Nutrient-Gene Interactions

Obesity Risk Is Associated with Carbohydrate Intake in Women Carrying the Gln27Glu β_2-Adrenoceptor Polymorphism

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ABSTRACT   Interindividual differences in the response to dietary intake are, in some cases, genotype dependent. Moreover, genotype-environment interactions may appear when the impact of lifestyle factors (e.g., diet) on a phenotype (e.g., BMI > 30 kg/m^2) differs by genotype. A case-control study (obese subjects vs. normal weight controls) was conducted to assess a possible effect modification on obesity risk of the Gln27Glu polymorphism for the β_2-adrenoceptor gene depending on dietary intake. The sample included 159 subjects with BMI > 30 kg/m^2 and 154 controls with BMI < 25 kg/m^2. The allele frequency for the Glu27 polymorphism, as assessed by the polymerase chain reaction-restriction fragment length polymorphism methodology, was 0.40 in cases (obese) and 0.37 in controls (lean), which was similar to that of other Caucasian populations. The dietary intake was estimated by using a previously validated food frequency questionnaire. Obesity incidence was not directly affected by the polymorphism [odds ratio (OR) = 1.40; $P = 0.246$]. However, a significant interaction (effect modification) between carbohydrate (CHO) intake and the presence of the Glu27 variant in the probability of obesity was apparent. Thus, females with the polymorphism and a higher CHO intake [$>$49% energy (E)] had a higher obesity risk (OR = 2.56, $P = 0.051$). The product-term introduced in the logistic model to assess effect modification revealed a marginally significant interaction ($P = 0.058$) between both factors. Furthermore, a high intake of CHO (E > 49%) was associated with higher insulin levels among women carrying the Gln27Glu polymorphism ($P < 0.01$). This gene-nutrient interaction emphasizes the importance of examining the outcome of some obesity-related mutations depending on lifestyle (including diet) and may explain the heterogeneity of findings from previous studies.   J. Nutr. 133: 2549–2554, 2003.

KEY WORDS: • β_2-adrenoceptor gene • Gln27Glu polymorphism • carbohydrate • obesity • gene-nutrient interactions

The onset and development of obesity have been associated with inadequate dietary and sedentary habits, as well as a genetic predisposition (1,2). The impact of at least 250 genes or chromosomal regions on body fat variability has been described in humans by means of association and linkage studies as well as through a number of different molecular genetic approaches (3). Furthermore, evidence from both genetic and molecular epidemiological studies suggests that genetic factors are involved in determining the susceptibility to gaining or losing fat in response to diet or in the higher risk of developing comorbidities generally observed in obese subjects (4–6).

Most studies concerning gene-diet interactions in humans have used lipid or lipoprotein phenotypes as the outcome (7–10), whereas less information is available concerning nutritional influences on gene expression affecting body weight homeostasis (11,12). Thus, despite the findings that genetic factors may play an important role in the etiology of obesity and the increasing number of related genes identified (3), relatively little is known about the role of genetic traits in the

response of different obesity genotypes to alterations in the energy balance or diet composition (13,14). Advances in this field have been delayed by the occurrence of polygenic (gene × gene) interactions affecting the obese phenotype and by the influence of environmental factors (gene × environment interactions) such as dietary intake and physical activity (15–18). In addition to the increasing number of genes apparently involved in obesity, a major difficulty arises from the fact that factors such as age, gender and physical activity patterns are likely to induce effect modifications (19,20), thus hampering interpretation of the data (21,22). Association studies of gene variants and assessments of the response to dietary challenges represent promising ways of investigating gene-nutrient interactions (23).

In this context, the role of a number of genes such as β_2- and β_3-adrenoceptors, fatty acid binding protein and peroxisome proliferator-activated receptor-γ $(PPAR\gamma)^2$ has been

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[2] Abbreviations used: β_2-AR, β_2-adrenergic receptor gene; CHO, carbohydrate; E, energy; MET, metabolic equivalent; OR, odds ratio; PCR, polymerase chain reaction; PPAR, peroxisome proliferator-activated receptor; UCP, uncoupling protein gene.

ascribed to the control of lipid metabolism and to the regulation of body fat variability (3,24). β_2-Adrenergic receptors affect lipolysis, and different β_2-adrenergic receptor gene (β_2-AR) polymorphisms have been associated with higher BMI (25), but not in all populations (26,27). Therefore, the aim of this study was to assess, using a case-control design, whether the macronutrient distribution of dietary intake may influence the risk of obesity in individuals carrying the Gln27Glu polymorphism of the β_2-AR gene.

