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ACP1 genotype, glutathione reductase activity, and riboflavin uptake affect cardiovascular risk in the obese

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Abstract

Erythrocyte acid phosphatase (ACP locus 1), also known as *low–molecular-weight protein tyrosine phosphatase*, has previously been associated to glycemia, dyslipidemia, and obesity. In this study, ACP1 genotype and activity were tested in 318 women aged 19 to 83 (mean, 51.74 ± 13.44) years. ACP1 genotype was found to directly correlate to glutathione reductase activity ($P < .001$) and levels of low-density lipoprotein cholesterol ($P = .038$). Glutathione reductase activity was in turn found to correlate to a series of cardiovascular risk factors such as systolic arterial pressure ($P < .001$), total cholesterol levels ($P = .018$), and low-density lipoprotein cholesterol levels ($P = .039$). A possible protective effect of ACP1 genotype AA against these cardiovascular risk factors was observed in this study. Furthermore, this work hypothesizes that nutritional riboflavin uptake becomes more crucial as body mass index increases, to counteract oxidative stress and minimize cardiovascular risk. This might be especially true in ACP1 genotypes AC, BC, and CC, which might possibly show the least endogenous protection against oxidative stress.

1. Introduction

Erythrocyte acid phosphatase (ACP locus 1), also known as *low–molecular-weight protein tyrosine phosphatase* (LMW-PTP), has previously been associated to hyperglycemia, dyslipidemia, and obesity [1-9]. Recently, Pandey et al [4] demonstrated for the first time that ACP1 is a key negative regulator of insulin signaling in vivo. In their work, the reduction of LMW-PTP expression with an antisense inhibitor ameliorated plasma glucose and insulin levels in 2 models of obese mice, suggesting improved glucose and insulin tolerance and offering a new target for the treatment of type 2 diabetes mellitus.

ACP1 is expressed ubiquitously, although the level of LMW-PTP messenger RNA (mRNA) has been found to be highest in liver and brain and lowest in skeletal muscle in mice [1,4,5,10]. ACP1's autosomal locus (2p25) shows polymorphism, resulting in 3 common alleles—ACP1∗A, ACP1∗B, and ACP1∗C—and their respective allozymes, as well as in a number of rare alleles [3,5,9,10]. Each of the 3 allozymes exists in 2 isoforms, termed either *fast* or *slow* according to their electrophoretic mobility. They are produced by alternate splicing of a single pre-mRNA [4,9,11]. Total enzymatic activity is determined by the fast to slow ratio and phenotype. Activity of the allozymes decreases in the order C > B > A, leading to phenotypic activities in the order AA < AB < BB < CA < BC < CC [5,6,11-14].

Functions suggested for LMW-PTP presently include phosphotyrosine phosphatase activity and flavin mononucleotide phosphatase action [1-4,6,12,13,15]. It has been

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proposed that, acting as a flavin mononucleotide phosphatase, LMW-PTP influences the activity of flavoenzymes such as glutathione reductase (GR) by affecting the cellular concentration of their cofactor flavin adenine dinucleotide [2,6,12,13,15]. Negative correlation of ACP1 enzymatic activity and GR activity has in fact been observed [13], suggesting that ACP1 genotype AA can be expected to show the highest levels of GR activity in white populations.

Significantly lower levels of GR activity and GR mRNA have previously been found in patients with untreated hypertension than in respective control groups [16,17]. Hence, it has been suggested that the inadequate antioxidant enzyme response may contribute to hypertension [16,17]. Inadequate antioxidant enzyme response, that is, low GR activity, may also contribute to cardiovascular risk. Erythrocyte GR activity coefficient is commonly used as an indicator for nutritional riboflavin uptake; and Gariballa and Ullegaddi [18] found that 51% of acute stroke patients were in fact biochemically riboflavin deficient, suggesting that they had inadequate antioxidant enzyme response.

This led us to hypothesize that ACP1 genotype AA, through its potential influence on GR activity, may act as a possible protection in developing hypertension by providing better antioxidant enzyme response. The findings of Gariballa and Ullegaddi, however, have also led us to consider the effect of nutritional riboflavin uptake on maintaining good antioxidant response and thus on the possible contribution to hypertension in individuals we considered to be genetically favored and those we did not.

As a result, we suggest the protective effect of genotype ACP1∗AA against oxidative stress, obesity-related hypertension, and high low-density lipoprotein (LDL) cholesterol levels, depending on nutritional riboflavin uptake.

2. Materials and methods

Three hundred eighteen women aged 19 to 83 years (mean, 51.74 ± 13.44) were tested for the following parameters: body mass index (BMI), ACP1 genotype, ACP1 enzymatic activity, total cholesterol, high-density lipoprotein (HDL), LDL, triglycerides, and GR and glutathione peroxidase activity. Informed consent regarding the participation in the study was obtained from each patient. This study was approved by the University of Lisbon's Committee on Ethics.

