High-Saturated-Fat Diet Increases Circulating Angiotensin-Converting Enzyme, Which Is Enhanced by the rs4343 Polymorphism Defining Persons at Risk of Nutrient-Dependent Increases of Blood Pressure

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Background—Angiotensin-converting enzyme (ACE) plays a major role in blood pressure regulation and cardiovascular homeostasis. Contrary to the assumption that ACE levels are stable, circulating ACE has been shown to be altered in obesity and weight loss. We sought to examine effects of a high-saturated-fat (HF) diet on ACE within the NUtriGenomic Analysis in Twins (NUGAT) study.

Methods and Results—Forty-six healthy and nonobese twin pairs initially consumed a carbohydrate-rich, low-fat diet over a period of 6 weeks to standardize for nutritional behavior prior to the study, followed by 6 weeks of HF diet under isocaloric conditions. After 6 weeks of HF diet, circulating ACE concentrations increased by 15% \( (P=1.6 \times 10^{-3}) \), accompanied by an increased ACE gene expression in adipose tissue \( (P=3.8 \times 10^{-8}) \). Stratification by ACE rs4343, a proxy for the ACE insertion/deletion polymorphism \( (I/D) \), revealed that homozygous carriers (GG) of the variant had higher baseline ACE concentrations \( (P=7.5 \times 10^{-8}) \) and additionally showed a 2-fold increase in ACE concentrations in response to the HF diet as compared to non- or heterozygous carriers \( (AA/AG, P=2 \times 10^{-5}) \). GG carriers also responded with higher systolic blood pressure as compared to AA/AG carriers \( (P=0.008) \). The strong gene-diet interaction was confirmed in a second independent, cross-sectional cohort, the Metabolic Syndrome Berlin Potsdam (MeSyBePo) study.

Conclusions—The HF-diet-induced increase of ACE serum concentrations reveals ACE to be a potential molecular link between dietary fat intake and hypertension and cardiovascular disease (CVD). The GG genotype of the ACE rs4343 polymorphism represents a robust nutrigenetic marker for an unfavorable response to high-saturated-fat diets.

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Key Words: angiotensin-converting enzyme • blood pressure • diet • gene-diet interaction • nutrigenomics genetics

The zinc metallopeptidase angiotensin-converting enzyme (ACE) plays an important role in blood pressure control and cardiovascular homeostasis as a central regulatory enzyme within the renin-angiotensin system (RAS). ACE catalyzes the generation of the vasoconstrictor angiotensin II from inactive angiotensin I and degrades the vasodilator bradykinin. Its pharmacological inhibition represents a standard of care in the therapy of hypertension and related cardiovascular disease (CVD). Carriers of a frequent insertion/deletion \( (I/D) \) polymorphism located in the 16th intron of the ACE gene \( (Alu I/D) \) are characterized by higher ACE levels and have been shown, albeit inconsistently, to be associated...
with higher blood pressure and increased risk for CVD.\textsuperscript{1-3} Despite huge interindividual variability in circulating ACE levels depending on the ACE genotype,\textsuperscript{4} circulating levels are reported to be stable within given individuals.\textsuperscript{5} However, ACE circulating concentrations or activity levels, which are highly correlated,\textsuperscript{6,7} were shown to be increased in obesity\textsuperscript{8} and decreased by weight loss.\textsuperscript{5,9,10} In addition to its predominant expression in pulmonary endothelium as a primary source of circulating levels, ACE is also expressed in adipose tissue\textsuperscript{11,12} with increased expression in response to overfeeding.\textsuperscript{13}

We investigated the effects of an isocaloric diet high in total and saturated fat on ACE within the framework of the NUTriGenomic Analysis in Twins (NUGAT) study, which aimed to identify nutrition-responsive genes and biomarkers and their heritability. The cross-sectional Metabolic Syndrome Berlin Potsdam (MeSyBePo) cohort, which includes nutritional assessments, was additionally investigated for reevaluation of gene-diet interactions.

**Methods**

**NUGAT Study**

The study protocol was approved by the independent ethics review committee of the Charité-Universitätsmedizin Berlin in accordance with the principles of the Helsinki Declaration of 1975, as revised in 2000. Written informed consent was obtained from all participants prior to the study, which was registered at ClinicalTrials.gov (Unique identifier: NCT01631123).

The study was conducted at the department of Clinical Nutrition at the German Institute of Human Nutrition Potsdam-Rehbrücke. Twin pairs were recruited either from a twin register (HealthTwiSt, Berlin, Germany) or by public advertisements. Exclusion criteria were consumptive diseases, diabetes mellitus, high-grade anemia, renal failure, moderate to severe heart diseases, angina pectoris, or stroke in the last 6 months, food allergy, eating disorders, body weight change $\geq 3$ kg within 3 months prior to the study, pregnancy or breastfeeding, drugs influencing metabolic homeostasis, lipid and liver metabolism, or inflammation (eg, systemic corticosteroids).

