
Interference of cross-reactive carbohydrates in the determination of specific IgE in alcohol drinkers and strategies to minimize it: the example of latex

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Background: Cross-reactive carbohydrate determinants (CCDs) are N-glycans in plant and invertebrate proteins that interfere with specific IgE determinations. The prevalence of IgE to Man₂XylFucGlcNAc₂ (MUXF), the CCD from bromelain, may be increased in heavy drinkers.

Objective: To further investigate the relationship of alcohol consumption to CCD specific IgE. Latex was used as an example for investigating CCD interference with in vitro allergy testing and how to minimize the interference by using nonglycosylated recombinant allergens and inhibition assays.

Methods: We determined the levels of IgE to CCD markers (MUXF and ascorbate oxidase) and natural rubber latex in 270 adults without a history of latex allergy (73 abstainers or occasional drinkers, 76 light drinkers, 47 moderate drinkers, and 74 heavy drinkers). In cases with latex reactivity, we performed inhibition assays with MUXF and screened for IgE to a panel of recombinant latex allergens. Fourteen-day serologic follow-up was available for a subset of individuals.

Results: Moderate to heavy drinkers displayed an increased prevalence of IgE to CCD markers. The presence of CCD specific IgE was closely associated with latex IgE reactivity. Inhibition studies and the absence of reactivity to nonglycosylated recombinant latex allergens indicated CCD interference in latex IgE determinations. Serum levels of specific IgE decreased with alcohol abstention.

Conclusions: In this population, alcohol consumption is associated with an increased prevalence of IgE reactivity to natural rubber latex due to CCD interference. The use of nonglycosylated recombinant allergens and inhibition assays may help to minimize CCD interference in populations in which IgE to CCDs is common.

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INTRODUCTION

Cross-reactive carbohydrate determinants (CCDs) are N-glycans (oligosaccharide side chains N-linked to asparagine residues) found in plant and invertebrate glycoproteins.^{1–3} CCDs are ubiquitous in nature, constituting the most prevalent set of epitopes to which humans are exposed.⁴ The existence of IgE to CCDs was first reported more than 25 years ago,⁵ and subsequent reports have emphasized the interference of N-

glycans in specific IgE (sIgE) determinations due to widespread cross-reactivity.^{1–7}

There are many forms of N-glycans, and there is not a single marker for detecting CCD sIgE.^{1–3} All N-glycans have 2 N-acetylglucosamines and 1 mannose as a backbone to which additions delineate different CCDs.^{1–3} Xylosylation and fucosylation are crucial for the antigenic properties of CCDs.^{1,4,8} Common glycan structures include Man₂XylFucGlcNAc₂ (MUXF; M, terminal α -mannose; U, unsubstituted 3-hydroxyl on the β -mannose; X, xylose; F, core α 1,3-fucose), which is found in plant glycoproteins^{1,3}; Man₃XylFucGlcNAc₂ (MMXF; the second M indicating the addition of a β ₃-mannose to the MUXF structure), which is found in plant and invertebrate glycoproteins^{1,4}; and nonxylosylated Man₃FucGlcNAc₂ or Man₃Fuc₂GlcNAc₂, which is mainly found in Hymenoptera venom allergens.^{1,9} Pineapple bromelain (which contains a single MUXF-type glycan), zucchini ascorbate oxidase, and horseradish peroxidase (which mainly contain MMXF-type glycans) are widely used as CCD markers in daily practice because they are regarded as nonallergenic proteins.^{1,2,7} Recently, the determination of IgE to the

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isolated glycan of bromelain (MUXF) has become commercially available.

The prevalence of CCD sIgE can be as high as 23% among Italian people with suspected respiratory allergy.⁷ In a Spanish general population, approximately 5% of adults had significant levels of MUXF sIgE.¹⁰ Intriguingly, the prevalence of MUXF sIgE was higher in heavy drinkers, who consequently displayed IgE to a variety of allergens due to CCD interference.¹⁰ The latter is consistent with a role of alcohol on IgE-mediated responses.^{11,12}

CCD interference is particularly important when determining IgE to high-risk allergens, such as latex and Hymenoptera venoms.^{1-3,7} Furthermore, natural rubber latex (NRL) glycoproteins and Hymenoptera venoms share glycan epitopes that confer cross-reactivity.^{13,14} Investigating CCD interference in NRL sIgE determinations offers the advantage that recombinant nonglycosylated allergens are commercially available.^{15,16} Epidemiologic studies have investigated the prevalence of NRL sIgE in populations of blood donors not at risk,¹⁷⁻²¹ but, to our knowledge, none of these epidemiologic studies has considered the possible interference of CCDs in NRL sIgE determinations or the role that alcohol consumption might play.