MATERIALS AND METHODS

Study population. The methods of this study have been previously reported (19–21). Thus, the surveyed population was recruited from the Endocrinology and Occupational Health Departments at the Navarra Hospital between January 1999 and June 2000 and was comprised of 313 Spanish subjects (66 men), aged 20–60 y (the mean age for the surveyed population was ~41 y). We based the study on a case-control design, defining cases of obesity as those individuals having BMI > 30 kg/m². Exclusion criteria were exposure to hormonal treatment or development of secondary obesity due to endocrine disease or serious intercurrent illness. Subjects with type 2 diabetes who were not receiving glucose-lowering agents were eligible as cases (9%). Controls were healthy subjects having a BMI < 25 kg/m² with no apparent disease and blood pressure < 120/90. In total, 159 obese patients (BMI, 37.7 ± 5.3 kg/m²) and 154 normal-weight subjects (BMI, 22.0 ± 1.8 kg/m²) were selected. Response rates were acceptable (65% for cases and 75% for controls), and the interviews were all conducted in a medical environment with little or no time pressure. The study was approved by the Ethics Committee of the University of Navarra, and all subjects provided written informed consent for participation. All reported investigations were carried out according to the principles of the Declaration of Helsinki II.

Dietary intake was assessed through a previously validated food frequency questionnaire for population studies including 136 items (28), and energy intake and macronutrient distribution were calculated from values obtained from two reliable Spanish food composition tables (29,30). Physical activity and time spent sitting during leisure time were estimated with a previously validated questionnaire and assessed as metabolic equivalents (MET) (MET · h/wk) or h/wk, respectively (31). MET represent the ratio of energy expended during a physical activity to the metabolic rate of sitting quietly, and are independent of body weight. The number of hours spent participating in each activity was multiplied by the MET score specific to each activity, thus obtaining the weekly MET · h.

Procedures. Weight and height were measured by conventional protocols as described elsewhere (32). Also, following a 12-h fast, venous blood samples were obtained, and the serum glucose and the lipid profile were measured by enzymatic methods (33). Serum insulin was measured by radioimmunoassay (TKIN1 kit; Diagnostic Products, Madrid, Spain) and plasma leptin by a commercial enzyme immunoassay (EIA-1863 kit; DRG Diagnostics, Marburg, Germany). Blood samples were taken for the extraction and characterization of genomic DNA from leukocytes as previously described (19–21). The DNA segment containing codon 27 of the β_2AR gene was amplified by polymerase chain reaction (PCR) carried out in a volume of 30 μL containing 200 ng of genomic DNA, 10 pmol of each primer (upstream, 5′-CCGCCGTGGGTCCGCC-3′, and downstream, 5′-CCATGACCAGATCAGCAC-3′), 200 μmol/L of deoxynucleotide triphosphate, 1.5 mmol/L of magnesium chloride, 3 μL of reaction buffer (10×: 160 mmol/L of $(NH_4)_2SO_4$, 670 mmol/L of Tris-HCl (pH 8.8 at 25°C) and 0.1% Tween 20) and 0.8 U of *Taq* polymerase (BIOTAQ; Bioline, London). The PCR began with denaturation at 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 65°C for 30 s and extension at 72°C for 30 s, with a final extension at 72°C for 10 min. Ten μL of PCR products (310 bp) were digested with the addition of 10 μL of a mixture containing 6 U of *Ita*I, a specific restriction enzyme for the sequence GC/NGC (Roche Diagnostics, Somerville, NJ) and the reaction buffer. This mixture was incubated at 37°C for 2 h, and the digested samples were separated by electrophoresis through a 2.5% agarose gel and visualized by staining with ethidium bromide.

Statistical analyses. A two-way factorial ANOVA (2 × 2) was used to assess the association between several anthropometric, dietary, metabolic and lifestyle characteristics of the sample and the presence of obesity (case) and/or the presence of the β_2-adrenoceptor mutation. The significance of the interaction between the presence of obesity and the presence of the polymorphism was also obtained.

