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Calorie Restriction in Biosphere 2: Alterations in Physiologic, Hematologic, Hormonal, and Biochemical Parameters in Humans Restricted for a 2-Year Period

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Four female and four male crew members, including two of the present authors (R. Walford and T. MacCallum)—seven of the crew being ages 27 to 42 years, and one aged 67 years—were sealed inside Biosphere 2 for two years. During seven eighths of that period they consumed a low-calorie (1750–2100 kcal/d) nutrient-dense diet of vegetables, fruits, nuts, grains, and legumes, with small amounts of dairy, eggs, and meat (~12% calories from protein, ~11% from fat, and ~77% from complex carbohydrates). They experienced a marked and sustained weight loss of 17 ± 5%, mostly in the first 8 months. Blood was drawn before entry into Biosphere 2, at many time-points inside it, and four times during the 30 months following exit from it and return to an ad libitum diet. Longitudinal studies of 50 variables on each crew member compared outside and inside values by means of a Bayesian statistical analysis. The data show that physiologic (e.g., body mass index, with a decrease of 19% for men and 13% for women; blood pressure, with a systolic decrease of 25% and a diastolic decrease of 22%), hematologic (e.g., white blood cell count, decreased 31%), hormonal (e.g., insulin, decreased 42%; T3, decreased 19%), biochemical (e.g., blood sugar, decreased 21%; cholesterol, decreased 30%), and a number of additional changes, including values for rT3, cortisol, glycated hemaglobin, plus others, resembled those of rodents or monkeys maintained on a calorie-restricted regime. Significant variations in several substances not hitherto studied in calorie-restricted animals are also reported (e.g., androstenedione, thyroid binding globulin, renin, and transferrin). We conclude that healthy nonobese humans on a low-calorie, nutrient-dense diet show physiologic, hematologic, hormonal, and biochemical changes resembling those of rodents and monkeys on such diets. With regard to the health of humans on such a diet, we observed that despite the selective restriction in calories and marked weight loss, all crew members remained in excellent health and sustained a high level of physical and mental activity throughout the entire 2 years.

BIOSPHERE 2 is a 3.15 acre, 7-million ft^3 (0.6-million m^3) closed ecological space near Tucson, Arizona. At the time of the present study it contained five wilderness and two domestic biomes (rain forest, savannah, desert, ocean, marsh, agricultural station, and living quarters), plus a large basement "technosphere." It could be likened to an aircraft carrier with a massive garden on top and extensive mechanical innards below decks. The entire complex rests on a 3/8-in (~0.9 cm) thick stainless-steel plate.

In September 1991, four men and four 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 using an air lock and limited to scientific items, no material entered or left for the 2-year period, except that, for reasons given elsewhere (1), oxygen had to be supplied on two occasions. All organic material was recycled or stored as "carbon banks," all water and virtually all air was recycled, and ~85% of food was raised inside (the rest coming from preentry 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 (1,2).

Before closure a daily intake in excess of 2500 kcal per person had been projected, to be supplied entirely by the agricultural system. In the actual event, however, as a result of crop problems, caloric intake during the first 6 months averaged only 1784 kcal/day, rising then to ~2000 kcal for most of the remaining time period. This intake 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 6–8 months (~21% for the men, 14% for the women), and this weight loss was maintained at that level until the last several months, when slightly more food became available. Despite the relative calorie deficit, 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 corresponded to what has been shown to retard aging, prevent or retard the development of most age-related diseases, and extend average and maximum life spans in rodents, as well as a variety of other species (3). Additionally, animals on such a diet have shown a substantial number of physiologic, hematologic, hormonal, and biochemical changes that for the most part seem to accord with an enhanced health status.

The situation of the Biosphere 2 crew members was recognized to be a serendipitous opportunity to study the responses of humans on such a diet and over a prolonged 2-year period under carefully monitored conditions.

The present report concerns the nutritional, health, and blood-response status of the crew members during the 2 years of closure, plus a 30-month follow-up period that served as a retrospective ad libitum control. Our primary aim was to determine how closely their physiologic, hematologic, hormonal, and biochemical responses resembled those of rodents and monkeys on nutritionally adequate but calorie-restricted diets. In this report we have also given brief emphasis and illustration as to how the large and hitherto mostly untapped knowledge base of clinical medicine may assist interpretation of calorie restriction results in humans.

Methods

Subjects and Examination Protocol

Four men and four women made up the crew of Biosphere 2. Ages at time of closure ranged from 27 to 42 years, except for one member (R. Walford) who was 67. All were in good health and physically active, having participated in the actual construction of Biosphere 2 and in worldwide field expeditions to collect specimens intended for Biosphere 2, and all were nonsmokers. During the 2 years inside Biosphere 2, all sustained physical activity in the agricultural biome, judged equivalent to 3–4 hours daily 6 days per week of heavy-duty manual farming, plus other activities required in the maintenance of a highly complex ecological system were performed by a limited number of people. Many of these activities were strenuous, such as ocean weeding demanding use of Scuba gear, climbing in the 65-ft (~19.8 m) high space frame to prune back excessive vine growth, and handling and repair of heavy equipment. A 70- to 80-hour work week was required of all crew members throughout the 2 years of closure.

All medical data was collected by one of us (R. Walford), who was the medical officer inside Biosphere 2 and also a crew member. Several 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 (blood count, glucose, blood lipids, a liver battery, blood urea nitrogen, uric acid, and creatinine). Beginning 2 weeks after closure, every 2 weeks one subject of each sex received a symptom review, physical examination, urinalysis, and fasting blood analysis (see below), all done by the medical officer inside the enclosure. Thus, each subject was evaluated every 8 weeks. In addition, chest x-rays, electrocardiography, and respiratory spirometry were performed at 6-month intervals. The study protocol was approved by the Human Use Committees of the University of Arizona and the University of California—Los Angeles (UCLA).

Medical Facility

The medical facility inside Biosphere 2 has been described elsewhere in detail (1). It consisted of a minor surgical suite, examination room, well-stocked pharmacy, and small clinical laboratory equipped for a number of hematological, bacteriologic, and clinical chemical analyses.

