

# IgE to the Mammalian Oligosaccharide Galactose- $\alpha$ -1,3-Galactose Is Associated With Increased Atheroma Volume and Plaques With Unstable Characteristics—Brief Report

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**Objective**—Emerging evidence suggests a link between coronary artery disease and type 2 immunity. We sought to test the hypothesis that IgE sensitization to the mammalian oligosaccharide galactose- $\alpha$ -1,3-galactose ( $\alpha$ -Gal)—the target allergen of delayed anaphylaxis to red meat—is associated with coronary artery disease.

**Approach and Results**—Total IgE and specific IgE to  $\alpha$ -Gal were assayed on sera from 118 subjects who presented for cardiac catheterization and underwent intravascular ultrasound. IgE to  $\alpha$ -Gal was detected in 26%, and atheroma burden was higher in sensitized subjects ( $P=0.02$ ). Because  $\alpha$ -Gal sensitization relates to an environmental exposure that could be a risk factor for early-onset coronary artery disease (ie, tick bites), we age stratified the cohort. In subjects  $\leq 65$  years of age, the strength of the association with atheroma burden was stronger ( $P<0.001$ ), and plaques in the sensitized group had less stable features based on intravascular ultrasound. To address the specificity of the association with IgE to  $\alpha$ -Gal, IgE to inhalants and peanut were assayed and were not associated with coronary artery disease. Total IgE and  $\alpha$ -Gal-specific IgE were strongly associated with each other, but the strength of the relationship with atheroma burden was stronger for  $\alpha$ -Gal-specific IgE. This association was significant when adjusted for sex, diabetes mellitus, hypertension, statin use, and total IgE (regression coefficient, 12.2; SE, 5.2;  $P=0.02$ ).

**Conclusions**—Increased atheroma burden and plaques with more unstable features were associated with IgE to  $\alpha$ -Gal—an effect most pronounced in subjects  $\leq 65$  years of age. IgE sensitization to  $\alpha$ -Gal may represent a novel, and potentially modifiable, risk factor for coronary atherosclerosis.



**Visual Overview**—An online [visual overview](#) is available for this article. (*Arterioscler Thromb Vasc Biol.* 2018;38:1665-1669. DOI: 10.1161/ATVBAHA.118.311222.)

**Key Words:** atherosclerosis ■ galactosyl-(1-3)galactose ■ immunoglobulin E ■ red meat ■ risk factors

Inflammation is integral to the pathogenesis of coronary artery disease (CAD), and a role for food-specific antibodies and mast cells has long been a subject of inquiry.<sup>1-3</sup> Recent reports have confirmed a role for mast cells and demonstrated an association between levels of total serum IgE and cardiovascular disease, though no specific allergens have been identified.<sup>4-6</sup> Galactose- $\alpha$ -1,3-galactose ( $\alpha$ -Gal) is a blood group-like oligosaccharide of nonprimate mammals and is recognized in humans as a target of natural IgM, IgA, and IgG antibodies.<sup>7,8</sup> In 2008, IgE specific to  $\alpha$ -Gal was identified as the cause of anaphylactic reactions to cetuximab.<sup>9</sup> Subsequently, it became clear that this IgE antibody is also the primary cause of delayed anaphylaxis to red meat ( $\alpha$ -Gal syndrome).<sup>10</sup> In the United States, sensitization to  $\alpha$ -Gal is recognized as a consequence of bites of the tick *Amblyomma americanum*, which is in keeping

with a prevalence of sensitization to  $\alpha$ -Gal as high as 20% in areas of the southeast.<sup>11</sup> Although  $\alpha$ -Gal is present in mammalian meat as both glycolipids and glycoproteins, the delay in reactions to red meat is best explained by the time taken for processing of lipids into chylomicrons and smaller lipoprotein particles, such as LDL (low-density lipoprotein) or HDL (high-density lipoprotein).<sup>12,13</sup> Taken together,  $\alpha$ -Gal represents a foreign epitope, which is regularly consumed as glycolipids and to which a subset of the population has a strikingly different type of immune response. Thus, it is possible that specific IgE (sIgE) on mast cells, including those in coronary arteries, could increase the inflammatory response to dietary glycolipids. Here, we sought to use intravascular ultrasound imaging in a population where sensitization is prevalent to investigate whether the presence of IgE sensitization to  $\alpha$ -Gal is related to the burden of CAD.<sup>14</sup>

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**Nonstandard Abbreviations and Acronyms**

<b>α-Gal</b>	galactose-α-1,3-galactose
<b>CAD</b>	coronary artery disease
<b>HDL</b>	high-density lipoprotein
<b>LDL</b>	low-density lipoprotein
<b>slgE</b>	specific IgE

**Methods**

Data are available on request from the authors.

