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Comparison of Biomarkers of Oxidative Stress and Cardiovascular Disease in Humans and Chimpanzees (*Pan troglodytes*)

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In the oxidative stress hypothesis of aging, the aging process is the result of cumulative damage by reactive oxygen species. Humans and chimpanzees are remarkably similar; but humans live twice as long as chimpanzees and therefore are believed to age at a slower rate. The purpose of this study was to compare biomarkers for cardiovascular disease, oxidative stress, and aging between male chimpanzees and humans. Compared with men, male chimpanzees were at increased risk for cardiovascular disease because of their significantly higher levels of fibrinogen, IGF1, insulin, lipoprotein a, and large high-density lipoproteins. Chimpanzees showed increased oxidative stress, measured as significantly higher levels of 5-hydroxymethyl-2-deoxyuridine and 8-iso-prostaglandin $F_{2\alpha}$, a higher peroxidizability index, and higher levels of the prooxidants ceruloplasmin and copper. In addition, chimpanzees had decreased levels of antioxidants, including α- and β-carotene, β-cryptoxanthin, lycopene, and tocopherols, as well as decreased levels of the cardiovascular protection factors albumin and bilirubin. As predicted by the oxidative stress hypothesis of aging, male chimpanzees exhibit higher levels of oxidative stress and a much higher risk for cardiovascular disease, particularly cardiomyopathy, compared with men of equivalent age. Given these results, we hypothesize that the longer lifespan of humans is at least in part the result of greater antioxidant capacity and lower risk of cardiovascular disease associated with lower oxidative stress.

Abbreviations: 5OHmU, 5-hydroxymethyl-2-deoxyuridine; 8isoPGF$_{2\alpha}$, 8-iso-prostaglandin F$_{2\alpha}$; HDL, high-density lipoprotein; IGF1, insulin-like growth factor 1; LDL, low-density lipoprotein; ROS, reactive oxygen species.

Aging is characterized as a progressive reduction in the capacity to withstand the stresses of everyday life and a corresponding increase in risk of mortality. According to the oxidative stress hypothesis of aging, much of the aging process can be accounted for as the result of cumulative damage produced by reactive oxygen species (ROS).[6,21,28,41,97] Endogenous oxygen radicals (that is, ROS) are generated as a byproduct of normal metabolic reactions in the body and subsequently can cause extensive damage to proteins, lipids, and DNA.[6,41] Various prooxidant elements, in particular free transition metals, can catalyze these destructive reactions.[6] The damage caused by ROS can be counteracted by antioxidant defense systems, but the imbalance between production of ROS and antioxidant defenses, over time, leads to oxidative stress and may contribute to the rate of aging.[28,97]

Oxidative stress has been linked to several age-related diseases including neurodegenerative diseases, ophthalmologic diseases, cancer, and cardiovascular disease.[21,28,97] Of these, cardiovascular disease remains the leading cause of adult death in the United States and Europe.[71] In terms of cardiovascular disease, oxidative stress has been linked to atherosclerosis, hypertension, cardiomyopathy, and chronic heart failure in humans.[55,78,84] Increases in oxidant catalysts (prooxidants)—such as copper, iron, and cadmium—have been associated with hypertension, coronary artery disease, atherosclerosis, and sudden cardiac death.[98,102,106] Finally, both endogenous and exogenous antioxidants have been linked to decreased risk of cardiovascular disease, although the mechanisms behind this relationship are unclear.[11,52,53] However, the oxidative stress hypothesis of aging aims to explain not only the mechanism of aging and age-related diseases (such as cardiovascular disease) in humans but also the differences between aging rates and the manifestations of age-related diseases across species.

The differences in antioxidant and ROS levels between animals and humans offer promise for increasing our understanding of human aging. Additional evidence supporting the oxidative stress hypothesis of aging has come from comparative studies linking differences in aging rates across taxa with both antioxidant and ROS levels.[4,17-21,58,71,86,105] In mammals, maximum lifespan potential is positively correlated with both serum and tissue antioxidant levels.[17,18,21,71,105] Research has consistently demonstrated that the rate of oxidative damage varies across species and is negatively correlated with maximum lifespan potential.[4,19,20,58,71,86] However, few studies involved detailed comparisons of hypothesized biochemical indicators of aging and oxidative stress between humans and animals.[6] This type of interspecies comparison has great potential for directly testing the oxidative stress hypothesis of aging.

Much evolutionary and genetic evidence supports remarkable similarity between humans and chimpanzees.[95,100] Despite

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this similarity, humans have a lifespan of almost twice that of chimpanzees.[3,16,47] Most comparative primate aging research has focused on the use of a macaque model,[62,81,88] and several biochemical markers of age-related diseases have been identified in both humans and macaque monkeys.[9,22,28,81,93,97] Several other species of monkeys have also been used in research addressing oxidative stress, antioxidant defenses, and maximum lifespan potential.[18,21,58,105] However, no study to date has examined biochemical indicators of oxidative stress and aging in chimpanzees and humans as a test of the oxidative stress hypothesis for aging. The purpose of this study is to compare biochemical markers for cardiovascular disease, oxidative stress, and aging directly between male chimpanzees and humans. Given the oxidative stress hypothesis for aging and the known role of oxidative stress in cardiovascular disease, we predict that chimpanzees will show higher levels of cardiovascular risk and oxidative stress than humans.