Laboratory Examination

This consisted of urinalysis performed by dip stick and microscopic examination; red and white blood cell counts, and hematocrits manually; and albumin and total protein, total cholesterol, high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol, triglycerides, blood urea nitrogen (BUN), creatinine, uric acid, alkaline phosphatase, alanine transferase (ALT, SGPT), aspartate aminotransferase (AST, SGOT), serum iron, and ferritin with a Kodak Ektachem DT (Rochester, NY) dry reagent chemistry system (1). Extra material from each bleeding was stored at −70ºC inside Biosphere 2. On three occasions, blood was transported outside Biosphere 2 via an air lock, to either UCLA or University of Arizona clinical laboratories to check the results of the inside determinations, and concordance was noted. In addition, aliquots of serum, plasma, and packed red blood cells were obtained at approximately 7 AM in a fasting state, at intervals from all crew members, tested with the dry reagent system, and frozen. Specimens were also obtained at four time periods up to 30 months following exit from Biosphere 2 and return of the crew to an ad libitum intake. All frozen specimens were later transported to UCLA on dry ice and analyzed at the UCLA clinical laboratories. At least three aliquots of specimens tested inside with the dry reagent system were retested at UCLA, and concordance was noted. For determination of glycated hemoglobin, frozen packed red blood cells were thawed, reconstituted to 2× volume with saline, and subjected to analysis. This gives a somewhat higher reading than found with freshly drawn blood, but comparisons over time remain valid. Additionally, and with the use of results of calorie-restriction studies in rodents as a general guide, specimens were examined or reexamined at UCLA for the following variables: albumin and total protein, alkaline phosphatase, ALT (SGPT), AST (SGOT), cholesterol, HDL, LDL, triglycerides, glucose, insulin, renin, triiodothyronine (T3), thyroxine (T4), reverse T3 (rT3), thyroid binding globulin (TBG), thyroid stimulating hormone (TSH), uric acid, urea nitrogen, aldosterone, androstenedione, estradiol (men only), prolactin, luteinizing hormone (LH; men only), sex hormone binding globulin (SHBG), total and free testosterone (men only), dehydroepiandrosterone sulfate (DHEA-S), growth hormone (GH), insulin-derived growth factor (IGF-1), parathyroid hormone (PTH), total and free cortisol, cortisol binding globulin (CBG), calcitonin, transferrin, iron, total iron binding capacity, and ferritin. Because of limitations in quantities of frozen sera, not all tests could be run at every time point. In general, for any one test on any one individual, when frozen specimens were available, all dates were run at the same time to avoid comparative errors that might occur by daily variations in the test procedure.

Nutritional Intake

The diet was largely but not exclusively vegetarian. Fruits were chiefly bananas and papaya but included smaller quantities of fig, guava, and lemons. Grains were chiefly wheat, rice, and sorghum. Peanuts, split peas, and several types of beans were raised, as well as 25 varieties of vegetables and greens. Bananas, sweet potatoes, and beets formed a significant portion of the carbohydrate source. 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 by means of a computer program (The Interactive Diet Planner, The Longbrook Co., Los Angeles, CA).

Three meals per day were eaten by the crew members, with equal portions given to each individual regardless of size or gender. Although 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 and 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 B12, folic acid, and vitamin D (the enclosure's glass transmitted only a trace of ultraviolet radiation); 400 international units of vitamin E; and 500 mg of ascorbic acid.

Statistical Analysis

Because the eight crew members were of different sizes but each received exactly the same amount of food, a statistical analysis whereby each person served as his or her own control, and the eight sets of comparisons combined by means of a meta-analysis, was considered optimal in assessing the data. A mathematical model using a Bayesian approach was therefore applied to the time series data (4). This approach makes fewer distributional assumptions than the classical statistical method, which would also deal poorly with the relatively small number of data points (i.e., only eight subjects) and inherent human biological variability with responses for effect (5). A meta-analytical Bayesian hierarchical approach served as a joint probability model for the individual effects on each subject for each metabolite. A prior distribution representing a sample of a theoretical whole population was generated by using each subject's individual baseline. Similarly, the posterior effects distribution was generated by considering the individual changes in effects levels compared with that baseline. Finally, the resulting parameters for all the distributions and the range estimations using bootstrapping methods were calculated (6).

A nonparametric additive model using a meta-analytic measure called the Fisher chi-square model was used to rank the likelihood and overall strength of the evidence for the sample population effect change for each metabolite (7). The experimental design involved using each subject as his or her own control and estimating the distributional range of values "inside" and "outside" of Biosphere 2. Values outside included those determined preentry (Figures 1–4), but either (a) considered the "transitional" period of ∼1 month postexit as lying with the outside values, or (b) excluded the transitional period altogether from the analysis. Both of these values were calculated because the speed of change of the parameter when the crew member went abruptly from a restricted (inside) to an ad libitum (outside) diet seemed to vary depending on the parameter being measured. Percent differences in both instances were transformed in an additive model and tested against separate null hypotheses with a chi-square distribution for significance.

Results

Tables 1 and 2 show the food intake and nutrient values averaged from 8 to 9 representative (random) days from each of eight consecutive 3-month periods (64+ days in all) inside Biosphere 2. These are exact (not estimated) values and indicate, for reasons given above, the precise intake of each crew member. The calorie intake over the first seven 3-month periods ranged from approximately 1750 to 2100 kcal. The increase to approximately 2400 kcal during the eighth period was because near the end of the experiment the crew were able to consume material that would otherwise have been set aside for future plantings.

Except for minor ailments described elsewhere (1), the health of the crew remained excellent throughout the 2 years. Only 5 off-work days of illness were recorded for the entire crew. Electrocardiographs, chest x-rays, respiratory spirometry, and urinalyses were normal. Quantitative data for body temperature inside Biosphere 2 are not at hand because the available clinical thermometers were not calibrated for values below 96°F. Before and after exit, average body temperatures of all crew members were in the normal range of 98.6°F; inside Biosphere 2 they were generally as a group in the 96–97°F range, and occasionally below 96°F on an individual basis.

Figures 1–4 show values for body mass index (BMI), systolic and diastolic blood pressures, and 27 of the 47 hematological, hormonal, or chemical parameters examined. Those not shown revealed no significant changes. In Figures 1–4, the Chi-sq.(1) values are the chi-square values for which the transitional values are included with the outside values; for the calculation of the Chi sq.(2) values, the transitional values were simply excluded from the analysis. Table 3 lists the parameters of Figures 1–4 according to the Chi-sq.(1) values in descending order. Those at the top are highly significant (10^{-10} or thereabouts) and those at the bottom may be regarded as borderline ($10^{-1.5}$–10^{-1}).