Participants were initially screened to determine their eligibility for enrollment in the intervention study. This screening visit comprised physical examination, medical history, anthropometric measurements, and blood analysis. Additionally, a standardized 3-hour, 75-g oral glucose tolerance test (OGTT) was performed. Participants’ resting energy expenditure (REE) was measured by indirect calorimetry, and physical activity level (PAL) was assessed by questionnaire to calculate individual daily energy requirements. A total of 46 healthy and nonobese twin pairs, 34 monozygotic and 12 dizygotic, age 18 to 70 years and body mass index (BMI) 18 to 29 kg/m$^2$ with BMI difference $<3$ kg/m$^2$ between twins were included in the study. The CONSORT Flow Diagram is shown in Figure 1. Participants went from a 6-week, carbohydrate-rich, low-fat diet (LF) to standardize for nutritional behavior prior to the study to a 6-week HF diet in a sequential design under isocaloric conditions (Figure 2). Three clinical investigation days (CID) were performed, after 6 weeks of low-fat diet (LF6) and after 1 and 6 weeks of high-saturated-fat diet (HF1 and HF6). At each CID, anthropometric measurements were performed. Three blood pressure readings were taken in a relaxed sitting position with an appropriate size cuff, and the average values were used for analysis. Blood samples were drawn in the fasted state for routine laboratory marker and biomarker analysis in plasma or serum and SNP array-based genotyping. Additionally, a biopsy of the subcutaneous adipose tissue was performed lateral to the umbilicus by needle aspiration for microarray gene expression analysis.

**Dietary Intervention**

All subjects completed a dietary record for 5 days prior to the study to encompass dietary habits. They commenced with the isocaloric dietary intervention for 6 weeks, receiving a high-carbohydrate, low-fat diet (LF: 55% carbohydrate, 30% fat, 15% protein) in accordance with accepted national dietary guidelines. After the first investigation day (LF6) the diet was changed to a low-carbohydrate, high-saturated-fat diet (HF: 40% carbohydrate, 45% fat, 15% protein) for 6 weeks with emphasis on foods high in saturated fat such as red meat, sausage, bacon, and full-fat dairy products. Participants received a list of 94 food items and individual daily meal plans on how to exchange and combine these foods, and energy intake was adjusted according to body weight if needed. To ensure compliance, participants were given intensive, regular, and detailed dietary guidance by a nutritionist over the entire period of intervention. For 1 week prior to each particular CID, $\sim 70\%$ of the food was provided with detailed daily meal plans to ensure a standardized dietary pattern for all participants. All subjects had to complete 5 dietary records during the 12 weeks of the dietary intervention period.

**Blood Parameters**

Determination of routine serum parameters (eg, total cholesterol, HDL-cholesterol, triglycerides) was performed using an automated analyzer (ABX Pentra 4000; ABX, Montpellier, France). LDL cholesterol concentrations were calculated using the Friedewald equation. ACE concentrations were measured in the serum of all participants at each CID using a human
ACE immunoassay ELISA kit (R&D Systems Inc, Minneapolis, MN) with an interassay variance <5%.

**Gene Expression Analysis**

About 500 mg of subcutaneous adipose tissue were homogenized using a Speed Mill (Analytik Jena, Jena, Germany), and total RNA was extracted by using the RNeasy Lipid Tissue Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer’s protocol. Quality was assessed by using RNA 6000 NanoLabChips and Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA). One microgram of each sample was amplified using the Amino Allyl MessageAmp™ II aRNA Amplification Kit (Ambion by Life Technologies, Carlsbad, CA) and hybridized onto Agilent Whole Human Genome 8×60K Gene Expression Microarrays (Agilent Technologies, Santa Clara, CA). Microarray data have been uploaded to NCBI GEO (http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE62199; Accession No: GSE62199). To validate microarray data, quantitative real-time PCR was performed. cDNA was synthesized from 1 μg of RNA of each sample by using the High-Capacity cDNA Reverse Transcription Kit™ (Applied Biosystems by Life Technologies, Carlsbad, CA). Samples were labeled by Power SYBR Green Master Mix and detected.

