Changes in Plasma Lipids and Lipoproteins in Humans During a 2-Year Period of Dietary Restriction in Biosphere 2

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Background: A cohort study was performed of 8 people sealed inside Biosphere 2 to evaluate the effects of dietary restriction in humans on lipid and lipoprotein levels and the relationship of these levels to energy, fat, and protein content of the diet, and body weight, weight change, and energy expenditure.

Methods: Eight healthy people aged 27 to 67 years, 4 women and 4 men, were sealed inside Biosphere 2 from September 26, 1991, to September 26, 1993, the longest sustained period in an “isolated confined environment” on record. They were studied throughout confinement and for more than 2 years after their exit and return to an ad libitum diet. Food available was severely restricted during most of the 2-year period inside Biosphere 2. High work output was maintained and food quality remained high, resulting in prolonged restriction of energy intake without malnutrition.

Results: Fasting plasma cholesterol, triglyceride, and high-density lipoprotein (HDL) cholesterol levels; HDL subfraction distribution; dietary energy, fat, and protein content intake; and height, weight, weight change, and energy expenditure were measured. Total plasma cholesterol and triglyceride levels decreased 30% and 45%, respectively. The HDL and low-density lipoprotein levels also decreased and, in some participants, levels of HDL2 subfractions were increased. Multivariate analysis showed that the major cause of these changes was energy restriction.

Conclusions: Energy restriction was the major factor leading to low lipid and lipoprotein levels. Energy restriction with adequate nutrition of young and middle-aged people may substantially reduce risk for atherosclerosis and consequent coronary artery and cerebrovascular disease.

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SUBJECTS AND METHODS

SUBJECTS

All 8 participants in the initial Biosphere 2 experiment were included in the follow-up study. Ages ranged from 27 to 67 years at the time of entry into Biosphere 2 on September 26, 1991. Methods to assess health status, including periodic physical examinations, routine laboratory evaluations, chest radiographs, and electrocardiograms, have been previously described.4,5 All participants were nonsmokers and were clinically healthy throughout the 2 years of confinement in Biosphere 2 despite the significant weight loss and metabolic changes described below. No unusual medications were taken except by 1 woman who took thyroid replacement at a stable dose. Throughout the time of study this subject was clinically euthyroid, and analysis of the data with this participant excluded did not change the conclusions. Informed consent was obtained for the measurements reported herein, following guidelines of the Human Subject Review Committees at both the University of Arizona, Tucson, and the University of California, Los Angeles.

DIET

Eighty-five percent of all food eaten was grown within Biosphere 2 during the 2 years of closure, and 15% was from food grown there before closure and stored before September 21, 1991. During the final 2 to 3 months, this storage depot was consumed in addition to what was available from current production. Thus, the first 20 to 21 months constituted the period of greatest energy restriction. Foods available in Biosphere 2 on representative days have been listed elsewhere.3 The diet was unique in the large quantities of green and yellow vegetables, including tubers, that were consumed. Animal products were limited to approximately 1 egg, 112 g of meat, and about 300 mL of goat milk per week for each crew member. Grain consumption was modest compared with that in other semimeanitarian or full vegetarian diets. Individual food items were weighed, and the same amount of food was allotted to each subject at every meal regardless of the individual’s weight or other consideration, by common consent. While this decision may seem surprising, the group felt that attempts to apportion food, labor, and certain other items according to body weight, sex, age, subjective sense of hunger or fatigue, or other considerations would prove hopelessly complex and lead to unrest. Food intake was recorded daily, and nutrient values were calculated daily by means of a commercially available nutrition software package (The Interactive Diet Planner, The Longbrook Co, Los Angeles, Calif). Detailed analysis of representative days in each 6-month interval showed that protein intake was 97% to 163% of the recommended daily allowance (RDA) with intake of all essential amino acids being greater than 200% of RDA, fat intake was 40% to 52% of RDA, cholesterol intake was 18 to 135 mg/d, intake of all vitamins ranged from 136% of RDA (nicacin, 1 day) to 3580% of RDA (vitamin A including carotene, 1 day) except for vitamin B12 (3%-21% of RDA), and intake of all minerals ranged from 78% of RDA (zinc, 1 day) to 323% of RDA (potassium, 1 day) except sodium (10%-32% of RDA), selenium (64%-91% of RDA), and calcium (48%-67% of RDA). In addition to vitamins and minerals obtained from the diet, all participants took a daily multivitamin and mineral supplement (Thompson Medical, West Palm Beach, Fla).