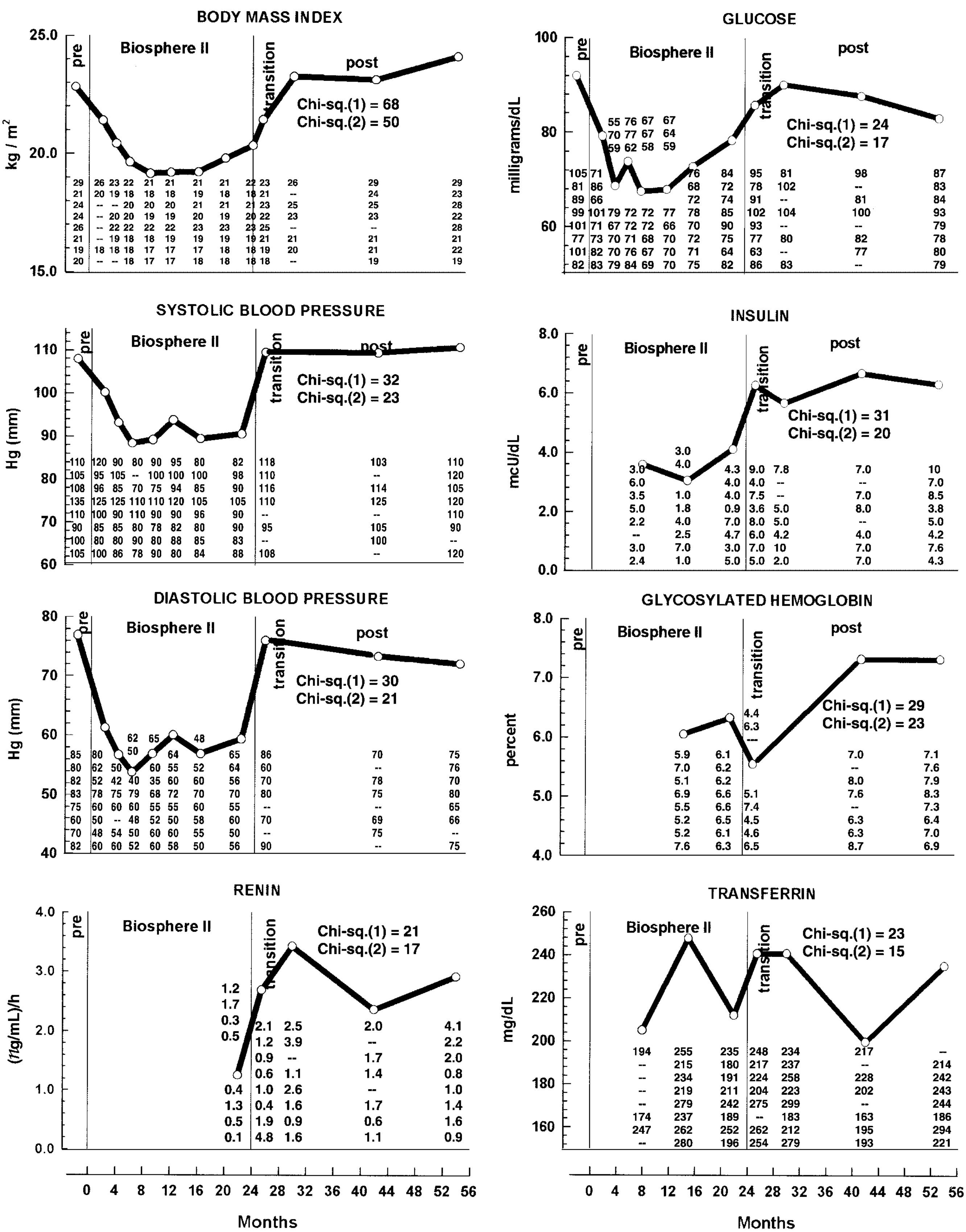

Figure 1. Individual and average values for 8 physiologic, hematological, hormonal, and biochemical parameters on the eight crew members of Biosphere 2 over a 56-month period (includes 2 months preentry). For each parameter, the columns of numbers are positioned so as not to obscure the line of averages, but in each column the first number corresponds to the same person, and so on for the rest. Chi-sq.(1) represents a comparison of all inside with all outside (i.e., preentry, and after exiting from Biosphere 2) values. Chi-sq.(2) excludes the transition value from the analysis.

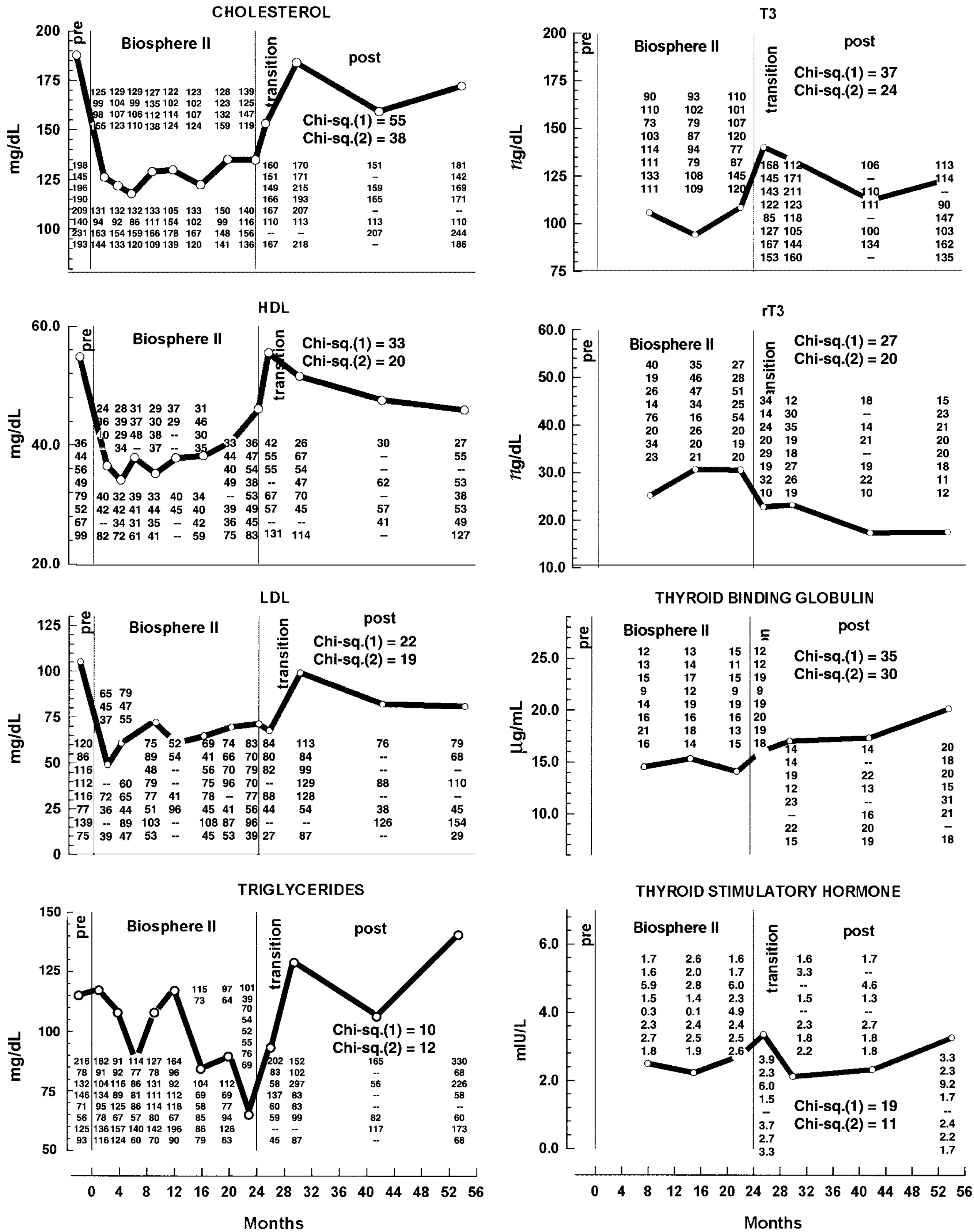

Figure 2. Individual and average values for 8 physiologic, hematological, hormonal, and biochemical parameters on the eight crew members of Biosphere 2 over a 56-month period (includes 2 months preentry). For each parameter, the columns of numbers are positioned so as not to obscure the line of averages, but in each column the first number corresponds to the same person, and so on for the rest. Chi-sq.(1) represents a comparison of all inside with all outside (i.e., preentry, and after exiting from Biosphere 2) values. Chi-sq.(2) excludes the transition value from the analysis. T3 = triiodothyronine; HDL = high-density lipoprotein; LDL = low-density lipoprotein; rT3 = reverse triiodothyronine.