**Figure 1.** CONSORT flow diagram of the NUGAT study. NUGAT indicates NUtriGenomic Analysis in Twins; HF, high-saturated-fat diet; LF, low-fat diet.
in triplicates in optical 384-well plates with the ABI ViiA™7 Real-Time PCR System (Applied Biosystems by Life Technologies, Carlsbad, CA). The samples were normalized to ribosomal protein L32 (RPL32), and the standard curve method was used for evaluation. The primer sequences were ACE forward primer CAAGCACCTGCACAGTCTCAAC, reverse primer TGATCGACGAGGTAGCTGAAGG; RPL32 forward primer CAACGTCAAGGAGCTGGAAGT, reverse primer TTGTGAGCGATCTCGCACAAT.

Genotyping
Genomic DNA was isolated from buffy coat using a commercial kit (NucleoSpin, Macherey-Nagel, Duren, Germany) and genotyped on HumanOmniExpressExome BeadChips (Illumina, Inc, San Diego, CA) at the Interdisciplinary Center for Clinical Research (IZKF, Leipzig, Germany).

Genotyping for the Metabolic Syndrome Berlin Potsdam (MeSyBePo) study was performed using a predesigned rs4343 TaqMan SNP Genotyping Assay on 384-well plates using the ABI ViiA™7 Real-Time PCR System (Applied Biosystems by Life Technologies, Carlsbad, CA) according to the manufacturer’s protocol.

Metabolic Syndrome Berlin Potsdam Study
The cross-sectional MeSyBePo study was approved by the ethics commissions of Berlin and Brandenburg, Germany. All individuals gave written informed consent prior to the study. Two thousand three hundred sixty-four white volunteers were randomly recruited from the Berlin and Potsdam areas. Four hundred seventy-nine out of the 2364 participants were excluded from the analyses due to missing data. Dietary data were collected from 671 participants via a 4-day estimated food record that comprised 18 categories with 151 food items. By use of the German Nutrient Database BLS, version 2.3, the mean daily energy and nutrient intakes were calculated. For evaluation of dietary fat intake, data on total fat intake but not on saturated fat were available.

To analyze the influence of a high-fat dietary pattern on ACE rs4343 genotype associations with blood pressure, we excluded misreporting as a main source of error by evaluating under-, normal, and overreporting of energy intake (EI). Therefore, the basal metabolic rate (BMR) was calculated using the Harris-Benedict equation. Based on the ratio of EI to BMR, underreporting, normal, and overreporting of energy intake were defined as <1.35, 1.35 to 2.39 and ≥2.4, respectively.

Statistical Analysis
For estimation of heritability, the “ACE” structural equation model was applied. This covariance analysis relies on comparing the degree of concordance within and between monozygotic versus dizygotic twin pairs and decomposes the proportion of variance into (A) additive genetic influences and (C) common environmental and (E) individual environmental influences. The “ACE” model was calculated using R 2.15.0 plus OpenMX package. Genotype frequencies were analyzed for deviation from the Hardy-Weinberg equilibrium by chi-square test using R 3.1.2 plus Hardy-Weinberg package 1.5.5. The evaluation of microarray data was performed using Agilent GeneSpring GX Version 11 Software (Agilent Technologies, Waldbronn, Germany). The data set was normalized to LF6, and genes with >1.5-fold changes were analyzed. The statistical significance of expression changes was calculated by one way analysis of variance (ANOVA) and post hoc analysis following a Mann-Whitney U test. P-values were adjusted for multiple testing via the Benjamini-Hochberg (FDR) method. The Kolmogorov-Smirnov test was used to assess variables for normal distribution. Continuous variables with skewed distribution were natural logarithm (ln)-transformed. One-way or repeated-measures ANOVA followed by Bonferroni post hoc test were used to compare mean values for continuous data. To verify significant results for nonnormally distributed...
data, the Kruskal-Wallis test was used. Additionally, linear regression analyses were performed with adjustment for main confounders. Correlation analyses were performed using Pearson and Spearman rank correlation coefficients for variables with normal and skewed distributions, respectively. Statistical significance was defined as $P<0.05$. Values are expressed as mean±SD unless otherwise stated. Statistical analyses were carried out using SPSS 20.0 (IBM SPSS, Chicago, IL).