WEIGHT AND PHYSICAL ACTIVITY

Subjects were weighed every 2 to 3 months after entry into Biosphere 2 and periodically after exit. The data are expressed as body mass index (BMI), expressed as kilograms per height in meters squared. Physical activity was constant and included manual farming, maintenance of Biosphere 2, collection of scientific data, and day-to-day activities of living. Basal energy expenditure (BEE) was calculated from weight, height, age, and sex by means of the Harris-Benedict equation.12 Total energy expenditure (TEE) was estimated by 2 techniques: calculation of work-associated energy expenditure, and calculation of the energy deficit needed to produce observed changes in weight according to the relationship published by the World Health Organization13: weight change/kcal=0.0292 g/kcal energy deficit (energy intake−TEE).

BLOOD SAMPLE COLLECTION AND STORAGE

Blood was obtained every 1 to 2 months after a 10- to 12-hour fast, and both EDTA anticoagulated plasma and serum were prepared immediately. Some analyses were performed on fresh plasma and serum, and aliquots of both serum and plasma were frozen and stored at −70°C for subsequent analysis. Inside Biosphere 2, cholesterol and triglyceride levels were determined by means of an autoanalyzer (Ektachem DT60 Autoanalyzer with side units DTE and DTSC, Kodak, Rochester, NY). Beginning approximately 6 months before exit, EDTA plasma was transported at 4°C to a laboratory outside of Biosphere 2 for analysis. This laboratory has continued to periodically measure lipid and lipoprotein levels in these subjects since their exit. Accuracy of analyses was confirmed (laboratory-to-laboratory coefficient of variation, <10%) by repeated analysis with the use of stored frozen serum after exit from Biosphere 2.

LIPID AND LIPOPROTEIN MEASUREMENT

Cholesterol and triglyceride levels were measured by standard enzymatic methods.14-16 The HDL levels and HDL subfractions were measured on fresh unfrozen EDTA plasma, and HDL and HDL2 were measured by means of double precipitation with dextran sulfate.17 The low-density lipoprotein (LDL) level was estimated by the Friedewalde formula.3 The HDL subfractions were measured by equilibrium ultracentrifugation followed by analysis with the use of gradient gel electrophoresis as previously described.18-20

DATA ANALYSIS AND STATISTICS

Results were entered into a computerized database and analyzed by means of a standard statistical package (SAS, Cary, NC). Means of continuous variables were compared by the paired t test. Regression analyses of lipid and lipoprotein levels occurring at various times were made with stepwise addition of potential independent variables. Multivariate analysis of linear models to explain observed variations was performed by both forward (addition of significant variables) and backward (subtraction of insignificant variables) analysis after age and sex were entered into the regression equations. Results were the same with either forward or backward analysis. Differences were considered significant at the P<.05 level in univariate analyses, and at the P<.15 level in the multivariate analyses. Results are presented as mean ± 1 SEM.
to weight, weight change, and energy expenditure. To determine whether the low lipid levels observed were caused by the restricted diet in the small number of subjects involved, a follow-up study of the same people has been conducted for more than 2 years after their exit from Biosphere 2. All subjects ate the same amount of food, which was weighed at every meal to ensure equal distribution during the period of restriction. Results of this follow-up study showed that changes in lipid and lipoprotein levels occurred after exit from Biosphere 2, demonstrating the significant decrease in BMI caused by weight loss that occurred during the period of restriction of energy intake. Data are means, and error bars indicate SE.

Table 1 gives age, sex, height, initial BMI, and TEE/BEE values of the 8 crew members. Figure 1 shows BMI before, during, and after exit from Biosphere 2. The BMI of the men significantly decreased 19%, from 23.7 ± 1.8 kg/m² to 19.3 ± 0.9 kg/m² (P < .01), and the BMI of the women significantly decreased 13%, from 21.2 ± 1.5 kg/m² to 18.5 ± 1.2 kg/m² (P < .01) during energy restriction in Biosphere 2; lost weight was completely restored after exit. Work output by individuals, indexed as TEE/BEE, varied from 1.20 to 1.65. The TEE/BEE was relatively constant for each individual throughout the 2 years. A TEE/BEE of 1.5 would correspond to a daily activity schedule of 8 hours of sleep, 4 hours of heavy agriculture, 4 hours of light activity, and 8 hours of desk work. The person with the lowest TEE/BEE had average daily work output (TEE/BEE) similar to that of other people in Biosphere 2, and ate the same amount as the lightest subject, but was the heaviest at entry and lost 25 kg, 25% of initial weight, during the stay in Biosphere 2.