Materials and Methods

Subjects included 10 healthy, young men (mean age, 24 y; age range, 22 to 30 y) and 10 healthy, young male chimpanzees (*Pan troglodytes*; mean age, 12 y; range, 11 to 16 y). The described mean ages were chosen to reduce, as much as possible, any age-dependent changes that may occur in the biochemical parameters measured. The mean age for chimpanzees in this study represents 1 to 2 y after the median age of skeletal,[32,39] dental,[1,59,96] body weight,[32,65] and reproductive[74,119] maturity for captive chimpanzees. In addition, the age range was selected to fall below the onset of physiologic aging in chimpanzees.[46,108] The age range of human subjects was chosen to equal twice that of the chimpanzee subjects, because the human lifespan is twice that of chimpanzees.[3,16,47] Only male subjects were chosen to minimize hormonal variation (that is, female menstrual cycles) that could alter the biochemical parameters measured.

The chimpanzees were housed at the Primate Foundation of Arizona, an AAALAC-accredited facility, and the protocol was approved by the facility's institutional animal care and use committee. No animals were exposed to hepatitis C or HIV, nor did they have hepatitis A or B antigen. Likewise, no animals were on infectious studies at the time of blood sampling. Chimpanzees were housed in compatible social groups of 4 to 7 animals, in housing that exceeds current standards and recommendations of the US Department of Agriculture and the *Guide for the Care and Use of Laboratory Animals*.[51] Chimps were provided water ad libitum and a varied diet of seasonal fruits, vegetables and pelleted monkey chow biscuits (Lab Diet 5045, PMI Nutrition International, Richmond, IN), in addition to vitamin supplementation (Bronson Chewable Multivitamin, Bronson Vitamins, Lindon, UT) and daily environmental enrichment and forage materials. The water source for the chimpanzees was an onsite well, and all tests showed that the water met Arizona state drinking water standards. Chimpanzees were healthy, with no sign of illness or disease at the time of the study, and body weights ranged from 51.0 to 73.6 kg.

All chimpanzees were examined while under anesthesia for routine biannual health checks. After a 12-h fast, each chimp was anesthetized by intramuscular injection of either ketamine HCl (5.0 to 7.5 mg/kg; Ketaset, Fort Dodge, IA) or tiletamine hydrochloride–zolazepam HCl (3.0 to 4.0 mg/kg; Telazol, Fort Dodge, IA). Injections were given by dart (Telinject USA, Agua Dulce, CA) or manually by syringe and hypodermic needle. Personnel used all appropriate personal protective equipment when interacting with chimpanzees, and standard precautions were taken. Blood and urine samples were collected for biochemical assays, including a cardiovascular risk factor profile and oxidative stress status profile (Tables 1 through 4). Blood was collected from the femoral vein into vacuum phlebotomy tube tubes (Vacutainer, Becton Dickinson, Rutherford, NJ). Urine was collected by using a catheter (Kendall–Sovereign, Mansfield, MA).

The men were paid volunteers, recruited from the greater Phoenix community by direct mail (from a database of subjects that had previously participated in research studies) and word of mouth. The research protocol was approved by the Arizona State University Institutional Review Board, and subjects gave written informed consent to participate. Participants were asked to complete a health history questionnaire to determine health status. Subjects chosen for the study had no major medical problems or signs of illness and body mass index scores less than 30. The human subjects were examined at Kronos Science Laboratories. Blood and urine samples were collected for biochemical assays as described for the chimpanzees. Blood samples were drawn via venipuncture, after a 12-h fast. Urine samples were first-morning voided urines and were collected on the same day as the blood draw.

All biochemical assays were performed at Kronos Science Laboratories. The cardiovascular risk factor profile was run by using standard clinical methodology on an automated protein chemistry analyzer (Beckman Array, Beckman Coulter, Fullerton, CA), a clinical chemistry system (Synchron LX20, Beckman Coulter), and an immunoanalyzer (Immulite 2000, Diagnostic Products Corporation, Los Angeles, CA). High-density lipoprotein (HDL) and low-density lipoprotein (LDL) subfractions were determined manually by using a lipograph (Kronos Science Laboratories, Phoenix, AZ).[26] The fatty acid profile was run by using custom (Kronos Science Laboratories) methodology on a gas chromatograph.[26] Urine creatinine was measured by using standard clinical methodology (Synchron LX20, Beckman Coulter). Urinary markers of oxidative damage were measured by using custom (Kronos Science Laboratories) methodology for liquid chromatography–tandem mass spectrometry.[42,67] Isoprostane levels were measured by using stable isotope dilution gas chromatography–negative ion chemical ionization mass spectrometry.[87] Prooxidant metals were measured by using custom (Kronos Science Laboratories) methodology for inductively coupled plasma–mass spectrometry. Antioxidants were measured by using custom (Kronos Science Laboratories) methodology for HPLC, and additional oxidative stress protection factors were determined by using standard clinical methodology (Synchron LX20, Beckman Coulter).