## Results

### Clinical Characteristics

Table 1 shows anthropometric and clinical characteristics of the 92 healthy and nonobese participants at screening. As calculated from dietary records, energy consumption from total fat/saturated fat was 29%/10% for LF and 45%/18% for HF diet, respectively. Energy consumption from carbohydrates and protein was 55% and 16% for the LF diet and 41% and 15% for the HF diet, respectively. Although the study was performed under isocaloric conditions, the weight of participants increased slightly (0.4±1.0 kg, $P=9\times10^{-6}$). As expected, LDL, HDL, and total cholesterol increased significantly in response to the HF diet ($P_{LDL}=4.7\times10^{-8}$, $P_{HDL}=1.7\times10^{-13}$, $P_{Chol}=8.5\times10^{-11}<0.001$; Table 2), confirming good compliance of the participants with the dietary instructions.\(^{15}\)

### Effect of HF Diet on ACE Serum Concentrations

In response to the HF diet, fasting serum concentrations of ACE increased by 15% (LF6 139±41 ng/mL vs HF6 161±49 ng/mL; repeated-measures ANOVA $P=1.6\times10^{-30}$; Figure 3). Changes in ACE were not influenced by changes in body weight (linear regression, $P=0.114$). At each CID, serum concentrations of ACE were significantly correlated with weight (LF6 $r=0.461$, HF1 $r=0.474$, HF6 $r=0.423$; $P<0.001$), height (LF6 $r=0.362$, HF1 $r=0.389$, HF6 $r=0.354$; $P<0.001$), and thus also with BMI (LF6 $r=0.315$, HF1 $r=0.289$, HF6 $r=0.302$; $P<0.01$), whereas no significant correlations were noted between ACE and systolic or diastolic blood pressure despite a weak correlation between ACE and systolic blood pressure at HF6 ($r=0.234$, $P=0.025$) and between ACE and pulse pressure at HF6 ($P=0.228$, $P=0.029$).

Both LDL and total cholesterol were not correlated with ACE concentrations (LF6 $P=0.765$ and $P=0.420$). However, the increase in ACCE (ΔACE, HF6-LF6) was modestly but significantly correlated with the increase in LDL cholesterol ($P=0.296$, $P=0.004$). Furthermore a modest negative correlation was shown for ACE concentrations and HDL cholesterol (LF6 $P=−0.278$, HF1 $P=−0.277$, HF6 $P=−0.350$; $P<0.01$).

### Effect of HF Diet on ACE mRNA Expression in Subcutaneous Adipose Tissue

Adipose tissue ACE gene expression was significantly increased in response to the HF diet (HF6 vs LF6 1.412-fold; ANOVA $P=3.8\times10^{-6}$, post hoc HF6 vs LF6 $P=0.023$, and HF6 vs HF1 $P=0.007$; Figure 4A).

To confirm microarray data, we performed quantitative real-time PCR where ACE mRNA expression was again significantly increased under HF diet conditions (repeated-measures ANOVA, $P=0.005$; Figure 4B).

### Table 1. Characteristics of the Participants Overall and Stratified for ACE rs4343 at Baseline in the NUtriGenomic Analysis in Twins Study

|                | Total  | AA Genotype | AG Genotype | GG Genotype | $P$ Value |
|----------------|--------|-------------|-------------|-------------|-----------|
| n              | 92     | 31          | 44          | 17          | 0.842     |
| Male/female    | 34/58  | 10/21       | 14/30       | 10/7        | 0.120     |
| Age, y         | 31±14  | 30±14       | 31±11       | 34±20       | 0.764     |
| BMI, kg/m$^2$  | 22.8±2.7 | 22.8±2.2  | 22.9±2.7   | 22.9±3.6    | 0.990     |
| SBP, mm Hg     | 118±13 | 116±12      | 116±14     | 125±8       | 0.044     |
| DBP, mm Hg     | 74±9   | 73±7        | 74±9        | 80±10       | 0.035     |
| PP, mm Hg      | 43±9   | 43±8        | 42±9        | 46±10       | 0.452     |
| Total cholesterol, mmol/L | 4.58±0.93 | 4.55±0.91 | 4.64±0.93 | 4.49±1.04 | 0.822 |
| HDL cholesterol, mmol/L    | 1.38±0.35 | 1.38±0.30 | 1.45±0.39 | 1.20±0.27 | 0.043 |
| LDL cholesterol, mmol/L     | 2.73±0.77 | 2.73±0.75 | 2.67±0.76 | 2.90±0.88 | 0.600 |
| Triglycerides, mmol/L        | 0.99±0.44 | 0.97±0.47 | 1.01±0.43 | 0.97±0.39 | 0.935 |