**Study Cohort**

The studied population consisted of subjects between the ages of 30 to 80 years who were undergoing medically warranted cardiac catheterization and were enrolled into ongoing studies at the University of Virginia as described previously.<sup>15</sup> Written consent was obtained from all subjects, and the studies were approved by the Institutional Review Board at the University of Virginia.

**Intravascular Ultrasound**

Intravascular ultrasound was conducted as previously described and in accordance with the standards of the American College of Cardiology for image acquisition on a noninfarct-related artery.<sup>14,15</sup> Analysis was conducted by an investigator (A.T.N. or A.M.T.) who was blind to IgE assay results. The fraction of atherosclerotic plaque that was fibrous, fibrofatty, necrotic, or calcified (as determined by intravascular ultrasound) was calculated as a percentage.

**Assays**

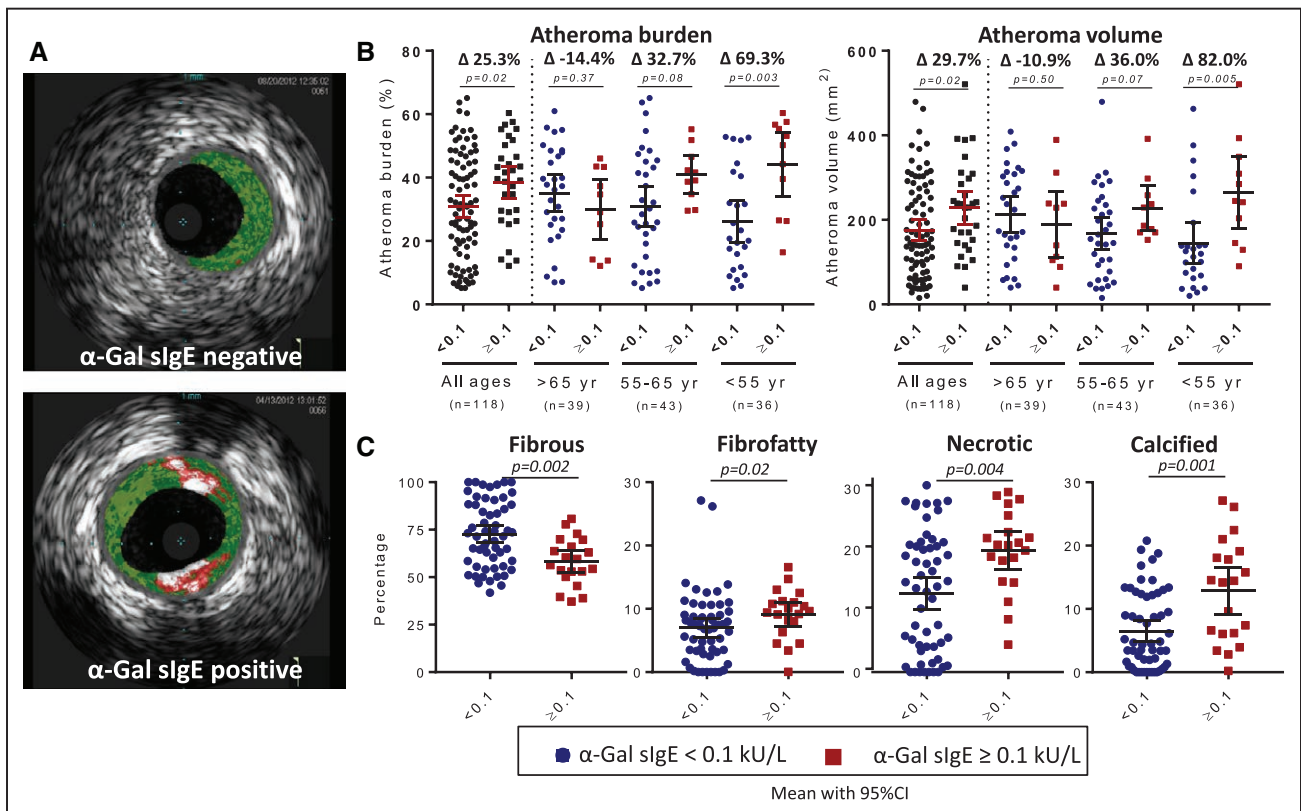
Total IgE and sIgE to α-Gal, dust mite (*Dermatophagoides pteronyssinus*), oak, timothy grass, ragweed, and peanut were assayed using ImmunoCAP 250 (Thermo-Fisher/Phadia, Kalamazoo, MI) as described previously.<sup>10</sup> The solid phase for the α-Gal assay utilized biotinylated cetuximab conjugated to streptavidin ImmunoCAP.<sup>9</sup>

