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Diet-induced early-stage atherosclerosis in baboons: lipoproteins, atherogenesis, and arterial compliance

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Abstract

Background—The purpose of this study was to determine if dietary manipulation can reliably induce early-stage atherosclerosis and clinically relevant changes in vascular function in an established, well-characterized nonhuman primate model.

Methods—We fed 112 baboons a high cholesterol, high fat challenge diet for two years. We assayed circulating biomarkers of cardiovascular disease (CVD) risk, at 0, 7, and 104 weeks into the challenge; assessed arterial compliance noninvasively at 104 weeks; and measured atherosclerotic lesions in three major arteries at necropsy.

Results—We observed evidence of atherosclerosis in all but one baboon fed the two-year challenge diet. CVD risk biomarkers, the prevalence, size, and complexity of arterial lesions, plus consequent arterial stiffness, were increased in comparison to dietary control animals.

Conclusions—Feeding baboons a high cholesterol, high fat diet for two years reliably induces atherosclerosis, with risk factor profiles, arterial lesions, and changes in vascular function also seen in humans.

Keywords

High fat diet; Cardiovascular disease; Nonhuman primate

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INTRODUCTION

Cardiovascular disease (CVD) is the leading cause of mortality worldwide[1–3], accounting for nearly 50% of the deaths (Global status report on noncommunicable diseases 2010; http://www.who.int/nmh/publications/ncd_report_full_en.pdf). CVD encompasses a broad range of disorders of the cardiovascular system, including coronary heart disease, cerebrovascular disease, and peripheral artery disease. The underlying cause of these diseases is atherosclerosis - a complex disorder in which a host of different intrinsic and extrinsic processes and factors contribute to the development of lesions that eventually compromise normal vascular function[4].

Baboons (*Papio hamadryas ssp.*) have been used to great advantage in studies of biological and environmental factors known to influence variation in risk for CVD in humans for over 60 years[5]. The baboon has been one of the more thoroughly genetically characterized nonhuman primate species during the past 3 decades and, as such, the goals of a majority of CVD-related studies utilizing baboons have been the detection, characterization, and identification of genes[6–8]. However, in the "post genomics" era their value may be more fully realized in experimental studies focused on mechanisms of development and progression of atherosclerosis with clear and direct translational implications for its diagnosis, prevention, and treatment. The baboon satisfies the majority of key criteria of an ideal animal model for many studies of CVD and related disorders. The species mimics the human subject developmentally, metabolically and pathophysiologically[9–11]; it is large enough to allow repeated whole organism physiological and metabolic studies; it naturally develops atherosclerosis and exhibits similar responses to an atherogenic diet, including dyslipidemia and susceptibility to atherosclerosis. Also like humans, baboons exhibit individual variation in the extent of atherosclerotic lesions and have been shown to develop end-stage disease(s) comparable to those in humans[9, 12, 13]. Moreover, as in humans, quantitative variation in traditional CVD risk factors is heritable in baboons[5]. Several studies have confirmed and identified novel genes that play roles in the mediation of response to atherogenic diets (e.g.,[6, 8, 14–16]). Further, baboon growth, development, maturation, and aging follow a generalized Old World primate pattern[17–20] shared with humans, and their longer lifespans compared to small animal models provide unique opportunities to better observe the development and progression of atherosclerosis, its comorbidities, and consequent disease end-points. These characteristics correspond to those of the "ideal animal model" for CVD-related studies – i.e., one likely to have potential for more immediate translation to humans[13].

Many researchers eschew the baboon and related nonhuman primates in favor of smaller, shorter-lived, less phenotypically and genetically variable animals like inbred mice, despite well recognized dissimilarities of the latter to the human condition[21]. The most frequently raised reasons for this are the greater time and expense associated with breeding and developing atherosclerosis in a nonhuman primate like the baboon[12, 13]. These concerns likely are amplified by a common misperception that genetic variability in non-inbred baboon breeding colonies makes model development unpredictable, necessitating larger initial sample sizes, which further increase total costs before data collection can even begin[21].

In this paper, we demonstrate that the baboon is a reliable animal model for studies related to onset, severity, and progression of atherosclerosis. We show that simple dietary manipulation (i.e., addition of cholesterol and saturated fat) for two years induces the classic features of early stage atherosclerosis – fatty streaks and fibrous plaques[22] – as well as increased stiffness in the large and medium arteries (decreased vascular compliance), a hallmark of arteriosclerosis. Thus, a salient link between atherosclerosis and consequent CVD can be produced predictably in a time frame that accommodates a majority of current study designs. The data presented are derived from a recently completed phase of a long-term study of the interaction between diet and genes underlying variation in atherosclerosis risk factors in baboons from a single, large, six-generation pedigree, the members of which have been extensively characterized – genetically and phenotypically[5].

MATERIALS AND METHODS

Humane Care Guidelines

All research procedures involving animals for this study were conducted in facilities certified by the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) International at the Southwest National Primate Research Center (SNPRC) in San Antonio, TX. All procedures were reviewed and approved by the Institutional Animal Care and Use Committee at the Texas Biomedical Research Institute, the host institution for the SNPRC.

Subjects and Diets

The subjects of the study were 173 baboons [olive baboons (*Papio hamadryas anubis*), yellow baboons (*P. h. cynocephalus*), and their hybrid descendants], all of which were members of a large pedigreed breeding colony developed and maintained at the SNPRC.

The study utilizes data from two groups of baboons distinguished by diet protocols. Baboons in the control diet group were fed a basal diet, low in cholesterol and fat (LCLF), for at least 7 years prior to, and for the duration of, this study. Baboons in the experimental diet group were fed the LCLF diet prior to beginning a two-year dietary challenge with a diet high in cholesterol and saturated fat (HCHF). Data for animals in this group were obtained just prior to beginning the two-year HCHF diet challenge (while on LCLF diet), at seven weeks, and at the end of two-year period. Table 1 shows the composition of the basal (LCLF) diet and the base diet used to prepare to atherogenic HCHF diet (respectively, “Monkey Diet 15%/5LEO” and “Monkey Diet 25/50456,” LabDiet, St. Louis, MO). To make the HCHF diet, we add a mix of lard, cholesterol, sodium chloride, vitamins [ascorbic acid and vitamin A (a retinyl acetate)], and water to the base diet (Table 1). Our analyses of the resulting atherogenic HCHF diet reveal the following[23]. Metabolizable energy is approximately 3.8 kcal/g; fats and carbohydrates each make up approximately 40% of calories, with proteins comprising 20%. The composition of total fatty acids, determined by gas-liquid chromatography of the fatty acid methyl esters [on DB-225 column (15 m), J&W Scientific], is saturated fatty acids: myristic (1.7%), palmitic (24.9%), and stearic (17.9%); monounsaturated fatty acids: palmitoleic (2%) and oleic (38.7%); and polyunsaturated acids: linoleic (13.9%) and linolenic (0.9%). All baboons in the study were fed daily and allowed

to eat *ad libitum.* The approximate mean per animal daily intake of the LCLF diet is 500 g (~1500 kcal) and that for the HCHF diet is 400 g (~1200 kcal). Respectively, the mean amount of cholesterol consumed daily by animals on each of these diets is approximately 30 mg and 2230 mg (the latter being equivalent to that in 10–12 large eggs).

Control diet groups—There were two control groups consisting of animals fed only the LCLF basal diet for most of their lives, including two years prior to data collection. As the control group for the arterial lesion study (see below), we selected 20 adult baboons: 9 females and 11 males (mean age = 12.6 years, range = 7.2 to 18.4 years). A second control group, for the arterial compliance study, was made up of 41 animals: 11 females and 30 males (mean age: 11.7 years, range: 8.9 to 17.4 years).

