The Insertion/Deletion Polymorphism of the ACE Gene Is Related to Insulin Sensitivity in Overweight Women

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OBJECTIVE — The ACE insertion/deletion (I/D) polymorphism has been identified as a genetic risk factor for coronary heart disease (CHD). The deletion (D) allele of the ACE gene may be associated with higher insulin sensitivity. Individuals who are homozygous for the DD allele have higher ACE levels and possibly more angiotensin II, which, when infused exogenously, causes an increase in insulin sensitivity. The purpose of this study was to investigate the association of the I/D polymorphism of the ACE gene with insulin sensitivity and CHD risk factors.

RESEARCH DESIGN AND METHODS — The study included 66 women (ages 57 ± 1 years) who were overweight or obese (means ± SEM, BMI = 33 ± 1 kg/m2) and sedentary (\(V_{\text{O}2\text{max}} = 19.6 ± 0.4 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}\)). Total body fat mass and percent fat were determined by dual-energy X-ray absorptiometry, and abdominal fat was by computed tomography. Insulin sensitivity was measured during the last 30 min of 3-h hyperinsulinemic-euglycemic clamps (40 \(\mu\text{mol} \cdot \text{m}^{-2} \cdot \text{min}^{-1}\)). Comparisons were made among women with the II (n = 9), ID (n = 36), and DD (n = 21) genotypes.

RESULTS — Age, percent body fat, waist-to-hip ratio, visceral and subcutaneous abdominal fat areas, plasma lipid levels, and systolic and diastolic blood pressures did not differ by ACE genotype. Fasting glucose and 2-h glucose levels were similar among genotypes, but fasting plasma insulin levels were lower in DD women than in ID women (P < 0.05). Glucose utilization was higher in women with the DD genotype than in women with the II genotype (53.1 ± 3.9 vs. 36.0 ± 3.8 \(\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{FFM} \cdot \text{min}^{-1}, P = 0.01\)) and was higher in ID women than in II women (48.5 ± 2.5 \(\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{FFM} \cdot \text{min}^{-1}, P = 0.04\)).

CONCLUSIONS — These data suggest that the I/D polymorphism is not associated with risk factors for CHD in overweight sedentary women; however, women who are homozygous for the D allele of the ACE gene are more insulin sensitive, whereas women who are homozygous for the I allele of the ACE gene have greater insulin resistance and potential risk for type 2 diabetes.

Diabetes Care 24:1646–1652, 2001

The ACE gene regulates vascular tone through the activation of angiotensin II, a potent vasoconstrictor (1,2), and inactivation of bradykinin (3), a nonapeptide belonging to a class of active peptides (kinins) that are released from tissue to produce a variety of effects, including arterial vasodilatation and venoconstriction (4). In young men, intravenous infusion of angiotensin II during a hyperinsulinemic-euglycemic clamp increases insulin sensitivity due to an enhanced blood flow to insulin-sensitive tissues (5). The insertion/deletion (I/D) polymorphism of the ACE gene is characterized by the presence (I) or absence (D) of a 287-bp alu repeat sequence within intron 16 of the ACE gene. The DD polymorphism is associated with higher plasma ACE levels (6). Therefore, because the I/D polymorphism affects ACE and angiotensin II levels, it may also affect an individual’s sensitivity to insulin. Published reports examining glucose metabolism in persons who are homozygous for the deletion allele (DD), compared with the heterozygote (ID) and homozygote insertion (II) genotypes, demonstrate conflicting findings. Glucose metabolism is not different between ACE genotypes in subjects when assessed by glucose tolerance tests or by fasting insulin and glucose concentrations with the homeostasis model assessment (HOMA) (7) calculation (8–10). However, quantification of resistance to insulin-mediated glucose disposal by the insulin suppression test indicates that subjects with the DD genotype are either more insulin-sensitive (11) or are not different than subjects with the ID or II genotypes (10). In select groups of patients with type 2 diabetes (9) and angina pectoris (12), insulin sensitivity is reportedly higher in those with the DD genotype.