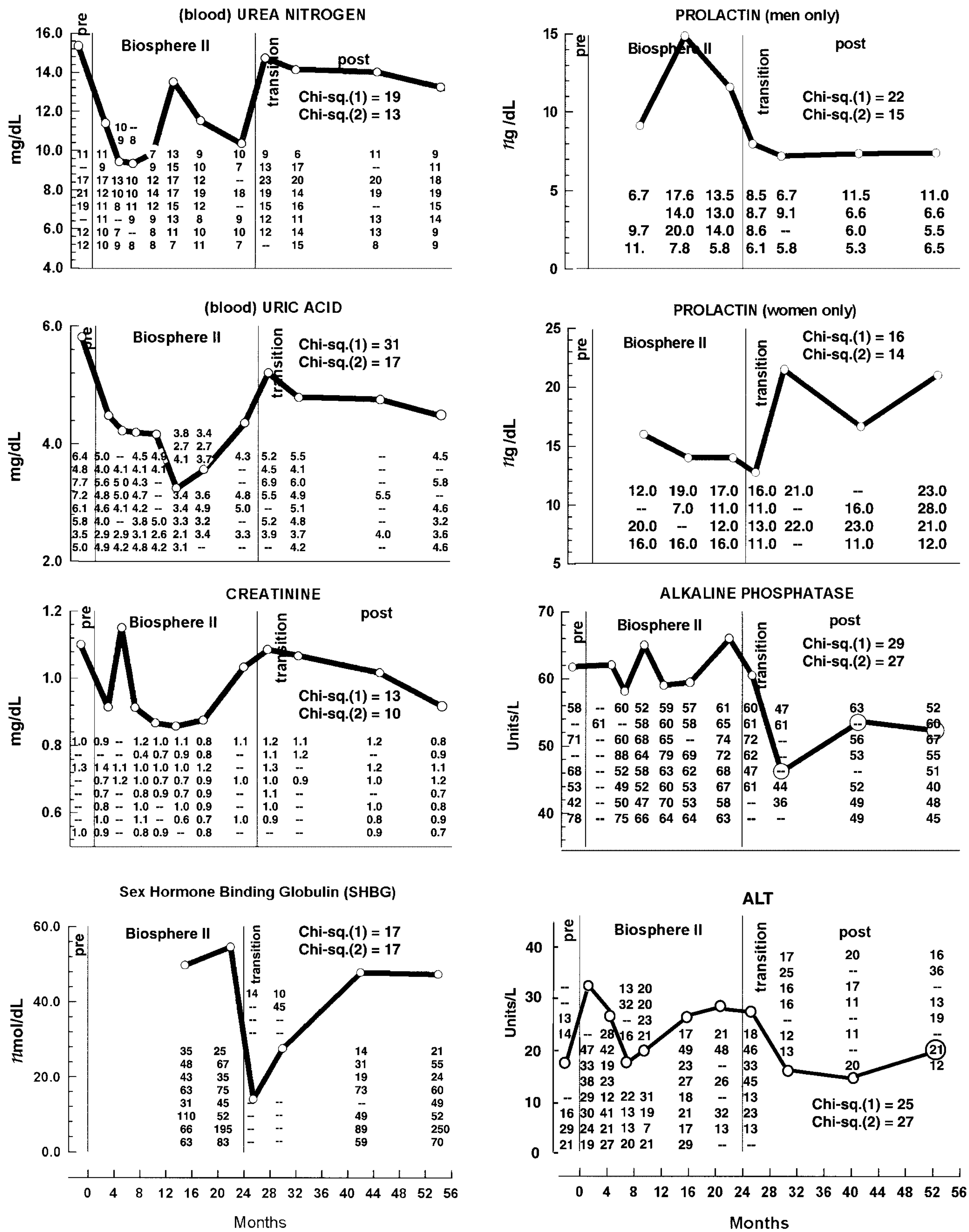

Figure 3. Individual and average values for 8 physiologic, hematological, hormonal, and biochemical parameters on the eight crew members of Biosphere 2 over a 56-month period (includes 2 months preentry). For each parameter, the columns of numbers are positioned so as not to obscure the line of averages, but in each column the first number corresponds to the same person, and so on for the rest. Chi-sq.(1) represents a comparison of all inside with all outside (i.e., preentry, and after exiting from Biosphere 2) values. Chi-sq.(2) excludes the transition value from the analysis. ALT = alanineaminotransferase.

