Long-term calorie restriction is highly effective in reducing the risk for atherosclerosis in humans

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Little is known regarding the long-term effects of caloric restriction (CR) on the risk for atherosclerosis. We evaluated the effect of CR on risk factors for atherosclerosis in individuals who are restricting food intake to slow aging. We studied 18 individuals who had been on CR for an average of 6 years and 18 age-matched healthy individuals on typical American diets. We measured serum lipids and lipoproteins, fasting plasma glucose and insulin, blood pressure (BP), high-sensitivity C-reactive protein (CRP), platelet-derived growth factor AB (PDGF-AB), body composition, and carotid artery intima–media thickness (IMT). The CR group were leaner than the comparison group (body mass index, 19.6 ± 1.9 vs. 25.9 ± 3.2 kg/m^2; percent body fat, 8.7 ± 7% vs. 24 ± 8%). Serum total cholesterol (Tchol), low-density lipoprotein cholesterol, ratio of Tchol to high-density lipoprotein cholesterol (HDL-C), triglycerides, fasting glucose, fasting insulin, CRP, PDFG-AB, and systolic and diastolic BP were all markedly lower, whereas HDL-C was higher, in the CR than in the American diet group. Medical records indicated that the CR group had serum lipid–lipoprotein and BP levels in the usual range for individuals on typical American diets, and similar to those of the comparison group, before they began CR. Carotid artery IMT was ≈40% less in the CR group than in the comparison group. Based on a range of risk factors, it appears that long-term CR has a powerful protective effect against atherosclerosis. This interpretation is supported by the finding of a low carotid artery IMT.

Atherosclerotic arterial disease is the leading cause of morbidity and mortality in Western societies and is rapidly increasing in the developing nations (1). During the Second World War the shortage of food in some North European countries led to a sharp fall in mortality from coronary artery disease; when the war ended mortality rose sharply (2). Data obtained on the eight subjects confined to Biosphere 2 for 2 years showed that caloric restriction (CR) with a low-protein diet can improve a number of risk factors for atherosclerosis, including blood pressure (BP), serum total cholesterol (Tchol), and triglyceride (TG) levels (3, 4). However, no information regarding the long-term effects of CR in humans eating a good quality diet is available.

CR has been shown to slow aging in rats, mice, fish, worms, and various insects (5). There are a small number of individuals who have been practicing CR for a number of years in the belief that CR will also extend their lifespan beyond the usual range. The availability of these individuals is making it possible for us to investigate the effects of long-term CR in humans. In this study we measured a range of risk factors for atherosclerosis and carotid artery intima–media thickness (IMT) in individuals who have been practicing CR for periods ranging from 3 to 15 years. In addition, we present serum lipid and lipoprotein data that some of the study participants obtained through their personal physicians before and during the period of CR.

Methods

Study Participants. Eighteen individuals who are practicing CR were recruited through the Caloric Restriction Optimal Nutrition Society. Three were from the St. Louis area and the others came to the Washington University Medical Center from other cities in the U.S. and Canada. Their average age was 50 ± 10 years (range 35–82 years). Three of the 18 are women. They were in good health, with no chronic diseases. None were smokers. Eighteen individuals eating a usual American diet, matched with the CR group in terms of age and socioeconomic status, were used as a comparison group. None of the participants in this study were taking lipid-lowering, antihypertensive, or other medications that could affect the variables that were measured. They were enrolled in this study after giving written informed consent and undergoing a physical examination, medical history, and laboratory evaluation that revealed no evidence of any health problems. All of the study participants were weight stable, i.e., less than 2 kg of weight change in the preceding 6 months. Twelve of the individuals on CR provided us with copies of their medical records, which included BP and serum lipid and lipoprotein concentrations, obtained by their personal physicians before and during the period of CR. This study was approved by the Human Studies Committee of Washington University School of Medicine.

Anthropometrics and Body Composition. Height was measured without shoes to the nearest 0.1 cm. Body weight was obtained on a balance scale in the morning after a 12-hr fast. Body mass index (BMI) was calculated by dividing body weight (in kilograms) by the square of height (in meters). Total body fat mass and lean mass were determined by dual-energy x-ray absorptiometry (DEXA) (QDR 1000/w, Hologic, Waltham, MA), as described by Salamone *et al.* (6).