This study aimed to further investigate the association of alcohol consumption with CCD sIgE. NRL was used as an example for investigating CCD interference and how to minimize the interference by using nonglycosylated recombinant allergens and inhibition assays. Furthermore, we studied the time course changes of CCD sIgE after alcohol abstention.

METHODS

Study Population

This study was performed in individuals attending the Estancia Termal do Gerês, a thermal spa in northern Portugal that is officially recognized as providing therapy for hepatobiliary and metabolic diseases. During a standard stay, individuals follow a supervised diet, drink the local mineral waters, and voluntarily stop alcohol consumption if relevant. These stays are a tradition in the country. The study included 270 individuals admitted on 9 selected days among the 690 individuals attending the spa during October 2005. Self-reported indications for attending were biliary disease (n = 86), liver disease (n = 58), dyslipidemia (n = 31), hypertension (n = 26), obesity (n = 23), diabetes (n = 6), stress or depression (n = 4), and primary prevention (n = 35). None of the study participants reported a history of allergy to latex or Hymenoptera venoms. On admission, all attendants underwent a routine checkup. Blood samples were taken, and serum samples were stored frozen until tested. In 211 individuals, a second sample was obtained after 14 days of stay. All individuals gave written consent to participate in the study, which was approved by the Portuguese institution. Determinations in coded samples were performed in the Hospital Clínico Universitario (Santiago, Spain) after approval from the institutional review board.

Alcohol Consumption

Alcohol consumption was recorded as the number of standard drinking units (glasses of wine, beer, aperitifs, and spirits, each of which contain approximately 10 g of alcohol) consumed per week.^{10,22} In a subsample of 151 individuals, alcohol consumption was found to be correlated with serum γ -glutamyl-transferase (ADVIA-2400; Siemens, Munich, Germany; $r = 0.42$, $P < .001$). Individuals with occasional alcohol consumption (<1 unit per week) and abstainers (n = 73) were included in the same group; habitual alcohol consumers were classified as light drinkers (1 to 14 units per week, n = 76), moderate drinkers (15 to 28 units per week, n = 47), and heavy drinkers (>28 units per week, n = 74). All moderate to heavy drinkers were wine consumers, and 62.9% consumed only wine. The proportion of moderate to heavy drinkers was greater in men (66.3%) than in women (7.1%).

sIgE Determinations

Depending on the allergen considered, sIgE determinations were performed by either enzyme-enhanced fluorescence in an ImmunoCAP-250 system (Phadia, Uppsala, Sweden) or enzyme-enhanced chemiluminescence in an Immulite-2000 immunoassay system (Siemens), which use solid-phase and liquid-phase allergens, respectively. According to the manufacturers, both systems are tested with negative samples spiked with high-concentration myeloma IgE, as a control of nonspecific binding. Following the manufacturer's instructions, any sIgE level of 0.1 kU/L or higher was considered a positive result. In all study participants, baseline determinations included the following: 2 CCD markers, MUXF (Ro214; Phadia) and ascorbate oxidase (k226; Siemens)^{1,3,4}; honeybee venom phospholipase-A2 (PLA2; k203; Phadia), which contains protein epitopes and CCDs that can be recognized by IgE antibodies^{1,9}; and NRL (k82; Phadia), a group of *Hevea brasiliensis* (Hev b) glycoproteins that are commercially spiked with recombinant Hev b 5 (rHev b 5).²³

In cases with NRL sIgE levels greater than 0.35 kU/L, the classic positivity threshold, we determined IgE to 8 commercially available (Phadia) recombinant latex allergens, including rHev b 1 (k215), rHev b 3 (k217), rHev b 5 (k218), rHev b 6.01 (k219), rHev b 6.02 (k220), rHev b 8 (k221), rHev b 9 (k222), and rHev b 11 (k224). According to the manufacturer specifications, these allergens are produced in an *Escherichia coli* strain and therefore are not glycosylated.