Different equations were modeled using logistic regressions to analyze the relationship between the macronutrient intake [dichotomized at the median of carbohydrate (CHO), lipid and protein consumption] and the polymorphism on the risk of obesity. All of the analyses were carried out separately in men and women and were adjusted for confounding factors such as age and physical activity during leisure time measured in MET · h/wk.

As we have reported previously (19), significant interactions have been found for the Glu27 polymorphisms of the β_2-AR and physical activity. Therefore, we conducted stratified analyses for the groups with or without the β_2-adrenergic mutation to facilitate the interpretation of the results.

Because of the presence of a marginally significant interaction between intake of CHO and the polymorphism among women, we presented the natural logarithm of the odds ratios (OR) of being obese according to the intake of CHO [% energy (E)] as a continuous variable separately in women with and without the polymorphism.

The association between CHO intake (dichotomized at the median or as quartiles) and the level of insulin was also assessed by Student's *t* test and a linear regression model only in women because of the small size of the sample of men (n = 66). Because the distribution of the insulin levels presented a positive skewness, its log transformation as the dependent variable was considered. All of the statistical analyses were carried out by using the SPSS package software (version 10.0). Differences with values of $P < 0.05$ were considered significant, and those with values of $P > 0.05$ and <0.10 were considered marginally significant.

RESULTS

The polymorphism distribution was similar for case and control subjects, with the Gln27Gln genotype found in 35.8% of the obese subjects and in 40.2% of the controls, and the Glu27Glu genotype found in 16.0% of the obese subjects and 14.3% in lean individuals. As expected, all obesity phenotype characteristics concerning anthropometric (e.g., weight and BMI) and biochemical markers (e.g., glucose, insulin, leptin and lipid profile) differed between obese and lean individuals in this population (**Table 1**). Data obtained from the food frequency questionnaire suggested that obese individuals (mutated and nonmutated) underreported dietary energy intake (~8800 kJ/d) compared with lean controls (~10,900 kJ/d) with a trend to report protein overconsumption at the expense of fat (**Fig. 1**). The reported CHO intakes did not differ between groups but were related to the triglyceride levels ($P < 0.07$), giving some support to the validity of the dietary assessment we used for this study. In addition to a likely underreporting, obese individuals declared a lower level of physical activity (Table 1), which may explain in part the reduced energy intake in these subjects.

Obesity incidence was not directly affected by the polymorphism (OR = 1.40, $P = 0.246$). The analysis of the association between CHO intake and the Glu27 allele on obesity risk was assessed through a multivariate logistic model. After introducing in the model the previously reported interaction between the β_2-adrenergic mutation and physical activity, a marginally significant interaction between the polymorphism and the CHO intake was found among women (**Table 2**). This finding suggested that a dietary intake higher than the median CHO consumption (>49% E) produced an increased obesity risk in those women carrying the Glu27 allele (OR = 2.56, $P = 0.051$) when adjusted for age and physical activity. A second model, using the CHO/fat ratio instead of the percent

TABLE 1

Anthropometric, metabolic and lifestyle variables, including energy intake and physical activity indicators, in obese (case) and normal weight (control) subjects stratified by the presence of the Gln27Glu polymorphism[1]

