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Serum glucose concentration and ACP_1 genotype in healthy adult subjects

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Abstract

Acid phosphatase locus 1 (ACP_1) or cytosolic low molecular weight protein tyrosine phosphatase is a polymorphic enzyme that can hydrolyze phosphotyrosine-containing peptides of the human insulin receptor and of band 3 protein. High-activity ACP_1 may favor an increase in serum glucose concentration through a depression of insulin action and through inactivation of aldolase, phosphofructokinase, and glyceraldehyde-3-phosphate dehydrogenase induced by dephosphorylation of band 3 protein. In diabetic subjects, we have previously reported lower serum glucose concentration in subjects with low-activity ACP_1 A and AB phenotypes.

We have now studied the relationship between serum glucose concentration and ACP_1 genotype in a sample of 137 healthy adult workers of our university.

In males, serum glucose concentration is significantly higher in medium-high– than in low-activity ACP_1 genotypes. With advancing age in males, there is a progressive increase in glycemic differential between medium-high– and low-activity ACP_1 genotypes.

The data suggest that normal variability of ACP_1 genotype influences serum glucose concentration in normal individuals. Such influence depends on sex and in males becomes more marked with advancing age.

1. Introduction

Acid phosphatase locus 1 (ACP_1) or cytosolic low molecular weight protein tyrosine phosphatase is a polymorphic enzyme composed of 2 isoforms, f and s, that show strong quantitative variation among genotypes [1,2]. Significant differences between f and s isoforms have been observed in both enzymatic and molecular properties, suggesting that they perform different physiological functions [3]. In white populations, 6 genotypes are present with total enzymatic activity increasing in the order *A/*A < *A/*B < *A/*C ≤ *B/*B < *C/*B < *C/*C.

At present, the functions of this enzyme in vivo have not been clarified [4] Biochemical studies suggest 2 different physiological functions for ACP_1: flavin-mononucleotide phosphatase and protein tyrosine phosphatase. As flavin-mononucleotide phosphatase, by regulating the concentration of flavin mononucleotide and flavin adenine dinucleotide, ACP_1 influences flavo-enzyme activity and energy metabolism. As protein tyrosine phosphatase, ACP_1 is able to hydrolyze phosphotyrosine-containing peptides of the human insulin receptor and of band 3 protein (B3P; see Ref [4] for a review). Therefore, high-activity ACP_1 may favor an increase in serum glucose concentration through a depression of insulin action. On the other hand, as phosphorylation of B3P is associated with increased glycolytic rate through activation of aldolase, phosphofructokinase, and glyceraldehyde-3-phosphate dehydrogenase [5], high-activity ACP_1 may favor an increase in serum glucose also through a decrease in glycolytic enzyme activity. Association studies are in favor of this hypothesis; in diabetic pregnancy, and in type 2 diabetes in fact we have shown that subjects with low-activity genotypes

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*A/*A and *A/*B have lower serum glucose concentrations as compared to medium-high ACP_1 genotypes [6,7].

In this article, we have investigated the possible effects of ACP_1 on serum glucose concentration in a sample of healthy adult subjects.

2. Subjects and methods

The study was performed on 137 healthy adult workers of the University of Rome Tor Vergata included in a project of the Division of Occupational Health Medicine approved by the Department of Biopathology and Imaging Diagnostics and by the Central Administrative Board of the university. Diabetic subjects and subjects with other clinically evident pathologies were excluded.

Table 1 shows some demographic data on the sample study. Significant differences between males and females are observed for age and serum glucose concentration.

Serum glucose concentrations are fasting values determined by standard laboratory method and are expressed in milligrams per deciliter.

Acid phosphatase locus 1 is a high polymorphic enzyme controlled by an autosomal locus showing 3 codominant alleles named ACP_1*A, *B, and *C [8]. The 3 ACP_1 alleles show single-base substitutions located at 3 specific sites: ACP_1*A and *B alleles differ for 2-base substitutions, a silent C-T transition at codon 41 (exon 4), and an A-G transition at codon 105 (exon 6). The ACP_1*C allele differs from the *A and *B alleles at codon 43 (exon 3) [9].