Experimental diet group—The experimental diet group included 112 baboons: 47 females and 65 males. Ages ranged from 8.1 years to 17.0 years. Mean age for the females was 12.6 years (range = 8.7 to 17.0 years) and that of the males was 11.4 years (range = 8.1 to 14.1 years).

Arterial lesions

We assessed lesion formation in three major arteries: the aortic arch, thoracic section of the descending aorta, and the common iliac artery. Atherosclerosis in these three arteries has been shown to be relevant to systemic risk of the disease and its complications in humans. Aortic arch atherosclerosis is an independent risk factor for ischemic stroke and recurrent vascular events[24–26]; atherosclerosis in the thoracic aorta is strongly predictive of generalized atherosclerosis[24] *and* coronary artery disease[27, 28]; and the common iliac arteries are the second most frequently affected blood vessels (after the arteries of the thigh) in atherosclerotic peripheral artery disease[29]. These associations have been replicated in earlier studies of baboons from this same pedigreed breeding colony[30, 31].

For the lesion studies, baboons in the control and experimental diet groups were humanely euthanized and subjected to a standard necropsy procedure. Baboons in the experimental group were euthanized after two-years on HCHF diet. The three above noted arteries were defatted and dissected longitudinally. Adventitial surfaces were adhered to chip-board, and the arteries were fixed in buffered 10% formalin and stained for 18 h with Sudan IV in 38% isopropanol (v/v) made up as a stock solution and diluted to a consistent optical density (i.e., A_{520nm}=0.22±0.01)[32].

A team of research support staff, trained by an experienced cardiovascular pathologist, first identified fatty streaks and raised fibrous lesions (plaques) by visual inspection of the stained arterial sections. The same team assessed all arteries from which data for this study were obtained. Members of this team, trained by an experienced histomorphometrist, quantified the extent of atherosclerotic lesions (i.e., percent area covered by fatty streaks, plaques, or both when each was present) on arterial surfaces using BioQuant Image Analysis software (Nashville, TN) as previously described[33]). Briefly, an entire artery was photographed and images imported into the BioQuant software. Subsequently, regions in the imager corresponding to lesion were scanned to obtain the fraction of pixels in affected regions compared to the entire artery template. The fraction of involved pixels represented the

percent of intimal surface area or the extent of a specific type of lesion in a given artery as explained[33]. Age- and sex-adjusted residuals of these measures were normalized (inverse Gaussian transformation).

Arterial compliance

We used a computerized central blood pressure and pulse wave analysis/pulse wave velocity (PWA/PWV) assessment system (AtCor Medical, Itasca, IL) to noninvasively assess parameters related to arterial compliance (stiffness) in two cohorts of baboons: 50 animals fed the HCHF atherogenic challenge diet for two years and 41 fed the LCLF basal diet for two years. Following the validated approach of Lazar et al[34] with bonnet macaques (*Macaca radiata*), the chosen peripheral artery was brachial and all assessments were done on the left side. We conducted evaluations in animals immobilized with an intramuscular injection of ketamine hydrochloride (10 mg/kg) after observing a stable state – i.e., stable pulse rate and resting blood pressures within one standard deviation of values observed in previous studies of non-anesthetized (tethered) baboons from this same pedigreed colony[35]. Note: Ketamine HCl has been shown to be "a suitable anesthetic for endocrine, metabolic, and cardiovascular studies," including vascular compliance in baboons[36, 37, 34] and other Old World Monkeys[38] because it generally does not significantly affect vascular resistance or systemic blood pressure[39]. We accepted data for analyses only if the operators observed consistent, acceptable waveform profiles for at least ten seconds. Acceptable waveforms were those with in-device quality indexes (a function of mean pulse height, variation in pulse height and diastolic pressure, and the maximum rise of the peripheral waveform) greater than 80%. All assessments were performed by research staff after training by both the device manufacturer and a veterinary researcher with experience using the device with nonhuman primates.

CVD-related biomarkers

We also obtained data on circulating CVD risk factors, including lipids and lipoproteins, lipoprotein-related enzymes, and biomarkers of inflammation and oxidative stress from fasting blood samples collected at three time points from all animals undergoing the two-year dietary challenge and at one-time point from animals in the basal LCLF diet control group for lesion studies.

Lipids and Lipoproteins—We quantified the concentrations of the following serum lipids and lipoproteins. Total serum cholesterol (TSC) and triglyceride (TG) concentrations were determined enzymatically using commercial reagents in a clinical chemistry analyzer. High density lipoprotein cholesterol (HDLC) was measured in the supernatant after heparin-Mn+2 precipitation, and non-HDLC (V+LDLC) was calculated as the difference between total and HDLC. Concentrations of apolipoproteins AI (APOAI), B (APOB), and E (APOE) were determined using an immunoturbidometric approach with commercial reagents in a clinical chemistry analyzer. We provide detailed descriptions of the methods used in these assays elsewhere[6, 40, 41].

We used composite gradient gels to further resolve LDLs and HDLs on the basis of size[37] and Sudan black B to quantify lipoprotein cholesterol size distributions. Fractional

cholesterol absorbance among APOB-containing lipoproteins was estimated for the following very low density lipoprotein (VLDL) fractions and LDL fractions: VLDL1C (36–33 nm), VLDL2C (33–30 nm), LDL1C (30–28 nm), LDL2C (28–27 nm), LDL3 (27–26 nm), and LDL4 (26–24 nm): and HDL fractions: HDL1AC (24–20 nm), HDL1BC (20–13 nm), HDL2C (13–9.9 nm), and HDL3C (9.9–8.2 nm). From these we estimated serum concentration for each fraction by multiplication with non-HDL or HDL cholesterol concentrations as appropriate. For cholesterol distributions only, we estimated median diameters for APOB-containing lipoproteins (Bmed; 24–36 nm) and HDLs (Hmed; 8.2–24 nm), which were defined as the diameter where half the absorbance was on larger, and half was on smaller, particles. We measured plasma oxidized LDL (OxLDL) concentrations (U/L) immunologically using a sandwich-style enzyme-linked immunoabsorbent assay (Mercodia Oxidized LDL ELISA; ALPCO Diagnostics, Salem, NH)[41].

Lipoprotein-related enzymes—We measured serum activity levels of two lipoprotein-associated enzymes, lipoprotein-associated phospholipase A_2 (Lp-PLA_2) and paraoxonase 1 (PON1) on two substrates. We determined the activity of the APOB associated enzyme, Lp-PLA_2 (μmol/min/L), using 2-thio-PAF as substrate in a commercial microplate-based colorimetric assay[42)]. We used a microplate-based colorimetric assay[43] to measure the activity (μmol/min/L) of HDL-associated enzyme, PON1, with paraoxon as substrate (PON1-para) in one case and arylesterase with phenylacetate as substrate (PON1-aryl) in the other.

Inflammation and Oxidative Stress—We assayed interleukin-8 (IL8; pg/mL) using a sandwich-style enzyme-linked immunoassay kit, as suggested by the manufacturer (R&D Systems, Inc.)[44]. Total antioxidant status (TAS), reflecting the overall antioxidant capacity of serum, was defined as the ability to prevent oxidation of 2,2'-azino-di-(3-ethylbenzthiazoline sulfonate) by metmyoglobin with the use of a kit from Calbiochem (San Diego, CA)[41]. We used a high-sensitivity assay kit (Kamiya Biomedical, Seattle), with a latex particle-enhanced immunoturbidometric method, to measure C-reactive protein (CRP) concentrations (mg/L). We quantified the Von Willebrand factor (vWF; expressed as percentage of an international standard value[41]) using a sandwich-style enzyme-linked immunoassay kit (Diagnostica Stago, Parsippany, NJ).