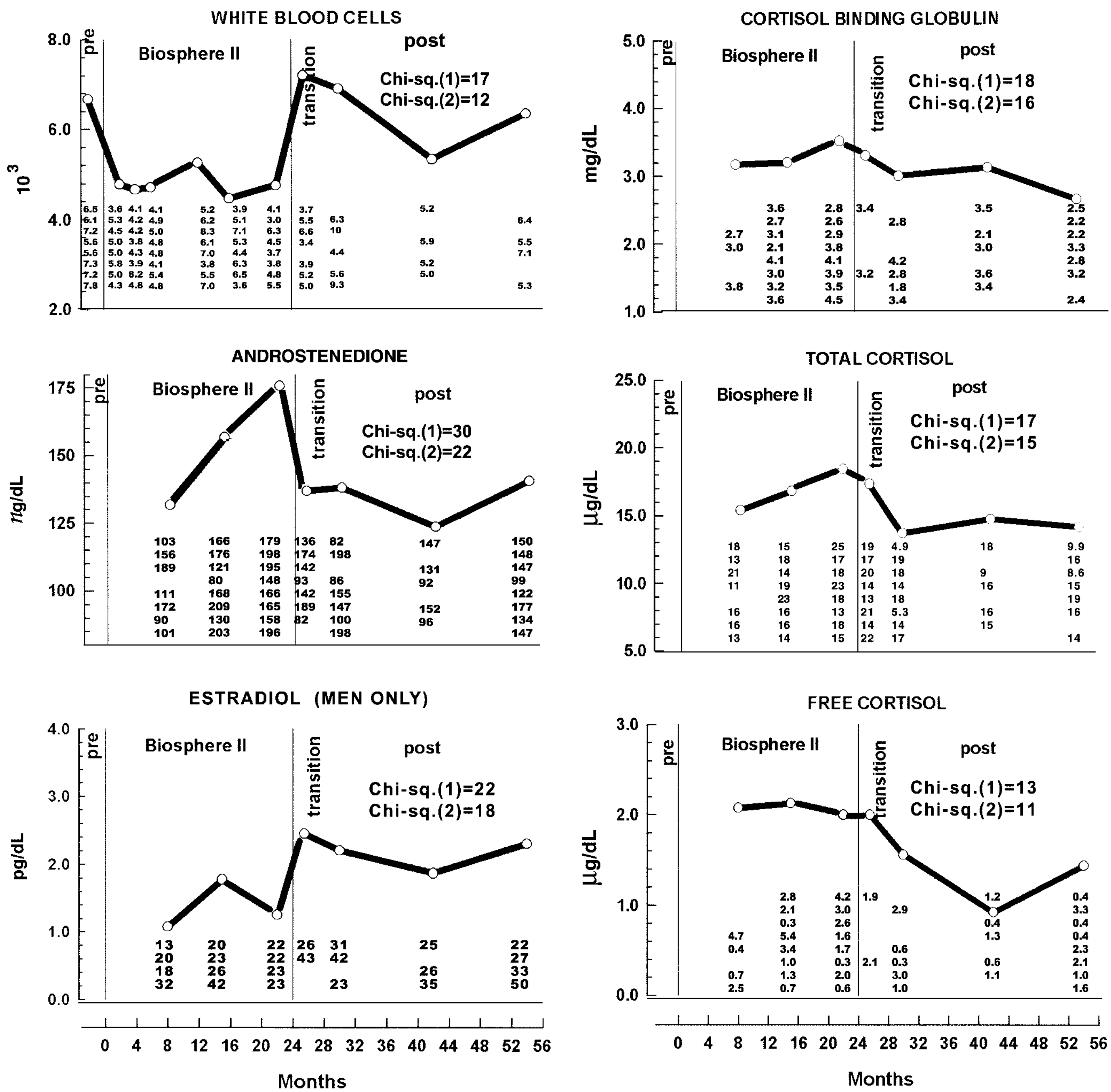

Figure 4. Individual and average values for 6 physiologic, hematological, hormonal, and biochemical parameters on the 6 crew members of Biosphere 2 over a 56-month period (includes 2 months preentry). For each parameter, the columns of numbers are positioned so as not to obscure the line of averages, but in each column the first number corresponds to the same person, and so on for the rest. Chi-sq.(1) represents a comparison of all inside with all outside (i.e., preentry, and after exiting from Biosphere 2) values. Chi-sq.(2) excludes the transition value from the analysis.

BMIs of the crew before, during, and after their stay in Biosphere 2 are shown as the first item of Figure 1. The BMI of the men decreased by 19%, from 23.7 ± 1.8 kg/m^2 to 19.3 ± 0.9 kg/m^2, and the BMI of the women by 13%, from 21.2 ± 1.5 kg/m^2 to 18.5 ± 1.2 kg/m^2 during calorie restriction in Biosphere 2. With the food intake equal for each person, the amount of weight lost was roughly proportional to the size of the person. In the order of preentry, prerestriction weights, the maximum weight loss within the first 8–12 months was 208 → 150, 165 → 140, 150 → 120, 148 → 119, 148 → 125, 130 → 111, 123 → 109, and 110 → 98 lb (where 1 lb = 0.453 kg). Lost weight was completely restored upon return to an ad libitum diet, and the regained weight was largely due to an increase in fat mass (8). As shown also in Figure 1, there occurred a prompt and substantial decrease in both systolic (by ~25%) and diastolic (22%) blood pressures for the crew members inside Biosphere 2, reverting to preentry levels soon after return to an

Table 1. Food Type and Quantity Eaten by Each Crew Member per Day

Food	Period 1st	2nd	3rd	4th	5th	6th	7th	8th
Fruit								
Banana	233	218	346	197	256	366	438	340
Fig	3	45	13	32	4			
Guava	3				9	6	3	
Lemon	2	2	15	2	30	5	21	10
Kumquat		2	1			1		
Papaya	104	91	160	231	233	100	107	143
Nuts								
Peanuts	18	21	14	15	9	17	18	26
Grains								
Rice	59	56	37	73	57	48		30
Sorghum	5			17	31	26		20
Wheat	100	111	145	107	114	85	92	133
Millet							12	6
Legumes								
Green beans			2	29	18			21
Hyacinth beans	7	34	42	26	11	4	15	19
Pinto beans	7	3			6	89	36	25
Red beans				2	7		50	
Soybeans	11				4	20	53	20
Split peas	17	5						
Meat, dairy, and eggs								
Chicken		13						
Pork		8	13	16			11	15
Goat	8			8	10			12
Fish (tilapia)	7	3						
Goat milk	103	79	75	146	136	99	95	166
Eggs	6	4	2	3	3		6	
Vegetables								
Beets	131	269	34			10	209	140
Beet greens	56	17				29	50	27
Bok choy				11	13			
Cabbage	28	94					56	34
Carrots	7	42	19	27		25	48	78
Chard	52	25	55	24	53	30	110	24
Corn		5			7			
Cucumber			5			5	35	34
Eggplant	74	25	8	33	46	27	9	48
Herb greens	4	4	6	4	4	5	4	5
Jicama		2	7	10				
Kale	4	8	16					
Lettuce (romaine)	39	62	19	21	46	30	46	27
Onions	10	88	105	33	20	12	7	13
Parsley	2	2	2	2	2	2	2	2
Pepper								
green	11	20	27	31	71	40	34	20
red	2	1					24	45
Potato								
sweet	313	110	240	512	472	318	188	538
greens, sweet			11	81	98	19		
white	49	224	143				18	
Radish	4		2	2		22	7	
Squash								
summer	93	177	161	203	248	29	38	140
winter			18	36	34			
Taro	26	33	70			15	26	13
Tomato	54	182	131	116	33	7	35	27

Note: There were eight consecutive 3-mo periods for 2 y inside Biosphere 2. Each column represents an average of 8–9 random days during the 3-mo period. Values are in grams.

ad libitum diet. The alterations in blood pressure were reflected by changes in renin (Figure 1).

Other values were as shown in Figures 1–4. It is noteworthy that by 6–8 months inside Biosphere 2, fasting blood sugar had decreased by 21%, fasting insulin by 42%, cholesterol by 30%, white blood cell count by 31%, and T3 by 19%. Of the 50 variables studied in this report, seven have been reported elsewhere in terms of average values ± standard error (8–10) but are repeated here to give individual values, to assemble all data in one publication, and to subject them to a uniform and more contemporary (Bayesian) statistical approach, as outlined above, than heretofore employed. The changes in the oldest crew member (67 years at time of entry), who might be considered in terms of age as an "outlier," the next oldest being 42 years of age at time of entry, in fact paralleled those of the rest of the crew.

Table 4 lists all physiologic, hematologic, hormonal, and chemical variables for which the crew were examined, comparing inside and outside values. The arrows in the column labeled "This Study" indicate direction (but not magnitude) of significant change inside Biosphere 2 compared with outside values (before or after residence), plus variables that were not changed by the low-calorie fare. For comparison with the animal (rodent and monkey) data, Table 4 also contains arrows indicating direction of significant change, or of no change, from relevant literature of calorie restriction in these species. This is a representative, not an exhaustive, citation. Furthermore, in Table 4 we have not distinguished between actual (usually prompt) elevation or decline in the parameter listed and merely the slower rate of change that might result from decelerated aging, so that eventually the parameter is different from normal values for that age and species. DHEA-S is an example of such a parameter.