In individuals with positive (≥ 0.1 kU/L) baseline sIgE levels and a serum sample after 14 days of stay, we again determined IgE to MUXF, PLA2, and NRL to investigate changes over time, specifically regarding the influence of alcohol abstention.

Inhibition Assays

For inhibition assays of sIgE results by CCD, the solid phase was either NRL (k82; Phadia) or PLA2 (k203; Phadia) and the inhibitor was a neoglycoprotein consisting of bromelain glycopeptides with an MUXF structure conjugated to bovine

serum albumin (BSA).⁴ The conjugate, which contains 3 to 4 MUXF-type glycans per molecule of BSA, was obtained from the Institute of Chemistry, Universitaet fuer Bodenkultur, Vienna, Austria. Serum samples (150 μ L) were preincubated with 150 μ L of the phosphate-buffered saline–buffered MUXF-BSA solution to give a final conjugate concentration of 100 μ g/mL, as previously described.¹⁰ After overnight coincubation at 4°C, sIgE results were evaluated in the ImmunoCAP-250 system (Phadia).

Multiallergen sIgE Tests

All serum samples were assayed using 2 commercial qualitative screening tests: Phadiatop (Phadia) and Al atop (Siemens). Both assay for IgE to mixtures of common inhalant allergens. The test results are considered positive when reactivity is higher than that of the reference control. The allergen composition of the Phadiatop is not revealed by the manufacturer. The allergens in the Al atop include *Dermatophagoides pteronyssinus*, cat epithelium, dog dander, Bermuda grass, timothy grass, *Penicillium notatum*, *Alternaria tenuis*, birch, Japanese cedar, common ragweed, English plantain, and pellitory.

Statistical Analyses

The χ^2 test (with Yates correction, when appropriate) was used to compare proportions. The χ^2 trend test was used to investigate the trends of proportions relative to independent variables in ordered categories. The Wilcoxon test was used to compare paired samples. The Spearman rank test was used to evaluate correlation. Logistic regression was used to adjust for confounders. Drinkers of less than 1 unit per week (occasional drinkers or abstainers) were used as the reference category.

RESULTS

Positivity of IgE to CCDs, PLA2, NRL, and Multiallergen Test Results in Relation to Alcohol Consumption

Heavy drinkers and moderate drinkers showed a higher prevalence of CCD, PLA2, NRL, and multiallergen sIgE positivity than occasional drinkers or abstainers (Table 1). Furthermore, a significant trend was seen toward increased CCD, PLA2, NRL, and multiallergen sIgE positivity as the level of alcohol intake increased ($P < .001$ in all cases). The association between heavy drinking and CCD, PLA2, NRL, and multiallergen sIgE positivity remained after adjusting for sex, age, and smoking (Table 2). Serum levels of sIgE were similar in individuals with and without a diagnosis of liver disease (data not shown). Serum γ -glutamyl-transferase levels were higher in CCD-positive than in CCD-negative individuals (median, 35 vs 18 IU/L; $P = .01$). Serum alanine aminotransferase levels were similar in CCD-positive and in CCD-negative individuals (median, 9 vs 8 IU/L; $P = .20$).

Anti-PLA2 was the most frequently encountered IgE positivity among the allergens studied (Table 1). A total of 5.4% of heavy drinkers had PLA2 sIgE levels of 3.5 kU/L or higher (class 3 or higher). Most heavy drinkers who showed CCD sIgE positivity also tested positive for PLA2. However, significant numbers of individuals with PLA2 sIgE positivity and CCD sIgE negativity were found (Fig 1). Among heavy drinkers with a PLA2 sIgE level greater than 0.35 kU/L, preincubation with MUXF-BSA induced more inhibition of PLA2 sIgE reactivity in individuals with positive MUXF sIgE results (median, 61%; range, 38%–87%; $n = 7$) than in individuals with negative MUXF-sIgE results (median, 0%; range, 0%–35%; $n = 10$; $P < .001$).