Data analysis

Summaries of raw data are presented as means, standard deviations, and medians. Unless otherwise indicated, data in all analyses were i-normalized residuals of the quantitative variables after adjustments for the significant mean effects of common covariates like sex, age, body weight, etc. Because the data are derived from related animals (mean kinship coefficient = 0.03, between second and first cousins), we used a maximum likelihood-based variance decomposition approach, implemented in SOLAR[46], that is designed to account for kinship in data from pedigrees to estimate both the mean effects of covariates and appropriately weighted correlations. We used likelihood ratio tests to assess significance of these parameter estimates.

We used a Bayesian model selection approach, also implemented in SOLAR, to identify the subset of the highly inter-correlated CVD risk factors contributing the most independent information to lesion extent. Using this method, we computed an approximate Bayes factor, the Bayesian Information Criterion (BIC), for each covariate model[47]. BIC differences greater than 2 provide positive evidence of support for one model over another (posterior probabilities, $P_{Posterior}$, ≥75%); differences of 6 units indicate stronger support ($P_{Posterior}$, = 95%); and differences greater than 10 units indicate posterior probabilities of at least 99%. The approach automatically eliminates multiple testing bias and the penalties for it[47, 48].

In all tests, $\alpha = 0.05$. When necessary to control for multiple testing bias in non-Bayesian analyses, we used Bonferroni adjusted p-values; even though they are overly conservative, given expected intercorrelations among the CVD risk factors. For descriptive analyses in which kinship was not an issue, we used statistical routines implemented in the commercially available software package NCSS, version 07.1.9[49].

RESULTS

Arterial lesions

We observe atherosclerotic lesions (fatty streaks and/or plaques) in the aortic arch, thoracic aorta, and common iliac artery (Figure 1). Lesions are observed in at least one artery from 111 of 112 animals (99%) that completed the two-year diet challenge; more than twice the 40% prevalence observed in the same three arteries harvested from 20 dietary control animals. All three arteries are affected in 109 baboons (Table 2). The mean percentages of the areas covered by lesions (fatty streaks plus plaques, when the latter are present) in all three arteries from the challenge diet group are approximately 2.6 times to 5.0 times larger than in those from controls (P<0.0004). Two-sample t-tests show no difference between the percentages of areas covered by lesions in the aortic arch and common iliac artery (*P = 0.694) but the means for each of these arteries differ significantly from that of the thoracic aorta (*P = 0.0002).

Extent of atherosclerotic lesions for the aortic arch and thoracic aorta, but not the common iliac artery, differ by sex. Mean percentages for areas affected in females are significantly greater than that in males in both the aortic arch (P = 0.00052) and the thoracic aorta (P = 0.000009), but not in the common iliac artery (P = 0.768). These analyses also reveal a significant effect of age on lesion extent in the aortic arch (P = 0.0023) and common iliac artery (P = 0.039), but not in the thoracic aorta (P = 0.42), even when accounting for the possible confounding effect of variation in days on the challenge diet which does exert a significant mean effect on percent area covered by fatty streaks in the thoracic aorta (P = 0.032).

The percentages of the areas in the three arteries covered by atherosclerotic lesions are significantly inter-correlated (P < 0.001). The correlations between their extent at the aortic arch and each of the other two sites (i.e., thoracic aorta: r = 0.488, $r^2 = 0.238$; common iliac artery: r = 0.541, $r^2 = 0.293$) are greater than that between the thoracic aorta and the common iliac artery (r = 0.317, $r^2 = 0.100$).

We observe plaques in only 29 baboons (12 females and 17 males) that completed the HCHF diet challenge, but in only four (20%) dietary control animals. The number of plaques per challenge diet baboon range from one to seven, *versus* one to four for the eight affected control diet animals.

While we observe no plaques in the thoracic aorta, three individuals (two females, one male) have plaques in the aortic arch and 26 (10 females, 16 males) have plaques in the common iliac artery. On average, plaques in the aortic arch are smaller than those in the common iliac artery (covering 0.2% to 1.0% vs. 0.6% to 6.1% of the intima, respectively).

Atherosclerotic lesion extent is only moderately predictive of the presence and numbers of plaques in the 112 animals that completed the two-year dietary challenge ($\chi^2_{[3]} = 7.96$, P = 0.047) and nominally correlated with plaque size in the common iliac artery (r = 0.353, r^2 = 0.125, P = 0.042).

Circulating biomarkers of CVD risk—Concentrations (or activities) of the majority of circulating biomarkers of lipid/lipoprotein metabolism and inflammation show significant mean changes in the animals fed the HCHF challenge diet for two years (Figures 2A–2J). Values for HDL3C, LDL3C, vWF, and TAS at two-years (Week 104) are not significantly different from those at baseline (Week 0). An interesting observation is that all variables, except IL8 and TAS, exhibited a significant increase in the first seven weeks of the HCHF diet challenge; with the values for the major lipoprotein cholesterol classes, OxLDL, $LpPLA_2$, PON-aryl, IL8, and vWF being greater than those after two years.

Concentrations of TSC and other biomarkers within or associated with the β-lipoprotein size range consistently show the strongest correlations with extent of lesions in the three arteries, as well as with PC1, the first principal component accounting for approximately 70% of the covariance among lesion sizes in all 3 arteries (Tables 3A–3C). Significant correlations are most pronounced for the aortic arch and common iliac artery. Taking into consideration only these significant correlations, the percent of the variance ($r^2 \times 100$) shared by the extent of lesions with TSC and key β-lipoprotein traits ranges from 9% to 20%. Compared to those measured at baseline, more CVD biomarkers assayed at either time point (seven weeks or two years) during the dietary challenge are significantly correlated with the extent of lesions in the three arteries. The number and means of correlations that are significant after Bonferroni corrections are greatest for those based on data from samples collected at seven weeks.

Bayesian model selection analyses of the subset of biomarkers exhibiting at least nominally significant correlations with the extent of lesions in any artery support the correlation analysis results and identify LDL_1C (i.e., large LDL particles between 28 and 30 nm in diameter), measured at seven weeks on the HCHF diet, as the single best predictor of variation in PC1. The model with the closest BIC value is the two-degree model that includes LDL_1C measured at both seven weeks and 104 weeks ($BIC_{\text{degree 1}} = -25.11$, $BIC_{\text{degree 2}} = -23.09$, difference = 2.02, $P_{\text{Posterior}} = 0.75$). Positive evidence of support for these two models over the model with the next highest BIC, a three-degree model including LDL_1C at seven and 104 weeks and LDL_3C at 104 weeks, respectively, is approximately

85% and 79%. All more saturated models have much larger BIC values (weaker evidence), indicating that no additional information is to be had by addition of any number of the other covariates (previously found to be correlated with PC1) to a model that only included LDL_1C at seven weeks.

An incidental, but still interesting, observation is suggestive evidence (P = 0.07) that some of the variation in the extent of lesions, as represented by PC1, is attributable to the additive effects of genes. Although, as we indicated in the Materials and Methods section, the mean kinship is low, there is sufficient variability in degrees of relationship among all possible pairs of baboons in these 112 to detect some evidence of genetic effects on the trait. The estimated heritability (h^2), or the proportion of the residual phenotypic variance in PC1 that is due the effects of genes, in the model with the lowest BIC (above) is $h^2 = 0.21 \pm 0.12$.

After Bonferroni correction, OxLDL concentration and the median diameter of HDL particles are significantly correlated with the sum of plaque extent ($P < 0.0018$) in all three arteries (respectively, $r = 0.603$, $r^2 = 0.364$ and $r = -0.575$, $r^2 = 0.331$). We find nominal evidence (i.e., $P < 0.05$) for correlations between plaque extent and several additional variables related to LDL (APOB, $r = 0.449$, $r^2 = 0.201$ and the APOB associated $LpPLA_2$, $r = 0.368$, $r^2 = 0.135$) and HDL (total HDLC, $r = -0441$, $r^2 = 0.194$; HDL1BC, $r = -0.475$, $r^2 = 0.226$; and PON-aryl, $r = 0.495$, $r^2 = 0.245$).