	Cases		Controls		2×2 ANOVA		
	Gln27Gln genotype $n = 57$	Gln27Glu polymorphism $n = 102$	Gln27Gln genotype $n = 62$	Gln27Glu polymorphism $n = 92$	P, Gln27 vs. Glu27	P, cases vs. controls	P, interaction
Men, %	7.0	15.7	30.6	23.9			
Age, y	40.1 ± 10.4	39.3 ± 9.1	37.5 ± 8.9	42.3 ± 10.6			
Current weight, *kg*	96.2 ± 15.9	95.3 ± 17.0	62.8 ± 10.7	60.6 ± 9.5	0.34	< 0.01	0.70
Weight at 20 y, *kg*	69.8 ± 16.6	68.1 ± 16.5	59.7 ± 12.2	57.9 ± 9.6	0.29	< 0.01	0.95
Maximum weight, *kg*	100.6 ± 16.0	98.8 ± 17.2	66.7 ± 12.6	64.4 ± 10.6	0.22	< 0.01	0.89
Body mass index, *kg/m2*	38.1 ± 5.6	37.3 ± 5.8	22.3 ± 1.9	22.3 ± 1.8	0.42	< 0.01	0.35
Glucose, *mmol/L*	103.6 ± 35.6	100.2 ± 20.1	91.7 ± 9.2	93.4 ± 14.4	0.75	< 0.01	0.30
Total cholesterol, *mmol/L*	209.5 ± 36.7	208.1 ± 39.6	192.0 ± 29.8	198.0 ± 37.6	0.59	< 0.01	0.39
Leptin, *ug/L*	34.5 ± 29.1	34.4 ± 32.4	7.3 ± 6.5	8.3 ± 8.3	0.86	< 0.01	0.82
Insulin, *pmol/L*	150.0 ± 162.0	147.6 ± 125.0	57.6 ± 34.8	52.2 ± 28.2	0.74	< 0.01	0.90
Energy, *kJ/d*	8907 ± 3243	8722 ± 2681	11444 ± 2807	10536 ± 4714	0.28	< 0.01	0.47
Sitting (*h/wk*)	32.2 ± 12.9	28.8 ± 12.9	20.5 ± 8.8	22.1 ± 8.3	0.48	< 0.01	0.05
Physical activity (*MET · h/wk*)[2]	9.3 ± 10.3	10.8 ± 14.8	24.1 ± 21.2	18.3 ± 19.9	0.28	< 0.01	0.07

[1] Values are means $\pm$ SD.

[2] Metabolic equivalents (MET) represent the ratio of energy expended during a physical activity to the resting metabolic rate. The number of hours spent participating in each activity during a week were multiplied by the MET score specific to each activity, thus obtaining the weekly MET · h.

derived from CHO supported these results, because a CHO/fat intake ratio of >1.77 was associated with a higher risk of obesity (OR = 3.21, P < 0.02) in women with the β_2-adrenoceptor gene polymorphism.

The natural logarithms of the OR of being obese for women with and without the polymorphism according to the intake of CHO were marginally significant for women with the polymorphism ($b = 0.074$, $P = 0.056$), using the lower level of intake as the reference and adjusted for age and physical activity (**Fig. 2**).

Additionally, a relationship between CHO intake and insulin levels (log transformed) among women was found, and this relationship showed that an intake of CHO above the median (>49% E) was associated with higher insulin levels (**Fig. 3**) among women carriers of the polymorphism in the
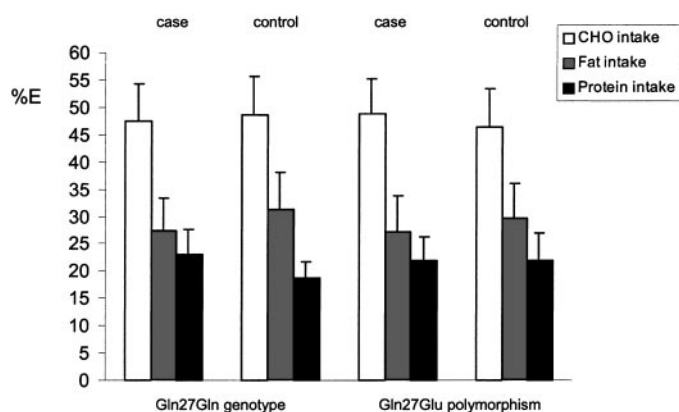

FIGURE 1 Macronutrient distribution as a percentage of energy intake (% E) in obese (case) and control individuals with and without the Gln27Glu polymorphism. Values are means $\pm$ SD ($n = 313$). Carbohydrate (CHO) intake did not differ between case and control subjects overall ($P = 0.42$). There were no differences between case and control subjects within genotype for CHO ($P = 0.73$), fat ($P = 0.38$) or protein ($P = 0.12$) intakes.

β_2-adrenoceptor gene ($P = 0.01$). Moreover, a positive linear trend (data not shown) for insulin levels was observed across quartiles of CHO intake within the mutated group ($P = 0.03$).

DISCUSSION

Obesity results from the combined effects of genes, environment and lifestyle (1,6). In this context, an understanding of the interactions between macronutrient intake and the genotype is important to provide a basis for determining the role of dietary intake and habits in the prevalence of different pathological conditions such as obesity (34). The effects of nutrition on the phenotype can be exerted at many stages between transcription of the genetic sequence and production of a functional protein (35).