Blood Analyses. A venous blood sample was taken to determine lipid and hormone concentrations after subjects had fasted for at least 12 hr. Measurement of serum lipid and lipoprotein concentrations was performed in the Core Laboratory for Clinical Studies at Washington University. Cholesterol and glycerol-blanked TG were measured by automated enzymatic commercial kits (Miles/Technicon, Tarrytown, NY). High-density lipoprotein cholesterol (HDL-C) was measured in plasma after precipitation of apolipoprotein B-containing lipoproteins by dextran sulfate (50,000 molecular weight) and magnesium (7). Low-density lipoprotein cholesterol (LDL-C) was calculated by using the Friedewald equation (8). These methods are continuously standardized by the Lipid Standardization Program of the Centers for Disease Control and Prevention. Plasma glucose was measured by the glucose oxidase

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Abbreviations: CR, caloric restriction; BP, blood pressure; Tchol, total cholesterol; TG, triglyceride; IMT, intima–media thickness; BMI, body mass index; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; CRP, C-reactive protein.

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method (Beckman Instruments, Fullerton, CA), and insulin was measured by RIA (9). C-reactive protein (CRP) was measured by using a high-sensitivity ELISA kit (ALPCO Diagnostics, Windham, NH). Platelet-derived growth factor AB was measured with an ELISA kit (R & D Systems, Minneapolis).

BP. BP was measured with a mercury sphygmomanometer, with the patient in the sitting position after 5 min of rest in a quiet environment, according to the recommendations of the American Hypertension Society. Four measurements of systolic and diastolic BP were made at ≈5-min intervals and averaged.

Ultrasonographic Measurement of Carotid Intima–Media Thickness (IMT). B-mode ultrasonographic imaging of the common carotid artery was performed by using high-resolution real-time ultrasonography with an 11-MHz transducer. The examinations and image analyses were performed by a trained sonographer who remained unaware of other data. In brief, the right and left carotid arteries were scanned for measurement of IMT in the longitudinal projections over an arterial segment including 30 mm of the distal common carotid artery (10). IMT was measured in the anterior wall of the vessel as the distance from the trailing edge of the adventitia to the leading edge of the intima–media, and in the posterior wall of the vessel it was measured as the distance from the leading edge of the intima–media to the trailing edge of the adventitia. The average of 16 measurements was taken as the mean IMT.

Dietary Assessment. All of the volunteers were instructed by a research dietitian to record for 7 consecutive days all food and beverages consumed, preparation methods, and approximate portion sizes in food diaries at the time of consumption. To assist with portion size determinations, measuring spoon and cup sets were provided to all participants, and all food diaries had a ruler imprinted on the back cover. The food record was analyzed by using the NDS-R program (version 4.03_31), which is the Nutrition Data System for Research from the Nutrition Coordinating Center at the University of Minnesota. The database has 117 nutrients. The nutrients of interest are calories, total fat, total carbohydrate, total protein, animal protein, vegetable protein, saturated fatty acids, monounsaturated fatty acids, polyunsaturated fatty acids, sucrose, starch, soluble fiber, insoluble fiber, folate, vitamin B_{12}, all of the different fatty acids (4:0 to 22:6), all of the amino acids, phytic acid, and *trans* fatty acids (11).

Statistical Analysis. For normally distributed variables with approximately equal standard deviations the unpaired Student *t* test was used. For variables not normally distributed, or with unequal standard deviations, the Wilcoxon two-samples test was used. Statistical significance was set at $P < 0.05$ for all tests. All data were analyzed by using SPSS FOR WINDOWS software, version 10.0 (SPSS, Chicago). All values are expressed as means ± SD.

Results

Body Weight and Composition. BMI was significantly lower in the CR group than in the comparison group (Table 1). BMI, based on body weight records kept by the participants, decreased from 24 ± 3 (range 29.6 to 19.4) kg/m^2 to 19.5 ± 2 (range 22.8 to 16.5) kg/m^2 during the period of CR, which averaged 6 ± 3 years (range 3 to 15 years), with nearly all of the decrease occurring during the first year. The BMI values for the individuals in the comparison group were similar to the mean range for middle-aged people in the U.S. (12). Total body fat and trunk fat, measured by dual-energy x-ray absorptiometry, were extremely low in the CR men (Table 1). Average values for the three women in the CR group were 19.8 kg/m^2 for BMI and 18.8% for total body fat, and for the three women in the comparison group the average values were 26 kg/m^2 for BMI and 31.9% for total body fat.