Results for all cardiovascular risk factors, oxidative stress markers, and oxidative stress protection factors were compared between chimpanzees and humans by using ANOVA. Significance was set at the 0.05 level, and all statistics were conducted by using JMP 6.0 (SAS Institute, Cary, NC).

Results

In terms of cardiovascular risk, chimpanzees had significantly higher levels of fibrinogen ($F_{19} = 4.55$, $P = 0.046$), insulin-like growth factor 1 (IGF1; $F_{19} = 54.99$, $P < 0.001$), insulin ($F_{19} = 5.20$, $P = 0.035$), lipoprotein a ($F_{19} = 77.30$, $P < 0.001$), and WBC ($F_{19} = 9.85$, $P = 0.006$; Table 1). All of the chimpanzees had lipoprotein

Table 1. Comparison of mean (1 SD) values of serum indicators of cardiovascular risk in male chimpanzees (n = 10) and men (n = 10)

Cardiovascular risk factor	Chimpanzee	Human	Human reference range[a]	Chimpanzee: human ratio	*P*
Cholesterol (mg/dL)	187.9 (34.1)	180.0 (39.8)	150.0–200.0	1.04	0.639
Triglycerides (mg/dL)	72.6 (28.1)	75.0 (47.0	35.0–160.0	0.97	0.891
HDL(mg/dL)	50.0 (10.0)	42.0 (7.2)	40.0–70.0	1.19	0.055
LDL (mg/dL)	120.4 (28.7)	122.3 (35.1)	50.0–160.0	0.98	0.896
LDLM (mg/dL)	**266.7 (2.9)**	**274.0 (2.4)**	**255.0–280.0**	**0.97**	**<0.001**
IGF1 (ng/mL)	**519.3 (124.7)**	**194.2 (60.5)**	**90.0–360.0**	**2.67**	**<0.001**
Insulin (μIU/mL)	**14.8 (9.2)**	**7.7 (3.7)**	**2.0–20.0**	**1.92**	**0.035**
Apolipoprotein A (mg/dL)	125.6 (28.5)	116.7 (19.9)	90.0–170.0	1.08	0.429
Apolipoprotein B (mg/dL)	100.0 (19.1)	92.7 (28.0)	56.0–162.0	1.08	0.505
Fibrinogen (mg/dL)	**367.9 (143.5)**	**263.5 (57.9)**	**200.0–400.0**	**1.40**	**0.046**
hs–CRP (mg/L)	4.4 (5.4)	2.1 (1.9)	0.0–2.5	2.00	0.233
Lipoprotein A (mg/dL)	**145.0 (38.2)**	**16.7 (24.7)**	**0.0–64.0**	**8.68**	**<0.001**
Folic acid (ng/dL)	*12.7 (2.5)*	*9.4 (2.5)*	*3.1–17.5*	*1.35*	*0.009*
Vitamin B12 (pg/mL)	*1500.0 (0.0)*	*519.8 (181.4)*	*180.0–914.0*	*28.86*	*<0.001*
Homocysteine (μmol/L)	*4.9 (0.9)*	*9.7 (1.6)*	*5.4–13.4*	*0.51*	*<0.001*
Coenzyme Q_{10} (μg/mL)	0.6 (0.2)	0.9 (0.3)	0.5–1.2	0.67	0.067
WBC ($\times 10^3/\mu L$)	**9.5 (2.8)**	**6.2 (1.8)**	**4.0–11.0**	**1.53**	**0.006**

HDL, high-density lipoproteins; hs-CRP, high-sensitivity C-reactive protein; IGF1, insulin-like growth factor 1; LDL, low-density lipoproteins; LDL_M, mean low-density lipoprotein particle size
Bold type indicates chimpanzee value significantly higher than human, and italics indicate human value significantly higher than chimpanzee.
[a]Reference ranges from the Kronos Science Laboratory.

Table 2. Comparison of mean (1 SD) values of biomarkers of oxidative stress in male chimpanzees (n = 10) and men (n = 10)

Oxidative stress factor	Chimpanzee	Human	Chimpanzee: human ratio	*P*
2,3dinorPFGα (μg/g)	*2.9 (1.3)*	*5.4 (1.6)*	*0.54*	*0.009*
5OHmU (μg/g)	**17.9 (4.6)**	**7.93 (2.5)**	**2.26**	**<0.001**
8OHdG (μg/g)	2.6 (1.4)	2.7 (0.7)	0.96	0.851
8isoPFGα (μg/g)	**0.39 (0.29)**	**0.03 (0.02)**	**13.09**	**0.003**
Peroxidizability index	**110.1 (10.6)**	**89.2 (4.8)**	**1.23**	**<0.001**

2,3dinorPFGα, 2,3-dinor 8-iso-prostaglandin F2α/creatinine; 5OHmU, 5-hydroxymethy l-2-deoxyuridine/creatinine; 8OHdG, 8-hydroxy-2-deoxyguanosine/creatinine; 8isoPFGα, 8-iso-prostaglandin F2α /creatinine
Peroxidizability index = (% monenoic × 0.025) + (% dienoic × 1) + (% trienoic × 2) + (% tetraenoic × 3) + (% pentaenoic × 4) + (% hexaenoic × 5)
Bold type indicates chimpanzee value significantly higher than human, and italics indicate human value significantly higher than chimpanzee.