**Statistics**

Prism 7.0 (GraphPad, San Diego, CA) was used for graphs and statistics. Categorical variables were compared with Fisher exact test. Continuous data were compared by the Mann-Whitney *U* test. Spearman rank correlation was used to compare the relationship between α-Gal IgE and total IgE. Regression modeling was performed with IBM SPSS statistics, version 24.0 (IBM Corp, Armonk, NY).

**Results**

Using a cutoff of ≥0.1 kU/L, 26.3% of the subjects had detectable levels of IgE to α-Gal, and, not unexpectedly, there was higher total IgE in the sensitized group. There was no difference in established risk factors or markers of CAD, such as hypertension, lipid levels, or diabetes mellitus, though the sensitized group had a higher percentage of males and black subjects (Table I in the [online-only Data Supplement](#)). Intravascular ultrasound revealed greater burden and volume of atheroma in α-Gal-sensitized subjects (Figure [A and B]). Because induction of IgE to α-Gal results from an environmental exposure that could disproportionately impact younger



**Figure. A**, Representative examples of intravascular ultrasound (IVUS) with fibrous (dark green), fibrofatty (light green), necrotic (red), and calcified plaques (white) evident. **B**, Results of IVUS in total cohort and when stratified by age. **C**, Results of IVUS virtual histology in subjects ≤65 y of age. Data in **B** and **C** show mean with 95% confidence interval (CI) where each data point represents 1 subject and comparison is with the Mann-Whitney *U* test. Percentage differences in the mean value between galactose-α-1,3-galactose (α-Gal)-positive and α-Gal-negative subjects within an age group are represented in **B** by Δ. slgE indicates specific IgE.

adults, we stratified the cohort into 3 age groups with roughly equal numbers of subjects. This stratification revealed a striking difference between subjects of relatively younger versus older age. The significant association of atheroma burden and volume in  $\alpha$ -Gal-sensitized compared with nonsensitized subjects was predominantly driven by results in subjects  $\leq 65$  years of age (Figure [B]), so additional analysis focused on this group (n=79). The demographic characteristics and risk factors in these subjects ( $\leq 65$  years) were similar to the cohort as a whole (Table I in the [online-only Data Supplement](#)), and atherosclerotic plaques had significantly more fibrofatty, necrotic, and calcified content but were less fibrous in  $\alpha$ -Gal-sensitized than nonsensitized subjects (Figure [C]). IgE to a panel of inhalant allergens (including dust mite, oak, timothy grass, and ragweed) and IgE to peanut, in contrast to IgE to  $\alpha$ -Gal, did not have a significant relationship with atheroma burden, atheroma volume, or maximal stenosis (Table 1). To model the association of CAD with  $\alpha$ -Gal sIgE status, we conducted linear regression of atheroma burden as a continuous dependent variable with the independent variable of presence or absence of  $\alpha$ -Gal sIgE. Although there was a significant association between  $\alpha$ -Gal sIgE and total IgE (Figure I in the [online-only Data Supplement](#)), in regression modeling, the strength of the relationship with atheroma burden was stronger for IgE to  $\alpha$ -Gal than for high levels of total IgE (using 100 kU/L as a cutoff; Table 2). Moreover, total IgE  $> 100$  kU/L did not have a significant association with atheroma volume or maximal stenosis (Table 1). Collectively, these results support the specificity of the relationship between  $\alpha$ -Gal sIgE and CAD. Finally, the relationship between  $\alpha$ -Gal IgE status and atheroma burden was preserved in regression analysis adjusted for sex, diabetes mellitus, hypertension, statin use, and total IgE (regression coefficient, 12.2; SE, 5.2;  $P=0.02$ ; Table 2).