Total daily energy intake, as well as energy derived from fat and from protein, at representative intervals centered around 3, 9, 12, and 18 months, is presented in Figure 2. As previously mentioned, the energy consumed per day was equal among the 8 subjects, leading to a wide range in energy and protein per body weight. The fat component of this nearly vegetarian diet was high in polyunsaturated fat (75% of fat energy) and low in saturated fat (10% of fat energy) and cholesterol (46 mg/d). During the 2 years in Biosphere 2, energy intake from fat increased from 12% to 13% during the first 6-month period to 16% in the third 6-month period and 20% in the fourth. Energy intake from protein was essentially constant (13%-15%) during the 2 years.
Energy deficit (food energy intake–TEE) calculated from weight, height, sex, and food intake and activity is presented in Figure 3. Throughout the stay in Biosphere 2, the men had a greater energy deficit than the women, doubtless because they were heavier on entry, yet they ate the same amount of food and performed the same amount of work. As TEE decreased because of decreasing weight, and food availability increased during the second year in Biosphere 2, the energy deficit diminished, resulting in a slight positive energy balance for both sexes by 12 months (Figure 3) and a slight weight gain for the women by 12 months, the men by 16 to 18 months (Figure 1). This can also be seen in the stabilization of weights during the second year. Only the heaviest man (subject 3 in Table 1) never stopped losing weight.

As shown in Figure 4, the restricted diet was associated with marked decreases in plasma cholesterol and triglyceride levels. Average cholesterol levels significantly decreased 36%, from 4.68 ± 0.26 mmol/L (181 ± 10 mg/dL) to 3.13 ± 0.16 mmol/L (121 ± 6 mg/dL) (P < 0.001); and triglyceride levels significantly decreased 42%, from 1.26 ± 0.18 mmol/L (112 ± 16 mg/dL) to 0.73 mmol/L (65 mg/dL) (P < 0.001). Within 6 months after exit from Biosphere 2 and return to preentry diet patterns, cholesterol and triglyceride levels had ascended to those seen before entry.

Lipoprotein levels are presented in Figure 5. Simultaneous with the changes in cholesterol level, there were parallel changes in LDL and HDL cholesterol levels, both decreasing with the onset of energy restriction and both returning to preclosure levels within a few months after exit. Decreases in LDL and HDL levels occurred simultaneously in all subjects, and LDL level decreased significantly from 2.72 ± 0.21 mmol/L (105 ± 8 mg/dL) to a low of 1.50 ± 0.18 mmol/L (58 ± 7 mg/dL) (P < 0.01), while HDL level decreased significantly from 1.55 ± 0.18 mmol/L (60 ± 7 mg/dL) to a low of 0.67 ± 0.05 mmol/L (26 ± 2 mg/dL) (P < 0.01). During the stay in Biosphere 2, the average LDL/HDL ratio decreased from 1.93 at the beginning of the experiment to 1.20 at exit. Average LDL/HDL ratio returned to 1.84 by 12 months after the subjects exited Biosphere 2.

Effects of energy restriction on HDL subfractions in responders are shown in Figure 6, top and bottom. There were significant variations between men and women, and from subject to subject. Two of the men, including 1 man who had practiced moderate energy restriction before entry into Biosphere 2, showed only minimal dietary effects of restriction or of further restriction, respectively, on the subfractions. Two of the women, including the woman taking thyroid hormone replacement, showed no significant effect of diet on HDL subfractions. The 2 remaining women showed higher response levels of HDL subfractions to the restriction than the 2 remaining men. These data suggest that energy restriction decreased HDL subfractions to the restriction than the 2 remaining men.

Energy Intake–Expenditure, kJ/d

<table>
<thead>
<tr>
<th>Energy Intake–Expenditure, kJ/d</th>
<th>0</th>
<th>420</th>
<th>840</th>
</tr>
</thead>
<tbody>
<tr>
<td>Women</td>
<td>-2520</td>
<td>-2100</td>
<td>-1680</td>
</tr>
<tr>
<td>Men</td>
<td>-2520</td>
<td>-2100</td>
<td>-1680</td>
</tr>
</tbody>
</table>

Figure 3. Difference between food intake and total energy expenditure of women and men, based on measured food intake and total energy expenditure calculated from weight changes and basal energy expenditure for each individual. Negative values occurred during times of energy intake restriction. The women had lower energy deficits than the men because they weighed less than the men at entry into Biosphere 2 but received the same food allocation while producing similar work output. Data are means, and error bars indicate SE.

Figure 4. Cholesterol and triglyceride levels before, during, and after confinement in Biosphere 2, showing the changes in cholesterol levels that paralleled changes in body mass index shown in Figure 1 and changes in triglyceride levels that reached a nadir just before exit, even though food intake increased during the second year. Dotted lines indicate dates of entrance into and exit from Biosphere 2. Data are means, and error bars indicate SE.