Increased body fat is associated with reduced rates of insulin-mediated glucose disposal (13). In addition, visceral fat is directly correlated with glucose intolerance (14) and severity of insulin resistance (15). In addition to type 2 diabetes, obesity is also associated with coronary heart disease (CHD), hypertension, and hypercholesterolemia (16–19). A sedentary lifestyle is also linked to insulin resistance, obesity, and CHD. Therefore, obesity, visceral fat, and physical fitness may play a role in differences in insulin sensitivity by the ACE genotype.

We hypothesized that women with the DD genotype would be more sensitive to insulin action, measured with
the hyperinsulinemic-euglycemic clamp, the “gold” standard for the determination of insulin sensitivity, than overweight and obese women with the II genotype. Moreover, because the ACE I/D polymorphism is related to the development of myocardial infarction and has been identified as a risk factor for CHD in some (20–23) but not all studies (9,10,24,25), it was also of interest to determine whether lipoprotein profiles and blood pressure, risk factors for CHD, may be associated with the ACE genotype in this population. Therefore, the purpose of this study was to determine the association of the I/D polymorphism of the ACE gene with insulin resistance and cardiovascular disease risk factors in overweight and obese sedentary women.

RESEARCH DESIGN AND METHODS—All subjects were healthy, overweight and obese (BMI >25 kg/m², range 25–40) women between the ages of 38 and 70 years. Only women who were weight stable (<2.0 kg change in weight during past year) and sedentary (<20 min of aerobic exercise two times per week) were recruited. Subjects were screened by medical history questionnaire, physical examination, fasting blood profile, and graded exercise treadmill testing to exclude those with CHD. The women underwent a 2-h 75-g oral glucose tolerance test (OGTT) (26). Blood samples were collected at baseline and at the end of the 2-h period for measurement of plasma glucose. All subjects were nonsmokers and had no evidence of cancer, liver, renal or hematological disease, or other medical disorders. A total of 67 women met all study criteria and were enrolled into the study. One woman was deleted from the analysis because her glucose utilization value was 5 SD higher than that of the group. Therefore, 66 women were included in the statistical analyses; 50 women were Caucasian and 16 were African-American. A total of 15 women had been on hormone replacement therapy for several years before participation in the study and remained on the therapy for the duration of the study. A total of 16 women were taking medications for hypertension, including calcium channel blockers (n = 8) and ACE inhibitors (n = 8). All methods and procedures for the study were approved by the institutional review board of the University of Maryland. Each participant provided written informed consent.

Maximal oxygen uptake
Maximal oxygen uptake (\(V_{O_{2max}}\)) was measured using a continuous treadmill test protocol as previously described (27). Validation for attainment of \(V_{O_{2max}}\) included meeting two of the following three criteria: 1) a plateau in oxygen uptake with an increased work load, as evidenced by a difference in oxygen uptake of <2 ml \(\cdot\) kg\(^{-1}\) \(\cdot\) min\(^{-1}\); 2) a respiratory exchange ratio >1.10; and 3) a maximal heart rate within 10 bpm of the age-predicted maximal value. Seated blood pressures were determined twice before the exercise test, and the average determination was used in the statistical analyses.

Body composition
Anthropometry. Height (in cm) and weight (in kg) were measured to calculate BMI as weight (kg)/height (m) squared. Waist circumference, measured at the narrowest point superior to the hip, was divided by the circumference of the hip, measured at its greatest gluteal protuberance, to obtain the waist-to-hip ratio (WHR).

Dual-energy X-ray absorptiometry. Fat mass, lean tissue mass, and bone mineral content (BMC) were determined by dual-energy X-ray absorptiometry (DEXA) (Model DPX-L; Lunar Radiation, Madison, WI). Fat-free mass (FFM) is reported as lean tissue plus BMC. All DEXA scans were analyzed using the Lunar Radiation Version 1.3z DPX-L extended analysis program for body composition analyses.

Computed tomography. A single 5-mm computed tomography (CT) scan of the abdomen taken at the L₄–L₅ region was performed using a PQ 6,000 Scanner (Marconi Medical Systems, Cleveland, OH) to quantitate the relative proportions of intra-abdominal fat and subcutaneous adipose tissue area and sagittal diameter (28). A second scan performed at the level of the mid-thigh was used to quantify muscle area and total fat area of the thigh, as previously described (28). Five CT scans of the abdomen and mid-thigh are missing in the 1D women.