Table 1. Anthropometric characteristics

Characteristic	Value: CR (n = 18)	Value: Controls (n = 18)	P value
Age, years	50.3 ± 10	50.3 ± 11	0.988
Height, m	1.7 ± 0.1	1.8 ± 0.1	0.562
Weight, kg	59.5 ± 5.5	80.9 ± 8.8	0.0001
BMI, kg/m^2 (men)	19.6 ± 1.9	25.9 ± 2.7	0.0001
Total body fat, % (men)	6.7 ± 4	22.4 ± 7	0.0001
Trunk fat, % (men)	3.4 ± 4	23.7 ± 9.2	0.0001
Lean mass, % (men)	93.3 ± 4	76.8 ± 7	0.0001

Values are means ± SD.

Risk Factors for Atherosclerosis. The serum Tchol, LDL-C, HDL-C, and TG concentrations of the comparison group (Table 2) fall close to the 50th percentile value for people in their age group in the U.S. (ref. 13, accessed Sept. 20, 2003 at www.nhlbi.nih.gov/guidelines/cholesterol/atp3xsum.pdf). The comparison group's BP and fasting plasma glucose levels (Table 2) were also similar to the average values found in middle-aged healthy people in the U.S. (14, 15). In contrast, the average serum Tchol and LDL-C concentrations of the CR group fall in the lowest 10% for people in their age group (13) (Table 2). Even more unusual are the CR group's TG levels, which are similar to the 5th-percentile values for 20-year-olds (13) (Table 2). Unlike the decrease in HDL-C that often occurs when individuals are placed on low-fat diets to lose weight, the CR group had high HDL-C levels, in the 85th to 90th percentile range for middle-aged men in the U.S. (13) (Table 2). As a consequence, their Tchol/HDL-C ratio is remarkably low. Twelve of the individuals in the CR group gave us records of their serum lipid and lipoprotein levels obtained through their personal physicians before starting, and during, the CR. As shown in Table 3, the average serum Tchol, LDL-C, HDL-C, and TG levels of the CR group before they began CR were close to the 50th percentile levels for middle-aged persons in the U.S. (13). Most of the decrease in body weight and improvement in serum lipid and lipoprotein levels occurred during the first year of CR (Table 3).

Both systolic and diastolic blood pressures in the CR group were remarkably low, with values in the range found in 10-year-olds (16) (Table 2). As shown in Table 3, the initial BP levels of

Table 2. Risk factors for atherosclerosis

Parameter	Value: CR (n = 18)	Value: Controls (n = 18)	P value
Tchol, mg/dl	158 ± 39	205 ± 40	0.001
LDL-C, mg/dl	86 ± 28	127 ± 35	0.0001
HDL-C, mg/dl	63 ± 19	48 ± 11	0.006
Tchol/HDL-C ratio	2.6 ± 0.5	4.5 ± 1.3	0.0001
TG, mg/dl	48 ± 15	147 ± 89	0.0001
TG/HDL-C ratio	0.8 ± 0.3	3.5 ± 2.8	0.0001
Systolic BP, mmHg	99 ± 10	129 ± 13	0.0001
Diastolic BP, mmHg	61 ± 6	79 ± 7	0.0001
Fasting glucose, mg/dl	81 ± 7	95 ± 8	0.0001
Fasting insulin, mIU/ml	1.4 ± 0.8	5.1 ± 2	0.0001
Hs-CRP, μg/ml	0.3 ± 0.2	1.6 ± 2.2	0.001

Values are means ± SD. IU, international unit; Hs-CRP, high-sensitivity CRP; 1 mmHg = 133 Pa.

Table 3. Serial measurements of risk factors for atherosclerosis in CR individuals

Parameter	Value		
	Pre-CR	≈1 yr CR	Present
BMI, kg/m^2	24.5 ± 2.6	20.9 ± 2.4	19.5 ± 2.1
Tchol, mg/dl	194 ± 45	161 ± 31	157 ± 38
LDL-C, mg/dl	122 ± 36	89 ± 24	86 ± 17
HDL-C, mg/dl	43 ± 8	58 ± 13	65 ± 24
Tchol/HDL-C ratio	4.1 ± 1	2.8 ± 0.5	2.5 ± 0.4
TG, mg/dl	149 ± 87	72 ± 35	54 ± 15
Systolic BP, mmHg	132 ± 15	112 ± 12	97 ± 8
Diastolic BP, mmHg	80 ± 11	69 ± 7	59 ± 5