Arterial Compliance (Stiffness)

Data on arterial compliance and blood pressures come from 50 animals sampled randomly from the 109 in which lesions of some size are seen *in all three arteries* after being fed the HCHF diet for two years and from 41 adult baboons fed the LCHF diet for two years (Table 4). The two groups do not differ significantly by age (p=0.39) or body weight (p=0.15), even when sex-adjusted residuals are analyzed (results not shown).

All measures from the pulse wave analyses show higher values in challenge-diet animals than in controls, but we find only nominally significant evidence of increased arterial stiffness (decreased arterial compliance) in analyses of PWV, augmentation index (Aix), and related measures: augmentation pressure (AugP) and aortic pulse pressure (APP), and aortic systolic pressure (ASP). In diet-challenged animals, mean PWV and mean AIx, respectively, are 23% (P = 0.0184) and 58% (P = 0.0073) greater than in control animals. Similarly, mean AugP and APP in animals fed the HCHF diet for two years, respectively, are 78% (P = 0.0019) and 14% (P = 0.0199) greater than in control group baboons.

PWV is significantly correlated with percent artery area covered by plaque ($r = 0.365$, $r^2 = 0.133$) and fatty streaks in the thoracic aorta ($r = 0.368$, $r^2 = 0.135$) after correction for multiple testing (Table 5). Evidence for correlation between AIx and extent of fatty streaks in the thoracic aorta is suggestive ($r = 0.309$, $r^2 = 0.095$). AugP shows similar, suggestive correlations with fatty streak extent in the thoracic aorta ($r = 0.282$, $r^2 = 0.080$); and with total number of plaques ($r = 0.285$, $r^2 = 0.081$).

We observe the strongest and most consistent correlations (significant after adjustment for multiple testing) between arterial pressures and lesion extent variables. Brachial and aortic

diastolic pressures are significantly correlated with extent of plaques, number of plaques, and the summary measure of lesion extent (PC1). These correlations range from r = 0.388 (r^2 = 0.151) to r = 0.580 (r^2 = 0.336). Mean brachial artery and aortic systolic pressures also show high correlations with PC1 (r = 0.431, r^2 = 0.186 and r = 0.396, r^2 = 0.157, respectively) and fatty streak size in aortic arch (r=0.59, r^2 = 0.348 and r =0.426, r^2 = 0.181, respectively).

DISCUSSION

Ultimately, successful treatment and/or prevention of atherosclerosis will require an understanding of mechanisms by which interactions between CVD risk factors and the cells of the vascular endothelium result in the development and progression of arterial lesions from simple fatty streaks to unstable plaques. Although recent advances in clinical diagnostic and research technologies (e.g., imaging modalities) have made it possible to detect and determine the extent of atherosclerosis in human research participants and patients with greater precision than ever before, many critical studies of disease onset and progression, and responses to therapy remain impractical, if not impossible, in humans. It is for this reason that we turn to animal models to elucidate pathogenetic steps and causalities in atherosclerosis[50].

Our study shows that the baboon is a suitable model for human atherosclerosis studies in that the disease can be reliably induced in a time frame which will facilitate rapid research progress from discovery to translational outcomes in our own species. Together, the range of inter-individual phenotypic variation in the extent of lesions and their association with biomarkers of CVD risk and pathophysiology observed in nearly 100% of the baboons studied correspond well with what is observed in the early stages of atherosclerosis in humans[22, 33]. All lesions observed following a two-year exposure to the atherogenic diet, including those underlying raised areas, include fatty streaks. These fatty streaks, observed and described in reports of studies conducted over the past 35 years with baboons from earlier generations of this same pedigreed breeding colony (e.g.,[31]), correspond to AHA Type II lesions[51], from which more complicated plaques arise. As other studies of biomaterials and data from these same baboons have shown, reliable, consistent production of sizable and widespread early stage lesions in two years makes the baboon a most appropriate model for studies designed to dissect and understand the biological mechanisms responsible for the earliest disruptions of vascular cellular function leading to atherosclerosis[52, 53]. Such studies can realistically be expected to further inform efforts to develop and evaluate interventions and individually tailored lifestyle recommendations to prevent early stage lesion formation.

In earlier studies of baboons from this breeding colony, raised lesions have been shown to be relatively uncomplicated fibrous plaques, made up primarily of vascular smooth muscle cells and connective tissue with varying amounts of lipid, most resembling AHA lesion Types Va or Vc[30, 31, 51, 54]. Our observation of these plaques in approximately 28% of the affected animals indicates that, under the described experimental conditions, pathology consistent with the progression of atherosclerosis can be induced in baboon as well.

Our observations not only reinforce the validity of baboon model for studies relevant to our own species but they also point to the utility of baboons for studies involving experimental manipulation and/or control, which may be impractical in humans, designed to assess genetic influences on development, severity, and progression of atherosclerosis, as well as responsiveness of the disease to therapeutic interventions.

As reported for this species in the past[9, 31], prolonged exposure to the HCHF diet significantly increased both prevalence and extent of atherosclerotic lesions. However, our observation of lesions in 40% of the dietary control group confirms that baboons, like humans but unlike mice, can naturally develop lesions without genetic manipulation. This is consistent with the results of previous studies, which have found that free-living baboons and captive baboons fed the LCLF diet also develop arterial lesions resembling those naturally observed in humans[55, 56] and further reinforces the validity of the baboon model of atherosclerosis for translational studies.

To a large degree, our observations of responses of "classical" CVD risk factors – i.e., circulating concentrations and/or activities of biomarkers of lipid and cholesterol metabolism – to the HCHF challenge diet reaffirm the relevance of the baboon for studies of CVD risk factors in humans. In addition to observing evidence of expected lipemic responses to the diet, the concentrations of these biomarkers, particularly those within the LDL and VLDL particle size ranges, are the best predictors of overall extent of lesion development in baboons, corresponding well to their known pro-atherogenic effects in humans. Evidence of expected anti-atherogenic effects of molecules within the HDLC particle size ranges, albeit weaker, is equally suggestive of the baboon-human correspondence. Similarly, observed positive and negative correlations of extent of plaques with biomarkers related to, respectively, LDL (e.g., oxidized LDLC, APOB, $LpPLA_2$) and HDL (e.g., HDLmed, total HDLC, HDL1BC, and PON-aryl) are consistent with well documented associations observed in human CVD epidemiology,[22, 31].

Results from our analyses of these "classical" risk factors may point also to areas for further study in baboons, particularly with respect to the duration of exposure to a HCHF diet necessary to induce atherogenesis. For example, concentrations of circulating biomarkers of lipid and lipoprotein metabolism measured at seven weeks and two years into the HCHF diet challenge are both elevated significantly compared to those at baseline. However, the 7-week values are both greater than those measured at the end of the challenge and equally or more predictive of lesion extent at the end of the study (approximately 22 months later). The pattern of correlations between biomarkers and the extent of raised plaques is consistent with these findings – i.e., measures at seven weeks into the atherogenic diet challenge are more highly correlated with plaque development than those at either baseline or at the end of the 2-year challenge. These observations, plus others from shorter term studies[57], suggest that circulating biomarkers obtained as early as seven weeks post diet challenge are predictive of future atherosclerosis burden. Also, as the concentrations of circulating risk factors like LDLC either peak or plateau at or before seven weeks, the model likely would be valuable for further studies of genetic and environmental (intrinsic and extrinsic) factors that influence variation in the norms of reaction for lipid and cholesterol homeostasis in the continued presence of dietary stressors. Previous studies in the larger pedigreed baboon

breeding colony from which these animals were selected have provided evidence for shared (pleiotropic) genetic effects, as well as diet-specific genetic effects on many of these circulating atherosclerosis risk factors in the two dietary environments studied here[58–60].