Table 1. sIgE Positivity in the Different Categories of Alcohol Consumption

Test result	No. (%) of study participants by alcohol consumption			
	<1 unit/wk (n = 73)	1–14 units/wk (n = 76)	15–28 units/wk (n = 47)	>28 units/wk (n = 74)
sIgE to markers of CCD sensitization				
MUXF sIgE positivity ^a	1 (1.4)	3 (3.9)	6 (12.8) ^b	10 (13.5) ^c
Ascorbate oxidase sIgE positivity ^a	2 (2.7)	2 (2.6)	5 (10.6)	16 (21.6) ^c
Any CCD positivity ^a	2 (2.7)	3 (3.9)	7 (14.9) ^b	17 (23.0) ^c
sIgE to CCD-bearing allergens				
Honeybee venom PLA2 sIgE positivity ^a	9 (12.3)	10 (13.2)	18 (38.3) ^d	34 (45.9) ^d
Natural rubber latex sIgE positivity ^a	1 (1.4)	6 (7.9)	8 (17.0) ^c	15 (20.3) ^d
Multiallergen sIgE tests				
Positive Al atop test result	2 (2.7)	9 (11.8)	9 (19.1) ^c	15 (20.3) ^c
Positive Phadiatop test result	8 (11.0)	12 (15.8)	12 (25.5) ^b	24 (32.4) ^c
Positive result on any multiallergen test	8 (11.0)	14 (18.4)	12 (25.5) ^b	25 (33.8) ^c

Abbreviations: CCD, cross-reactive carbohydrate determinant; MUXF, Man₂XylFucGlcNAc₂; PLA2, phospholipase-A2; sIgE, specific IgE.

^a Positive result is 0.1 kU/L or greater.

^b $P < .05$.

^c $P \leq .01$.

^d $P \leq .001$, with respect to the reference category (drinkers of <1 unit per week).

Table 2. Multivariate Analyses of Factors Associated With sIgE Positivity^a

Factor	Odds ratio (95% confidence interval)			
	CCD sIgE positivity ^b	Multiallergen sIgE positivity ^c	PLA2 sIgE positivity ^d	NRL sIgE positivity ^e
Male sex	3.22 (0.62–16.6)	1.60 (0.69–3.69)	1.24 (0.53–2.89)	2.54 (0.63–10.2)
Age	0.97 (0.93–1.00)	0.98 (0.95–1.01)	0.99 (0.96–1.02)	0.98 (0.94–1.01)
Smoking	0.74 (0.23–2.31)	1.19 (0.48–2.93)	1.39 (0.57–3.35)	0.62 (0.17–2.17)
Alcohol consumption				
<1 unit/wk (referent)	1	1	1	1
1–14 units/wk	1.33 (0.21–8.48)	1.74 (0.67–4.52)	1.04 (0.39–2.76)	5.6 (0.64–48.5)
15–28 units/wk	4.17 (0.73–23.9)	2.26 (0.76–6.73)	3.93 (1.39–11.0) ^f	10.2 (1.12–91.6) ^f
>28 units/wk	5.94 (1.14–30.9) ^f	2.80 (1.01–7.79) ^f	4.71 (1.73–12.8) ^g	11.6 (1.34–99.7) ^f

Abbreviations: CCD, cross-reactive carbohydrate determinant; NRL, natural rubber latex; PLA2, phospholipase-A2; sIgE, specific IgE.

^a Logistic regression analyses. All listed factors were forced to enter the equation. Data were available for all 270 individuals.

^b Positive (≥ 0.1 kU/L) sIgE to any of the markers of CCD sensitization (MUXF and/or ascorbate-oxidase).

^c Positivity to any of the multiallergen-sIgE tests (Phadiatop or Al atop).

^d Specific IgE to honeybee venom PLA2 ≥ 0.1 kU/L.

^e Specific IgE to NRL ≥ 0.1 kU/L.

^f $P < .05$.

^g $P < .01$.

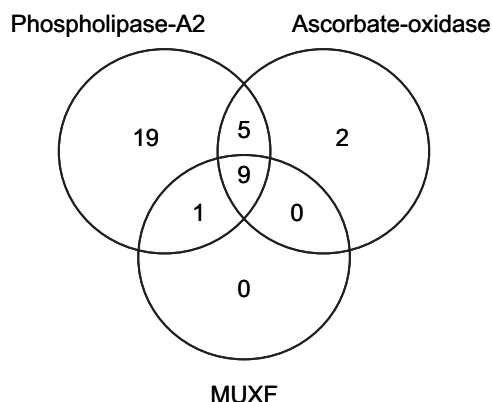


Figure 1. Venn diagram showing the relation of specific IgE positivity (≥ 0.1 kU/L) to cross-reactive carbohydrate determinant markers (ascorbate oxidase and Man₂XylFucGlcNAc₂ [MUXF]) and to honeybee venom phospholipase-A2 in heavy drinkers (>28 units per week, $n = 74$). A total of 38 heavy drinkers tested negative for all the 3 allergens represented.