a and IGF1 levels that were considerably above the human reference maximum (Table 1). Men had significantly higher levels of homocysteine ($F_{19} = 33.43$, $P < 0.001$) and lower levels of both folic acid ($F_{19} = 8.60$, $P = 0.009$) and vitamin B12 ($F_{19} = 291.85$, $P < 0.001$; Table 1). The fatty acid profiles (Figure 1) revealed that men had significantly higher levels of saturated (palmitic acid, C16:0, $F_{19} = 18.49$, $P < 0.001$) and monounsaturated fats (C18:1, $F_{19} = 32.57$, $P < 0.001$). Men also had significantly lower levels of unsaturated fats, both linoleic and α-linoleic (C18:2, C18:3) acid ($F_{19} = 5.36$, $P = 0.035$), and docosahexaenoic (C22:6) acid ($F_{19} = 4.60$, $P = 0.049$).

Chimpanzees and men did not differ significantly in either total HDL cholesterol ($F_{19} = 4.20$, $P = 0.055$) or total LDL cholesterol ($F_{19} = 0.02$, $P = 0.896$); however, chimpanzees had significantly smaller mean LDL particle size ($F_{19} = 77.30$, $P < 0.001$; Table 1). In terms of lipid subfractions, chimpanzees had significantly higher levels of both HDL_{IIa} ($F_{19} = 28.09$, $P < 0.001$) and HDL_{IIb} ($F_{19} = 11.17$, $P = 0.004$) and significantly lower levels of HDL_{III} ($F_{19} = 12.15$, $P = 0.003$; Figure 2). Men had significantly higher levels of IDL_3 ($F_{19} = 15.76$, $P = 0.001$; Figure 3). Chimpanzees had significantly lower levels of LDL_1 ($F_{19} = 7.11$, $P = 0.016$) and significantly higher levels of LDL_2 ($F_{19} = 17.25$, $P < 0.001$) and LDL_3 ($F_{19} = 9.18$, $P = 0.007$; Figure 4).

In terms of oxidative stress, chimpanzees had significantly higher levels of 5-hydroxymethyl-2-deoxyuridine (5OHmU; $F_{19} = 27.76$, $P < 0.001$) and 8-iso-prostaglandin $F_{2\alpha}$ ($8isoPGF_{2\alpha}$; $F_{19} = 13.09$, $P = 0.003$) but significantly lower levels of 2,3-dinor-8-iso-prostaglandin $F2_\alpha$ ($F_{19} = 9.81$, $P = 0.009$; Table 2). The amount of $8isoPGF_{2\alpha}$ in chimpanzees was more than 13 times that of men (Table 2). Chimpanzees also had significantly higher levels of ceruloplasmin ($F_{19} = 23.93$, $P < 0.001$) and copper (Cu, $F_{19} = 31.43$, $P < 0.001$) and a higher peroxidizability index ($F_{19} = 23.21$, $P < 0.001$) (Tables 2 to 3). In terms of oxidative protection, chimpanzees had significantly lower levels of albumin ($F_{19} = 98.98$, $P < 0.001$), uric acid ($F_{19} = 173.53$, $P < 0.001$), and both direct ($F_{19} = 8.10$, $P = 0.011$) and total bilirubin ($F_{19} = 24.44$, $P < 0.001$) (Table 4). In half of the chimpanzees, the amounts of total bilirubin and uric acid were

Table 3. Comparison of mean (1 SD) levels of prooxidants (oxidative stress catalysts) in male chimpanzees (n = 10) and men (n = 10)

Oxidative stress factor	Chimpanzee	Human	Human reference range[a]	Chimpanzee: human ratio	*P*
Cadmium (μg/L)	0.03 (0.04)	0.01 (0.01)	0.15–0.19	3.00	0.236
Ceruloplasmin (mg/dL)	**44.7 (7.7)**	**29.3 (6.3)**	**25.0–63.0**	**1.53**	**<0.001**
Copper (μg/L)	**1567.2 (285.5)**	**963.3 (151.4)**	**498.0–1945.0**	**1.63**	**<0.001**
Ferritin (ng/mL)	73.5 (31.6)	96.8 (45.5)	24.0–336.0	0.76	0.200
Glucose (mg/dL)	85.7 (11.4)	88.6 (7.6)	74.0–118.0	0.97	0.512
Hemoglobin A1C (%)	4.8 (0.2)	4.9 (0.2)	4.0–6.0	0.98	0.050
Iron (μg/dL)	111.1 (46.1)	92.3 (25.5)	45.0–182.0	1.20	0.274
Iron saturation (%)	33.0 (13.9)	29.1 (8.6)	10.0–36.0	1.13	0.506
Nickel (μg/L)	3.3 (1.0)	3.0 (1.0)	1.5–7.9	1.10	0.533

[a]Reference ranges from the Kronos Science Laboratory.