Although not previously investigated in baboons, our PWV/PWA results comport with those from a series of studies conducted more than two decades ago with two other Old World Monkey species, cynomolgus macaques (*Macaca fascicularis*) and rhesus macaques (*Macaca mulatta*). Limited in sample size, those studies variously found evidence for altered aortic distensibility, elasticity, and composition, increased arterial stiffness, and altered central blood pressures in macaques fed diets high in cholesterol and fat for 26 to 36 months, as well as some evidence for association with extent of atherosclerotic lesions in both progression and regression[61–65]. Specific observations relevant to this study include an inverse correlation with peak compliance in 12 monkeys fed a high cholesterol diet for 26 months[65] and increased arterial stiffness in small groups of monkeys fed an HCHF diet (40% of calories from butter) for up to 30 months[47].

More important, results from the current study with pedigreed baboons are concordant with associations of decreased arterial compliance with atherogenic diets, atherosclerosis risk factors, the disease itself, and CVD outcomes in general in a sizeable number of cross-sectional and mixed longitudinal epidemiological studies in humans, and in smaller case-control studies and randomized trials as well[66–72)]. The degree of correspondence between observations on arterial stiffness (arteriosclerosis) in this study and those in humans strongly supports the value of the pedigreed baboon as a model for research on the effects of long-term exposures to diet and other disease risk factors on atherosclerosis and its vascular consequences, which underlie variation in risk, severity, and progression of CVD.

While the pedigreed baboon model for early stage atherosclerosis certainly could be exploited for an analog of a human CVD epidemiological study (e.g., to mimic the effects of an *average* diet on the cardiovascular health of a human population), that is not our objective here. For our purposes, the principal value of the model derives from our ability to use diet as a tool to reliably and quickly induce atherosclerotic lesions on which we can conduct studies of the molecular and cellular determinants of variation in their initiation, development, severity, and progression; all in a species exhibiting greater genetic, anatomic, and physiological proximity to our own than most other animal models.

It is true that the relative amounts of cholesterol and fats in the HCHF atherogenic diet exceed those consumed on a daily basis by the *average* person in the US[73]. This diet has been designed to overcome the effects genes on inter-individual variation in susceptibility to endothelial damage and it does: increasing the probability of atherogenesis to nearly 100% in our studies.

Elevated though they are, the amounts of fat and cholesterol in the HCHF atherogenic diet are not so extreme as to compromise the relevance of the model to the human condition. Recent estimates place the mean fat intake for US men and women at approximately 33% of daily calories[73], just above the recommended upper limit of 30% in the Dietary Guidelines for Americans[74]. With 40% of total calories due to fat, the HCHF diet fed to the baboons

certainly would be considered an unhealthy one, but not so extreme as those used in some short-term diet studies with human participants (e.g., 55%[75] and 73%[76]). The HCHF diet also corresponds well to high fat diets, deriving 35% to 40% of calories from fats, used recently by others in cardiometabolic disease-related studies with related nonhuman primate species[77–79]; as well as widely-used experimental diets formulated by several companies to model atherogenic "Western" diets: e.g., 42% (Teklad TD88137, Harlan Laboratories, Inc., Indianapolis, IN) and 40% (TestDiet 5342, LabDiet, St. Louis, MO).

Likewise, at approximately 17.6% of total calories, the percentage of calories attributable to saturated fats in our HCHF diet is 33%–40% higher than the 11% recommended in the Dietary Guidelines for Americans[74]; with the percentage due to the highly atherogenic 16:0 palmitic acid exceeding the estimated mean daily intake by Americans at all ages[73]: i.e., about 10% versus 6.2%, respectively.

Relative to that consumed on a daily basis by the average American, the amount of cholesterol in the HCHF atherogenic diet also is very high. As indicated in our description of the diet (Materials and Methods), the average amount consumed daily by each baboon was equivalent to that found in ten to twelve large eggs. However, compared to humans, many nonhuman primate species exhibit substantial variability in their responses to dietary cholesterol[80]. Consequently, investigators working with several species have used very high dietary cholesterol relative to standard American diets (from 0.5 – 2 mg/kcal or 1250 – 5000 mg/2500 kcal) to enhance the effects of dietary fats and accelerate atherogenesis.

Because we repeatedly have shown that inter-individual variation in most "traditional" CVD risk factors – and now, likely lesion extent – has a heritable component, the considerable variation we observe in lesion development in both experimental and control baboons is a desirable characteristic, rather than a conundrum as some may describe it[21]. Indeed, it makes a pedigreed breeding colony of baboons, such as those from which these animals are derived, valuable for a broader range of studies than any single inbred line of most smaller mammalian species. Knowledge of pedigree relationships can be exploited to identify and select animals based on degrees of both phenotypic and *relevant* genetic similarity (even prior to, or without, molecular genetic characterization) for case-control type study designs (for example). It also allows for production of offspring that are phenotypically and genetically similar for study. Further, it makes possible larger-scale, pedigree-based studies with sufficient statistical power for detecting the effects of individual genes, gene-by-environment (e.g., diet, pharmaceutical agent) interactions, and more. All these approaches have benefited CVD research projects with baboons from this pedigreed colony[35, 57, 60, 81, 82].

CONCLUSIONS

Based on our results, we conclude that a diet high in cholesterol and fat can be employed to reliably produce a baboon model of human atherosclerosis that has greater translational potential than other frequently used species. This potential is attributable to the fact that the development of atherosclerotic lesions and their functional consequences (e.g., decreased arterial compliance), as well as interrelationships among "classical" CVD risk factors,

closely mirror those in humans. In addition, our study shows that baboons exhibit variation in extent of lesions as seen in humans. The potential for translation is further enhanced by the phylogenetic proximity of baboons to humans, which results in a high degree of genetic, anatomic, and physiological correspondence between the two species.

Given that the HCHF diet alone can induce a disease state in nearly 100% of adult baboons in two years or less, the baboon is a practical model for research into the pathobiology of atherosclerosis and its consequences for CVD and comorbidities. Considering the time frames associated with most independent investigator-initiated, NIH-funded research projects, this relatively brief interval allows for development and study of the disease state within a single funding cycle. The interval could be shortened further, thus facilitating more rapid progress towards research objectives using the baboon model, by augmenting the HCHF diet with other atherogenic components – e.g., simple carbohydrates[83, 84]. Additionally, dietary manipulation could be used to establish a standing nonhuman primate CVD research resource comprising adult baboons in which atherogenesis is nearly certain to have been initiated and progressed to a stage in which changes in vascular endothelial function (for example) are observable at the beginning of any project. An NIH-sponsored National Primate Research Center, like the SNPRC, would be well situated to develop and maintain such a resource.