Blood samples were collected in heparin-coated tubes. Erythrocytes were separated and centrifuged (500*g*, 12 minutes, 4°C) to obtain a final concentration of 10 g hemoglobin (Hb) per 100 mL H_2O in hemolysates. ACP1 genotype testing was carried out using a previously described method developed at our laboratory [19,20]. For testing, genomic DNA was extracted from 5 mL of peripheral blood leucocytes following a nonenzymatic extraction method published by Lahiri and Nurnberger [21]. ACP1 enzymatic activity was determined in erythrocytes as described by Dissing et al (1979) [22]. Plasma LDL cholesterol, HDL cholesterol, and total cholesterol levels were assayed following standard protocol. Erythrocyte GR activity was determined by a spectrophotometric method at 340 nm in the absence of FAD, following an established protocol [23].

Statistical testing was carried out using SPSS (Chicago, IL) version 14. Tests included the 1-way analysis of variance, Student *t* test, χ^2 test, Kolmogorov-Smirnov test, and Pearson and Spearman correlations.

To carry out statistical testing, women were grouped according to their level of BMI. Group 1 corresponded to women we considered to be of normal weight, presenting values of BMI up to 24.99 kg/m^2. Group 2 corresponded to women who were considered overweight; values of BMI were from 25.00 up to 29.99 kg/m^2. Group 3 comprised the obese, and values of BMI were higher than 30.00 kg/m^2.

In this study, *hypertension* was defined as a systolic arterial blood pressure of at least 140 mm Hg and/or a diastolic arterial blood pressure of at least 90 mm Hg according to the Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure (JNC 7).

3. Results

ACP1 genotype AB was found to be present in 41.2% (n = 131) of women, making it the most frequent. The frequencies of the remaining genotypes were as follows: BB = 30.8% (n = 98), AA = 15.1% (n = 48), BC = 6.9% (n = 22), and AC = 6.0% (n = 19).

Erythrocyte acid phosphatase genotype influenced ACP1 activity to a significant degree ($P < .001$). Mean enzymatic activities for ACP1 were observed to increase in the order AA < AB < BB < AC < BC (Table 1).

Significant ($P < .001$) differences were found in GR activities per ACP1 genotype. In ACP1 genotype AA, the highest mean values of GR activity were observed, decreasing in the order AA > BC > AB > AC > BB.

Low–molecular-weight protein tyrosine phosphatase genotype influenced blood LDL cholesterol levels to a significant degree ($P = .038$). Genotypes with the lowest ACP1 enzymatic activities also showed the lowest blood LDL cholesterol levels. Mean LDL cholesterol levels were

Table 1
ACP1 genotype and the values it affects

ACP1 genotype	ACP1 (mol/[min∗g Hb])	GR (μmol/[min∗g Hb])	LDL cholesterol (mg/dL)
AA	269.67 ± 85.99	66.20 ± 18.84	119.39 ± 35.97
AB	285.40 ± 85.40	45.45 ± 18.15	132.20 ± 30.84
BB	308.79 ± 104.72	41.82 ± 16.86	134.05 ± 38.49
AC	407.48 ± 104.23	45.22 ± 8.30	133.82 ± 33.88
BC	423.91 ± 133.26	56.78 ± 22.21	148.21 ± 37.15

ACP1 genotype AA shows the highest GR activity, the lowest LMW-PTP activity, and the lowest LDL cholesterol levels.

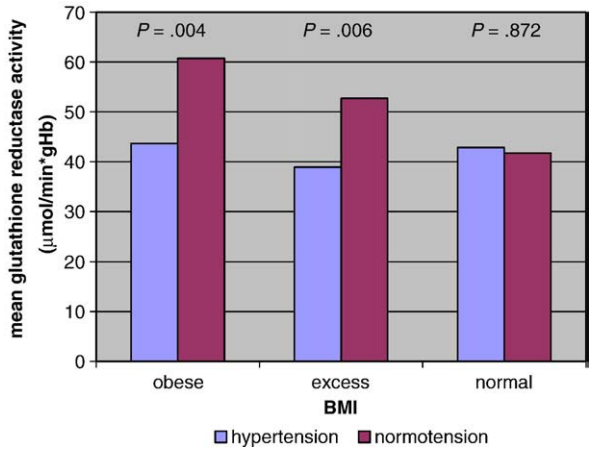

Fig. 1. Mean GR activity in normotensive and hypertensive women with respect to the level of BMI. In the overall population, GR activities have been found to be significantly (P = .001) lower in hypertensive women. However, for women with a BMI within the normal range, no statistically significant drop in GR activity could be observed in the hypertensive.

