Lack of carotid stiffening associated with MTHFR 677TT genotype in cardiorespiratory fit adults

Motoyuki Iemitsu,1,2 Haruka Murakami,1 Kiyoshi Sanada,2 Kenta Yamamoto,1 Hiroshi Kawano,3 Yuko Gando,3 and Motohiko Miyachi1

1Health Promotion and Exercise Program, National Institute of Health and Nutrition, Tokyo; 2Faculty of Sport and Health Science, Ritsumeikan University, Shiga; and 3Faculty of Sport Sciences, Waseda University, Saitama, Japan

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Iemitsu M, Murakami H, Sanada K, Yamamoto K, Kawano H, Gando Y, Miyachi M. Lack of carotid stiffening associated with MTHFR 677TT genotype in cardiorespiratory fit adults. Physiol Genomics 42: 259–265, 2010. First published April 20, 2010; doi:10.1152/physiolgenomics.00039.2010.—The TT genotype of C677T polymorphism in 5,10-methylenetetrahydrofolate reductase (MTHFR) induces elevation of homocysteine level and leads to atherosclerosis and arterial stiffening. Furthermore, cardiorespiratory fitness level is also associated with arterial stiffness. In the present study, a cross-sectional investigation of 763 Japanese men and women (18–70 yr old) was performed to clarify the effects of cardiorespiratory fitness on the relationship between arterial stiffness and MTHFR C677T gene polymorphism. Arterial stiffness was assessed by carotid β-stiffness with ultrasonography and tonometry. The study subjects were divided into high-cardiorespiratory fitness (High-Fit) and low-cardiorespiratory fitness (Low-Fit) groups based on the median value of peak oxygen uptake in each sex and decade. The plasma homocysteine level was higher in the TT genotype of MTHFR C677T polymorphism compared with CC and CT genotype individuals. MTHFR C677T polymorphism showed no effect on carotid β-stiffness, but there was a significant interaction effect between fitness and MTHFR C677T polymorphism on carotid β-stiffness (P = 0.0017). In the Low-Fit subjects, carotid β-stiffness was significantly higher in individuals with the TT genotype than the CC and CT genotypes. However, there were no such differences in High-Fit subjects. In addition, β-stiffness and plasma homocysteine levels were positively correlated in Low-Fit subjects with the TT genotype (r = 0.71, P < 0.0001), but no such correlations were observed in High-Fit subjects. In CC and CT genotype individuals, there were also no such correlations in either fitness level. These results suggest that the higher cardiorespiratory fitness may attenuate central artery stiffening associated with MTHFR C677T polymorphism.

peak oxygen uptake; arterial stiffness; homocysteine; 5,10-methylenetetrahydrofolate reductase

ELEVATED PLASMA HOMOCYSTEINE level is considered a risk factor for cardiovascular events and is associated with arterial stiffness and atherosclerosis in subjects with some cardiovascular risk factors (7, 15, 30, 36). High homocysteine levels may impair endothelial function, increase oxidative stress, and alter protein structure (5, 6, 37). Exposure of endothelial cells to elevated homocysteine levels results in decreased availability of nitric oxide (NO), which has vasodilatory and antiplatelet effects, and impaired vascular function, which are early events in atherogenesis (6, 29, 33, 35). Homocysteine metabolism represents an interesting model of gene-environment interaction (34, 38). Elevations in homocysteine may be caused by genetic and environmental factors and by gene-gene and/or gene-environment interactions. The enzyme 5,10-methylenetetrahydrofolate reductase (MTHFR) catalyzes the irreversible conversion of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate (4). A polymorphism of C677T (Ala→Val) in the gene encoding MTHFR is associated with decreased activity of the enzyme due to thermolability (1). In individuals homozygous for the T (Val) allele, a relative deficiency in the remethylation process of homocysteine into methionine leads to mild to moderate hyperhomocysteinemia, a condition recognized as an independent risk factor for arterial stiffness and atherosclerosis (1, 34). Thus the variation in MTHFR genetic sequence was shown to be associated with differences in the development of cardiovascular disease and related conditions, such as arterial stiffness and atherosclerosis.