Values are means ± SD for 12 individuals.

the 12 individuals in the CR group who gave us copies of their medical records were similar to those of the comparison group. Large decreases in systolic and diastolic BP occurred during the first year of CR with a further decrease to extremely low levels occurring during the subsequent period. Surprisingly, none of the individuals in the CR group had symptoms of postural hypotension. Fasting plasma insulin concentration was 65% lower and fasting plasma glucose concentration was also significantly lower in the CR than in the comparison group (Table 2). The plasma CRP concentrations of the individuals in the CR group are extremely low (Table 2). The average value for high-sensitivity CRP in the CR group was only 16% as high as that of the comparison group. Serum platelet-derived growth factor AB concentration was also much lower in the CR than in the comparison group (8,567 ± 2,642 pg/ml in the CR and 17,727 ± 4,926 pg/ml in the comparison group; $P = 0.0001$).

Carotid IMT. IMT of the common carotid artery was ≈40% less in the CR than in the comparison group (0.5 ± 0.1 mm in the CR and 0.8 ± 0.1 mm in the comparison group; $P = 0.0001$). None of the individuals on CR had evidence of atherosclerotic plaque, defined as an IMT of more than 1.0 mm and an increase of at least 100% compared to an adjacent wall segment (17).

Nutrient Intake. Nutrient intakes differed significantly between the diet groups. The CR subjects designed their diets to consume a balance of foods that supply more than 100% of the Recommended Daily Intake (RDI) for all of the essential nutrients, while minimizing energy content (1,112–1,958 kcal/day). They eat a wide variety of vegetables, fruits, nuts, dairy products, egg whites, wheat and soy proteins, and meat (≈26% of calories from protein, ≈28% from fat, and ≈46% from complex carbohydrates). All of CR group strictly avoid processed foods containing *trans* fatty acids and high glycemic foods (e.g., refined carbohydrates, desserts, snacks, and soft drinks). The comparison group ate typical U.S. diets containing nearly twice as many calories as the CR subjects (1,976–3,537 kcal/day; ≈18% calories from protein, ≈32% from fat, and ≈50% from carbohydrates).

A wide range of supplements were taken by both CR and comparison subjects, ranging from one multivitamin per day to combinations of antioxidants, vitamins, selenium, and folate (Table 4). Four CR and three comparison subjects were not taking any supplements. Serum lipid, lipoprotein, and CRP concentrations, BP and IMT of subjects not taking supplements were not statistically significantly different from those who were taking supplements (data not shown). Serum concentrations of several antioxidants, including vitamin E and β-carotene, and minerals, including selenium and magnesium, measured in 10 of the 18 CR subjects, were in the normal range (Table 5).

Table 4. Supplements taken by the participants

Supplements	CR	Controls
None	4	3
Only multivitamin	2	7
Only vitamin E	1	3
Only vitamin C	1	0
Vitamin E + vitamin C	2	0
Vitamin E + multivitamin	2	1
Vitamin C + E + multivitamin	2	1
Folate	1	0
Vitamin C + E + folate + multivitamin	1	0
Vitamin C + E + β-carotene + multivitamin	1	0
Vitamin C + E + β-carotene + folate	1	0
Vitamin C + E + selenium	1	0
Vitamin C + E + selenium + folate	0	2

Vitamin E range 200–800 international units (IU)/day; vitamin C range 100–2,650 mg/day; folic acid 400–1,600 μg/day; selenium 50–300 μg/day; vitamin A 5,000–50,000 IU/day.

Discussion

Little is known regarding the effects of long-term CR on metabolic health in nonobese humans. In this study, we evaluated the metabolic profile of 18 men and women who had been on self-imposed CR for 3–15 years. Our data show that CR results in profound and sustained beneficial effects on the major atherosclerosis risk factors, serum Tchol, LDL-C, HDL-C, TG, and BP, that usually increase with advancing age. They further show that CR provides a powerful protective effect against obesity and insulin resistance, and provide evidence for a decrease in inflammation, as reflected in extremely low CRP levels.