## Discussion

To our knowledge, this is the first report that has described an association between the IgE response to  $\alpha$ -Gal, or any specific allergen, and the burden of CAD. A link between a food allergen and CAD may not seem a priori obvious, but there are several elements of the immune response to  $\alpha$ -Gal that argue for biological plausibility. Because many, or perhaps most, subjects with IgE to  $\alpha$ -Gal do not have allergic symptoms, an implication is that many people, despite mounting a unique and potentially detrimental immune response to  $\alpha$ -Gal, continue to consume foods that contain the oligosaccharide allergen.<sup>16</sup> In regard to the cause-effect relationship, we are not aware of any evidence that dietary exposure to mammalian products can promote IgE to  $\alpha$ -Gal, and it is similarly unlikely that atherosclerosis itself could contribute to this response.<sup>11</sup>

The study design limits mechanistic insight, but we hypothesize that chronic ingestion of  $\alpha$ -Gal-glycosylated mammalian products, particularly the glycolipid form of the allergen, can lead to recurrent release of inflammatory products from mast cells bearing sIgE.<sup>4</sup> A role for other cells, which express members of the Fc $\epsilon$  or Fc $\gamma$  family of receptors, such as macrophages or basophils, cannot be discounted, especially given that IgG<sub>1</sub> often increases in parallel with IgE.<sup>17</sup> The

**Table 1. Comparison of Atheroma Measurements Based on sIgE Status to  $\alpha$ -Gal, a Panel of Common Inhalants,\* Peanut, or Total IgE  $> 100$  kU/L**

Intravascular Ultrasound Atheroma Measurements	Negative	Positive	P Value
$\alpha$ -Gal sIgE, n (% total)	58 (73%)	21 (27%)	...
Atheroma burden, %	28.8 (24.3–33.3)	42.7 (37.1–48.2)	$< 0.001$
Atheroma volume, mm <sup>2</sup>	157.7 (128.8–186.7)	246.8 (199.5–294.1)	$< 0.001$
Maximum stenosis, %	48.3 (42.8–53.7)	62.3 (55.1–69.5)	0.006
Inhalant sIgE, n (% total)*	54 (68%)	25 (32%)	...
Atheroma burden, %	31.3 (26.8–35.7)	34.5 (27.3–42.5)	0.38
Atheroma volume, mm <sup>2</sup>	171.1 (143.6–199.7)	202.4 (145.8–259.1)	0.52
Maximum stenosis, %	49.6 (44.1–55.1)	57.2 (48.7–65.8)	0.10
Peanut sIgE, n (% total)	73 (92%)	6 (8%)	...
Atheroma burden, %	31.8 (27.9–35.7)	40.4 (17.4–63.4)	0.26
Atheroma volume, mm <sup>2</sup>	176.7 (150.8–202.6)	238.7 (75.6–401.8)	0.33
Maximum stenosis, %	51.5 (46.8–56.1)	58.6 (30.6–86.6)	0.32
Total IgE $\geq 100$ kU/L, n (% total)	59 (75%)	20 (25%)	...
Atheroma burden, %	30.1 (25.7–34.6)	39.3 (32.2–46.4)	0.03
Atheroma volume, mm <sup>2</sup>	172.6 (142.8–202.3)	207.5 (153.4–261.6)	0.16
Maximum stenosis, %	49.9 (44.5–55.2)	58.4 (49.4–67.3)	0.10

Mean values (95% CI) compared by the Mann–Whitney *U* test.  $\alpha$ -Gal indicates galactose- $\alpha$ -1,3-galactose; CI, confidence interval; and sIgE, specific IgE.

\*Defined as detectable IgE to dust mite, oak tree, timothy grass, or ragweed (cutoff of 0.35 kU/L).

induction of sIgE (and IgG<sub>1</sub>) to the same antigen that is the target of abundant natural antibodies, as occurs with  $\alpha$ -Gal, is also interesting because it is in keeping with prior reports that natural antibodies to oxidation-specific epitopes are protective in atherosclerosis, whereas class-switched antibodies can be proatherogenic.<sup>18</sup> Relatedly, an inverse association between levels of  $\alpha$ -Gal-specific IgM and IgG<sub>2</sub> antibodies and CAD has been reported previously.<sup>19</sup>

There are several limitations to consider. We do not know the allergic histories or dietary habits of the participating subjects. Our assumption, based on clinical experience and other cohorts, is that most subjects in this study did not have a history of symptomatic  $\alpha$ -Gal syndrome (ie,

**Table 2. Results of Univariate and Multivariate Regression Analysis of Relationship With Atheroma Burden in Subjects  $\leq 65$  y of Age (n=79)**