Habitual exercise results in higher cardiorespiratory fitness and reduced risk of cardiovascular disease, such as arterial stiffness and atherosclerosis (8, 11, 12, 31). There have been several cross-sectional studies regarding the relationship between cardiorespiratory fitness and homocysteine status. These factors were reported to be independent regardless of sex (10) or to be negatively associated in women but not in men (19). Therefore, genetic variations in MTHFR, such as C677T polymorphism, may influence the effects of regular exercise and plasma homocysteine status on arterial stiffness. Recently, plasma homocysteine levels were shown to be associated with cardiorespiratory fitness after controlling for potential confounders, including MTHFR C677T, in a cross-sectional study of Swedish children and adolescents (26). However, it remains unclear whether cardiorespiratory fitness level affects the relationship between arterial stiffness and genetic variations in MTHFR.

We hypothesized that single-nucleotide polymorphism (SNP) genotypes of C677T (Ala→Val) in exon 5 of MTHFR on chromosome 1 and cardiorespiratory fitness level may affect arterial stiffness in healthy Japanese subjects. The present study represents a cross-sectional investigation of 763 Japanese men and women (18–70 yr) to clarify the effects of cardiorespiratory fitness on the relationship between arterial stiffness and MTHFR C677T gene polymorphism.

METHODS

Subjects. A total of 763 Japanese subjects (239 men and 524 women) between 18 and 70 yr of age participated in this cross-sectional study (mean: 40 ± 1 yr). The study population consisted of sedentary or moderately active subjects who participated in swimming, stretching, and healthy gymnastics programs (at least 60 min/wk) and did not participate in any other vigorous sports activities. Subjects were divided into low-cardiorespiratory fitness (Low-Fit) and high-cardiorespiratory fitness (High-Fit) groups, with the dividing line set at the median value of peak oxygen uptake (V̇O2peak), as an
index of cardiorespiratory fitness, in each sex and decade [median value of \( V_{\text{O2peak}} \) (ml kg\(^{-1}\) min\(^{-1}\)) for 18–30 yr old: men 47.1, women 36.7; 31–40 yr old: men 37.1, women 35.6; 41–50 yr old: men 34.7, women 31.9; 51–60 yr old: men 31.8, women 29.3; 61–70 yr old: men 31.0, women 27.2]. The median values of \( V_{\text{O2peak}} \) in the present study were similar to the reference values included in the exercise guidelines established by the Ministry of Health, Labor, and Welfare of Japan for prevention of lifestyle-related diseases (http://www.mhlw.go.jp/en/health/programs/environment/201606.pdf). Subjects were recruited for the present study by advertisement. All subjects were free of any overt signs or symptoms of chronic disease, and all were nonsmokers. Carotid \( \beta \)-stiffness (\( \beta \)-stiffness) and common carotid intima-media thickness (ccIMT) were determined as indexes of arterial stiffness in all subjects. Systolic blood pressure (SBP), diastolic blood pressure (DBP), percent body fat, and MTHFR gene C677T polymorphism were determined in all subjects. Body fat mass was determined for the whole body with a dual-energy X-ray absorptiometry (DXA) (Hologic QDR-4500A scanner, Hologic, Waltham, MA). SBP and DBP were measured at rest with a vascular testing device (Colin Medical Technology, Tokyo, Japan). Serum cholesterol, triglyceride, and folic acid levels and plasma glucose and homocysteine levels were also measured.

The study was approved by the Ethical Review Board of the National Institute of Health and Nutrition. Written informed consent was obtained from all subjects before inclusion in the study.

**Measurement of \( V_{\text{O2peak}} \).** \( V_{\text{O2peak}} \) was measured by an incremental cycle exercise test using a cycle ergometer (828E; Monark, Varberg, Sweden). The incremental cycle exercise began at a work rate of 90 W (40–120 W) in men and 60 W (30–90 W) in women, and power output was increased by 15 W/min until the subjects could not maintain a fixed pedaling frequency of 60 rpm. The subjects were encouraged during the ergometer test to exercise at the level of maximum intensity. Heart rate and rating of perceived exertion (RPE) were monitored minute by minute during exercise. RPE was obtained by 10.220.32.246 on April 20, 2017 http://physiolgenomics.physiology.org/ Downloaded from