Discussion

This study represents the seizure of a serendipitous opportunity. Because the crew entering Biosphere 2 were not expected to undergo calorie restriction, preentry baseline values for only a few of the final variables tested were obtained. However, the follow-up after exit and return to a normal diet for 30 months provides retrospectively an adequate baseline. Although the physical exertion required to maintain Biosphere 2 may have contributed to the effects noted, exercise alone does not cause changes approaching the magnitudes encountered in this study (73). Although only eight subjects were available, our study is unique because no normal, nonobese volunteers have been so carefully observed under conditions of long term, rigidly monitored calorie restriction since the seminal studies of Keys and colleagues (74) following World War II. Our study differs fundamentally from that of Keys in that the Biosphere 2 crew members were calorie restricted but well supplied with all other essential nutrients (Tables 1 and 2). The dietary intake used in the Keys studies was patterned after the postwar intake of certain semistarved European populations and may have been deficient in many nutrient factors other than calories. (The Keys report is not very clear on this issue.) Also, the period of dietary deprivation in the Keys investigation lasted only 6 months, in contrast to the 2 years

Table 2. Nutrient Contents of the Daily Food Intakes Represented in Table 1

Nutrient Content	Period							
	1st	2nd	3rd	4th	5th	6th	7th	8th
Calories, kcal	1735	1834	1926	2109	2017	1914	2107	2402
Protein, g	54	62	59	67	63	64	84	81
% cal from protein	12%	13%	12%	12%	12%	13%	15%	13%
Total fat, g	23	24	20	25	21	25	33	36
% cal from fat	12%	11%	9%	10%	9%	11%	13%	13%
Carbohydrate, g	351	369	408	429	424	386	404	471
% cal from carb.	76%	76%	80%	77%	79%	76%	72%	75%
Fiber, g	33	50	40	42	38	51	62	56
Vit. A, % RDA	1439	805	1222	2332	2050	1511	1239	2744
Vit. B6	191	211	241	219	226	221	278	263
Vit. B12	18	13	8	17	12	3	10	18
Vit. C	524	642	675	788	926	534	728	865
Vit. E	210	139	199	307	288	213	196	333
Thiamine	152	175	172	197	181	170	210	215
Folacin	281	455	340	368	357	436	635	490
Riboflavin	118	104	114	158	158	131	168	173
Niacin	122	161	141	155	142	116	124	162
Pantothenic acid	144	143	143	172	159	130	139	177
Calcium	76	72	74	86	86	73	99	99
Copper	153	153	200	143	140	150	207	185
Iron	198	184	245	169	182	201	304	233
Magnesium	180	196	199	195	196	189	257	220
Manganese	384	393	447	394	384	331	365	427
Phosphorus	178	209	208	219	209	205	242	252
Potassium	283	346	319	303	310	288	415	358
Selenium	57	40	43	42	50	69	74	51
Sodium	104	95	62	57	63	54	131	92
Zinc	63	71	68	71	67	65	85	86

Notes: Values from Vitamin A downward are expressed as % RDA. The RDAs given here represent averages of values for medium-framed men and women, ages 25–50 y, given by The National Research Council, *Recommended Dietary Allowances*, 10th ed.

of restriction inside Biosphere 2. The Keys diet contained only 1500 kcal, whereas the Biosphere 2 diet ranged from 1800 to ~2100 kcal (Table 2). The subjects of the Keys report (all male) suffered lethargy, mental confusion, weakness, and peripheral edema, that is, the classic signs of a starving malnourished population, and they 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 (1,2). The degree of physical activity is reflected by the fact that even on the 1750–2100 kcal daily intake, the lightest woman, weighing in at 110 lb (~50 kg), lost 12 lb (~5.5 kg), whereas the heaviest man, weighing in at 208 lb (~94 kg), lost 58 lb (~26 kg). (It is also noteworthy that despite the severe weight loss, none of the four women crew members missed a menstrual period.)

In a short-term study of healthy volunteers by Velthuis-te Wierik and colleagues (19), 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. Apart from this, plus our own investigations, to our knowledge no prior clinical studies of comparable calorie intake restriction on normal, healthy nonobese persons have been reported. There have, indeed, been numerous, usually short-term studies concerning the effects of low-fat and reduced calorie intake in the control of obesity, largely focusing on lipid values (see 9 for a review). The subjects have for the most part been obese, or obese and diseased, generally with cardiovascular disease, diabetes, or both.

Beginning with the report on the status of the crew members of Biosphere 2 at the initial 6-month interval (15), ours is the first appraisal of calorie restriction in humans of a nature that can be directly compared to observations in rodents (3), and more recently in monkeys (15,41,72). Furthermore, because the variety of clinical laboratory tests that can be performed on peripheral blood is greater and better standardized for humans than for any other species, our data contain not only items that can be compared to rodent and monkey data but also a number of parameters not hitherto examined (Table 4). For reasons given in prior studies (8,9), it seems that the changes reported here were largely attributable to the calorie restriction, and that the low-fat nature of the diet (Table 2) played at most a minor role.

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 (3, 75–77). Among the major questions of today are (a) Will it do so in humans?, (b) If properly followed, is it hazardous to health and function in humans?, and (c) What is the mechanism whereby a selective decrease in calorie intake, with other nutrients in adequate supply, exerts such wide-ranging, global effects?

Table 3. Fisher's Inverse Chi-Squared Values for Parameters Significantly Altered by Calorie Restriction

Parameter	Chi-sq.(1)	Chi-sq.(2)
Body mass index	68.3	49.9
Cholesterol	54.5	42.3
Triiodothyronine	37.4	24.0
Thyroid binding globulin	35.4	30.3
High-density lipoprotein	32.8	20.0
Systolic blood pressure	31.9	23.2
Insulin	31.5	20.1
Uric acid	31.1	17.0
Diastolic blood pressure	30.5	20.5
Androstenedione	29.5	22.5
Glycosylated hemoglobin	28.7	23.2
Alkaline phosphatase	28.6	26.8
Alanine transferase	27.2	25.0
Reverse triiodothyronine	26.9	19.8
Glucose	24.0	16.9
Transferrin	22.6	14.7
Low-density lipoprotein	22.5	19.4
Estradiol (men)	22.0	17.8
Prolactin (men)	21.7	14.6
Renin	21.0	17.1
Blood urea nitrogen	19.3	13.1
Thyroid stimulating hormone	18.7	10.9
Cortisol binding globulin	17.9	15.9
White blood cell count	17.4	12.2
Sex hormone binding globulin	17.0	17.0
Total cortisol	16.9	14.8
Prolactin (women)	16.4	13.9
Free cortisol	13.3	11.0
Creatinine	13.1	9.7
Triglycerides	11.7	10.1

Note: Chi-sq.(1) are the transition values included with the outside values; Chi-sq.(2) are the transition values excluded from the analysis.

Our data and the experience inside Biosphere 2 reflect principally upon the first two of these questions.

Whether calorie restriction will retard aging in humans cannot be answered definitively short of very long-term (life-long) studies of a cohort of humans on a calorie-restriction regime. Such a study has been ongoing but only for ~4 years by means of an Internet calorie-restriction group of volunteers (B. Delaney and R. L. Walford, unpublished data, 2001). Studies in nonhuman primates (Rhesus and squirrel monkeys) subjected to calorie restriction, now ongoing in four laboratories, will be at completion much sooner, although still not for a period of years (41–43, 78); however, they are beginning to show evidence of a decreased incidence of age-related diseases.