Although the specific role of the dietary macronutrient intake in the prevalence of obesity is still controversial (2,33,36), genotype-environment interactions arise when the phenotypic response (e.g., fat mass) to lifestyle habits (e.g., diet) is modulated by the genotype of the individual (5). It is well established that individual responses to different dietary interventions may be genotype dependent (6,37); however, most studies have ignored the gene-environment interactions due to the difficulty of examining the role of common polymorphisms in the absence of data concerning nongenetic exposures (14,38).

Some evidence for gene-environment interactions has been obtained not only by comparing the influence of a gene on a given phenotype in populations with different dietary habits or by categorizing on the basis of dietary variables that potentially affect the phenotype, but also by assessing the response to a dietary intervention among individuals with different genotypes at a given candidate gene or marker locus (6). Case-control approaches therefore provide a unique opportunity to explore multicausality for a given gene-diet interaction (39,40) by using a homogenous population with selected criteria of inclusion (BMI > 30 kg/m^2 for cases and BMI < 25

TABLE 2

Association (OR) between carbohydrate intake and obesity (multivariate logistic regression model) categorized by the Gln27Glu polymorphism adjusted for age and physical activity during leisure time (metabolic equivalents · h/wk)[1]

Carbohydrate intake (%E)	Gln27Gln genotype			Gln27Glu polymorphism		
	OR	95% CI	P	OR	95% CI	P
Women						
<49	1 (ref.)			1 (ref.)		
≥49	0.53	(0.15–1.86)	0.32	2.56	(1.00–1.16)	0.051
Interaction (effect modification)						0.058
Men						
<49	1 (ref.)			1 (ref.)		
≥49	0.13	(0.001–19.42)	0.42	1.39	(0.12–15.55)	0.79
Interaction (effect modification)						0.504

[1] Abbreviations used: OR, Odds ratio; E, energy; 95% CI, 95% confidence interval; ref., reference category.

kg/m² for controls) despite the difficulties that usually arise in recruiting the study population (31). The association in these studies is commonly assessed through the OR calculated by logistic regression analyses to perform multivariate adjustments for confounding factors and to take into account effect modifiers (39).

A limitation in these kinds of studies is that the tendency for the obese individuals to underestimate their dietary energy intake influences the macronutrient distribution pattern, particularly the dietary protein and lipid values (41,42). Therefore, we focused only on the CHO intake, because we found no statistical differences between obese and lean subjects, but an association between insulin and triglyceride levels was observed, which indirectly supports the validity of the CHO intake data.

The β-adrenoreceptors are involved in adipocyte lipid mobilization (7,43), and a number of genetic polymorphisms (Gln27Glu, Arg16Glu, Thr164Ile and Val34Met) have been associated with the risk of obesity (6,44,45) through changes in the receptor function (7,25,46). The Glu27 allelic frequency of the β_2-adrenoceptor gene polymorphism was high in both case (0.40) and control (0.37) groups, being in Hardy-Weinberg equilibrium. This distribution is similar to that of other European populations (7,47,48), but different from the Japanese allelic frequency (49). However, not all studies have demonstrated a significant association between the Gln27Glu polymorphism and obesity (26,27). These controversial findings could be explained, in some cases, by not having taken into account potential effect modifiers such as age, gender or physical activity as well as the dietary pattern (20,21).

Moreover, although the mutation cannot be considered as the definite cause of obesity, the polymorphism has shown in some circumstances a different effect in men than in women (19,50,51). The current analysis revealed that the effect of the Gln27 mutation on the obesity risk was modified by the macronutrient composition of the diet, when adjusted by age and physical activity. That is, a higher CHO intake may actually increase the obesity risk in women carrying the Glu27 allele, which may be associated not only with a hyperinsulinemic response in these subjects but also with changes in the CHO/fat proportions oxidized as a consequence of an impaired β_2-adrenoceptor function. Among female subjects bearing the Glu27 polymorphism, an association between high CHO intake and higher insulin levels ($P < 0.03$) was apparent, which may contribute to the greater likelihood of an onset of obesity among these subjects.