**RESULTS**

**Comparison of characteristics in low- and high-cardiorespiratory fitness groups.** In the High-Fit group, body weight, %Fat, and triglyceride levels were significantly lower than those in the Low-Fit group. High-density lipoprotein (HDL) level was significantly higher in the High-Fit group than in the Low-Fit group (Table 1). There were no significant differences in age, height, SBP, DBP, \( \beta \)-stiffness, ccIMT, total cholesterol, glucose, homocysteine, or folic acid levels between the High-Fit and Low-Fit groups (Table 1).
Table 1. Characteristics of subjects in high-cardiorespiratory fitness and low-cardiorespiratory fitness groups

<table>
<thead>
<tr>
<th></th>
<th>High-Fit</th>
<th>Low-Fit</th>
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<tbody>
<tr>
<td>Age, yr</td>
<td>39 ± 1</td>
<td>38 ± 1</td>
</tr>
<tr>
<td>Body weight, g</td>
<td>58 ± 1*</td>
<td>60 ± 1</td>
</tr>
<tr>
<td>Height, cm</td>
<td>163 ± 1</td>
<td>163 ± 1</td>
</tr>
<tr>
<td>%Fat</td>
<td>21.2 ± 0.3*</td>
<td>26.4 ± 0.4</td>
</tr>
<tr>
<td>SBP, mmHg</td>
<td>112 ± 1</td>
<td>112 ± 1</td>
</tr>
<tr>
<td>DBP, mmHg</td>
<td>65 ± 1</td>
<td>66 ± 1</td>
</tr>
<tr>
<td>β-Stiffness, AU</td>
<td>8.4 ± 0.2</td>
<td>8.8 ± 0.3</td>
</tr>
<tr>
<td>ccIMT, mm</td>
<td>0.59 ± 0.01</td>
<td>0.59 ± 0.01</td>
</tr>
<tr>
<td>Total cholesterol, mg/dl</td>
<td>191 ± 2</td>
<td>190 ± 2</td>
</tr>
<tr>
<td>HDL cholesterol, mg/dl</td>
<td>69 ± 1*</td>
<td>63 ± 1</td>
</tr>
<tr>
<td>Triglycerides, mg/dl</td>
<td>67 ± 1*</td>
<td>72 ± 2</td>
</tr>
<tr>
<td>Glucose, mg/dl</td>
<td>89 ± 1</td>
<td>90 ± 1</td>
</tr>
<tr>
<td>Homocysteine, mmol/l</td>
<td>7.7 ± 0.2</td>
<td>7.6 ± 0.2</td>
</tr>
<tr>
<td>Folic acid, ng/ml</td>
<td>9.8 ± 0.3</td>
<td>9.3 ± 0.2</td>
</tr>
<tr>
<td>V̇O₂peak, ml·kg⁻¹·min⁻¹</td>
<td>40.9 ± 0.5*</td>
<td>31.1 ± 0.4</td>
</tr>
</tbody>
</table>

Values are means ± SE. High-Fit, high cardiorespiratory fitness; Low-Fit, low cardiorespiratory fitness; SBP, systolic blood pressure; DBP, diastolic blood pressure; β-stiffness, carotid β-stiffness; AU, arbitrary units; ccIMT, common carotid intima-media thickness; HDL, high-density lipoprotein; V̇O₂peak, peak oxygen uptake. *P < 0.05 vs. Low-Fit.

We next compared the characteristics of subjects with different gene polymorphisms (Table 3). In the MTHFR C677T genotypes, plasma homocysteine level was significantly higher in the TT genotype than in the CC and CT genotypes. There were no significant differences in age, body weight, height, %fat, SBP, DBP, β-stiffness, ccIMT, total cholesterol, HDL cholesterol, triglycerides, glucose, homocysteine, folic acid, or V̇O₂peak between these groups.

Comparison of characteristics between genotypes and cardiorespiratory fitness groups. We compared the characteristics of subjects with different genotypes and fitness levels (Table 4). In the MTHFR C677T genotypes in the High-Fit group, body weight (vs. CT and TT genotypes), %fat (vs. all genotypes), and triglycerides (vs. TT genotypes) were significantly lower than those in the Low-Fit group, and HDL cholesterol level (vs. CT and TT genotypes) and V̇O₂peak (vs. all genotypes) were significantly higher than those in the Low-Fit group. V̇O₂peak in the High-Fit group with TT genotype was lower than those in the CC and CT genotypes. There were no significant differences in age, height, SBP, DBP, ccIMT, total cholesterol, HDL cholesterol, glucose, or folic acid between genotypes or cardiorespiratory fitness groups.