Table 4. Comparison of mean (1 SD) levels of factors protective against oxidative stress in male chimpanzees (n = 10) and men (n = 10)

Protective factor	Chimpanzee	Human	Human reference range[a]	Chimpanzee: human ratio	*P*
Carotenoids					
α-Carotene (ng/mL)	**8.3 2.8)**	**36.3 (23.3)**	**20.0-400.0**	**0.23**	**0.001**
β-Carotene (ng/mL)	**21.0 (42.2)**	**119.8 (64.8)**	**50.0-710.0**	**0.18**	**<0.001**
β-Cryptxanthin (ng/mL)	**8.4 (3.8)**	**76.7 (36.5)**	**5.0-200.0**	**0.11**	**<0.001**
Lycopene (ng/mL)	**10.3 (4.7)**	**174.6 (46.3)**	**42.0-435.0**	**0.06**	**<0.001**
Lutein (ng/mL)	*138.5 (48.9)*	*95.1 (36.9)*	*40.0-600.0*	*1.46*	*0.038*
Retinol (ng/mL)	*964.9 (186.5)*	*541.5 (91.5)*	*400.0-1300.0*	*1.78*	*<0.001*
Retinyl palmitate (ng/mL)	*47.4 (13.9)*	*10.6 (1.0)*	*5.0-27.0*	*4.47*	*<0.001*
Zeaxanthin (ng/mL)	30.0 (6.5)	25.8 (4.9)	10.0-150.0	1.16	0.121
Tocopherols					
α-Tocopherols (μg/mL)	9.7 (1.9)	11.1 (3.2)	7.2-22.4	0.87	0.238
β-Tocopherols (μg/mL)	**0.01 (0.00)**	**0.05 (0.03)**	**0.05-0.20**	**0.20**	**<0.001**
γ-Tocopherols (μg/mL)	**0.2 (0.1)**	**1.7 (0.5)**	**0.1-2.2**	**0.12**	**<0.001**
Other Antioxidants					
Albumin (g/dL)	**3.2 (0.3)**	**4.4 (0.2)**	**3.5-4.8**	**0.73**	**<0.001**
Ascorbate (μg/mL)	19.0 (8.9)	12.3 (5.4)	5.0-28.0	1.54	0.057
Direct bilirubin (mg/dL)	**0.08 (0.04)**	**0.14 (0.05)**	**0.0-0.5**	**0.57**	**0.011**
Total bilirubin (mg/dL)	**0.32 (0.09**	**0.75 (0.26)**	**0.4-2.0**	**0.43**	**<0.001**
Total thiols (μmol/L)	193.5 (94.1)	412.0 (0.0)	318-578	0.47	0.309
Uric acid (mg/dL)	**2.6 (0.3)**	**6.1 (0.8)**	**2.8-8.0**	**0.43**	**<0.001**

Bold type indicates that the chimpanzee value was significantly higher than that in men, and italics indicate that the human value was significantly higher than that in chimpanzee.
[a]Reference ranges from the Kronos Science Laboratory.

below the human reference minimum, and nearly all of the chimpanzees had albumin levels that were below the human reference minimum (Table 4). The chimpanzees also had significantly lower levels of α- (F_{19} = 14.25, P = 0.001) and β-carotene (F_{19} = 16.33, P < 0.001), β-cryptoxanthin (F_{19} = 34.64, P < 0.001), lycopene (F_{19} = 124.84, P < 0.001), and tocopherols δ (F_{19} = 15.06, P < 0.001) and γ (F_{19} = 71.22, P < 0.001; Table 4). In all of the chimpanzees, the levels of α-carotene, β-carotene, lycopene, and tocopherol were below the human reference minima (Table 4). However, men had significantly lower levels of lutein (F_{19} = 5.01, P = 0.038), retinol (F_{19} = 41.59, P < 0.001), and retinyl palmitate (F_{19} = 69.69, P < 0.001; Table 4).

Discussion

In humans, cardiovascular disease takes many forms, including coronary artery disease, atherosclerosis, coronary heart disease, cardiomyopathy, and chronic heart failure. The chimpanzees in this study showed increased levels of lipoprotein a, fibrinogen, and IGF1, which indicate a high risk for premature cardiovascular disease, particularly coronary heart disease and cardiomyopathy.[27,43,118] In particular, levels of lipoprotein a that are greater than 30 mg/dL are associated with a 200% increase in cardiovascular disease risk.[35,118] Cardiomyopathy-associated congestive heart failure has been documented in chimpanzees and is the leading cause of death in captive chimpanzees.[40,50,61,62,64] Elevated levels of lipoprotein a, fibrinogen, and IGF1 are all strongly associated with cardiomyopathy in humans.[7,8,13,14,72] The data pre-