Values are shown as mean±SD. BMI indicates body mass index; DBP, diastolic blood pressure; HDL, high-density lipoprotein; LDL, low-density lipoprotein; PP, pulse pressure; SBP, systolic blood pressure.
**Table 2.** Characteristics of the Participants After the Standardization (LF6) and After 1 and 6 Weeks of High-Saturated-Fat Diet (HF1, HF6)

|               | LF6    | HF1     | HF6     | P Value |
|---------------|--------|---------|---------|---------|
| Weight, kg    | 66.6±11.7 | 66.5±11.6 | 67.0±11.8 | <0.001 |
| BMI, kg/m²    | 22.5±2.7  | 22.5±2.6  | 22.6±2.7  | <0.001 |
| SBP, mm Hg    | 110±12   | 109±12   | 110±12   | 0.681   |
| DBP, mm Hg    | 70±9     | 69±9     | 70±9     | 0.709   |
| PP, mm Hg     | 41±9     | 40±8     | 40±8     | 0.899   |
| Total cholesterol, mmol/L | 4.29±0.85 | 4.47±0.87 | 4.70±0.91 | <0.001 |
| HDL cholesterol, mmol/L | 1.27±0.33 | 1.32±0.34 | 1.41±0.37 | <0.001 |
| LDL cholesterol, mmol/L | 2.59±0.71 | 2.71±0.73 | 2.86±0.79 | <0.001 |
| Triglycerides, mmol/L | 0.95±0.43 | 0.89±0.35 | 0.91±0.37 | 0.449   |

Values are shown as mean±SD. BMI indicates body mass index; DBP, diastolic blood pressure; HDL, high-density lipoprotein; HF1, investigation day after 1 week of the high-saturated-fat diet; HF6, investigation day after 6 weeks of the high-saturated-fat diet; LDL, low-density lipoprotein; LF6, investigation day after 6 weeks of the low-fat diet; PP, pulse pressure; SBP, systolic blood pressure.

Repeated measures ANOVA with Bonferroni post hoc test: *P<0.05 compared to LF6; †P<0.001 compared to LF6.

**Heritability of Serum ACE**

Figure 5 shows strong intrapair correlation of circulating ACE concentrations in monozygous twins (LF6 =0.867, P=3.3×10⁻¹¹; Figure 5A), whereas intrapair ACE concentrations were not correlated in dizygous twins (LF6 =0.287, P=0.366; Figure 5B). Based on the “ACE” structural equation model, estimated additive genetic effects contributed to 86%, and shared environmental influences to 14% of the variance of circulating ACE concentrations, respectively. ACE concentrations appeared to be one of the most highly heritable markers in the NUGAT study, with similar heritability estimates seen for bone mineral content (BMC) and height (Figure 5C).

**Influence of the ACE rs4343 Polymorphism on ACE Concentrations and Blood Pressure in Response to the HF Diet**

Because heritability is linked to genetics, we stratified our analysis by genotypes of the ACE rs4343 variant; due to strong linkage disequilibrium,16 this serves as a surrogate marker for the ACE I/D polymorphism, where A2350 corresponds to the I-allele and 2350G corresponds to the D-allele.17,18 Genotype frequencies for ACE rs4343 polymorphism were AA=31, AG=44, and GG=17 and did not deviate from values predicted by the Hardy-Weinberg equilibrium (χ²=0.04, P=0.842). As shown in Figure 6A, ACE serum concentrations significantly differed depending on the genotype, with the lowest concentrations for homozygous noncarriers (AA), intermediate concentrations for heterozygous (AG), and highest concentrations for homozygous carriers of the variant (GG), respectively (ANOVA, LF6 P=4.7×10⁻¹³, HF1 P=5.1×10⁻¹⁵, and HF6 P=1.0×10⁻¹⁴). The rs4343 genotype accounted for 47% of the variance in ACE serum concentrations (adjusted R²=0.466, P=4.2×10⁻¹⁴). After sex (P=4.5×10⁻⁵) and BMI (P=0.031) had been included in the model, rs4343 accounted for 60% of the variance (adjusted R²=0.602, P=3.4×10⁻¹⁵). An increase in ACE serum concentrations in response to the HF diet was confirmed independently of genotypes (repeated-measures ANOVA; AA P=1.6×10⁻¹¹, AG P=2.6×10⁻¹⁸, and GG P=3.1×10⁻⁹), although the increase was doubled for GG carriers compared to AA or AG carriers (Figure 6B; P=2×10⁻⁶, P-value adjusted for sex, age, and BMI Padj=1×10⁻⁵).