Interference of CCDs With NRL sIgE Results in Alcohol Drinkers

Titers of NRL sIgE were generally low and were correlated with those of CCD sIgE, including ascorbate oxidase ($r = 0.44$, $P < .001$) and MUXF (Fig 2). The latter was particularly strong among heavy drinkers ($r = 0.80$, $P < .001$). Among the 30 individuals with NRL sIgE levels of 0.1 kU/L or higher, 24 (80.0%) were positive for at least 1 CCD marker, and 26 (86.6%) were positive for PLA2.

Nine individuals (3.3%) had NRL sIgE levels greater than 0.35 kU/L (Table 3). None were either occupationally or medically exposed. Eight individuals were habitual alcohol drinkers. In 8 individuals, MUXF-BSA induced a strong inhibition of NRL sIgE reactivity (median, 90%; range, 60%–96%), and IgE to recombinant latex allergens was negative or

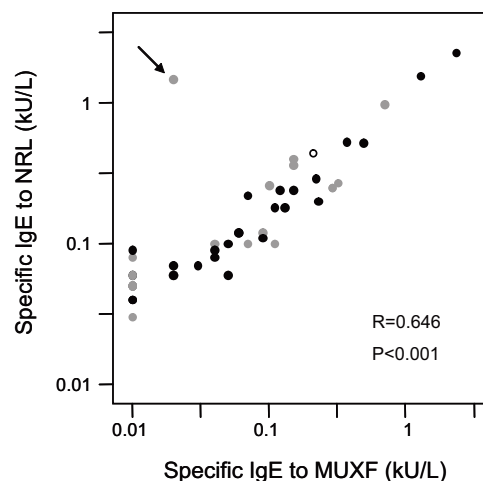


Figure 2. Scatterplot showing the correlation between IgE to Man₂XylFucGlcNAc₂ (MUXF) and IgE to natural rubber latex (NRL). Unfilled circles indicate abstainers or occasional drinkers (<1 unit per week), gray circles indicate light to moderate drinkers (1 to 28 units per week), and black circles indicate heavy drinkers (>28 units per week). Only cases with detectable specific IgE are represented. Only 1 true outlier was observed (arrow). Coefficient of correlation includes all cases except the outlier. The correlation coefficient including the outlier is 0.651.

low titer. The exception corresponded to the only outlier in Figure 2 (arrow), who displayed IgE positivity to rHev b 8 and seemed therefore sensitized to the profilin protein. MUXF-BSA induced no inhibition of NRL sIgE reactivity in a control sample of 4 patients with symptomatic latex allergy from an allergy clinic (data not shown).

Most individuals with positive (≥ 0.1 kU/L) NRL sIgE (25 of 30, 83.3%) results had at least 1 positive multiallergen sIgE test result (Phadiatop or Al atop). All 9 individuals with NRL sIgE levels greater than 0.35 kU/L showed positivity to

Table 3. Demographic Data, Alcohol Intake, sIgE Results, and the Inhibition of NRL Reactivity by CCD in the 9 Individuals With a NRL sIgE Level Greater Than 0.35 kU/L^a

Participant No./ sex/age, y	Weekly alcohol intake, g	sIgE in serum, kU/L											Inhibition of IgE to NRL by MUXF- BSA, %	
		CCDs		NRL	Recombinant latex allergens									
		MUXF	ASO		rHev b 1	rHev b 3	rHev b 5	rHev b 6.01	rHev b 6.02	rHev b 8	rHev b 9	rHev b 11		
1/M/62	280	<0.1	<0.1	1.47	<0.1	<0.1	<0.1	<0.1	<0.1	3.01	<0.1	<0.1	0	
2/M/46	350	2.34	3.90	2.28	<0.1	<0.1	<0.1	0.10	0.20	<0.1	0.11	0.14	96	
3/M/37	490	0.37	1.37	0.53	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	96	
4/F/65	0	0.21	0.66	0.44	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	81	
5/M/48	280	0.15	<0.1	0.40	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	60	
6/M/59	280	0.70	2.62	0.98	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	93	
7/M/71	140	0.15	<0.1	0.36	<0.1	<0.1	<0.1	<0.1	<0.1	0.15	<0.1	<0.1	72	
8/M/50	700	0.49	0.91	0.52	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	87	
9/M/57	350	1.29	4.18	1.56	<0.1	<0.1	<0.1	0.10	<0.1	<0.1	0.11	0.14	96	