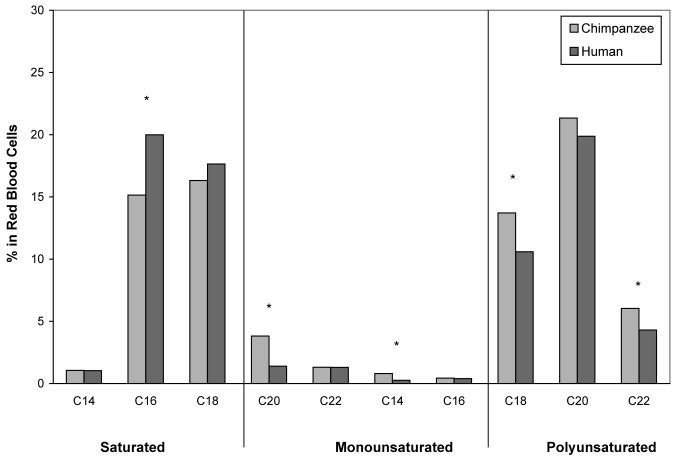

Figure 1. Comparison of mean (± SE) saturated, monounsaturated, and polyunsaturated fatty acids in male chimpanzees (n = 10) and men (n = 10). Asterisks indicate significant ($P < 0.05$) difference between chimpanzee and human values.

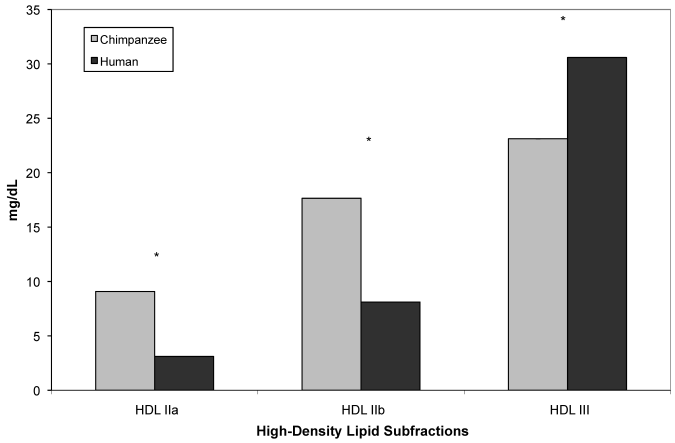

Figure 2. Comparison of mean (± SE) high-density lipid subfractions in male chimpanzees (n = 10) and men (n = 10). Asterisks indicate significant ($P < 0.05$) difference between chimpanzee and human values.

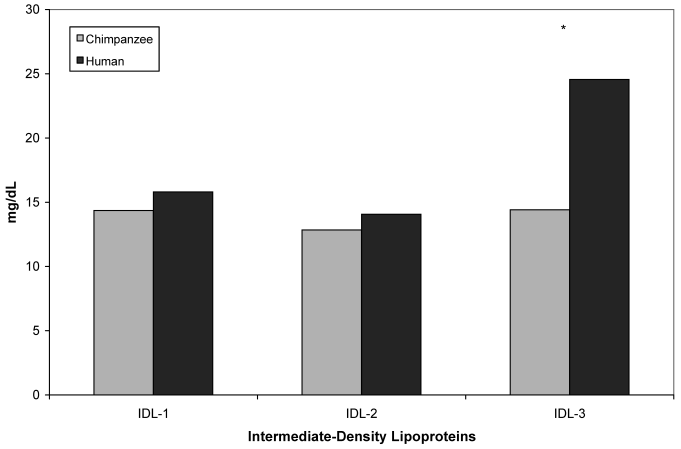

Figure 3. Comparison of mean (± SE) intermediate-density lipid subfractions in male chimpanzees (n = 10) and men (n = 10). Asterisks indicate significant ($P < 0.05$) difference between chimpanzee and human values.

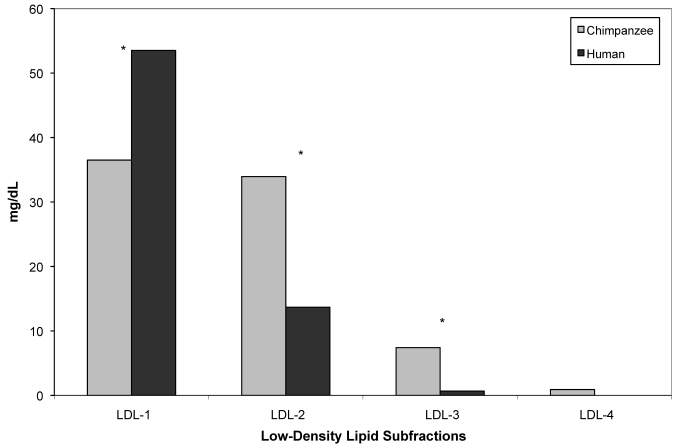

Figure 4. Comparison of mean (± SE) low-density lipid subfractions in male chimpanzees (n = 10) and men (n = 10). Asterisks indicate significant ($P < 0.05$) difference between chimpanzee and human values.