We further performed a bootstrap analysis to exclude bias due to relatedness of twins, randomly assigning both subjects of a twin pair to 1 of 2 data records, and both records were analyzed separately. Random assignment of the twins was repeated 10 000 times to prevent a bias, and group means, standard deviations, and mean P-values were calculated. The increase in circulating ACE levels differed significantly between rs4343 genotypes in both separately analyzed data records (recessive model AA/AG vs GG: 18±1 ng/mL vs 38±4 ng/mL, P₁=0.038 and P₂=0.039).
In Table 1, clinical characteristics at screening are summarized, stratified by genotype. Compared to AA/AG carriers, homozygous carriers of the polymorphism had lower HDL cholesterol (recessive model, $P=0.019$, $P_{adj}=0.028$) and higher systolic and diastolic blood pressure (recessive model, $P=0.012$ and $P=0.011$, $P_{adj}=0.087$ and $P_{adj}=0.033$, respectively). This difference in systolic and diastolic blood pressure did not persist at LF6 (AA/AG vs GG, 109±13 mm Hg vs 115±9 mm Hg, $P=0.077$, $P_{adj}=0.296$; 69±9 mm Hg vs 72±7 mm Hg, $P=0.154$, $P_{adj}=0.346$) and HF1 (AA/AG vs GG, 109±12 mm Hg vs 113±11 mm Hg, $P=0.120$, $P_{adj}=0.434$; 69±9 mm Hg vs 71±8 mm Hg, $P=0.304$, $P_{adj}=0.562$). However, after the 6-week HF diet, systolic blood pressure differed significantly between genotypes (AA/AG vs GG, 108±12 mm Hg vs 117±9 mm Hg, $P=0.008$, $P_{adj}=0.033$), whereas no differences in diastolic blood pressure were detected (AA/AG vs GG, 69±9 mm Hg vs 73±7 mm Hg, $P=0.105$, $P_{adj}=0.158$). Genotype-dependent

Figure 4. Results of ACE gene expression in subcutaneous adipose tissue by (A) microarray analysis and (B) quantitative real-time PCR. A, Values are presented as fold changes (HF1 vs LF6 and HF6 vs LF6). $P$-values are shown after Benjamini-Hochberg correction ($^*P<0.05$, $^{**}P<0.01$). B, Values are shown as mean±SD. Bonferroni post hoc test was used to compare main effects (HF6 vs LF6, $P=0.010$) of repeated-measures ANOVA ($P=0.005$). ACE indicates angiotensin-converting enzyme; LF6, investigation day after 6 weeks of the low-fat diet; HF1, investigation day after 1 week of the high-saturated-fat diet; HF6, investigation day after 6 weeks of the high-saturated-fat diet.

Figure 5. Intrapair correlation of ACE serum concentrations in monozygotic (A) and dizygotic (B) twins ($^{**}P<0.01$). Estimated heritability (C) for ACE serum concentrations in comparison with estimates for bone mineral content (BMC) and height: “A” additive genetic effects, “C” common environmental influences, and “E” unique environmental influences. ACE indicates angiotensin-converting enzyme.
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Figure 6. A, ACE serum concentrations at LF6, HF1, and HF6 stratified for ACE rs4343 genotype and (B) ΔACE (HF6–LF6) stratified for ACE rs4343 genotype (mean±SD; ***P<0.001). ACE indicates angiotensin-converting enzyme; LF6, investigation day after 6 weeks of the low-fat diet; HF1, investigation day after 1 week of the high-saturated-fat diet; HF6, investigation day after 6 weeks of the high-saturated-fat diet.

Validation of ACE rs4343 Genotype Effects on Blood Pressure in the MeSyBePo Study

A group of 1885 participants (1256 female and 629 male, age 52±14, BMI 29.4±6.3 kg/m²) of the cross-sectional MeSyBePo cohort were included in the analysis to further validate differences in pulse pressure (PP) values failed to reach statistical significance after adjustment for sex, age, and BMI (AA/AG vs GG, 39±8 mm Hg vs 44±7 mm Hg, P=0.026, Padj=0.081).

By performing a bootstrap analysis to exclude bias due to relatedness of twins, we could prove that systolic blood pressure differed significantly between genotypes in both data sets (recessive model: 108±1 mm Hg vs 117±2 mm Hg, P1=0.039 and P2=0.038). However, pulse pressure did not differ significantly between genotypes (recessive model: 39±1 mm Hg vs 44±2 mm Hg, P=0.179 and P2=0.176).

Validation of ACE rs4343 Genotype Effects on Blood Pressure in the MeSyBePo Study

A group of 1885 participants (1256 female and 629 male, age 52±14, BMI 29.4±6.3 kg/m²) of the cross-sectional MeSyBePo cohort were included in the analysis to further validate the interaction of dietary fat intake and ACE rs4343 genotype. ACE rs4343 genotype frequencies were AA=346, AG=1023, and GG=516, which differed significantly from values predicted by the Hardy-Weinberg equilibrium (χ²=16.77, P=4.2x10⁻⁵). Neither systolic nor diastolic blood pressure (SBP, DBP) nor pulse pressure (PP) was significantly associated with ACE genotype (additive model: PSBP=0.524, PDBP=0.391, and PP=0.309; recessive model PSBP=1.300, PDBP=0.973, and PP=0.144).