Comparison of arterial stiffness and plasma homocysteine levels between genotypes and cardiorespiratory fitness groups. There was a significant interaction effect of fitness and MTHFR C677T polymorphism on β-stiffness (P = 0.0017) but not on ccIMT (P = 0.6020). The β-stiffness of subjects with the TT genotype of MTHFR C677T in the Low-Fit group was significantly higher than that of individuals with the CC and CT genotypes, but there were no significant differences in β-stiffness of subjects with CC, CT, and TT genotypes in the High-Fit group (Fig. 1). However, there were no significant differences in ccIMT between MTHFR genotypes in the Low-Fit and High-Fit groups (Table 4). In addition, there was a significant association between plasma homocysteine level and MTHFR C677T polymorphism (P < 0.0001). The plasma homocysteine concentrations in subjects with the TT genotype of MTHFR in both Low-Fit and High-Fit groups were significantly higher than those of individuals with the CC and CT genotypes in each fitness group (Fig. 2).

To further explore the possible relationship between arterial stiffness (β-stiffness) and plasma homocysteine levels, we performed regression analyses between β-stiffness and plasma homocysteine level (Fig. 3). In the Low-Fit group, there were positive and significant correlations between β-stiffness and plasma homocysteine level in the individuals with the TT genotype of MTHFR (y = 0.78x + 2.80, r = 0.71, P < 0.0001). There were no significant correlations for the CC and CT genotypes. In the High-Fit group, there were no significant correlations for any of the MTHFR genotypes (Fig. 3). The slopes of the regression lines were significantly different between High-Fit and Low-Fit groups in TT genotype of MTHFR (P < 0.05). There was a slight significant correlation between plasma homocysteine and V̇O₂peak (y = 0.04x + 6.06, r = 0.16, P < 0.05).

**DISCUSSION**

The present cross-sectional study demonstrated the associations among arterial stiffness, cardiorespiratory fitness, and polymorphisms in the MTHFR gene in Japanese subjects. Plasma homocysteine concentrations were significantly higher in individuals with the TT genotype of MTHFR than in those with the CC and CT genotypes in each fitness group. Interestingly, in the Low-Fit subjects carotid β-stiffness was higher in the TT genotype individuals than in those with the CC and CT genotypes.
genotypes of MTHFR C677T. However, there were no such differences in High-Fit subjects. In addition, β-stiffness and plasma homocysteine levels were positively correlated in the Low-Fit subjects with the TT genotype ($r = 0.71, P < 0.0001$) but were not correlated in the other groups.

The TT genotype at C677T of the MTHFR gene was associated with elevated plasma homocysteine level but showed no effect on carotid arterial stiffness in the present study. Elevated plasma homocysteine level is associated with vascular function and increased risk of arterial stiffness (7, 15, 30, 36), because exposure of endothelial cells to elevated homocysteine levels leads to decreased availability of NO and results in impairment of endothelium-dependent vasodilation in humans (6, 29, 33, 35). In subjects with lower fitness, the TT genotype at C677T of the MTHFR gene increased arterial stiffness, but this was not seen in higher-fitness subjects. Moreover, homocysteine level was positively associated with arterial stiffness only in lower-fitness subjects with the TT genotype. Regular exercise improves endothelial function through increased NO production and decreased endothelin-1 concentration (22). Hayward et al. (14) reported that exercise training improved endothelium-dependent vasodilation under conditions of homocysteine exposure, and this may contribute to the increased endothelial nitric oxide synthase (eNOS) protein levels and eNOS activity in the aorta of rats. Exercise training induced changes in expression levels of vasodilation-related molecules, including eNOS, in the aorta of rats with improvement of arterial stiffness (21). Therefore, regardless of elevated homocysteine level induced by the T allele of the MTHFR C677T polymorphism, regular exercise is considered to decrease stiffening in the central artery via improvement of endothelial function. Thus regular exercise, which can maintain and obtain sufficient cardiorespiratory fitness, may be needed to cancel the genetic negative effects of MTHFR polymorphism in subjects with the TT genotype at C677T of the MTHFR gene.

