, pre-entry baseline values were obtained for only a few of the final variables tested. However, the follow-up after exit and return to a normal diet for $2\frac{1}{2}$ years provides, retrospectively, an adequate baseline. Although only 8 subjects were studied, the study is unique, because no volunteers have been so carefully observed under conditions of rigidly monitored calorie restriction since the studies of Keys *et al.* (1950). However, the present study differs fundamentally from that of Keys *et al.*, in that the Biosphere 2 crew members were calorically restricted but well supplied with all essential nutrients. By contrast, the dietary

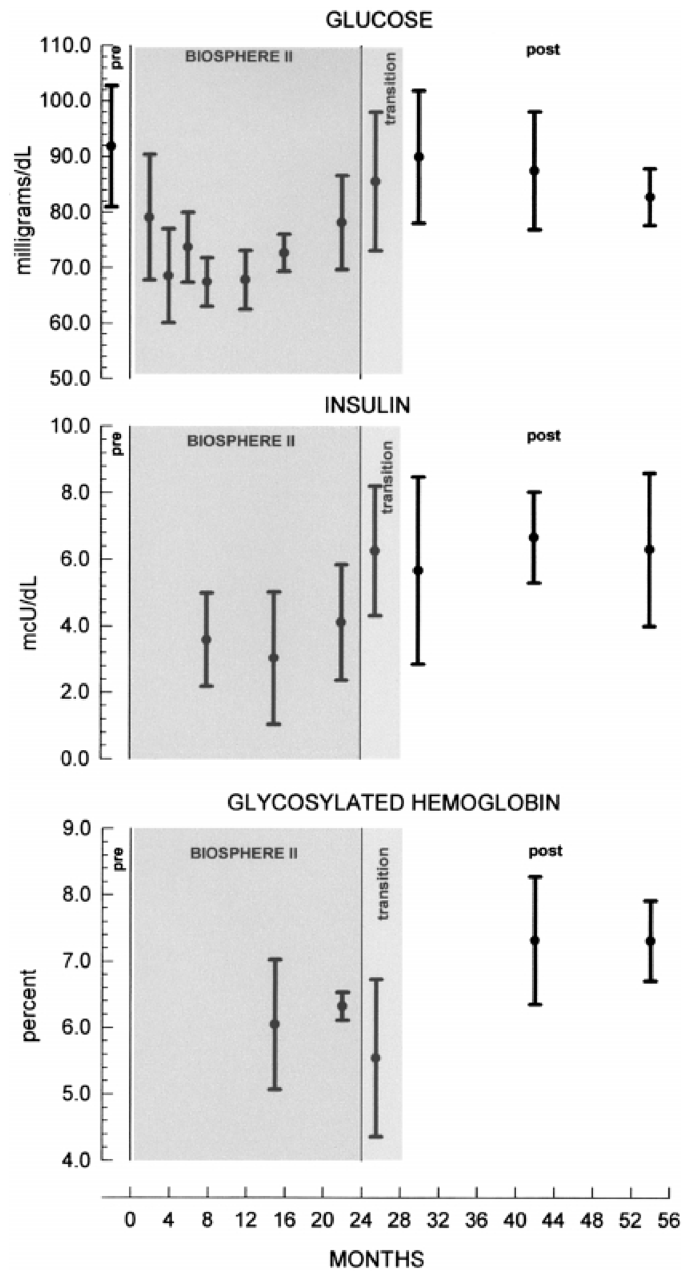

FIG. 3. Values for blood glucose, insulin, and glycated hemoglobin ± SD of crew members during residence in, and following exit from Biosphere 2.

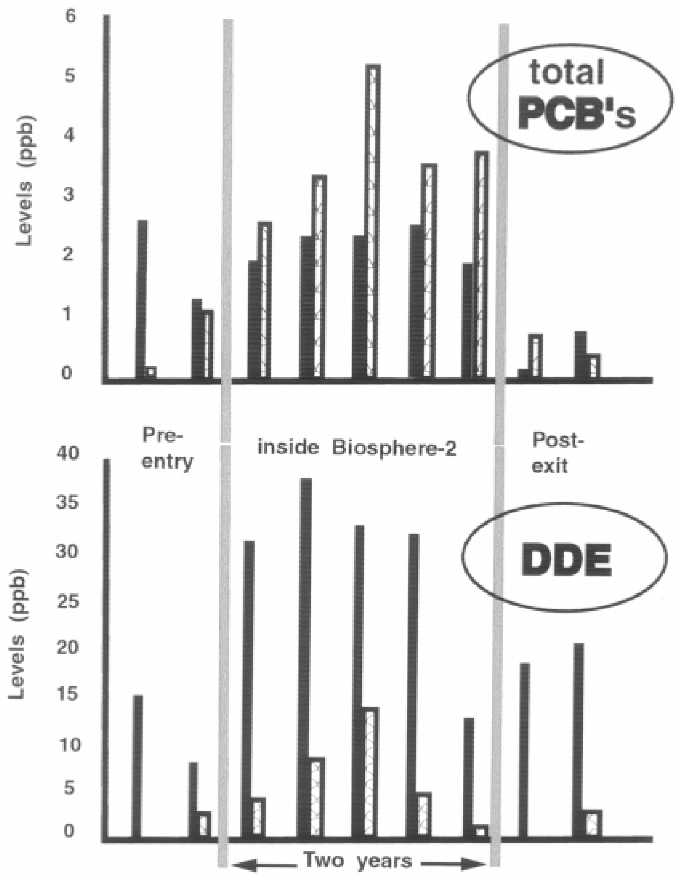

FIG. 4. Levels of DDE and of "total PCBs" in the sera of 2 crew members before entry, during residence in, and after exit from Biosphere 2.

intake of the Keys *et al.* (1950) study was patterned after the post-World War II intake of certain semi-starved European populations, and so was deficient in many nutrient factors other than calories. Furthermore, the period of dietary privation in the study by Keys *et al* (1950) lasted only 6 months, in contrast to the 2-year restriction inside Biosphere 2. Finally, the subjects of Keys *et al.* (1950) suffered lethargy, some mental confusion, weakness, and peripheral edema, i.e., the classic signs of a starving population, and performed no work activities. None of these signs appeared in the Biosphere 2 crew members, who in fact performed extensive physical and mental labor throughout the 2 years.