Metabolic testing
To control nutrient intake before the metabolic studies, all subjects were provided with a eucaloric diet for 2 days before testing by a registered dietitian. The composition of this diet was 50–55% carbohydrate, 15–20% protein, ≤30% fat, 300–400 mg of cholesterol per day, and a polyunsaturated-to-saturated fat ratio of 0.6–0.8. The diet was composed of at least 150 g carbohydrate per day for the 2 days before testing (29). The number of calories given to each woman was estimated from the 7-day food records, and estimates of energy expenditure were based on the Harris-Benedict equation (30). All testing was performed in the morning after a 12-h overnight fast. All subjects were weight stabilized (<1 kg) for at least 2 weeks before metabolic testing. All metabolic testing was performed during the same phase of the menstrual cycle for the premenopausal women.

Lipoprotein lipids. The average of two to three fasting blood samples collected on separate days was used in the determination of lipoprotein lipids. Blood samples were transferred into chilled tubes containing 1 mg EDTA per ml of blood. Plasma was separated by centrifugation at 4°C for 15 min at 2000g. Total cholesterol, triglyceride, HDL cholesterol, and HDL₃ cholesterol levels were measured as previously described (28), and LDL cholesterol was calculated using the Friedewald equation (31).

Hyperinsulinemic-euglycemic clamps.
Peripheral tissue sensitivity to exogenous insulin was measured using the hyperinsulinemic-euglycemic clamp technique (32). An intravenous catheter was inserted by percutaneous venipuncture for the infusion of glucose and insulin. A second catheter was inserted in a retrograde fashion into a dorsal hand or wrist vein, and the hand was enclosed in a grounded, insulated chamber and warmed to 70°C to “arterialize” (33) the blood obtained for all samples. For the assessment of basal glucose and insulin levels, three arterialized blood samples were collected at 10-min intervals. Blood samples were collected every 5 and 10 min thereafter for determination of plasma glucose and insulin levels. A 10-min priming and continuous infusion of insulin (240 pmol \(\cdot\) m\(^{-2}\) \(\cdot\) min\(^{-1}\) (Humulin; Eli Lilly, Indianapolis, IN) was performed for 180 min. This resulted in a square wave of hyperinsulinemia at a level of 502 ± 11 pmol/l among the women. A 20% dextrose solution was used, which was measured as 18%.

The mean plasma glucose level during 10–180 min of the euglycemic clamp...
was computed for each individual study and expressed as a percentage of the desired goal. The plasma glucose levels during each clamp period averaged 5.17 ± 0.14, 5.51 ± 0.12, and 5.42 ± 0.09 mmol/l for the II, ID, and the DD groups, respectively. This was 97.6 ± 0.4, 97.6 ± 0.2, and 97.4 ± 0.2% of the desired goal, with a coefficient of variation of 3.9 ± 0.3, 5.1 ± 0.3, and 4.8 ± 0.3% for the II, ID, and DD groups, respectively. Plasma insulin concentrations during 150–180 min of the hyperinsulinemic-euglycemic clamps were comparable among genotypes (II versus ID versus DD 500 ± 42 vs. 512 ± 14 vs. 477 ± 18 pmol/l; P = NS).  

Indirect calorimetry. To quantitate carbohydrate oxidation, continuous indirect calorimetry was performed before the initiation of the glucose infusion and during the last 30 min of the insulin infusion by the open-circuit dilution technique using a SensorMedics DeltaTrac cart (Yorba Linda, CA). Rates of glucose oxidation were calculated from measurements of carbon dioxide production and oxygen consumption using established equations (34), with correction for protein oxidation determined from 24-h urinary urea nitrogen. Nonoxidative glucose metabolism was calculated as the difference between total glucose uptake and glucose oxidation.