Possible misreporting of dietary intake was evaluated for a total of 671 participants among whom 136 participants (20.3%) underreported, 365 (54.4%) reported normally, and 170 (25.3%) overreported EI.

ACE rs4343 genotype frequencies for participants with plausible reported energy intakes (n=365) were AA=66, AG=197, and GG=102, which did not differ from values predicted by the Hardy-Weinberg equilibrium (χ²=2.96, P=0.09). ACE rs4343 genotype was again not associated with differences in SBP, DBP, or PP.

To examine the effects of dietary fat intake, we stratified subjects into HF diet consumers with total energy from fat greater than or equal to 37% (representing the average fat intake in Western countries) and normal or LF diet consumers (<37% energy from fat). Mean total fat intake accounted for 41% in the HF group and 31% in the normal/LF group, respectively. Also, in this case, genotype frequencies did not deviate from the Hardy-Weinberg equilibrium (Table 3). For a reported dietary fat intake of <37%, no genotype-specific differences were found, whereas for subjects with dietary fat intake ≥37%, increased systolic blood pressure and pulse pressure values were seen for GG carriers compared to AA/AG carriers (additive model: PSBP=0.011 and PP=0.023, respectively, Table 3; recessive model: PSBP=0.008, AA/AG vs GG 122±16 mm Hg vs 131±21 mm Hg, respectively; PP=0.017 AA/AG vs GG 46±12 mm Hg vs 52±14 mm Hg, respectively). In a linear regression model adjusted for sex, age, and BMI, the association of the ACE rs4343 polymorphism with systolic blood pressure and pulse pressure was highly significant (recessive model: βSBP=0.25 and PSBP=0.002; βPP=0.24 and PP=0.002). This model explained 27% of the variation in systolic blood pressure (r²SBP=0.270) and 29% of the variation in pulse pressure (r²PP=0.291). As expected, age (βSBP=-0.418 and PSBP=3.45x10⁻⁷; βPP=0.469 and PP=1.09x10⁻⁴) and BMI (βSBP=0.208 and PSBP=0.010; βPP=0.179 and PP=0.025) had a significant effect on systolic and pulse pressure, whereas sex had no effect. The association of the ACE rs4343 polymorphism with systolic blood pressure and pulse pressure remained significant after further adjustment for total energy intake, alcohol intake, smoking, and activity energy expenditure (recessive model: βSBP=0.26 and PSBP=0.002; βPP=0.26 and PP=0.003).

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Table 3. Dietary Fat Modified ACE rs4343 Genetic Effects on Blood Pressure in the Metabolic Syndrome Berlin-Potsdam Study

|               | Fat Intake <37% |          |          |          | Fat Intake ≥37% |          |          |          |
|---------------|-----------------|----------|----------|----------|-----------------|----------|----------|----------|
|               | AA   | AG   | GG   | P Value | AA   | AG   | GG   | P Value |
| n             | 39   | 131  | 68   | 0.070   | 27   | 66   | 34   | 0.632   |
| SBP, mm Hg    | 127±17 | 123±17 | 124±19 | 0.385   | 126±17 | 120±15 | 131±21 | 0.011   |
| DBP, mm Hg    | 77±11   | 78±10   | 78±10   | 0.885   | 77±10   | 75±8   | 79±10   | 0.132   |
| PP, mm Hg     | 50±11   | 45±11   | 46±13   | 0.061   | 49±13   | 45±11   | 52±14   | 0.023   |

Values are shown as mean±SD. ACE indicates angiotensin-converting enzyme; DBP, diastolic blood pressure; PP, pulse pressure; SBP, systolic blood pressure.

Discussion

The NUGAT study demonstrated that circulating ACE concentrations increase in response to a 6-week, high-saturated-fat diet in healthy, nonobese subjects with a parallel increase in adipose tissue ACE mRNA expression. To our knowledge, this is the first study reporting increased circulating ACE concentrations independent of body weight gain or obesity. Thus, next to the well-known HF diet-induced increases in LDL-cholesterol as an established CVD risk factor, we identified ACE as a second parameter that is closely linked with cardiovascular risk and that increases in response to HF diets.

Perhaps even more importantly, we identified the ACE rs4343 polymorphism as a strong nutrigenetic marker that powerfully modulates the extent of HF diet-induced increases in ACE with parallel increases in blood pressure even in healthy, nonhypertensive subjects. The frequent ACE rs4343 variant might therefore be linked to an increased risk of cardiovascular disease, whereas ACE itself might constitute a molecular link between total and saturated fat intake and cardiovascular disease. Thus, our study shows that a nutrigenetic approach offers real potential for providing personalized nutritional advice for disease prevention.