Based on the data of Biosphere 2, what can be said at present about the potential effects of calorie restriction in humans? Our data are limited to physical examination and to analyses of samples of peripheral blood. With this limitation, numerous comparisons can be made (Table 4). Considering the variations between species, duration and degrees of calorie restriction, levels of physical activity, and possibly other variables, we find that there is a substantial degree of across-species concordance, including humans.

Blood lipids are markedly sensitive to calorie restriction, with variations as defined in the literature and illustrated in Table 4. The lipid changes are health enhancing in terms of cardiovascular risk factors. Although both LDL and HDL declined in the Biosphere 2 crew members, the LDL:HDL ratio underwent a significant decline, and in some crew members, levels of HDL_2 were increased (9), as has been recorded also in calorie-restricted monkeys (63,72).

Reference is made to Figures 1–4 for the following observations. Insulin, blood glucose, and glycated hemoglobin were all significantly decreased in the crew members inside Biosphere 2. Similar changes have been found in rodents and monkeys (Table 4), except that in monkeys glycated hemoglobin has not been shown to be decreased (35,72). Leucopenia in response to calorie restriction occurs in all the species illustrated, including humans.

In the present study of humans the thyroid-related hormones, T3, T4, and rT3, responded similarly to calorie restriction as in most studies of other species (Figure 2 and Table 4). TSH decreased somewhat in the calorically restricted crew members, whereas rodents have been reported to show no change in this variable (Table 4) (3,45). TBG was significantly decreased in the Biosphere 2 crew members (Table 4) but has not been studied in other species on calorie restriction.

In short-term (~6 weeks) calorie restriction or fasting of normal or obese humans, the subjects remain euthyroid with normal TSH and T4 values, whereas T3, the metabolically active hormone, decreases, and rT3, an inactive form, increases (see 79 for review). Experiments in humans completely fasted for 4 weeks indicated that calorie deprivation may shunt peripheral T4 metabolism from activating (T3) to inactivating (rT3) pathways; hence the decrease in T3 and increase in rT3 (80). These changes contribute to the decreased metabolic rate seen in short-term fasting. Our new finding in this regard is that in calorie-restricted humans, the alterations in thyroid hormones are maintained for much longer periods (well over 1 year) than have been previously described.

Certain so-called nonthyroidal systemic conditions (infectious disease, surgery, trauma, some metabolic disorders, etc.) may show alterations in thyroid hormones with no other evidence of thyroid disease (79). These illnesses are generally characterized by normal T4, normal TSH, decreased T3, and increased rT3. In contrast to the fasting state, the increased rT3 results from decreased clearance. The patients are considered to have altered thyroid metabolism but to be euthyroid; they have no clinical evidence of hypothyroidism. The low T3 state might be considered a form of "adaptive hypothyroidism" in that evidence from measurements of enzyme markers suggests that the low T3 in these illnesses reflects a special condition in which thyroid status varies from one tissue to another and involves alternate routes of T3 metabolism (79,80).

Our cortisol values reflect only early-morning-drawn specimens, whereas those summarized in Table 4 in rodents (as corticosterone in these species) and monkeys mostly represent diurnal studies, and demonstrate an increase for rodents, compared with controls, later in the daily cycle; but there is no increase in monkeys (25,72). We found definite increases in early-morning total cortisol and CBG in the crew members, and a marginal increase in free cortisol (Figure 4). Free cortisol has been reported to be increased in ro-

Table 4. Variables for Which the Crew Was Examined

Variable	This Study	Nonprimate Mammals (chiefly rodents)	Monkeys	Humans
Blood pressure				
Systolic	↓	↓ (11–13)	↓ (14)	↓ (15)
Diastolic	↓	↓ (11–13)	↓ (14)	↓ (15)
Hematologic indices				
Hematocrit	↔	↔ (3)		
Total WBC count	↓	↓ (3,16,17)	↓ (16,18)	↓ (15,19)
Hormones				
Aldosterone	↔			
Androstenedione	↑			
Calcitonin	↔	↓ (20)		
Cortisol				
total	↑	↑ (21–24) as corticosterone	↔ (25,72)	
free	↑	↑ (22–24)		
binding globulin	↑	↓ (23,26)		
DHEA-S	↔		↓ (27,28) ↔ (72)	
Estradiol	↓ (in men)		↔ in females (27)	
FSH	↔	↓ (3)	↔ in females (29)	
Growth hormone	↔	↓ (30–32,37,56) ↑ (33,34)	↓ (35)	
IGF-1	↔	↓ (30,31,34,36–38)	↓ (27,39)	
Insulin	↓	↓ (27,31,32,40,41)	↓ (25,43,72) ↔ (42,44)	
Luteinizing hormone	↔	↓ (3)	↔ in females (29)	
Prolactin	↑ (in women)	↓ in females (45–47)		
	↓ (in men)	↔ in males (47)		
Parathyroid hormone	↔	↓ (3,16,48) ↔ (49,50)	↔ (29,33,51,52)	
Renin	↓			
SHBG	↑	↔ (3)		
Testosterone, total	↔	↓ (45) ↔ (3) ↓ (24)	↔ (53)	
Testosterone, free	↔			
Thyroid-related				
triiodothyronine	↓	↓ (3,16,54–56)	↔ (57,72) ↓ (58)	↓ (8)
thyroxine	↔	↔ (3,54)	↓ (57) ↔ (58)	↔ (8)
rT3	↑	↑ (3,16)		
TBG	↑			
TSH	↓	↔ (3,45)		
Lipids				
Cholesterol	↓	↓ (3,59,60)	↔ (43,61,62)	↓ (9,15,19)
Lipoprotein				
high density	↓	↓ (60)	↔ (61,62)	↑ (9,19)
low density	↓		↔ (61,62)	↓ (9,19)
Triglycerides	↓	↓ (3,59)	↓ (61–63) ↔ (43)	↓ (9)
Miscellaneous				
Albumin	↔			
Blood urea nitrogen	↓		↓ (64)	
Calcium	↔	↔ (50)	↔ (51)	
Creatinine	↓	↓ (49) ↔ (50)		
Enzymes				
alkaline phos.	↑	↑ (65)	↑ (51) ↔ (64)	
ALT (SGPT)	↑	↑ (66)	↑ (64)	
AST (SGOT)	↔	↑ (65)	↑ (64)	
Glycated hemoglobin	↓	↓ (67)	↔ (22,44)	
Glucose	↓	↓ (59,67–69)	↓ (16,25,41,72) ↔ (44)	↓ (15)
Iron-related				
serum iron	↔	↓ (70)		
ferritin	↔			
TIBC	↔			
transferrin	↑			
Protein, total	↔	↓ (66)		
Uric acid	↓	↓ (3) ↑ (71)	↓ (64)	

Notes: Relevant literature is given parenthetically. WBC = white blood cell; DHEA-S = dehydroepiandrosterone sulfate; FSH = follicle stimulating hormone; IGF-1 = insulin-derived growth factor; SHBG = sex hormone binding globulin; rT3 = reverse triiodothyronine; TBG = thyroid binding globulin; TSH = thyroid stimulating hormone; ALT (SGPT) = alanine transferase; AST (SGOT) = aspartate aminotransferase; TIBC = total iron binding capacity; ↓ = decreased by calorie restriction; ↑ = increased by calorie restriction; ↔ = unchanged by calorie restriction.

dents on calorie restriction, but it was not examined in restricted monkeys; to our knowledge CBG has not been examined in other species on calorie restriction.