Analysis of blood samples. Blood samples were collected in heparinized syringes and placed in prechilled test tubes containing 1.5 mg EDTA per ml of blood in a total volume that was 4% of the sample volume. The blood samples were centrifuged at 4°C, and a 1-ml aliquot of plasma was rapidly frozen (80°C) for subsequent hormone analysis. All determinations were performed in duplicate. Plasma glucose was measured with the glucose oxidase method (Beckman Instruments, Fullerton, CA). Immunoreactive insulin was determined by an insulin-specific double-antibody system as previously described (35) using human insulin standards and tracer (Linco, St. Louis, MO).

Genotyping
Genomic DNA was extracted from whole blood using Qiagen miniprep columns (Qiagen, Valencia, CA) according to the manufacturer’s instructions and was used to genotype the ACE I/D polymorphism following the method of Tiret et al. (6).

Statistical analyses
The mean concentration of glucose and insulin was calculated for each sample time point during the clamp. The trapezoidal rule was used to calculate the integrated response during 30-min intervals from 30 to 180 min for each subject. The integrated response was divided by its time interval to compute mean concentrations. Glucose utilization (M) for 30-min intervals was calculated from the amount of glucose infused after correction for glucose equivalent space (glucose space correction). Insulin sensitivity was expressed as M/I, which represents the amount of glucose metabolized per unit of plasma insulin (I), and was calculated by dividing the glucose used by the insulin concentration during the last 30 min of the clamp for each subject. Statistical significance among genotype groups was determined by one-way analysis of variance and post hoc least significant difference testing. Relationships between variables were determined by linear regression analyses and calculation of Pearson correlation coefficients. All data were analyzed using SPSS statistical software (SPSS, Chicago, IL).

Data are expressed as mean ± SEM, and significance was set at P < 0.05.

RESULTS — A total of 14% of the women were classified as II (n = 9), 54% were ID (n = 36), and 32% had the DD genotype (n = 21). The frequency of the I and D alleles were 0.41 and 0.59, respectively. The separation of the women by ethnicity revealed that of the 16 African-American women, 2 (12.5%) had the II genotype, 10 (62.5%) had the ID genotype, and 4 (25%) had the DD genotype. A total of 15 women were on hormone replacement therapy (II, n = 3 [20%]; ID, n = 9 [60%]; DD, n = 3 [20%]). Of the 17 women taking medication for hypertension, 3 (17%) were in the II group, 10 (59%) were in the ID group, and 4 (24%) were in the DD group.

The physical characteristics of the women are presented in Table 1. Age, body weight, BMI, waist and hip circumferences, WHR, and VO2max (ml · kg–1 · min–1) were similar among the three genotype groups. The three groups also did not differ in percent fat, fat mass, FFM, intra-abdominal fat, subcutaneous abdominal adipose tissue area, or sagittal diameter (Table 1). Mid-thigh muscle area and mid-thigh subcutaneous fat were not different between genotypes (Table 1).

The association of ACE genotype on blood pressure and lipoprotein lipids are reported in Table 2. Systolic and diastolic blood pressures were similar among the three genotypes. In addition, total cholesterol, LDL cholesterol, HDL cholesterol, HDL2 cholesterol, and triglyceride levels

**Table 1—Physical characteristics and total and regional body composition of overweight sedentary women by ACE genotype**