Figure 5. Low-density lipoprotein (LDL) and high-density lipoprotein (HDL) cholesterol levels before, during, and after confinement in Biosphere 2, showing that levels of LDL and HDL changed in parallel with changes in cholesterol levels illustrated in Figure 4. Dotted lines indicate dates of entrance into and exit from Biosphere 2. Data are means, and error bars indicate SE.
levels and increased HDL₂ levels, particularly in people whose HDL patterns responded to changes in energy intake, and that there may be a separation into “responders” and “nonresponders” to such a regimen with regard to these subfractions.

Since lipid and lipoprotein levels are considered to be variously affected by dietary intake of energy, fat, and cholesterol, and by body weight and composition, we estimated the relative contributions of diet and body composition to the observed changes in lipid and lipoprotein levels by multivariate analyses, accounting for age and sex, using lipid and lipoprotein levels as dependent variables and BMI, fat intake, cholesterol intake, and body mass index (BMI) were evaluated by means of stepwise addition of significant independent variables as described in the “Subjects and Methods” section. When energy deficit and energy intake were included in linear models, fat and cholesterol intake had no significant effect on prediction of observed lipid and lipoprotein levels.

Cholesterol, triglyceride, HDL, and LDL levels of the 8 normal subjects, with restricted energy intake during their 2-year stay in Biosphere 2, were decreased by energy restriction. In addition to energy restriction, the diet consumed was also extremely low in fat (12%-20% of energy intake) and cholesterol (18-135 mg/d) for the entire 2 years. On the basis of multivariate analyses, the variable in the diet that best explained the decreases in lipid and lipoprotein levels, independent of age and sex of the participants, was the energy deficit per se. To a lesser extent, dependent on sex and individual variability, levels of the antiatherogenic HDL₂b subfractions were increased by the same energy restriction. However, the multivariate analysis supports the thesis that it was energy, not fat, that made the major difference in these variables.

All of these changes reversed with normalization of subjects’ diet on exit from Biosphere 2, when subjects went from fat- and energy-restricted, near-vegetarian diets to eating their regular unrestricted diets. Discussions with individual participants suggest no systematic dietary restriction after exit from Biosphere 2 except in 1 participant (R.L.W.), who maintained an energy-restricted diet, albeit less severe than that inside Biosphere 2, a habit that preceded his entry into Biosphere 2. These observations indicate that energy restriction with adequate nutrition led to significant decreases in cholesterol and triglyceride levels and a lesser, although significant, increase in levels of HDL₂, particularly HDL₂b cholesterol.

This study has limitations, in part because this study of participants in the Biosphere 2 experiment represents the seizure of a serendipitous opportunity. Because participants entering Biosphere 2 were not expected to undergo energy restriction, baseline records of their food intake and longitudinal measurements of lipid

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**Table 2. Results of Multivariate Regression Analysis of Predictors of Lipid and Lipoprotein Levels*\)**

<table>
<thead>
<tr>
<th>Dependent Variables</th>
<th>Significant Independent Variables</th>
<th>Energy Deficit</th>
<th>Energy Intake</th>
<th>BMI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cholesterol</td>
<td>F (1,44)=9.65</td>
<td>F (1,43)=4.37</td>
<td>F (1,42)=2.86</td>
<td></td>
</tr>
<tr>
<td></td>
<td>P=.003</td>
<td>P=.04</td>
<td>P=.10</td>
<td></td>
</tr>
<tr>
<td>Triglyceride</td>
<td>F (1,44)=9.58</td>
<td>F (1,42)=0.04</td>
<td>F (1,43)=0.06</td>
<td></td>
</tr>
<tr>
<td></td>
<td>P=.003</td>
<td>P=.83</td>
<td>P=.81</td>
<td></td>
</tr>
<tr>
<td>HDL cholesterol</td>
<td>F (1,44)=3.40</td>
<td>F (1,43)=0.13</td>
<td>F (1,42)=0.03</td>
<td></td>
</tr>
<tr>
<td></td>
<td>P=.07</td>
<td>P=.72</td>
<td>P=.87</td>
<td></td>
</tr>
</tbody>
</table>