Prolactin was increased among the male crew members and decreased among female crew members (Figure 3). Similar findings have been reported in rodents (Table 4). LH was normal in the crew members; it has been so reported in monkeys, but elevated in calorically restricted rodents (Table 4).

Androstenedione was increased among the Biosphere 2 crew members of both sexes (Figure 4). In premenopausal women an inverse relationship between calorie intake and levels of plasma androstenedione has been reported (81). In a cross-sectional study of 2300 middle-aged men, Field and colleagues (82) found that low weight was associated with higher levels of androstenedione and was generally unrelated to free testosterone levels. The Biosphere 2 male crew members demonstrated no alterations in total or free testosterone. Both sexes showed an increase in SHBG. Serum estradiol was decreased in male crew members; it was not tested in women (Figure 4). To our knowledge, there have been no prior long-term longitudinal studies of androgen levels in humans on a weight loss regime, but the Biosphere 2 experience is consistent with the previous short-term studies. Findings to date in other species on calorie restriction are illustrated in Table 4.

IGF-1 has been reported to be decreased by calorie restriction in both rodents and in one out of three studies in monkeys, and GH has been reported to be, in most studies, decreased (Table 4). We found no significant changes in these variables (only early morning GH levels were measured) in restricted humans. These are the only items that seem discordant with most of the reports in other species. We note that in the higher levels of physical fitness, in older persons with reduced abdominal fat, the 24-hour GH concentrations overlap those of young adults (48). It is possible therefore that the high level of physical exertion required to maintain Biosphere 2 may have influenced these variables in the calorie-restricted crew members.

The decrease in BUN and creatinine observed in the crew members, and the increases in alkaline phosphatase and ALT, resemble findings in other species on a calorically restricted regime (Table 4). Uric acid, significantly decreased in the crew members, has been reported as increased in rodents on calorie restriction but decreased in monkeys (Table 4).

The few discordances between our findings and those recorded for rodents and monkeys on calorie restriction can in some instances be attributed to the comparative lengths of time the animals or humans have been on this restriction in relation to their characteristic life spans. For example, the age-related increase in calcitonin in rodents, and the age-related decrease in DHEA-S in some studies in monkeys, is apparently attenuated by calorie restriction. However, because differences in calcitonin and DHEA-S between calorically restricted and control animals have been slow to appear, we interpret them as manifestations of retarded aging rather than as being primarily influenced by calorie restriction. Therefore, our failure to note differences in humans following a 2-year period of restriction would not be contradictory to the rodent–monkey findings.

For illustrative purposes, Figure 5 compares photographs of the senior author (R. Walford) taken after 15 months resi-

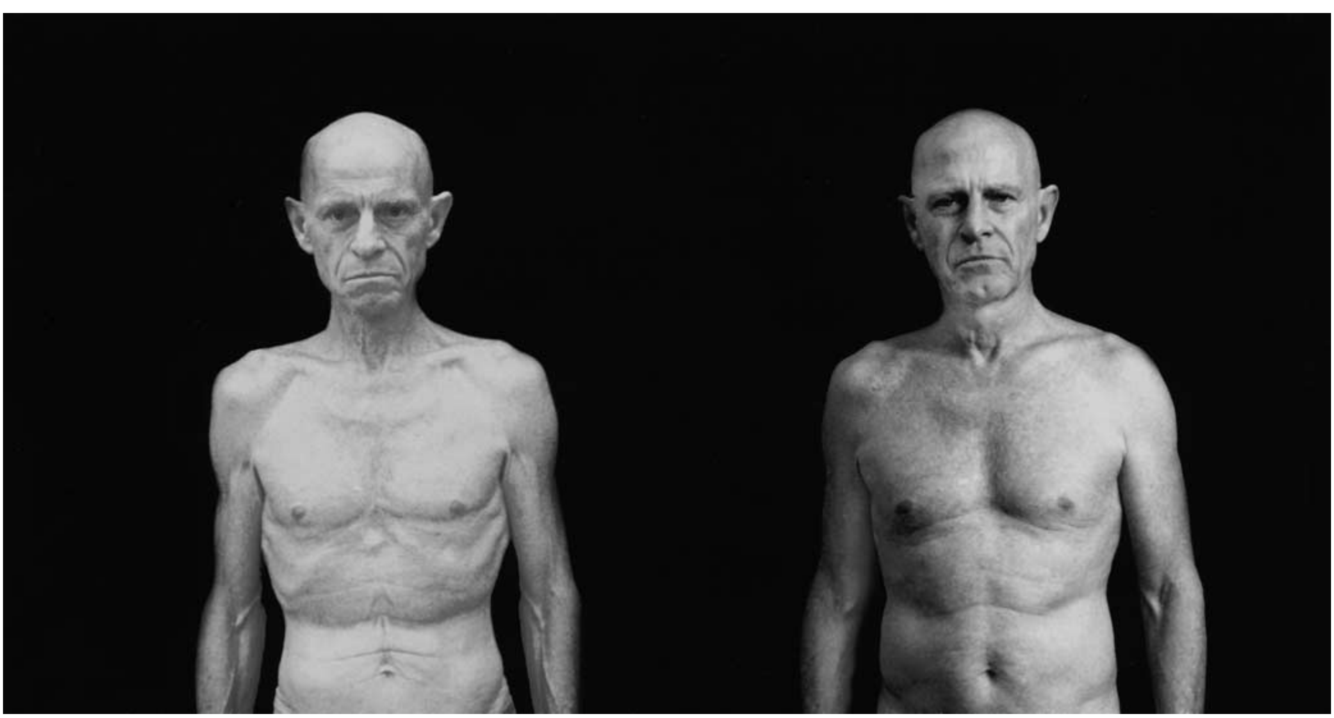

Figure 5. Composite photograph of the senior author (R. Walford) after 15 months residence inside Biosphere 2 (on the left: weight 119 lb or ~54 kg), and 18 months after exiting Biosphere 2 (on the right: weight 150 lb, or ~68 kg; normal weight when on an ad libitum diet).

dence inside Biosphere 2 (on the left: weight 119 lb, or ~54 kg), and 18 months after exiting Biosphere 2 and consumption of an ad libitum diet, to serve as a retrospective control (on the right: weight 150 lb, or ~68 kg, his normal weight).

In summary, we believe our overall data, insofar as physical examination and blood changes are concerned, support the supposition of a similar response of rodents, monkeys, and humans to calorie restriction. Our observations of lowered body temperatures in calorically restricted crew members would also support this similarity (10). Our analysis suggests that the changes in calorie restriction reflect a complex adaptive response that merits further study (83), with humans offering some interpretive advantages over other species because of the large database of clinical medicine. The Biosphere 2 experience strongly suggests that moderately severe calorie restriction in humans (Figures 1 and 5) is not detrimental to health as long as other aspects of nutrition are adequate, but in fact is health enhancing in terms of a number of risk factors, and that calorie-restricted humans can sustain a continuous high level of performance activity even in a challenging environment.

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