To our knowledge, the only other information regarding the effects of CR in nonobese people was that obtained on the eight individuals sealed inside Biosphere 2 for 2 years. As a result of technical problems, food availability was reduced and caloric intake averaged ≈1,780 kcal/day during the first 6 months (3). Like the CR individuals in the present study, the Biospherians underwent large reductions in Tchol, LDL-C, and BP (3, 4). However, in contrast to the increase in HDL-C that occurred in our CR group, the Biospherians had a large decrease in HDL-C (3, 4), presumably because of differences in composition of the diets. The Biospherians' largely vegetarian diet was very low in fat (≈10% of energy intake) and high in carbohydrate (≈76% of energy intake), with protein supplying ≈14% of energy intake. It is well documented that energy-restricted high-carbohydrate diets result in a decrease in HDL-C (18). In contrast, the CR group in the present study restricted their carbohydrate intake to, on average, 46% of energy intake.

Studies of the effects of CR in rhesus monkeys have shown reductions in fasting plasma glucose and insulin (19–21) but not in serum Tchol (22). The individuals on CR in the present study also had low normal fasting plasma glucose concentrations despite remarkably low fasting plasma insulin levels. The difference in serum cholesterol responses between humans and monkeys is probably explained by the monkeys' low initial cholesterol level.

Of the risk factors documented by epidemiological studies, elevated serum cholesterol has the distinction that it can induce development of atherosclerosis in the absence of other risk factors. This is evident in people with familial hypercholesterolemia (23) and has been demonstrated repeatedly in studies on animals (24). Furthermore, lowering cholesterol levels has been shown to reduce cardiovascular disease mortality (25). However, factors other than Tchol, such as HDL-C (26), BP (27), and inflammation, as reflected in CRP (28), are also of major

Table 5. Blood levels of antioxidants and minerals

Participant	Vitamin E, mg/liter	Vitamin A, mg/liter	β-Carotene, mg/liter	Selenium, ppm packed RBC	Zinc, ppm packed RBC	Magnesium, ppm packed RBC
CR1	32.02	0.75	0.52	0.21	7.19	40.19
CR2	6.54	0.25	1.06	0.12	6.42	25.27
CR3	10.19	0.49	1.05	0.25	10.04	48.25
CR4	14.22	0.86	2.57	0.12	6.75	34.17
CR5	11.89	0.45	2.16	0.11	6.83	32.16
CR6	12.12	0.46	2.84	0.11	7.23	44.10
CR7	17.45	1.13	2.21	0.26	6.44	35.32
CR8	25.39	1.14	1.62	0.24	6.51	45.34
CR9	11.32	0.85	3.18	0.25	6.32	40.00
CR10	17.53	1.29	1.88	0.35	7.23	35.35
Reference range	12–50	0.5–1.2	0.4–3.5	0.12–0.4	6–11	40–80

The levels of vitamin E, β-carotene, and minerals, including selenium, zinc, and magnesium, have been measured in 10 of the 18 CR subjects (these measurements were provided by Richard S. Lord, Metametrix Clinical Laboratory, Norcross, GA).

importance, as evidenced by the marked variability in the development of clinical atherosclerotic disease in individuals with similar cholesterol levels.

A large percentage of both CR and comparison subjects were taking a variety of supplements. However, this should not interfere with the interpretation of our findings, because it has been shown that supplementation with antioxidant vitamins does not lower serum lipid and lipoproteins (29–34) or BP (29). Moreover, a recent study, conducted in more than 20,000 subjects, found that vitamin E, vitamin C, and β-carotene supplementation resulted in small but significant increases in plasma Tchol, LDL-C, and TG concentrations (30). In the present study we found no significant differences in serum lipid, lipoprotein, and CRP concentrations, BP, or IMT between supplemented and not-supplemented subjects.

In addition to the roles of the lipoprotein fractions to which cholesterol is bound, i.e., decreased risk with high HDL-C levels, and increased risk with high LDL-C levels, and BP, it has become evident that inflammation plays a major role in atherosclerosis (25, 35). Atherosclerotic plaque formation appears to be mediated by a chronic inflammatory process resulting from interactions between macrophages, oxidized cholesterol, and cellular components of the arterial wall (35). A key component of this process is the proliferation and migration of smooth muscle cells and deposition of extracellular matrix. This proliferative effect appears to be mediated by high levels of insulin and other growth factors, including platelet-derived growth factor (36).