*After accounting for age and sex, linear models describing variations in total cholesterol level, triglyceride level, and high-density lipoprotein (HDL) cholesterol levels as resulting from variations in the potential independent variables, energy deficit, energy intake, fat intake, cholesterol intake, and body mass index (BMI) were evaluated by means of stepwise addition of significant independent variables as described in the “Subjects and Methods” section. When energy deficit and energy intake were included in linear models, fat and cholesterol intake had no significant effect on prediction of observed lipid and lipoprotein levels.
and lipoprotein levels before the experiment were not obtained. However, the follow-up after exit for more than 2 years provides, retrospectively, an adequate baseline. The number of participants was small. Nonetheless, the changes in lipid and lipoprotein levels that were observed occurred in all participants and were clearly statistically significant. Although variability from subject to subject in response of HDL subfractions to energy restriction was seen, the changes that did occur were in the same direction (increasing HDL₂b subfractions in those participants who responded) and are the same as those seen in energy-restricted nonhuman primates.⁹

Although only 8 subjects were studied, the present report is unique because no volunteers have been so carefully observed under conditions of rigidly monitored energy restriction since the seminal studies of starvation performed by Keys et al²¹ in the 1940s. However, differing from the conditions of the Keys et al study, energy restriction in Biosphere 2 occurred in the absence of nutritional deficiency except for energy intake, and while vigorous work output continued. Also, the period of dietary deprivation in the study by Keys et al lasted 6 months, in contrast to the 2 years’ restriction inside Biosphere 2.

Apart from the studies of Keys et al, no other clinical studies of effects of energy restriction on normal, healthy, nonobese people have been reported. The changes in lipid and lipoprotein levels observed are similar to changes seen in low-fat, energy-restricted diets aimed at reducing atherosclerosis risk in overweight men.²²,²³ The degree of lowering of cholesterol levels in our study is similar to that observed by Ornish et al²⁴ and Gould et al²⁵ in their studies of fat and energy restriction in human subjects. Low-fat diets (typically 25%-30% of energy as fat) that are effective in reducing lipid and lipoprotein levels are also low in energy intake;²⁶ our results, with a very-low-fat diet (<20% of energy as fat) support the conclusion that it is the low energy intake, rather than the low fat intake, that plays the predominant role in the reduction of such risk factors.²⁷ Studies based on responses to food frequency questionnaires have found low lipid and lipoprotein levels in populations that traditionally consume low-fat and vegetarian diets.²⁸ However, a recent meta-analysis that evaluated several equations predicting changes in lipid and lipoprotein levels caused by changes in dietary fat intake and composition suggested that reductions in just saturated fat and cholesterol would be expected to cause at most a 5% to 10% reduction in cholesterol level,²⁹ whereas we observed cholesterol levels to decrease by 36% and triglyceride levels by 42%. Pariza,³⁰ who assessed the effect of energy restriction on cancer risk, also concluded that energy restriction, rather than changes in diet composition, was responsible for the observed benefit. The increased survival of women with very low BMIs, as shown in the Nurses’ Health Study,³¹ may also accord with the improved risk factors shown in the present study.

Energy restriction has been studied extensively in rodents and to a lesser extent in nonhuman primates. In rodents, energy restriction leads to decreased body weight and lower levels of cholesterol and triglyceride,³²–³⁴ although the effects of energy restriction on HDL in rodents has been variable.³³,³⁴ Data from the rodent studies do indicate that as long as there is no nonenergy nutritional deficiency, it is the total energy intake, not its distribution among carbohydrate, fat, and protein components, that exerts the health-enhancing, age-retarding effect of energy restriction that has been well documented in these species.³⁵ The studies of energy restriction in nonhuman primates are still under way.³⁶,³⁷ Initial reports were presented in abstract form,³⁷,³⁸ and a complete report from 1 group of monkeys was recently published.³⁹ These results are similar to those in people described in the present study, ie, triglyceride and cholesterol levels are generally reduced by energy restriction, and HDL level shows an increase in the HDL₂b subfraction, although nonhuman primates, like humans, have large individual variation in HDL subfraction levels.

When these data are combined with data previously reported on the subjects in Biosphere 2, it can be seen that almost all modifiable cardiovascular risk factors were favorably affected by the energy-restricted, near-vegetarian diet.²⁴ Blood pressure decreased to a very low level. Body weight and percentage of body fat decreased markedly and fasting glucose level was significantly reduced, along with the changes in lipids and lipoproteins. It is emphasized that these various changes occurred not in high-risk patients but in healthy, active individuals. These results lead to the clear conclusion that prolonged ingestion of low-energy diets by normal people is safe and reduces almost all risk factors for atherosclerosis and, possibly, for other age-associated diseases. While the effect on life prolongation is unknown, at least for healthy young people, an energy-restricted diet that provides adequate nutrition and leads to a relatively low BMI can be expected to improve health.

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REFERENCES