There have been numerous studies of the effects of low-fat, reduced-calorie intake in the control of obesity, largely focusing upon lipid values (reviewed by Verdery and Walford, 1998). The subjects, for the most part, had been obese, or obese and diseased, generally with cardiovascular disease and/or diabetes. In the study of healthy volunteers by Velthuis-te Wierik *et al.* (1994), 10 weeks of a 20%-reduced calorie intake led to a 10% loss in body weight, with decreases in blood pressure, metabolic rate, and T3. The subjects of the present study were healthy, active, non-obese adults maintained on reduced calories for 2 years.

Beginning with the report on the status of the crew members of Biosphere 2 at the initial 6-month interval (Walford *et al*, 1992), ours is the first study of calorie restriction in humans of a nature that can be directly compared to corresponding observations in rodents (Weindruch and Walford, 1988), and more recently in monkeys (Kemnitz *et al*, 1994; Lane *et al*, 1997).

Calorie restriction in rodents has been shown to increase average and maximum life spans, decrease the incidence and delay the time of onset of most age-related diseases, and alter the physiology of the animals as evidenced by changes in a number of measurable variables (Masoro, 1995; Weindruch and Walford, 1988; Weindruch and Sohol, 1997; Yu, 1995). The major questions of today are (1) Will it do so in humans? and (2) What is the mechanism whereby a *selective* decrease in calorie intake, with other nutrients in adequate supply, has these wide-ranging, global effects? In this communication, we address principally the first question, under the assumption that similarity of changes in the physiologic variables might accord with similar effects on aging and disease. Studies in non-human primates (rhesus and squirrel monkeys), now ongoing in 3 laboratories, will, at completion, yield direct evidence as to an effect of CR on aging in primates (Bodkin *et al.*, 1995; Kemnitz *et al.*, 1994; Lane *et al.*, 1997).

Our data are limited to physical examination and analyses of samples of peripheral blood. With this limitation, numerous comparisons can be made (Verdery and Walford, 1998; Walford *et al.*, 1992). Considering the variations between species, duration, and degrees of calorie restriction, levels of physical activity, and possibly other variables, there is a substantial degree of across-species concordance, including humans.

Calorie restriction in the crew members was associated with substantial weight loss (Fig. 1) and a significant drop in both systolic and diastolic blood pressure, plus a decrease in renin (Fig. 2). Blood lipids are markedly sensitive to calorie restriction, with variations in the Biosphere 2 crew members reported in detail in a prior publication (Verdery and Walford, 1998). These changes are health enhancing in terms of cardiovascular risk factors. Figure 3 reveals that blood glucose, insulin, and glycated hemoglobin were all significantly decreased in the crew members inside Biosphere 2. The same changes have been found in rodents and monkeys, except that in monkeys glycated hemoglobin did not decrease. Leucopenia in response to CR occurs in both rodents and monkeys, and was also found in the humans in Biosphere 2 (Walford *et al*, 1992). In the Biosphere 2 crew members, thyroid-related hormones T3, T4, rT3, and TSH responded similarly to CR in humans as in rodents (Walford, R. L., unpublished observations). Thyroid binding globulin (TBG) was significantly decreased in humans, but has not been studied in other species (Walford, R. L., unpublished observations). To our knowledge, no data are available as to thyroid-related hormones in monkeys.

The little discordance between our findings and those recorded for rodents and monkeys can, in some instances, be attributed to the comparative lengths of time the animals or humans have been on CR, in relation to their characteristic life spans. For example, the age-related increase in calcitonin in rodents, and the age-related decrease in DHEA in monkeys is apparently alleviated by CR in these species. However, since the differences in calcitonin and DHEA between CR and control animals were slow to appear, we interpret them as manifestations of retarded aging rather than as primary (causative) factors. We did not observe differences in DHEA or calcitonin in humans following a 2-year period of restriction (Walford, R. L., unpublished observations). A 2-year period would not, with present techniques, be sufficient to show changes limited to signs of retarded aging.

Our overall data, as far as physical examination and serum chemical changes are concerned, support the supposition of a similar response of rodents, monkeys, and humans to calorie restriction. Our finding of a lowered body temperature in crew members on CR would also support this similarity. Furthermore, the Biosphere 2 study suggests that severe calorie restriction in humans is not detrimental to health, so long as other aspects of nutrition are adequate. In fact, it is health enhancing in terms of a number of risk factors, and calorie-restricted humans can continue to sustain a high level of performance activity even in a challenging environment.

Our finding with the lipophilic toxicants DDE and the PCBs, known to be stored in body fat, is of special interest (Fig. 4). With the prompt (first six months) and severe weight loss of the crew members, these materials appeared substantially increased in the peripheral blood. The DDE level slowly abated during the second year of confinement. Upon exiting from Biosphere 2, and re-exposure to the outside environment, the level of DDE increased slightly. Levels of "total PCBs" also increased in the blood inside Biosphere 2, but clearance was less evident. These combined data suggest that alternating periods of weight loss and weight gain (so-called "yo yo dieting"), with flushing of lipophilic toxicants in and out of the body, may be harmful in terms of internal exposure to toxic substances.

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