In the present study, higher cardiorespiratory fitness did not seem to be associated with elevated plasma homocysteine

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**Table 4. Characteristics of subjects in each cardiorespiratory fitness and genotype of MTHFR C677T group**

<table>
<thead>
<tr>
<th></th>
<th>CC</th>
<th>CT</th>
<th>TT</th>
<th>CC</th>
<th>CT</th>
<th>TT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>39±1</td>
<td>38±1</td>
<td>37±2</td>
<td>38±1</td>
<td>38±1</td>
<td>41±2</td>
</tr>
<tr>
<td>Body weight, g</td>
<td>60±1</td>
<td>60±1</td>
<td>61±2</td>
<td>59±1</td>
<td>58±1</td>
<td>57±1*</td>
</tr>
<tr>
<td>Height, cm</td>
<td>164±1</td>
<td>162±1</td>
<td>164±1</td>
<td>164±1</td>
<td>163±1</td>
<td>162±1</td>
</tr>
<tr>
<td>%Fat</td>
<td>25.3±0.7</td>
<td>26.9±0.5</td>
<td>27.1±1.1</td>
<td>20.7±0.6*</td>
<td>21.0±0.5*</td>
<td>22.7±0.8*</td>
</tr>
<tr>
<td>Systolic blood pressure (SBP), mmHg</td>
<td>111±1</td>
<td>112±1</td>
<td>115±3</td>
<td>113±1</td>
<td>112±1</td>
<td>111±2</td>
</tr>
<tr>
<td>Diastolic blood pressure (DBP), mmHg</td>
<td>66±1</td>
<td>66±1</td>
<td>69±1</td>
<td>66±1</td>
<td>65±1</td>
<td>66±1</td>
</tr>
<tr>
<td>cIMT, mm</td>
<td>0.60±0.01</td>
<td>0.59±0.01</td>
<td>0.58±0.01</td>
<td>0.58±0.01</td>
<td>0.58±0.01</td>
<td>0.60±0.02</td>
</tr>
<tr>
<td>Total cholesterol, mg/dl</td>
<td>190±3</td>
<td>189±3</td>
<td>193±6</td>
<td>189±3</td>
<td>191±3</td>
<td>195±5</td>
</tr>
<tr>
<td>HDL cholesterol, mg/dl</td>
<td>65±1</td>
<td>63±1</td>
<td>65±1</td>
<td>67±1</td>
<td>70±1*</td>
<td>70±2*</td>
</tr>
<tr>
<td>Triglycerides, mg/dl</td>
<td>75±3</td>
<td>70±2</td>
<td>75±4</td>
<td>68±3</td>
<td>67±2</td>
<td>64±4*</td>
</tr>
<tr>
<td>Glucose, mg/dl</td>
<td>89±1</td>
<td>90±1</td>
<td>90±1</td>
<td>90±1</td>
<td>89±1</td>
<td>89±1</td>
</tr>
<tr>
<td>Folic acid, ng/ml</td>
<td>9.8±0.4</td>
<td>9.2±0.3</td>
<td>8.4±0.8</td>
<td>9.9±0.4</td>
<td>9.9±0.4</td>
<td>9.0±0.6</td>
</tr>
<tr>
<td>$\mathrm{V}<em>{\mathrm{O}</em>{2} \text {peak}}, \text{ml-kg}^{-1} \cdot \text{min}^{-1}$</td>
<td>31.1±0.8</td>
<td>30.6±0.5</td>
<td>32.9±1.4</td>
<td>41.4±0.9*</td>
<td>41.2±0.8*</td>
<td>38.1±1.1*†</td>
</tr>
</tbody>
</table>