Predictors	$\beta$ -Coefficient	SE	P Value
<b>Model 1</b>			
$\alpha$ -Gal sIgE positive	13.9	4.1	0.001
<b>Model 2</b>			
Total IgE >100 kU/L	9.2	4.3	0.036
<b>Model 3</b>			
$\alpha$ -Gal sIgE positive	12.2	5.2	0.02
Sex (female)	-4.8	3.6	0.20
Diabetes mellitus	3.5	3.7	0.34
Hypertension	4.0	4.4	0.37
Statin use	12.4	4.2	0.01
Total IgE >100 kU/L	-2.0	5.2	0.71

Regression modeling is reported where  $\alpha$ -Gal sIgE status, sex, diabetes mellitus, hypertension, statin use, and total IgE >100 kU/L are categorical variables.  $\alpha$ -Gal indicates galactose- $\alpha$ -1,3-galactose; and sIgE, specific IgE.

urticaria or anaphylaxis) and consequently are regularly consuming mammalian products.<sup>16</sup> The known association of total IgE with atherosclerosis is a potential confounder; however, in this cohort, the association with CAD is stronger for  $\alpha$ -Gal sIgE than total IgE. Moreover, in areas where delayed anaphylaxis to red meat is common, it is likely that elevated total IgE levels are often directly attributable to the same environmental trigger that generates IgE to  $\alpha$ -Gal (ie, tick bites).<sup>11,20</sup> The specificity of the relationship with  $\alpha$ -Gal sIgE is also supported by the finding that sIgE antibodies to a panel of inhalants and peanut did not have a significant relationship with CAD. Finally, our current cohort is relatively small, and the effect was predominantly in subjects  $\leq 65$  years of age.

In summary, we report here that a specific antibody response to the dietary antigen  $\alpha$ -Gal is associated with an increased burden of atherosclerosis and plaques with less stable characteristics. As the causal agent of a delayed allergic reaction to mammalian meat, which is IgE mediated, the glycolipid form of  $\alpha$ -Gal may be particularly relevant to understanding its role in atherosclerosis. Mechanistic studies in animal models are suggested, but ultimately, efforts to translate this finding into clinical relevance will benefit from analysis of larger cohorts, including disparate geographic areas and prospective studies of adult subjects, which include information on diet, allergic history, and detailed investigation into sIgE antibodies.

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### Disclosures

Dr Commins has been on the speaker's bureau for Genentech. Dr Platts-Mills has a patent on ImmunoCAP IgE assays to galactose- $\alpha$ -1,3-galactose ( $\alpha$ -Gal) and has received assay support from ThermoFisher/Phadia. Drs McNamara and Taylor have had research grants with Astra-Zeneca.