Total genomic DNA was extracted from frozen whole-blood samples collected in Na–ethylenediaminetetraacetic acid using the procedure described by Kunkel et al [10] with slight modifications. All polymerase chain reactions (PCRs) were set up in 30 μL and 0.2 μmol/L of both primers, 0.1 mmol/L dNTPs, 1.5 mmol/L $MgCl_2$, 0.5 U of Taq polymerase (AmpliTaq, Applied Biosystem, Mannheim, Germany), 1 × AmpliTaq buffer (PE), and 50 ng of DNA template. The amplification conditions consisted of an initial denaturation at 94°C for 2 minutes, followed by 35 cycles of 94°C for 45 seconds, 54°C for 45 seconds, 72°C for 45 seconds, and a final extension at 72°C for 5 minutes. The annealing temperature, extension time, and primer concentration for the 2-kb amplification product were 57°C, 120 seconds, and 0.1 μmol/L, respectively.

Table 1
Demographic data on the sample study

	Males	Females	Differences between means (P)
Age			
Mean	42.18	35.73	<.001
SE	1.48	0.99	
N	55	81	
Serum glucose concentration (mg/dL)			
Mean	96.51	86.31	<.001
SE	1.55	0.82	
N	55	81	
Male proportion	40.9%		

Table 2
Primers used for ACP_1 SNP analysis

Primer number	Target amplification	Nucleotide sequence 5'-3'
263	Exon 3	AGGCCAACCTGAACTCCTCT
264	Exon 3	CCTGTCTTGCTTTATGGGCT
267	Exon 6	TTCAGAAGACCCTAGCAGATG
268	Exon 6	TGGCAAAACCTGCATAACAA

SNP indicates single-nucleotide polymorphism.

Oligonucleotide primers used for PCR amplification are reported in Table 2.

The C-T transition at codon 43 and the A-G transition at codon 105 generate a Cfo and a Taq 1 restriction site that, together, were used for PCR-based genotyping (modified from Lazaruk [9]), respectively. A 341-bp segment spanning the entire exons 3 and 4 was amplified using primers 263 to 264 (Table 1). A 299-bp segment including exon 6 was amplified using primers 267 and 268. Ten microliters of the 341-bp exon 3 amplicon was fully cleaved by Cfo 1 at 37°C for 1 hour according to the manufacturer's instructions and then electrophoresed on 1.8% agarose gels. The digestion created 2 fragments of 255 and 86 bp for the ACP_1*A and ACP_1*B alleles, whereas the ACP_1*C allele was not cut. Similarly, the 299-bp PCR product was digested by Taq 1 at 65°C for 1 hour according to the manufacturer's instructions, generating 2 fragments of 100 and 199 bp for the ACP_1*A allele but not for the *B and *C alleles (modified from Lazaruk [9]).

Statistical analyses were performed using the SPSS package [11].

3. Results

Table 3 shows the distribution of ACP_1 genotypes. No difference was observed with respect to Hardy-Weinberg expectation. No significant difference was observed between males and females.

As males differ from females for both age and serum glucose concentration, the relationship of ACP_1 with serum glucose will be considered separately in the 2 sexes.

Table 4 shows the mean values of serum glucose concentration in relation to ACP_1 genotype and sex. Acid phosphatase locus 1 genotypes have been grouped into low activity and medium-high activity. Serum glucose is higher in males than in females; such difference is statistically significant in both groups of genotypes but more marked in medium-high– than in low-activity ACP_1 genotypes. Males with high-activity ACP_1 genotype show a statistically significant higher levels of serum glucose concentration than males with low-activity genotype ($P < .05$), whereas in females the pattern is reversed with medium-high–activity genotypes showing a concentration of serum glucose

Table 3
Distribution of ACP_1 genotypes

		ACP_1 genotypes					Total number
		*A/*A	*B/*B	*A/*B	*A/*C	*B/*C	
Whole sample	Observed	11	61	48	4	13	137
	Hardy-Weinberg expected	9.98	61.50	49.57	4.44	11.01	
χ^2 Test of goodness of fit for Hardy-Weinberg expectation							P = NS
Males	Observed	2	22	23	2	6	
Females	Observed	9	39	24	2	7	
χ^2 Test of independence							P = NS

Table 4
Mean value of serum glucose concentration

ACP_1 genotypes	Males			Females			Males vs females	
	Mean	SE	N	Mean	SE	N	Levene test for equality of variances (P)	t Test for equality of means (P)
*A/*A + *A/*B (low activity)	93.19	1.33	26	87.18	1.38	33	NS	<.01
Other genotypes (medium-high activity)	99.37	2.50	30	85.71	1.02	48	<.02	<.001
Low-activity ACP_1 vs medium-high–activity ACP_1								
Levene test for equality of variances (P)	.035			NS				
t Test for equality of means (P)	.036			NS				

Table 5
Serum glucose concentration (mg/dL) in relation to ACP_1 and age in males