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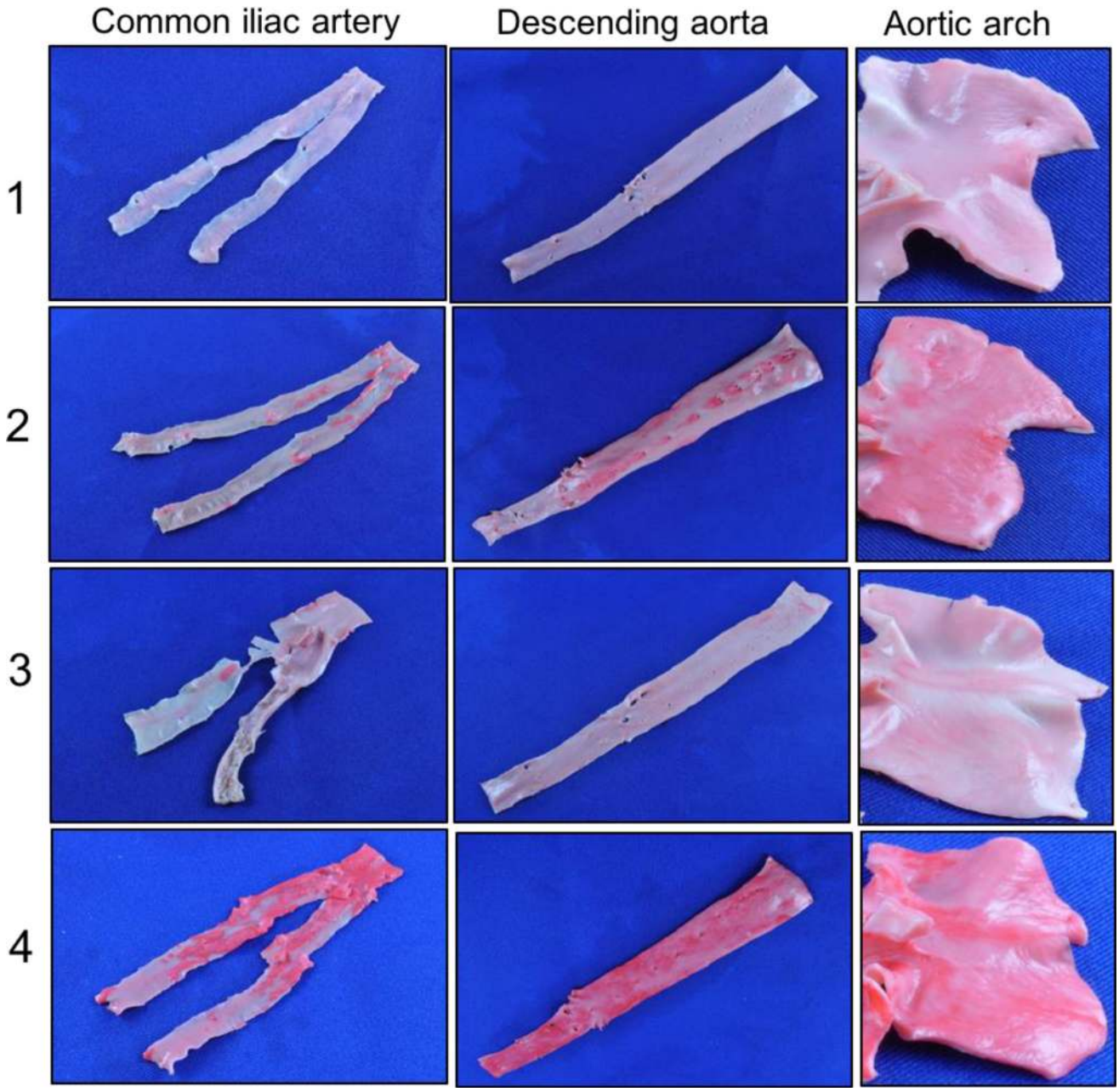

Figure 1.

Atherosclerotic lesions in three arteries from baboons after being stained with lipophilic Sudan IV. Columns: Common iliac artery (Left), thoracic aorta (center), and aortic arch (right). Rows 1 and 2: Control diet animals with no (row 1) and highest (row 2) total percent area affected. Experimental diet animals with lowest (row 3) and highest (row 4) percent total area affected.

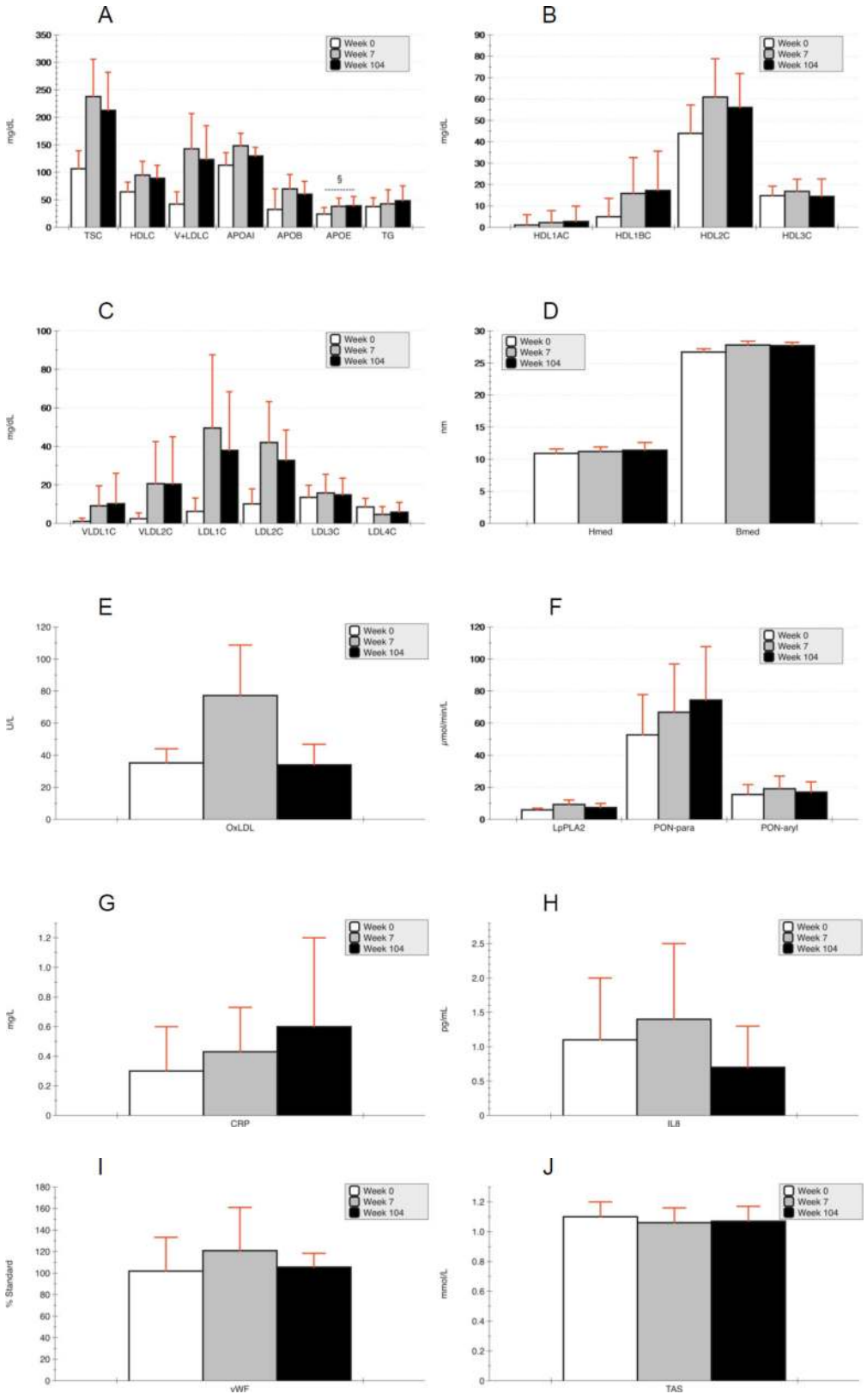

Fig. 2.

A–2J. Serum biomarkers of lipid/lipoprotein metabolism, inflammation, and oxidative stress measured at three time-points in 112 baboons in the two-year HCHF diet challenge study: at baseline (Week 0, LCLF diet), seven weeks into the HCHF diet challenge (Week 7), and at the end of the HCHF dietary challenge (Week 104). Figure 2A, concentrations of major lipoproteins, apolipoproteins, and triglycerides; Figure 2B. concentrations of HDLC fractions; Figure 2C, concentrations of V+LDLC fractions; Figure 2D, Hmed and Bmed; Figure 2E, concentration of OxLDL; Figure 2F, activity of lipoprotein associated enzymes, $LpPLA_2$, PON-para, and PON-aryl; Figure 2G, concentrations of CRP; Figure 2H, concentrations of IL8; Figure 2I, vWF as percentage of international standard value; and Figure 2J, TAS.