Abbreviations: ASO, ascorbate oxidase; BSA, bovine serum albumin; CCDs, cross-reactive carbohydrate determinants; MUXF, Man₂XylFucGlcNAc₂; NRL, natural rubber latex; rHev b, recombinant allergen from latex (*Hevea brasiliensis*); sIgE, specific IgE.

^a Figures for inhibition indicate the maximum percentage of inhibition obtained with 100 µg/mL of inhibitor protein, a conjugate of MUXF coupled to BSA. Percentages of inhibition have been approximated to the nearest whole number.

both Phadiatop and Al atop. The increases in multiallergen sIgE reactivity in moderate and heavy drinkers were related to CCD sIgE positivity (Fig 3).

Serum Levels of NRL and CCD sIgE After Alcohol Abstinence

In cases with baseline positive NRL sIgE results (≥ 0.1 kU/L), sIgE concentrations tended to decrease after 14 days in the institution and alcohol abstinence (Fig 4). Mean reduc-

tions in NRL sIgE levels tended to be correlated with baseline alcohol consumption. Reduction in NRL sIgE levels was statistically significant in the group of heavy drinkers ($P = .01$). Likewise, MUXF sIgE levels and PLA2 sIgE levels tended to decrease after alcohol abstinence in heavy drinkers ($P = .06$ and $P < .001$, respectively).

DISCUSSION

This study confirms that alcohol drinkers have a high prevalence of IgE to CCD markers, including ascorbate oxidase (which bears MMFX-type glycans) and MUXF. This association between alcohol consumption and CCD sIgE seems not to be an assay-related artifact because it was observed with 2 different laboratory methods. Our results also indicate that moderate to heavy alcohol consumption is associated with multiallergen sIgE test positivity and NRL sIgE positivity in relation to CCD interference. These findings were independent of potential confounders, such as age, sex, smoking, and liver disease. Furthermore, there was a dose-response effect: the prevalence of these abnormalities increased as the level of alcohol consumption increased. Importantly, cessation of alcohol consumption was shortly followed by reductions in serum levels of IgE to CCDs and CCD-bearing allergens, including PLA2 and NRL. Finally, we have shown that the use of nonglycosylated recombinant allergens and inhibition assays may be of help in minimizing CCD interference in this population.

Methodologic issues include the evaluation of alcohol consumption, which has been validated and allows for comparisons among studies.²⁴ Skin prick tests were not performed in this study, which focused on sIgE determinations. The sIgE assays show reasonably good agreement and allow for sIgE determination at concentrations less than 0.35 kU/L, which in some settings might be significant.²⁵ Time-course analyses of sIgE levels were restricted to cases that were positive at

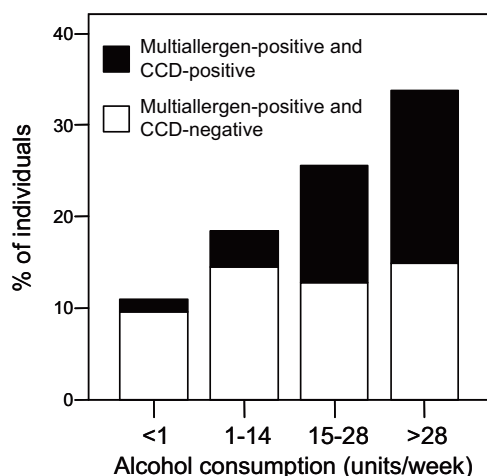


Figure 3. Proportion of individuals with a positive multiallergen specific IgE (sIgE) test result (Phadiatop and/or Al atop) in relation to alcohol consumption. The proportion of individuals with a positive multiallergen sIgE test result and a negative IgE result to cross-reactive carbohydrate determinants (CCDs) (Man₂XylFucGlcNAc₂ [MUXF] or ascorbate oxidase) is similar in all categories of alcohol consumption ($P = .77$). However, the proportion of individuals with a positive multiallergen sIgE test result and a positive IgE result to CCDs tends to increase as the level of alcohol consumption increases ($P = .001$).