The fact that some studies have shown that a polymor-
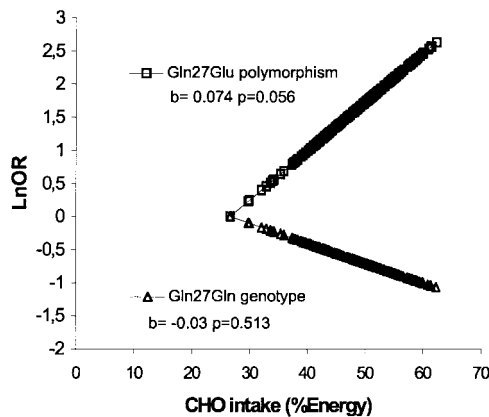
FIGURE 2 Natural logarithms of the odds ratios (LnOR) of being obese for women with and without the polymorphism (logistic regression model) according to the intake of carbohydrates (CHO) (% energy) and adjusting for age and physical activity during leisure time (metabolic equivalents · h/wk).
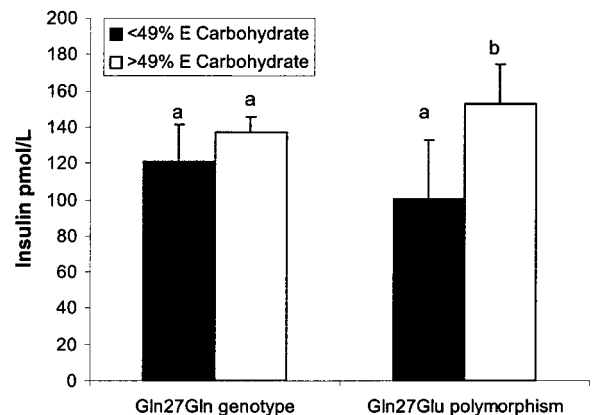
FIGURE 3 Mean insulin levels according to carbohydrate (CHO) intake among women ($n = 247$) categorized according the presence of the Gln27Glu polymorphism (P interaction = 0.17). Values are means ± SEM. Means with a different letter differ, $P < 0.01$. E, Energy.

phism on the β_2-AR adversely reduces fat oxidation (16,48), which may consequently be associated with hyperlipidemia, insulin resistance and hyperinsulinemia (52), also helps to explain that carriers of the Glu27 allele may show an impaired response after high CHO intake leading to obesity. This suggestion is confirmed not only by the positive association between a high CHO intake and insulin levels observed in this case-control study, as assessed by the quartiles of the CHO intake in Glu27 allele carriers, but also by the marginal correlation between CHO intake and triglyceride levels (53).

Apparently, this is the first time that a gene × diet interaction has been described for this gene polymorphism, although a number of studies have reported the influence of dietary intake on other polymorphisms such as Pro12Ala for the *PPAR* locus (14) or different *Apo* gene variants (8–10) and for other mutations (54). Furthermore, some examples of the role of the genotype affecting the weight-loss response to low-energy diets have been reported in obese women for uncoupling protein 1 gene (*UCP1*) (51), leptin receptor gene or leptin gene (55,56) and conjoint β_3-adrenoceptor or *UCP* gene polymorphism (57,58). Additionally, it has been shown that a β_3-adrenergic receptor gene polymorphism may affect the anatomical distribution (subcutaneous vs. visceral) of the body fat diet-induced loss (59) and predict the outcome of dietary interventions (60). Moreover, genetic variations of the β_2-adrenergic receptor locus have been associated with interindividual differences in the response to overfeeding (61). A previous finding obtained from this Spanish population showed that individuals bearing the Gln27Glu polymorphism are somewhat resistant to weight loss induced by physical activity and are less able to use fat stores as a source of fuel after a period of exercise (16).

In any case, the current data should be carefully interpreted, because they rely on self-reported dietary data, which may be influenced by the weight status of the individuals and by their physical activity patterns. Although the studied gene polymorphism did not have a direct effect on obesity risk, heterogeneous responses to different dietary situations (e.g., macronutrient distribution and weight-reducing methods) support the possibility that there are individual differences in the susceptibility to dietary intake and confirm that gene-diet interactions may have a role in the onset, prevalence and dietary management of obesity (62,63).

ACKNOWLEDGMENTS

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