### References

- Hansson GK, Hermansson A. The immune system in atherosclerosis. *Nat Immunol*. 2011;12:204–212. doi: 10.1038/ni.2001.
- Davies DF, Davies JR, Richards MA. Antibodies to reconstituted dried cow's milk protein in coronary heart disease. *J Atheroscler Res*. 1969;9:103–107.
- Constantinides P. Mast cells and susceptibility to experimental atherosclerosis. *Science*. 1953;117:505–506.
- Sun J, Sukhova GK, Wolters PJ, Yang M, Kitamoto S, Libby P, MacFarlane LA, Mullen-St Clair J, Shi GP. Mast cells promote atherosclerosis by releasing proinflammatory cytokines. *Nat Med*. 2007;13:719–724. doi: 10.1038/nm1601.
- Wang J, Cheng X, Xiang MX, et al. IgE stimulates human and mouse arterial cell apoptosis and cytokine expression and promotes atherogenesis in Apoe<sup>-/-</sup> mice. *J Clin Invest*. 2011;121:3564–3577. doi: 10.1172/JCI46028.
- Kounis NG, Hahalis G. Serum IgE levels in coronary artery disease. *Atherosclerosis*. 2016;251:498–500. doi: 10.1016/j.atherosclerosis.2016.05.045.
- Galili U, Rachmilewitz EA, Peleg A, Flechner I. A unique natural human IgG antibody with anti- $\alpha$ -galactosyl specificity. *J Exp Med*. 1984;160:1519–1531.
- Galili U. *The Natural Anti-Gal Antibody as Foe Turned Friend in Medicine*. 1st ed. Cambridge, MA: Elsevier; 2017.
- Chung CH, Mirakhor B, Chan E, et al. Cetuximab-induced anaphylaxis and IgE specific for galactose- $\alpha$ -1,3-galactose. *N Engl J Med*. 2008;358:1109–1117. doi: 10.1056/NEJMoa074943.
- Commins SP, Satinover SM, Hosen J, Mozena J, Borish L, Lewis BD, Woodfolk JA, Platts-Mills TA. Delayed anaphylaxis, angioedema, or urticaria after consumption of red meat in patients with IgE antibodies specific for galactose- $\alpha$ -1,3-galactose. *J Allergy Clin Immunol*. 2009;123:426–433. doi: 10.1016/j.jaci.2008.10.052.
- Commins SP, James HR, Kelly LA, Pochan SL, Workman LJ, Perzanowski MS, Kocan KM, Fahy JV, Nanga LW, Ronmark E, Cooper PJ, Platts-Mills TA. The relevance of tick bites to the production of IgE antibodies to the mammalian oligosaccharide galactose- $\alpha$ -1,3-galactose. *J Allergy Clin Immunol*. 2011;127:1286–1293.e6. doi: 10.1016/j.jaci.2011.02.019.
- Commins SP, Jerath MR, Cox K, Erickson LD, Platts-Mills T. Delayed anaphylaxis to  $\alpha$ -gal, an oligosaccharide in mammalian meat. *Allergol Int*. 2016;65:16–20. doi: 10.1016/j.alit.2015.10.001.
- Commins SP, James HR, Stevens W, Pochan SL, Land MH, King C, Mozzicato S, Platts-Mills TA. Delayed clinical and ex vivo response to mammalian meat in patients with IgE to galactose- $\alpha$ -1,3-galactose. *J Allergy Clin Immunol*. 2014;134:108–115. doi: 10.1016/j.jaci.2014.01.024.
- Mintz GS, Nissen SE, Anderson WD, Bailey SR, Erbel R, Fitzgerald PJ, Pinto FJ, Rosenfield K, Siegel RJ, Tuzcu EM, Yock PG. American College of Cardiology clinical expert consensus document on standards for acquisition, measurement and reporting of intravascular ultrasound studies (IVUS). A report of the American College of Cardiology task force on clinical expert consensus documents. *J Am Coll Cardiol*. 2001;37:1478–1492.
- Manichaikul A, Rich SS, Perry H, Yeboah J, Law M, Davis M, Parker M, Ragosta M, Connelly JJ, McNamara CA, Taylor AM. A functionally significant polymorphism in ID3 is associated with human coronary pathology. *PLoS One*. 2014;9:e90222. doi: 10.1371/journal.pone.0090222.
- Fischer J, Lupberger E, Hebsaker J, Blumenstock G, Aichinger E, Yazdi AS, Reick D, Oehme R, Biedermann T. Prevalence of type I sensitization to  $\alpha$ -gal in forest service employees and hunters. *Allergy*. 2017;72:1540–1547. doi: 10.1111/all.13156.
- Rispens T, Derksen NI, Commins SP, Platts-Mills TA, Aalberse RC. IgE production to  $\alpha$ -Gal is accompanied by elevated levels of specific

- IgG1 antibodies and low amounts of IgE to blood group B. *PLoS One*. 2013;8:e55566. doi: 10.1371/journal.pone.0055566.
18. Rosenfeld SM, Perry HM, Gonen A, Prohaska TA, Srikakulapu P, Grewal S, Das D, McSkimming C, Taylor AM, Tsimikas S, Bender TP, Witztum JL, McNamara CA. B-1b cells secrete atheroprotective IgM and attenuate atherosclerosis. *Circ Res*. 2015;117:e28–e39. doi: 10.1161/CIRCRESAHA.117.306044.
19. Mosedale DE, Chauhan A, Schofield PM, Grainger DJ. A pattern of anti-carbohydrate antibody responses present in patients with advanced atherosclerosis. *J Immunol Methods*. 2006;309:182–191. doi: 10.1016/j.jim.2005.12.003.
20. Wilson JM, Schuyler AJ, Schroeder N, Platts-Mills TA. Galactose- $\alpha$ -1,3-galactose: atypical food allergen or model IgE hypersensitivity? *Curr Allergy Asthma Rep*. 2017;17:8. doi: 10.1007/s11882-017-0672-7.

### Highlights

- IgE to galactose- $\alpha$ -1,3-galactose ( $\alpha$ -Gal) is common in adults in Central Virginia who are clinically judged to be at cardiovascular risk.
- In subjects  $\leq 65$  years of age, sensitization to  $\alpha$ -Gal is associated with greater burden of atheroma and with plaques that have less stable characteristics.
- As an oligosaccharide that is present on glycolipids in mammalian meat,  $\alpha$ -Gal may represent a novel risk factor for coronary artery disease in IgE-sensitized subjects.