<table>
<thead>
<tr>
<th></th>
<th>II Genotype</th>
<th>ID Genotype</th>
<th>DD Genotype</th>
</tr>
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<tbody>
<tr>
<td>n</td>
<td>9</td>
<td>36</td>
<td>21</td>
</tr>
<tr>
<td>Age (years)</td>
<td>61 ± 2</td>
<td>57 ± 1</td>
<td>55 ± 2</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>84.6 ± 2.8</td>
<td>86.2 ± 2.2</td>
<td>89.5 ± 3.1</td>
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<tr>
<td>BMI (kg/m2)</td>
<td>32.6 ± 1.1</td>
<td>32.6 ± 0.7</td>
<td>32.6 ± 1.0</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>96.8 ± 3.1</td>
<td>94.9 ± 1.9</td>
<td>96.0 ± 2.1</td>
</tr>
<tr>
<td>Hip circumference (cm)</td>
<td>116.2 ± 2.4</td>
<td>116.5 ± 1.5</td>
<td>116.6 ± 2.3</td>
</tr>
<tr>
<td>WHR</td>
<td>0.83 ± 0.02</td>
<td>0.81 ± 0.01</td>
<td>0.83 ± 0.02</td>
</tr>
<tr>
<td>VO2max (ml · kg · min–1)</td>
<td>19.1 ± 0.9</td>
<td>19.1 ± 0.6</td>
<td>20.7 ± 0.6</td>
</tr>
<tr>
<td>Percent body fat</td>
<td>46.6 ± 1.7</td>
<td>46.5 ± 0.6</td>
<td>46.4 ± 1.3</td>
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<td>Fat mass (kg)</td>
<td>38.9 ± 2.5</td>
<td>39.8 ± 1.3</td>
<td>41.7 ± 2.3</td>
</tr>
<tr>
<td>FFM (kg)</td>
<td>43.9 ± 0.8</td>
<td>45.5 ± 1.0</td>
<td>47.1 ± 1.2</td>
</tr>
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<td>Intra-abdominal adipose tissue area (cm²)</td>
<td>437.9 ± 27.2</td>
<td>459.0 ± 19.4</td>
<td>490.9 ± 25.3</td>
</tr>
<tr>
<td>Subcutaneous abdominal adipose tissue area (cm²)</td>
<td>25.9 ± 0.7</td>
<td>25.9 ± 0.5</td>
<td>26.6 ± 0.6</td>
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<tr>
<td>Sagittal diameter (mm)</td>
<td>162.1 ± 10.9</td>
<td>189.9 ± 8.6</td>
<td>199.5 ± 16.5</td>
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<tr>
<td>Mid-thigh fat (cm²)</td>
<td>84.2 ± 5.6</td>
<td>84.3 ± 3.3</td>
<td>79.3 ± 4.2</td>
</tr>
<tr>
<td>Mid-thigh muscle (cm²)</td>
<td>156.1 ± 14.2</td>
<td>159.2 ± 13.0</td>
<td>159.2 ± 13.0</td>
</tr>
</tbody>
</table>

Data are means ± SEM.
Table 2—Effects of ACE genotype on blood pressure, lipoprotein lipids, and insulin sensitivity in overweight sedentary women

<table>
<thead>
<tr>
<th>Genotype</th>
<th>n</th>
<th>Systolic blood pressure (mmHg)</th>
<th>Diastolic blood pressure (mmHg)</th>
<th>Total cholesterol (mmol/l)</th>
<th>LDL cholesterol (mmol/l)</th>
<th>HDL cholesterol (mmol/l)</th>
<th>Triglycerides (mmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>II</td>
<td>9</td>
<td>140 ± 5</td>
<td>84 ± 2</td>
<td>5.44 ± 0.35</td>
<td>3.45 ± 0.26</td>
<td>1.23 ± 0.14</td>
<td>0.13 ± 0.06</td>
</tr>
<tr>
<td>ID</td>
<td>36</td>
<td>133 ± 2</td>
<td>79 ± 2</td>
<td>5.14 ± 0.15</td>
<td>3.22 ± 0.17</td>
<td>1.26 ± 0.06</td>
<td>0.17 ± 0.03</td>
</tr>
<tr>
<td>DD</td>
<td>21</td>
<td>136 ± 4</td>
<td>81 ± 2</td>
<td>5.41 ± 0.16</td>
<td>3.35 ± 0.16</td>
<td>1.37 ± 0.06</td>
<td>0.19 ± 0.05</td>
</tr>
</tbody>
</table>

Data are means ± SEM. *P < 0.05, significantly different than DD genotype; †P < 0.05, ‡P < 0.01, significantly different than II genotype.

When women on hormone replacement therapy and women taking antihypertensive medications were excluded, the results for plasma insulin, M, and M/I remained the same and statistically significant. Furthermore, the results for M and M/I did not change when only Caucasian women were analyzed.