Values are means ± SE. *$P < 0.05$ vs. each genotype in Low-Fit; †$P < 0.05$ vs. CC and CT in High-Fit.
levels in individuals with the TT genotype at C677T of the MTHFR gene. There have been several studies regarding the association with homocysteine status according to varied cardiorespiratory fitness levels and age. The relationship between $V_{\text{O2peak}}$ and plasma homocysteine is unaffected in men or women aged 30–59 yr (10) and inversely associated in women (mean age 33.5 yr) but not in men (mean age 33.1 yr) (19). Moreover, the relationship was unaffected in children and adolescents (26), and a negative association was observed in women but not in men (27). Inconsistent results were also reported in athletes, in whom plasma homocysteine levels were elevated (25) or decreased compared with untrained control subjects (13). Thus the relationship between cardiorespiratory fitness and homocysteine status was not consistent. This discrepancy may be influenced by differences in physical fitness level, age, and genetic effects, such as MTHFR C677T, in each study. Although we extended our research effort to the association with MTHFR genotype, the present results could not account for the discrepancy. Therefore, further studies are required to examine differences in the relationship between different physical fitness levels and plasma homocysteine levels in various age groups.

In the present study, subjects were divided into Low-Fit and High-Fit groups, with the dividing line set at the median value of $V_{\text{O2peak}}$, as an index of cardiorespiratory fitness, in each sex and decade as a cutoff. Carotid $\beta$-stiffness was higher in the TT genotype at C677T of the MTHFR gene in subjects with lower cardiorespiratory fitness but was not altered in those with CC and CT genotypes. However, there were no effects of SNP on arterial stiffness in individuals with higher cardiorespiratory fitness. In contrast, ccIMT, evaluated as the thickness of the carotid arterial wall, was unaffected by the relationship between MTHFR C677T genotype and fitness level. Previous studies demonstrated the relationship between ccIMT and homocysteine levels in female smokers (17), whereas no relationship was observed in patients with atherosclerotic disease (28). de Bree et al. (10) reported no effect of ccIMT or pulse wave velocity on increases in plasma homocysteine levels in healthy middle-aged French subjects. Thus measurements using carotid $\beta$-stiffness may be a sensi-
active means of detecting the effect of cardiorespiratory fitness on stiffening in the central artery induced by MTHFR C677T polymorphism in healthy subjects.

A previous study indicated the effects of plasma homocysteine level on the association between C677T and A1298C or G1793A (3). Further studies are required to determine the effects of fitness on the association between arterial stiffness and MTHFR haplotype. In addition, Labayen et al. (20) recently reported the effects of polymorphisms in the UCP3 gene on plasma homocysteine levels during youth. Plasma homocysteine level was higher in the TT and CT genotypes of the rs1800849 polymorphism in the UCP3 gene compared with individuals with the CC genotype after adjustment for sex, age, pubertal status, folate and vitamin B12 intake, and MTHFR C677T polymorphism. Moreover, the T allele of the rs1800849 polymorphism was associated with elevated homocysteine levels in young subjects with low fitness, but not with moderate or high cardiorespiratory fitness, indicating that cardiorespiratory fitness modifies the association between the rs1800849 polymorphism and homocysteine. The UCP3 gene polymorphism-induced increase in plasma homocysteine level in subjects with low fitness may affect arterial stiffness. Therefore, further studies are required to examine the effects of UCP3 gene polymorphism on the relationships among homocysteine, fitness, and arterial stiffness. Furthermore, although homocysteine is affected by endothelial function, we did not measure endothelial function, such as flow-mediated diameter, plasma NO, plasma endothelin-1, etc., in the present study. Therefore, further studies are required to determine the endothelial function parameters and the effects of gene polymorphism and fitness on homocysteine and endothelial function. Although it is well known that dietary folate intake is a major determinant of plasma homocysteine level, it could not be assessed in all subjects in the present study. Further studies are required to determine the effects of folate intake. Finally, the present study population included only Asian (Japanese) subjects; therefore, our data may not be applicable to other populations because genotypic distribution appears to show ethnic differences.

We investigated the associations among cardiorespiratory fitness, arterial stiffness, and C677T polymorphism of the MTHFR gene in healthy Japanese subjects. The results of this study indicated a lack of arterial stiffening associated with the TT genotype at C677T of the MTHFR gene in cardiorespiratory fit subjects. Thus habitual exercise-induced cardiovascular fitness may affect cardiovascular adaptations to molecular variation in the MTHFR gene in Japanese subjects. However, further studies are required to clarify the effects of fitness or physical activity on the risk of cardiovascular disease associated with genetic factors.

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DISCLOSURES
No conflicts of interest, financial or otherwise, are declared by the author(s).

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