	Low-activity ACP_1		Medium-high–activity ACP_1		Serum glucose concentration differential
	Mean	SE	Mean	SE	
Age					
≤35 y	91.56	3.00	90.50	2.66	−1.06
36-45 y	92.42	1.54	98.29	3.77	+5.87
>45 y	98.00	1.65	106.77	4.16	+8.77
Variance analysis (P)	0.209		0.013		
Tukey HSD test (P)			≤35 vs >45 y, 0.010		

Table 6
Correlation analysis between enzymatic activity of ACP_1, serum glucose concentration, and age in males and females separately

Correlation	ACP_1 activity					
	Total ACP_1 activity		f Isoform		s Isoform	
	r	P	r	P	r	P
Males						
Serum glucose vs ACP_1 activity	0.267	.047	0.204	.131	0.098	.474
Serum glucose vs age	0.482	.000	0.482	.000	0.482	.000
ACP_1 activity vs age	0.159	.240	−0.029	.828	0.194	.151
Partial serum glucose vs ACP_1 activity	0.220	.107	0.249	.066	0.005	.973
Females						
Serum glucose vs ACP_1 activity	−0.123	.274	−0.079	.484	−0.072	.525
Serum glucose vs age	0.437	.000	0.437	.000	0.437	.000
ACP_1 activity vs age	−0.259	.019	−0.177	.113	−0.138	.220
Partial serum glucose vs ACP_1 activity	−0.011	.922	−0.001	.989	−0.013	.911

slightly lower than low-activity genotypes, but such difference is not statistically significant.

Table 5 shows the values of serum glucose concentration in relation to ACP_1 and age in males. With advancing age, there is a progressive increase in serum glucose differential between medium-high– and low-activity ACP_1 genotypes. Such phenomenon is lacking in females (data not shown). In ACP_1 genotypes with medium-high activity, there is a statistically significant increase in serum glucose concentration with advancing age. Such increase is much less evident and statistically not significant in low-activity *A/*A and *A/*B genotypes.

In consideration of the complexity of ACP_1 polymorphism due to the presence of 2 isoforms that have different concentrations among genotypes and have different biochemical properties, the analysis of association between ACP_1 and physiological parameters or pathological situation should be performed considering both genotypes and isoforms.

Table 6 shows a correlation analysis between ACP activity parameters (total activity, f and s isoform concentrations), serum glucose concentration, and age in males and females separately. In males, serum glucose is positively correlated with total enzymatic ACP_1 activity: most of the effect of ACP_1 on serum glucose concentration persists controlling for age. The effect of f isoform on serum glucose concentration is much greater than that of s isoform; when controlling for age, the effect of f isoform increases whereas that of s isoform disappears. This indicates that the association between ACP_1 and serum glucose concentration in males is practically due to f isoform. In females, no significant association of serum glucose concentration is observed with either total enzymatic activity or ACP_1 isoforms.

4. Discussion

The tendency toward lower serum glucose concentrations shown by ACP_1 genotypes with low enzymatic activity previously observed in diabetic subjects [6,7] is present in healthy male adults only. In females, we have not observed such association.

Recent interest has been focused on sex differences concerning common diseases, especially on those involving the immunological system [12]. Recently, we have reported in females with type 1 diabetes and medium-high–activity ACP_1 genotypes an onset of disease significantly earlier than in males [13]. At present, it is not known whether ACP_1 alleles, in particular the *A allele associated with low enzymatic activity, are differently expressed between sexes.

In type 2 diabetes [7], we have observed that serum glucose concentration is positively correlated with f isoform and not with s isoform. Stefani et al [3] have shown that a synthetic-phosphotyrosine–containing peptide corresponding to the 5 to 16 sequence of B3P is more efficiently hydrolyzed by the f component than by the s component of ACP_1. As dephosphorylation of B3P decreases the glycolytic rate [5], the fact that the association with serum glucose is practically due to f isoform concentration suggests that modulation of B3P phosphorylation is probably the more important mechanism underlying the association between ACP_1 and serum glucose concentration.

It is likely that "normal" genetic variability of enzymes plays an important role in determining the metabolic characteristics of individuals. However, it is also likely that in normal condition and in young people, the effect of polymorphic systems on the variability of metabolic parameters is very small and difficult to detect. Pathological situations and advancing age may enhance the relative effect of genetic polymorphisms with important consequences on clinical variability of diseases and survival. In fact, the differences in serum glucose concentration observed between ACP_1 genotypes are more evident in diabetic subjects [6,7] than in healthy subjects, and within healthy subjects more evident in older than in young people.

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