Glucose utilization during the last 30 min of the hyperinsulinemic-euglycemic period in the total group was negatively correlated with fat mass (r = −0.25, P < 0.05), waist circumference (r = −0.52, P < 0.001), intra-abdominal fat (r = −0.31, P < 0.05), sagittal diameter (r = −0.45, P < 0.001), and mid-thigh muscle area (r = −0.59, P < 0.001). These relationships persisted after controlling for the ACE genotype (fat mass r = −0.37, P < 0.01; waist circumference r = −0.59, P < 0.001; intra-abdominal fat r = −0.37, P < 0.01; sagittal diameter r = −0.49, P < 0.001; and mid-thigh muscle area r = −0.49, P < 0.001). M/I was negatively correlated with waist circumference (r = −0.47, P < 0.001), intra-abdominal fat (r = −0.34, P < 0.05), sagittal diameter (r = −0.42, P < 0.01), and mid-thigh muscle area (r = −0.44, P < 0.05).
ACE gene and insulin resistance

 increases in blood pressure, increases glucose uptake in normotensive men (5,37). The mechanism by which angiotensin II increases insulin sensitivity involves the redistribution of blood flow to skeletal muscles (5). An acute infusion of angiotensin II increases insulin sensitivity in normotensive patients with type 2 diabetes as well (38). The I/D polymorphism of the ACE gene is also associated with stiffening of the arteries, such that patients with type 2 diabetes with the II genotype have increased stiffness of the carotid arteries and the thoracoabdominal aorta (39). Because arterial stiffness indexes of the carotid and femoral arteries are associated with insulin resistance (40), it is conceivable that the link between the renin-angiotensin system and insulin resistance is such that vasodilation is reduced and arterial stiffness is increased in individuals with the ACE II genotype, resulting in a reduction in insulin sensitivity. Studies of blood flow and/or arterial stiffness during a clamp in subjects characterized by ACE genotyping are needed to test this hypothesis. Moreover, additional work is needed to determine the cellular mechanisms, including the early steps in the insulin signaling pathway, which may contribute to variations in insulin sensitivity among the ACE gene polymorphisms.

Because insulin resistance is related to total body fat and visceral fat (41), it is possible that variations in obesity could contribute to the observed differences in insulin sensitivity among the genotypes. Nevertheless, little is known about the potential contribution of obesity to the ACE gene effect on insulin sensitivity. Katsuya et al. (11) reported that subjects with the DD genotype had a lower BMI than individuals with the ID genotype. However, we and others (36,42) found no evidence to suggest that the three ACE genotypes differ in BMI or WHR. Moreover, we add that total body fat mass, visceral fat, and regional fat distribution do not differ by ACE genotype in women, and the relationships between glucose utilization and insulin sensitivity with body composition persist after controlling for the ACE genotype. These results suggest that total and central obesity do not contribute to the variation observed in insulin resistance by ACE genotypes in this homogenous population of overweight women. Although age, race, and fitness also influence insulin sensitivity, these factors did not differ among the genotypes. Therefore, we do not believe they account for the differences in glucose utilization and insulin sensitivity among the genotypes.

In the present study, we found no association of the ACE genotype with lipoprotein lipid risk factors in healthy women with IGT and type 2 diabetes. In previously published reports, lipoprotein levels did not differ by genotype in individuals from Finland (8), England (9), the U.S. (11), and the Pima Indians (36). Prior studies report that blood pressure levels do not differ between the II, ID, and DD genotypes (8,9,36,42). The lack of association between the ACE genotype and hypertension is also true in patients with type 1 and type 2 diabetes (43). Although our results confirm that blood pressure levels do not differ by ACE genotype in overweight sedentary women, some of the women in our study were taking medications for hypertension; therefore, further studies are warranted to clarify this finding.

In summary, our results show greater insulin resistance in overweight and obese sedentary women with the II genotype than in women of comparable physical characteristics with the ID and DD genotypes. These differences are not due to greater body fat or visceral obesity. These results must be confirmed in a larger sample of women, and these associations should be examined in men. Further studies are needed to determine the cellular mechanisms that account for the differences in insulin sensitivity between the ACE genotypes. Thus, overweight and sedentary individuals with the II genotype may require treatment and would benefit from diet-induced weight loss or an exercise program to enhance insulin sensitivity.
References

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