In studies of the slowing of aging by CR in rats and mice, it was found that CR results in a marked decreases in cellular proliferation and inflammation (37–39). The present finding of extremely low CRP levels in the CR group provides preliminary evidence that CR also reduces inflammation in humans. CRP is a marker for inflammation (40) and may also play a role in the inflammatory process (41, 42). This may explain why CRP is a strong predictor of cardiovascular events such as myocardial infarction (28). The present findings of very low plasma insulin and serum platelet-derived growth factor levels in the CR group provide preliminary evidence that CR also results in a decreased stimulus for cell proliferation in humans. Platelet-derived growth factor is a potent chemoattractant and mitogen for vascular smooth muscle cells, and some drugs that counteract its biological actions have been developed for the treatment of atherosclerosis (43, 44).

In conclusion, the results of this study on 18 individuals who had been on severe, self-imposed CR for 3–15 years provide evidence that CR is highly effective in lowering the risk of developing atherosclerosis.

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1. Yusuf, S., Reddy, S., Ounpuu, S. & Anand, S. (2001) *Circulation* **104,** 2746–2753.
2. Strom, A. & Jensen, R. A. (1951) *Lancet* **i,** 126–129.
3. Walford, R. L., Harris, S. B. & Gunion, M. W. (1992) *Proc. Natl. Acad. Sci. USA* **89,** 11533–11537.
4. Verdery, R. B. & Walford, R. L. (1998) *Arch. Intern. Med.* **158,** 900–906.
5. Weindruch, R. & Sohal, R. S. (1997) *N. Engl. J. Med.* **337,** 986–994.
6. Salamone, L. M., Fuerst, T., Visser, M., Kern, M., Lang, T., Dockrell, M., Cauley, J. A., Nevitt, M., Tylavsky, F. & Lohman, T. G. (2000) *J. Appl. Physiol.* **89,** 345–352.
7. Warnick, G. R., Benderson, J. & Albers, J. J. (1982) *Clin. Chem.* **28,** 1379–1388.
8. Friedewald, W., Levy, R. & Fredrickson, D. (1972) *Clin. Chem.* **18,** 499–502.
9. Kuzuya, H., Blix, P. M., Horwitz, D. L., Steiner, D. F. & Rubenstein, A. H. (1977) *Diabetes* **26,** 22–29.
10. Handa, N., Matsumoto, M., Maeda, H., Hougaku, H., Ogawa, S., Fukunaga, R., Yoneda, S., Kimura, K. & Kamada, T. (1990) *Stroke* **21,** 1567–1572.
11. Schakel, S. F., Sievert, Y. A. & Buzzard, I. M. (1988) *J. Am. Diet. Assoc.* **88,** 1268–1271.
12. Kuczmarski, R. J., Carroll, M. D., Flegal, K. M. & Troiano, R. P. (1997) *Obes. Res.* **5,** 542–548.
13. National Heart, Lung, and Blood Institute (2001) *Third Report of the Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III)* (Natl. Inst. Health, Bethesda), DHHS Publ. No. (NIH) 01-3670, Executive Summary, Appendix III-A.
14. Burt, V. L., Whelton, P., Roccella, E. J., Brown, C., Cutler, J. A., Higgins, M., Horan, M. J. & Labarthe, D. (1995) *Hypertension* **25,** 305–313.
15. National Institute of Diabetes and Digestive and Kidney Diseases (1995) *Diabetes in America* (Natl. Inst. Health, Bethesda), 2nd Ed., DHHS Publ. No. (NIH) 95-1465.
16. Williams, C. L., Hayman, L. L., Daniels, S. R., Robinson, T. N., Steinberger, J., Paridon, S. & Bazzarre, T. (2002) *Circulation* **106,** 143–160.
17. Lemne, C., Jogestrand, T. & de Faire, U. (1995) *Stroke* **26,** 34–39.
18. Turley, M. L., Skeaff, C. M., Mann, J. I. & Cox, B. (1998) *Eur. J. Clin. Nutr.* **52,** 728–732.
19. Kemnitz, J. W., Roecker, E. B., Weindruch, R., Elson, D. F., Baum, S. T. & Bergman, R. N. (1994) *Am. J. Physiol.* **266,** E540–E547.
20. Bodkin, N. L., Ortmeyer, H. K. & Hansen, B. C. (1995) *J. Gerontol. Biol. Sci.* **50,** B142–B147.
21. Lane, M. A., Ball, S. S., Ingram, D. K., Cutler, R. G., Engel, J., Read, V. & Roth, G. S. (1995) *Am. J. Physiol.* **268,** E941–E948.
22. Verdery, R. B., Ingram, D. K., Roth, G. S. & Lane, M. A. (1997) *Am. J. Physiol.* **273,** E714–E719.
23. Goldstein, J. L., Hobbs, H. H. & Brown, M. S. (2001) in *The Metabolic & Molecular Bases of Inherited Disease*, eds. Scriver, C. R., Beaudet, A. L., Sly, W. S. & Valle, D. (McGraw-Hill, New York), pp. 2863–2913.
24. Reardon, C. A. & Getz, G. S. (2001) *Curr. Opin. Lipidol.* **12,** 167–173.
25. Gotto, A. M., Jr., & Grundy, S. M. (1999) *Circulation* **99,** 1e-7.