sented here support the observation that chimpanzees appear to be predisposed to cardiovascular disease, particularly cardiomyopathy. We hypothesize that among chimpanzees diagnosed with early cardiomyopathy, levels of lipoprotein a, fibrinogen, and IGF1 will be significantly higher than those in otherwise healthy chimpanzees. In addition, such increased levels likely are present in other great ape species, because (as in chimpanzees) cardiovascular disease is the leading cause of death among captive gorillas, bonobos, and orangutans.[12,81,92,112] The predisposition for cardiovascular disease may be 1 of the factors accounting for the shorter lifespan of chimpanzees, and potentially other great ape species, compared with humans. In contrast, the men in our study showed slightly higher risk for atherosclerosis because of their increased homocysteine levels.[118] The lower homocysteine levels in the chimpanzees could be due to the extremely high levels of folic acid and vitamin B_{12}, both of which have been shown to lower homocysteine levels.[68] Recent research[25,54] has suggested that increased homocysteine as a causal risk factor for cardiovascular disease may be overrated. Given that the level of homocysteine in the men was below the recommended maximum (13.4 µmol/L), we do not consider that this result in any way contradicts the remaining evidence that chimpanzees are at higher risk for cardiovascular disease.

At first glance, the fatty acid profiles seem to present contradictory results suggesting a higher risk for cardiovascular disease in humans, which is due to increased levels of saturated fatty acids and decreased levels of unsaturated fatty acids. Previous human research has shown that low levels of saturated fats (that is, palmitic acid) and high levels of polyunsaturated fats (that is, linoleic acids) can result in decreased levels of fibrinogen and reduced risk of coronary heart disease.[89,118] However, these results have been inconsistent, and supplementation with n3 and n6 polyunsaturated fatty acids does not consistently lower cardiovascular risk.[89,91,113] That these same effects are not seen reflected in the chimpanzees' cardiovascular risk profile provides further support that the relationship between fatty acid profiles and cardiovascular risk is unclear. Additional research on fatty acid profiles and cardiovascular risk factors among chimpanzees and other great ape species is needed to address this issue.

The lipid profile indicated that the chimpanzees had higher levels of large, less-dense HDL subfractions (HDL_{IIa} and HDL_{IIb}), compared with humans. Increased levels of large HDL subfractions have been correlated with reduced risk of coronary artery disease.[107,118] However, chimpanzees had significantly higher levels of small LDL subfractions (LDL_3 and LDL_4) and lower levels of the larger LDL subfractions (LDL_1 and LDL_2), indicating increased risk for both coronary artery disease and cardiomyopathy.[2,56,57,101,107,115] Overall, these data balance out as increased risk for cardiomyopathy in chimpanzees, a pattern that matches observations of increased cardiomyopathy-associated heart failure in captive chimpanzees.[40,50,61,62,64] We hypothesize that among chimpanzees diagnosed with early cardiomyopathy, levels of small LDL subfractions are significantly higher than in other chimpanzees. In addition, such elevated levels likely also are present in other great ape species, given that cardiovascular disease is the leading cause of death among captive gorillas, bonobos, and orangutans also.[12,81,92,112] Finally, the chimpanzees had a smaller mean LDL particle size than did the human subjects. Among humans, members of families exhibiting extreme longevity (that is, offspring of centenarians) have larger mean LDL particle size than age-matched controls.[44,104] This finding adds further support to the hypothesis that the small mean LDL particle size in chimpanzees is related to high disease risk and early death, compared with humans. All of these results support our hypothesis that chimpanzees are naturally at higher risk of cardiovascular disease compared with humans.

The chimpanzees in this study exhibited significantly increased levels of DNA oxidative damage, in the form of 5OHmU and $8isoPGF_{2\alpha}$. In fact, half of the chimpanzees had 5OHmU levels that exceeded the recommended maximum (19.6 mg/g). DNA oxidative damage has been linked to both cardiomyopathy and heart failure, but the biomarker typically measured when evaluating cardiovascular disease risk is 8-hydroxy-2-deoxyguanosine.[54,82] In addition isoprostanes (such as $8isoPGF_{2\alpha}$) are strongly linked to oxidative damage in heart valves and the risk of both coronary heart disease and cardiomyopathy.[42,78,84,116] Recent research has suggested that 5OHmU and $8isoPGF_{2\alpha}$ are better indicators of oxidative damage than 8-hydroxy-2-deoxyguanosine.[42,87] We hypothesize that increased levels of DNA oxidative damage are present among other great ape species, which also show decreased longevity and increased cardiovascular risk.[12,80,92,112] Increased oxidative stress among chimpanzees is further evident in their higher peroxidizability index. Because polyunsaturated fatty acids are the most sensitive to oxidative damage, an increased peroxidizability index is indicative of increased oxidative stress and a potentially decreased lifespan.[19,85] In this regard, it is important to note that comparative gene expression studies between humans and chimpanzees have indicated very high levels of peroxiredoxin (PRDX2, PRDX5, PRDX6) in humans. Specifically, peroxiredoxin 6 is overexpressed 10-fold or more in humans than in chimpanzees (data not shown). The lower expression of peroxiredoxin in chimpanzees may have contributed to their higher peroxidizability index, but additional research is needed to confirm this possibility. Levels of the prooxidants ceruloplasmin and copper also were significantly higher in the chimpanzees, suggesting increased risk of cardiovascular disease and cardiac death.[29,34,90,94,106] The elevated levels of 5OHmU and $8isoPGF_{2\alpha}$, together with elevated levels of ceruloplasmin and copper and a high peroxidizability index in the chimpanzees, provide additional support for our hypothesis that chimpanzees have higher levels of oxidative stress and are at higher risk for cardiovascular disease than are humans.