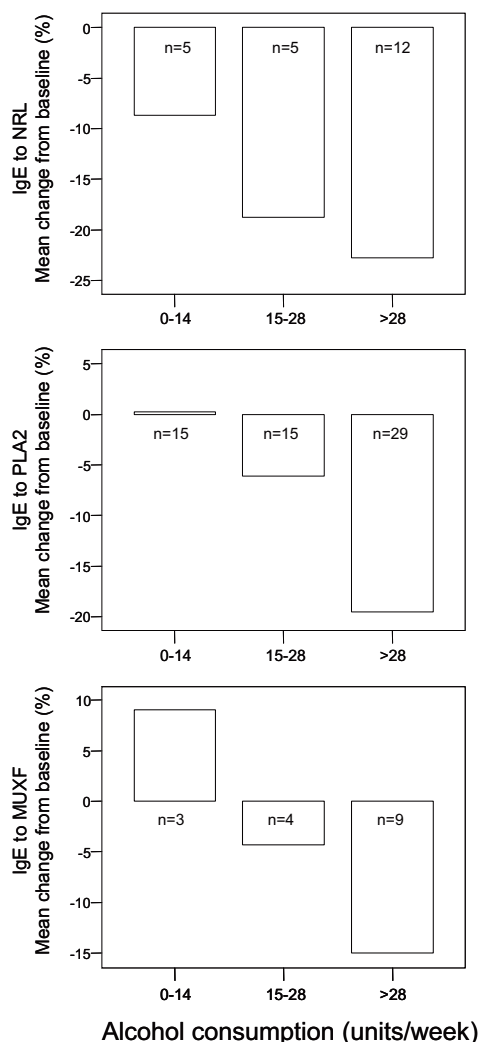


Figure 4. Relative changes in IgE to natural rubber latex (NRL), honeybee venom phospholipase-A2 (PLA2), and $\text{Man}_2\text{XylFucGlcNAc}_2$ (MUXF) (the N-glycan of bromelain) who had positive (≥ 0.1 kU/L) baseline values after 14 days. Note that nonregular drinkers and light drinkers were grouped in the same category because of the small number of individuals. Changes in the group of heavy drinkers (>28 units per week) are statistically significant for NRL ($P = .01$) and PLA2 ($P < .001$) and also tend to be significant for MUXF ($P = .06$).

baseline. Therefore, part of the observed decrease could be due to a regression toward the mean effect. However, the relative decrease was greater in heavy drinkers, suggesting that alcohol abstinence played a role.

The mechanisms underlying the association between alcohol consumption and CCD sIgE are unknown. Alcohol consumption in humans is consistently related to high total IgE levels.^{26–29} In rodents, alcohol administration increases IgE concentrations.^{30–33} The mechanisms of alcohol-induced IgE synthesis could include a cytokine imbalance caused by the effect of alcohol on immune cells, either directly^{30,32} or indi-

rectly by facilitating endotoxin absorption.²² It seems unlikely that enhanced CCD sIgE reactivity reflects nonspecific binding due to increased total IgE, because the correlation between serum levels of IgE and CCD sIgE was low (data not shown).⁷ Furthermore, heavy drinkers display a variable prevalence of IgE to allergens, depending on their CCD content.¹⁰ In this study, alcohol consumption was associated with positivity to glycosylated NRL but not to nonglycosylated recombinant latex allergens.