26. Assmann, G. & Nofer, J. R. (2003) *Annu. Rev. Med.* **54,** 321–341.
27. Lewington, S., Clarke, R., Qizilbash, N., Peto, R. & Collins, R. (2002) *Lancet* **360,** 1903–1913.
28. Ridker, P. M., Rifai, N., Rose, L., Buring, J. E. & Cook, N. R. (2002) *N. Engl. J. Med.* **347,** 1557–1565.
29. Hodis, H. N., Mack, W. J., LaBree, L., Mahrer, P. R., Sevanian, A., Liu, C. R., Liu, C. H., Hwang, J., Selzer, R. H. & Azen, S. P. (2002) *Circulation* **106,** 1453–1459.
30. Collins, R., Armitage, J., Parish, S., Sleight, P. & Peto, R. (2002) *Lancet* **360,** 23–33.
31. Brown, B. G., Zhao, X. Q., Chait, A., Fisher, L. D., Cheung, M. C., Morse, J. S., Dowdy, A. A., Marino, E. K., Bolson, E. L., Alaupovic, P., *et al.* (2001) *N. Engl. J. Med.* **345,** 1583–1592.
32. Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto miocardico (1999) *Lancet* **354,** 447–455.
33. Waters, D. D., Alderman, E. L., Hsia, J., Howard, B. V., Cobb, F. R., Rogers, W. J., Ouyang, P., Thompson, P., Tardif, J. C., Higginson, L., *et al.* (2002) *J. Am. Med. Assoc.* **288,** 2432–2440.
34. Fang, J. C., Kinlay, S., Beltrame, J., Hikiti, H., Wainstein, M., Behrendt, D., Suh, J., Frei, B., Mudge, G. H., Selwyn, A. P., *et al.* (2002) *Lancet* **359,** 1108–1113.
35. Ross, R. (1999) *N. Engl. J. Med.* **340,** 115–126.
36. Dzau, V. J., Braun-Dullaeus, R. C. & Sedding, D. G. (2002) *Nat. Med.* **8,** 1249–1256.
37. Spaulding, C. C., Walford, R. L. & Effros, R. B. (1997) *Mech. Ageing Dev.* **93,** 87–94.
38. Matsuzaki, J., Kuwamura, M., Yamaji, R., Inui, H. & Nakano, Y. (2001) *J. Nutr.* **131,** 2139–2144.
39. Guo, Z., Mitchell-Raymundo, F., Yang, H., Ikeno, Y., Nelson, J., Diaz, V., Richardson, A. & Reddick, R. (2002) *Mech. Ageing Dev.* **123,** 1121–1131.
40. Gabay, C. & Kushner, I. (1999) *N. Eng. J. Med.* **340,** 448–454.
41. Pasceri, V., Willerson, J. T. & Yeh, E. T. H. (2000) *Circulation* **102,** 2165–2168.
42. Verma, S., Wang, C. H., Li, S. H., Dumont, A. S., Fedak, P. W., Badiwala, M. V., Dhillon, B., Weisel, R. D., Li, R. K., Mickle, D. A., *et al.* (2002) *Circulation* **106,** 913–919.
43. Heldin, C. H. & Westermark, B. (1999) *Physiol. Rev.* **79,** 1283–1316.
44. Maresta, A., Balducelli, M., Cantini, L., Casari, A., Chioin, R., Fabbri, M., Fontanelli, A., Monici Preti, P. A., Repetto, S. & De Servi, S. (1994) *Circulation* **90,** 2710–2715.