The strong gene-diet interaction we have identified may help to explain inconsistent results in the relationship between ACE levels or ACE genotype associations and blood pressure and CVD.1,3,20-24 The assumption that ACE levels are associated with blood pressure is due to the facts that ACE catalyzes the production of angiotensin II and ACE inhibitors effectively reduce blood pressure; however, this is not consistently supported by all studies.7,25 A stratification of total and saturated fat intake by ACE genotype would be expected to improve the risk estimates obtained in these studies. Remarkably, a recent epidemiological meta-analysis did not observe an association between saturated/high fat intake and risk of cardiovascular disease26 and therefore requested a change in the dietary recommendations of the American Heart Association. This data set should be particularly suitable for assessing the interaction of ACE rs4343, diet, and cardiovascular risk.

With an estimated heritability of 86%, ACE appears to be 1 of the most heritable markers in our study, proving a high genetic component in ACE concentrations. This heritability estimate is considerably higher than the moderate values being reported by both a family-based and twin-based study.27,28 but is most likely explained by the controlled diet conditions underlying our study and might, therefore, be more accurate. Moreover, our study possibly increased the concordance among the monozygous twin pairs by excluding twins with significant differences in body weight.

A large proportion of theheritable variation of ACE concentrations is strongly linked to the ACE gene, which contains a series of frequent polymorphisms in strong linkage disequilibrium with each other.17,29 The best known is the ACE I/D polymorphism, which accounts for almost 50% of the interindividual variance in circulating ACE levels and represents a quantitative trait locus (QTL).1,4,30,31 Numerous studies have explored associations of the ACE I/D polymorphism, albeit inconsistently, with increased blood pressure and increased risk for CVD.1,3,20-24 The rs4343 SNP that was analyzed in our study is a silent coding SNP expressed at the mRNA level and, due to complete linkage disequilibrium, serves as a surrogate marker for the I/D polymorphism.16-18 At baseline, homozygous carriers of rs4343 (GG) had higher diastolic and by trend higher systolic blood pressure compared to heterozygous carriers and noncarriers (AA/AG). This difference did not persist after 6 weeks of LF diet. This is most likely due to the fact that blood pressure values improved in response to the LF diet, which was in accordance to general national dietary guidelines. After a challenge with the HF diet, genotype-dependent differences in systolic blood reappeared again at HF6. These results were indicative of an underlying gene-diet interaction. To validate these data, we analyzed associations between rs4343 genotype and blood pressure in a subcohort of the cross-sectional MeSyBePo study for whom reliable nutritional data were available. Indeed, whereas no associations between genotype and blood pressure were detected for the whole cohort or for subjects with normal fat intake, significant genotype differences were found in subjects with high dietary fat intake, with the GG carriers having significantly higher
systolic blood pressure and pulse pressure in comparison to AA/AG carriers. Thus, we provided an independent confirmation of the gene-diet interaction.

The mechanism by which diets high in total and saturated fat signal increases in ACE levels is unclear, but it was shown that a diet with high lipid content activates the renin-angiotensin system with increased ACE expression in adipose tissue in mice. The high-lipid diet used was based on soybean oil, which is rich in unsaturated fatty acids, and therefore stands in contrast to high-fat diets with characteristic high content of saturated fat. However, palmitic acid, the major saturated fatty acid, was shown to induce activation of the renin-angiotensin system in 3T3-L1 adipocytes through toll-like receptor 4 and NF-κB signaling.

A limitation of our NUGAT intervention study is the moderate number of participants with respect to genotype-related data analysis. Nevertheless, we reduced confounding factors by including only metabolically healthy, nonobese, and rather young participants in the study, and finally, we demonstrated that a healthy cohort might be favorable to study gene-diet interactions that affect metabolic and/or cardiovascular risks. Nevertheless, the results might be different in obese subjects, and the results may not be applicable in other ethnicities because ACE genotypes are more variable in nonwhites.

Furthermore, nutritional intake information of our MeSyBePo study cohort is limited in that only data on total fat intake but not on saturated fat were available. Nevertheless, consuming high-fat diets typically stands for increased saturated fat intake.

Our data suggest that a high total and saturated fat intake alters concentrations of ACE in a nutrigenetic manner and provides a potential pathway through which high intake of total and saturated fats contributes to the pathogenesis of cardiovascular diseases. Presumably, dietary strategies to lower LDL cholesterol, which are reducing dietary total and saturated fat (DASH diet, Dietary Approaches to Stop Hypertension), are equally efficient in reducing ACE concentrations. Next to lowered LDL cholesterol concentrations, reduced ACE concentrations might contribute concomitantly to beneficial effects.

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