Hypothetically, increased CCD sIgE levels in heavy drinkers could derive from the combination of enhanced CCD exposure and enhanced IgE synthesis. Digestive exposure could result from increased gut permeability³⁴ or impaired protein digestion^{31,35} due to alcohol. A peculiar exposure could occur after modification of self-glycoproteins, which become abnormally desialylated³⁶ and fucosylated³⁷ in alcohol abusers. It has been suggested that Hymenoptera stings are a major source of CCD sensitization.^{5,38} In keeping with this, Hymenoptera venom sIgE was the most prevalent and the highest in magnitude among all CCD-bearing allergens in previous studies in heavy drinkers.¹⁰ In this study, nearly all alcohol drinkers showing CCD sIgE also showed honeybee PLA2 sIgE, but a number of drinkers with PLA2 sIgE did not have evident CCD sensitization. Reactivity to PLA2 was inhibited by preincubation with CCD (MUXF-BSA) in the former but not in the latter, who therefore seemed to be sensitized to the Hymenoptera venom protein. Unfortunately, the lifetime history of Hymenoptera stings was not recorded. To our knowledge, there is no rationale to support the theory that alcohol drinkers are more frequently stung, but it could be argued that drinkers could develop IgE to Hymenoptera venom epitopes more efficiently when exposed. In addition, a report from Spain suggests that Hymenoptera allergens may be present in young wines.³⁹ Glycoproteins are normal constituents of grape juice and wines,⁴⁰ including those from the studied area,⁴¹ and the proportion of sugars in glycoproteins is higher in young wines.⁴² Drinkers both from this Portuguese population and the formerly published Spanish population¹⁰ are uniformly wine consumers, and consumption of home-made young wines is common in both regions. Thus, the effects of alcohol cannot be separated from the potential effects of additional substances contained in wine in these studies. Further studies are therefore needed to confirm the generalizability of the findings in populations with different patterns of alcohol consumption.

Sensitization to CCDs is misleading in the diagnosis of allergy.^{1–7} NRL, a high-risk allergen for which clinicians often rely on in vitro tests, exemplifies this issue. Of the more than 240 NRL polypeptides, 13 are considered allergenic,^{43,44} and most of them are glycosylated.⁴⁴ The overall prevalence of an NRL sIgE level greater than 0.35 kU/L observed in this population not at risk (3.3%) is in the range of those observed in blood donor surveys.^{17–21} Of note, NRL reactivity has been found to be associated with male sex¹⁷ and smoking.²¹ In this study, the association between male sex and both CCD sIgE and NRL sIgE positivity decreased after adjusting for alcohol

consumption (data not shown), suggesting that sex influence was partly due to the confounding effect of alcohol. In most drinkers with NRL sIgE levels higher than 0.35 kU/L, inhibition with MUXF-BSA and IgE to a panel of recombinant allergens indicated that NRL sIgE positivity was due to CCD interference. None of the study participants reported symptoms of latex allergy, which is in agreement with the current thought that CCDs have poor biological activity.^{1,45} The false positivity of NRL sIgE in this population serves to emphasize the need for strategies that minimize the interference of CCDs in determinations of sIgE.^{1,2,46} Inhibition assays are too complex to be routinely used in clinical practice, unless automated devices are designed.⁴⁶ The use of nonglycosylated recombinant allergens is simpler, but not all allergenic proteins are commercially available. Furthermore, the glycan part of an allergen may have some relevance in clinical allergy.⁴⁵ The sound use of native allergens,⁴⁵ recombinant allergens,^{15,16,47} and inhibition assays with specific glycans^{46,47} could help to reduce CCD interference in epidemiologic studies or in clinical settings where CCD sensitization is common. Taking a history of alcohol drinking habits may also be a low-cost measure for the detection of individuals at high risk of false-positive sIgE determinations due to carbohydrate cross-reactivity.

Cross-reactivity between carbohydrates is broader than between peptide epitopes.^{1,2,46} Furthermore, the risk of CCD interference is higher when heterogeneous allergens are mixed in the same reagent,² as in multiallergen sIgE tests. Of note, heavy drinkers display an increased prevalence of multiallergen sIgE test positivity,^{22,48} but the association between heavy drinking and skin test positivity is more controversial.⁴⁹ According to our results, this discrepancy could be due to CCD interference from plant allergens contained in multiallergen sIgE tests. Likewise, CCD sIgE could underlie the association between NRL sIgE and multiallergen sIgE test positivity reported in previous surveys.⁵⁰ Taken together, these results serve to emphasize that a positive multiallergen test result or a positive IgE test result should be questioned even in patients with relatively high sIgE levels but without obvious clinical history.

In summary, our results indicate that alcohol consumption is associated with an increased prevalence of positive NRL sIgE and multiallergen sIgE test results due to CCD interference. Furthermore, the presented data support the use of nonglycosylated recombinant allergens and, in selected cases, inhibition assays to minimize CCD interference in settings where CCD sIgE is common. Furthermore, a history of alcohol drinking seems recommendable in these cases. Further studies are needed to elucidate the mechanisms involved in the increased prevalence of CCD sIgE in this population of alcohol drinkers.

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