The chimpanzees in this study exhibited significantly lower antioxidant levels (that is, α- and β-carotene, β-cryptoxanthin, lycopene, and tocopherols) compared with their human counterparts. This status likely is not due to dietary deficiency, because the chimpanzees daily consumed, through diet and supplementation, more than 15,000 IU of vitamin A (1000 IU as β-carotene) and more than 30 IU of vitamin E (as α-tocopherol). Therefore, the significantly lower levels of antioxidants likely do not reflect dietary deficiency but rather a decreased ability to absorb certain antioxidants or increased degradation of them. This situation has a profound effect on both oxidative stress and cardiovascular risk. Tocopherols reduce levels of biomarkers of oxidative damage and reduce the risk of coronary heart disease.[11,49,53] Carotenoids, particularly α- and β-carotenes and lycopene, reduce the risk of mortality from cardiovascular disease.[52] In addition, β-carotene and lycopene alter fatty acid profiles and β-cryptoxanthin reduces inflammation, thereby decreasing cardiovascular disease risk.[57,117] However, results of studies examining the relationship between antioxidants and cardiovascular disease have been inconsistent.[45,53,69,76,120] Further, our chimpanzees exhibited increased levels of retinol and lutein, which some may interpret as reduced risk of cardiovascular disease. These antioxidant levels are within the range published for other captive chimpanzees, but because serum antioxidant levels are unavailable for wild chimpanzees, we cannot eliminate the possibility that the levels published for captive chimpanzees are artificially inflated due to diet.[15,31] These antioxidant data should be interpreted remembering that most randomized trials and meta-analyses of the effects of antioxidants on morbidity and mortality typically do not involve an integrated approach (using multiple antioxidants) and fail to examine the effects of antioxidant supplementation on measures of oxidative stress.[73] In addition, some research suggests that retinol and lutein levels are not good predictors of future coronary heart disease or cardiac death.[37,103,117] We hypothesize that chimpanzees are at increased risk for oxidative stress and, likely, cardiovascular disease, due to their decreased levels of tocopherols and carotenoids. However, additional research is needed.

Both albumin and bilirubin are viewed to have antioxidant properties by preventing the production of damaging free radicals and protecting free fatty acids from peroxidation.[38,99] Decreased levels of serum albumin and bilirubin are related to an increased risk of coronary heart disease and heart failure.[23,33,60,75] The decreased levels of albumin and bilirubin in our chimpanzees therefore add further support that chimpanzees are at high risk for oxidative stress and cardiovascular disease, and we hypothesize that chimpanzees diagnosed with early cardiomyopathy have lower levels of albumin and bilirubin than do other chimpanzees.

The lower levels of uric acid in chimpanzees could be interpreted as reducing risk of cardiovascular disease. Several studies have reported that increased uric acid levels are a risk factor for cardiovascular disease.[10,30,111] However, other authors have concluded that there is no independent association between uric acid and cardiovascular disease risk.[79,109,110,114] In addition, some studies suggest that uric acid has potent antioxidant properties and potential lifespan-enhancing benefits,[5,18,24,36,48,83] whereas others have found no relationship between uric acid levels and maximum lifespan potential.[71,77] In addition, uric acid levels are influenced

heavily by nutrition and environmental circumstance[109] and perhaps less so by oxidative stress. Therefore, the significance of the decreased uric acid in our chimpanzees is unclear. We hypothesize that the higher uric acid levels of humans may be unrelated to cardiovascular risk, because in studies in which levels were predictive of cardiovascular disease, uric acid levels exceeded 8 mg/dL,[10,66] well above the levels in the current study. However, additional research is needed, and no definitive conclusion regarding uric acid can be made at this time.

The oxidative stress hypothesis of aging predicts that differences in aging rates between closely related species are the result of differences in both oxidative stress (that is, ROS) and antioxidant levels.[4,17-21,84,96] If chimpanzees experience an aging rate twice that of humans, one would predict that chimpanzees would show correspondingly higher levels of oxidative stress and cardiovascular risk and lower levels of antioxidant capacity. Results of the current study supported this hypothesis, with chimpanzees exhibiting cardiovascular risk and oxidative stress levels twice that, on average, of humans. The data from this study generally support the idea that the extended lifespan in humans is, at least in part, the effect of genetic changes resulting in increased antioxidant capacity and decreased cardiovascular risk and oxidative stress. Further, our study suggests that throughout its lifespan, the chimpanzee may have a higher level of oxidative stress, which leads to acceleration of its aging process compared with that of humans. Fibrinogen, IGF1, lipoprotein a, 5OHmU, 8isoPGF$_{2\alpha}$, and ceruloplasmin are all good candidates for aging biomarkers in hominoids (that is, great apes and humans). Additional research is needed to test these hypotheses. Clearly, more studies are needed to explore the genetic basis for the differences observed in this study, as well as the longitudinal differences between these species in terms of oxidative stress and cardiovascular disease.

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