Table 1

Characteristics of the LCLF Diet and HCHF Base Diet

	LCLF	HCHF base diet
Energy, kcal/g provided by		
Carbohydrate, % kcal *	67.3	63.5
Protein, % kcal *	19	31.5
Fat, % kcal *	13.7	5.0
Cholesterol, mg/kcal	0.02	0.0
Nutrients, % of ration		
Protein, %	15.5	25.7
Arginine, %	0.85	1.40
Cystine, %	0.23	0.37
Glycine, %	0.63	1.05
Histidine, %	0.41	0.62
Isoleucine, %	0.65	1.27
Leucine, %	1.33	2.48
Lysine, %	0.74	1.22
Methionine, %	0.32	0.48
Phenylalanine, %	0.77	1.31
Tyrosine, %	0.53	0.93
Threonine, %	0.56	0.96
Tryptophan, %	0.19	0.31
Valine, %	0.83	1.31
Serine, %	0.80	1.42
Aspartic acid, %	1.46	2.56
Glutamic acid, %	3.71	6.22
Alanine, %	0.890	1.49
Proline, %	1.37	2.18
Taurine, %	0.00	0.01
Primary fat source	Vegetable	Vegetable
Cholesterol, ppm	49	0.0
Fat (ether extract), %	5.0	1.8
Fat (acid hydrolysis), %	6.2	3.1
Fatty acid composition, % †		
Total saturated fatty acids, %	1.17	0.5
Total monounsaturated fatty acids	1.33	0.5
C18:2 linoleic	2.1	1.3
C18:3 linolenic	1.9	0.1
Other omega-3 polyunsaturated fatty acids	0.23	0.1
Fiber (crude), %	8.3	4.7
Neutral detergent fiber, %	22.7	17.1

	LCLF	HCHF base diet
Acid detergent fiber, %	10.2	6.8
"Nitrogen-Free Extract" (by difference), %	54.7	51.8
Starch, %	28.5	32.2
Glucose, %	0.25	0.1
Fructose, %	0.27	0.1
Sucrose, %	1.73	2.6
Lactose, %	0.15	1.7
Total digestible nutrients, %	76	75.3
Gross energy, kcal/g	3.84	3.97
Physiological fuel value, kcal/g	3.25	3.26
Metabolizable energy, kcal/g	3.01	3.01
Minerals		
Ash, %	6.1	5.8
Calcium, %	1.00	1.03
Phosphorus, %	0.70	0.60
Phosphorus (non-phytate), %	0.40	0.24
Potassium, %	0.80	0.98
Magnesium, %	0.21	0.23
Sulfur, %	0.21	0.28
Sodium, %	0.35	0.10
Choline, %	0.55	0.11
Fluorine, %	24.3	5.12
Iron, ppm	378	387
Zinc, ppm	131	162
Manganese, ppm	118	145
Copper, ppm	37	22
Cobalt, ppm	0.76	0.53
Iodine, ppm	1.54	1.75
Chromium, ppm	1.79	0.42
Selenium, ppm	0.37	0.48
Vitamins		
Carotene, ppm	1.1	1
Vitamin K (as menadione), ppm	3.2	3.0
Thiamin hydrochloride, ppm	11.0	17.0
Riboflavin, ppm	12.0	8.9
Niacin, ppm	123	113
Pantothenic acid, ppm	61	62
Choline chloride, ppm	1200	1800
Folic acid, ppm	2.2	10.8
Pyridoxine, ppm	15.0	14.75
Biotin, ppm	0.2	0.2

	LCLF	HCHF base diet
Vitamin B-12, mcg/kg	33	48
Vitamin A, IU/g	40	43
Vitamin D-3 (added), IU/g	7.0	7
Vitamin E, IU/kg	49	110
Ascorbic acid, ppm	541	500

*Based on specifications of the manufacturer, Ralston Purina Co.
"Monkey Diet 15%"/5LEO, Ralston Purina Company/LabDiet
"Monkey Diet 25"/50456, Ralston Purina Company/LabDiet

†Fatty acid composition of the diets was determined by using gas-liquid chromatography of the fatty acid methyl esters on a DB-225 column (15 m) (J&W Scientific) with temperature programming from 100°C to 200°C at 3.25 °C/min.[23]

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Table 2

Total extent of atherosclerotic lesions and plaque extent (percent of artery area covered) in three arteries in 112 baboons fed HCHF diet and 20 Control baboons fed a baseline LCLF diet for two years.

	HCHF Diet 104 Weeks (n=112)				LCLF Diet Controls (n=20)				
Artery/Trait	**n**	**x̄ (SD)**	**min**	**max**	**n**	**x̄ (SD)**	**min**	**max**	**p*1***
Aortic arch									
Lesion %	110	13.6 (11.0)	0.3	55.5	4	2.7 (3.0)	0.2	7.0	0.0004
Plaque %	3	0.6 (0.4)	0.2	1.1	0	-	-	-	-
Thoracic aorta									
Lesion %	110	24.7 (17.6)	0.3	73.1	4	8.7 (3.2)	4.09	11.2	<0.0001
Plaque %	0	-	-	-	0	-	-	-	-
Common iliac									
Lesion %	109	14.2 (17.2)	0.2	77.5	6	5.4 (2.6)	0.9	8.0	<0.0001
Plaque %	29	3.1 (1.8)	0.4	6.1	4	3.6 (2.8)	0.4	7.3	ns

HCHF, high cholesterol, high fat atherogenic challenge diet; LCLF, low cholesterol, low fat baseline diet; n, number of individuals with affected artery; x̄ (SD), mean and (standard deviation); min, minimum observed value; max, maximum observed value.

*1*P values derived from comparison between extent of fatty streaks in two-year diet challenged group and control group by means of two-sample t-tests.

Table 3

Correlation[1] between extent of atherosclerotic lesions and serum biomarkers of lipid/lipoprotein metabolism and inflammation in 112 pedigreed baboons at three time-points before and during HCHF atherogenic diet challenge.

A. Baseline (week 0, LCLF diet) prior to the two-year HCHF atherogenic diet challenge study.

Trait	PC1	Aortic Arch	Thoracic Aorta	Common Iliac
TSC	0.309**	0.230*	0.205*	0.315**
HDLC	0.195*	0.165	0.212*	0.091
HDL1AC	0.101	0.064	0.110	0.072
HDL1BC	0.235*	0.166	0.250*	0.154
HDL2C	0.133	0.115	0.183	0.021
HDL3C	−0.095	−0.060	−0.186*	0.016
Hmed	0.209*	0.154	0.244*	0.107
V+LDLC	0.278*	0.177	0.093	0.409**
VLDL1C	0.179	0.077	0.081	0.285*
VLDL2C	0.385**	0.261*	0.354**	0.322**
LDL1C	0.373**	0.242*	0.287*	0.381**
LDL2C	0.255*	0.148	0.112	0.366**
LDL3C	0.031	0.014	−0.100	0.164
LDL4C	−0.087	−0.054	−0.172	0.014
Bmed	0.250*	0.157	0.246*	0.205*
APOAI	0.028	0.031	0.065	−0.037
APOB	−0.020	−0.078	−0.052	0.104
APOE	0.029	0.075	0.061	−0.084
TG	−0.091	−0.114	−0.115	0.017
CRP	0.162	0.109	0.162	0.121
IL8	−0.100	−0.136	0.019	−0.109
LpPLA2	0.168	0.170	0.004	0.227*
OxLDL	0.226*	0.159	0.029	0.364**
PON1-para	0.077	0.053	0.029	0.104
PON1-aryl	0.025	0.030	−0.028	0.057
TAS	−0.022	0.038	0.007	−0.106
VWF	−0.053	−0.063	−0.001	−0.055

B. After seven weeks on the HCHF atherogenic diet.

Trait	PC1	Aortic Arch	Thoracic Aorta	Common Iliac
TSC	0.499**	0.425**	0.300**	0.440**
HDLC	0.098	0.142	0.036	0.046
HDL1AC	0.121	−0.038	0.286	0.050

B. After seven weeks on the HCHF atherogenic diet.

Trait	PC1	Aortic Arch	Thoracic Aorta	Common Iliac
HDL1BC	0.353	0.368**	0.263	0.186*
HDL2C	−0.043	0.078	−0.168	−0.024
HDL3C	−0.189*	−0.250*	−0.062	−0.120
Hmed	0.252*	0.242*	0.203	0.139
V+LDLC	0.481**	0.414**	0.319**	0.386**
VLDL1C	0.365**	0.242*	0.166	0.448**
VLDL2C	0.365**	0.236*	0.168	0.453**
LDL1C	0.491**	0.445**	0.267	0.428**
LDL2C	0.009	0.136	0.106	−0.231*
LDL3C	−0.125	−0.007	−0.001	−0.292*
LDL4C	0.058	0.113	0.168	−0.149
Bmed	0.318**	0.196*	0.178	0.373**
APOAI	0.077	0.137	0.108	−0.072
APOB	0.378	0.321**	0.267	0.292*
APOE	0.170	0.052	0.075	0.277*
TG	−0.300**	−0.278*	−0.185	−0.234*
CRP	0.103	0.152	0.022	0.059
IL8	−0.178	−0.263*	−0.131	−0.011
LpPLA2	0.250*	0.190*	0.107	0.287*
OxLDL	0.068	0.130	−0.138	0.158
PON1-para	−0.135	−0.077	0.127	−0.114
PON1-aryl	−0.119	−0.077	−0.088	−0.116
TAS	−0.165	−0.079	−0.226	−0.088
VWF	−0.171	−0.207*	−0.051	−0.099

C. After 104 weeks on the HCHF atherogenic diet.

Trait	PC1	Aortic Arch	Thoracic Aorta	Common Iliac
TSC	0.345**	0.327**	0.108	0.379**
HDLC	0.001	−0.037	−0.050	0.088
HDL1AC	0.098	0.004	0.154	0.094
HDL1BC	0.086	0.047	0.096	0.070
HDL2C	0.022	0.001	−0.072	0.117
HDL3C	−0.177	−0.154	−0.193*	−0.087
Hmed	0.114	0.054	0.181	0.055
V+LDLC	0.410**	0.403**	0.200*	0.372**
VLDL1C	0.249*	0.245*	0.019	0.315**
VLDL2C	0.383**	0.347**	0.113	0.443**

C. After 104 weeks on the HCHF atherogenic diet.

Trait	PC1	Aortic Arch	Thoracic Aorta	Common Iliac
LDL1C	0.451 **	0.440 **	0.241 *	0.391 **
LDL2C	0.053	0.029	0.113	−0.003
LDL3C	−0.279 *	−0.244 *	−0.014	−0.393 **
LDL4C	−0.069	−0.039	0.074	−0.189 *
Bmed	0.363 **	0.368 **	0.063	0.413 **
APOAI	−0.037	−0.038	−0.088	0.030
APOB	0.280 *	0.300 **	0.131	0.232 *
APOE	0.112	0.113	0.032	0.117
TG	−0.168	−0.184	−0.065	−0.146
CRP	0.136	0.065	0.178	0.099
IL8	0.112	0.045	0.164	0.076
LpPLA2	0.275 *	0.27 *2	0.140	0.241 *
OxLDL	0.187 *	0.212 *	0.070	0.157
PON1-para	0.007	0.016	0.001	0.001
PON1-aryl	0.046	0.048	0.031	0.030
TAS	0.086	0.037	−0.130	0.281 *
VWF	−0.031	−0.049	−0.082	0.065

[1] Pearson product-moment correlations

[*] At α = 0.05 with nominally significant P = 0.05, |r| = 0.186 (2-tailed test).

[**] At α = 0.05 with Bonferroni corrected P = 0.00185, |r| = 0.292 (2-tailed test).

Table 4

Arterial compliance examined by pulse wave analysis using applanation tonometry in controls fed a basal LCLF diet and in challenge animals fed a HCHF diet for two years.

	Controls (n = 41) LCLF Diet			Challenge (n = 50) HCHF Diet			
Traits	**mean**	**sd**	**med**	**mean**	**sd**	**med**	**Δ**
Age (years)	11.7	1.8	11.3	11.6	1.7	11.1	ns
Weight (kg)	25.8	6.2	27.4	23.9	6.6	23.3	ns
Brachial systolic pressure	102.7	16.8	103.0	107.9	18.0	109.5	ns
Brachial diastolic pressure	51.1	11.2	52.0	51.9	14.7	48.0	ns
Brachial pulse pressure	51.6	13.8	50.0	55.6	14.9	53.0	ns
Brachial mean pressure	68.7	13.2	69.0	72.4	15.4	69.0	ns
Aortic systolic pressure	86.5	15.0	86.0	92.3	16.2	94.5	↑*
Aortic diastolic pressure	53.2	11.4	54.0	54.3	15.1	49.5	ns
Aortic pulse pressure	33.4	9.3	32.0	38.0	11.9	35.0	↑*
Aortic heart rate	86.2	15.9	83.0	86.9	17.0	84.5	ns
Augmentation pressure	5.1	5.2	5.0	9.1	7.6	7.0	↑**
Augmentation index (%)	13.8	14.3	14.0	21.9	12.7	21.5	↑**
Pulse wave velocity	5.5	1.9	5.1	6.8	3.2	6.1	↑*

All blood pressures: mm Hg; heart rate: beats per minute; augmentation pressure: difference between peak aortic pressure at second and first systolic peak; augmentation index: augmentation pressure/pulse pressure × 100; Pulse wave velocity: m/s).

Mean, arithmetic mean; sd, standard deviation; med, median; Δ, Direction of mean differences between two groups of baboons (i.e., controls fed only the LCLF diet and challenge animals fed the HCHF challenge diet for two years);

*P = 0.05; and

**P = 0.01; ns, no nominally significant difference – i.e., P ≥0.05.

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Table 5

Arterial compliance-related measures from pulse wave analysis in 50 pedigreed baboons following a two-year HCHF atherogenic diet challenge: Correlations [1] with plaque and lesions.

	Plaque		Lesion extent			
	Sum of areas	**Total number**	**PC1**	**Aortic Arch**	**Thoracic Aorta**	**Common Iliac**
			Brachial			
Systolic Pressure	0.169	0.070	0.301 *	0.341 *	0.144	0.287 *
Diastolic Pressure	0.388 **	0.448 **	0.394 **	0.580 **	0.090	0.316 *
Pulse Pressure	0.252 *	0.331 *	0.138	0.268 *	−0.036	−0.025
Mean Pressure	0.259 *	0.366 **	0.431 **	0.590 **	0.149	0.344 *
			Aortic			
Systolic Pressure	0.264 *	0.163	0.396 **	0.426 **	0.282 *	0.378 **
Diastolic Pressure	0.429 **	0.473 **	0.390 **	0.574 **	0.082	0.320 *
Pulse Pressure	0.237 *	0.347 *	−0.082	0.269 *	0.080	0.010
Heart Rate	0.165	0.424 **	0.195	0.283 *	0.121	0.078
Augmentation Pressure	0.224	0.285 *	0.020	0.211	0.282 *	−0.087
Augmentation index (%)	0.156	0.226	0.005	0.153	0.309 *	−0.091
Pulse wave velocity	0.365 **	0.184	0.238 *	−0.079	0.367 *	0.192

[1] Partial correlations between sex- and age-adjusted (when appropriate) residuals, conditional on body weight. Correlations with plaque measures are Spearman rank-order correlations. Correlations with lesion extent measures are Pearson product-moment correlations.

At $\alpha = 0.05$, * nominal P = 0.05 and $|r| = 0.235$; at $\alpha = 0.05$,

** Bonferroni corrected P = 0.0064 and $|r| = 0.35$ (1-tailed tests).