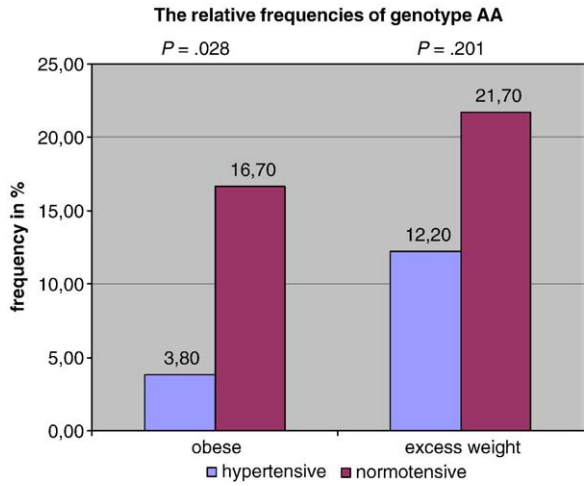

Fig. 2. The relative frequencies of genotype AA in the obese and overweight for hypertension and normotension, respectively. Overall, genotype AA was significantly less frequently found in overweight and obese hypertensive patients (P = .010).

found to increase in the order AA < AB < AC < BB < BC (Table 1).

Total cholesterol and HDL cholesterol levels were not significantly influenced by ACP1genotype in this study. However, a nonsignificant trend showed total cholesterol values to increase in the order AA < AB < AC < BB < BC, the same order that values of LDL cholesterol were found to increase. High-density lipoprotein cholesterol, however, was found to decrease in the order BB > AA > AB > AC > BC.

Homeostasis model assessment of insulin resistance (HOMA-IR) was found to be negatively correlated to GR activity in the overall (r = −0.389, P < .001) and normotensive (r = −0.380, P < .001) population but not in hypertensive patients. Furthermore, HOMA-IR showed direct correlation to BMI (r = 0.376, P < .001). Homeostasis model assessment of insulin resistance could not be correlated to ACP1 activity, and mean HOMA-IR did not differ between ACP1 genotypes to any significant degree.

Interestingly, ACP1 genotype AA was found to have the lowest mean ACP1 activity, highest GR activity, lowest LDL cholesterol levels, lowest total blood cholesterol levels, and lowest HOMA-IR.

Overall, 193 (60.7%) women were classified as normotensive and 103 (34.3%) as hypertensive. A significant difference (P = .001) was found in the overall mean GR activities when comparing hypertensive and normotensive patients. Normotensive women had mean GR activities of 52.24 ± 19.71 µmol/(min∗g Hb), whereas hypertensive women had a mean value of 41.54 ± 15.08 µmol/(min∗g Hb).

Of the women tested, 72 (22.6%) were of normal weight, 132 (41.5%) were overweight, and 112 (35.2%) were obese. Glutathione reductase activity was found to increase as BMI increased in normotensive (P = .008) but not in hypertensive women (P = .573) (Fig. 1). In the normotensive women, GR activity and BMI also showed direct correlation (P = .006) using the Spearman test.

Obese and hypertensive women had significantly lower GR values than obese and normotensive women (P = .004). The same was observed for overweight women (P = .006). In the obese, mean values dropped from 60.71 ± 16.85 to 43.65 ± 20.16 µmol/(min∗g Hb) in hypertension; and in the overweight, values dropped from 52.71 ± 21.41 to 38.89 ± 7.75 µmol/(min∗g Hb).

Table 2
The frequencies of ACP1 genotypes in obese, overweight, and normal-weight women with regard to hypertension

ACP1 genotype	Obese (BMI >30.00 kg/m^2) (n = 107)		Overweight (BMI = 25.00-29.99 kg/m^2) (n = 124)		Normal weight (BMI <24.99 kg/m^2) (n = 71)	
	Normotensive (n = 54)	Hypertensive (n = 53)	Normotensive (n = 83)	Hypertensive (n = 41)	Normotensive (n = 56)	Hypertensive (n = 15)
AA	16.7	3.8	21.7	12.2	7.1	33.3
BB	20.4	22.6	27.7	39.0	48.2	20.0
AB	35.2	66.0	38.6	39.0	35.7	33.3
AC	11.1	1.9	7.2	4.9	3.6	13.3
BC	16.7	5.7	4.8	4.9	5.4	0.0

All values are given in percentage. As only a total number of 15 patients were observed, we did not consider the results obtained for the group of normal-weight and hypertensive women statistically representative and excluded them from any further graphics or statistical testing. Frequencies of ACP1 genotype AA observed have been shown to drop significantly in the hypertensive, suggesting that carriers of genotype AA are less likely to develop hypertension.

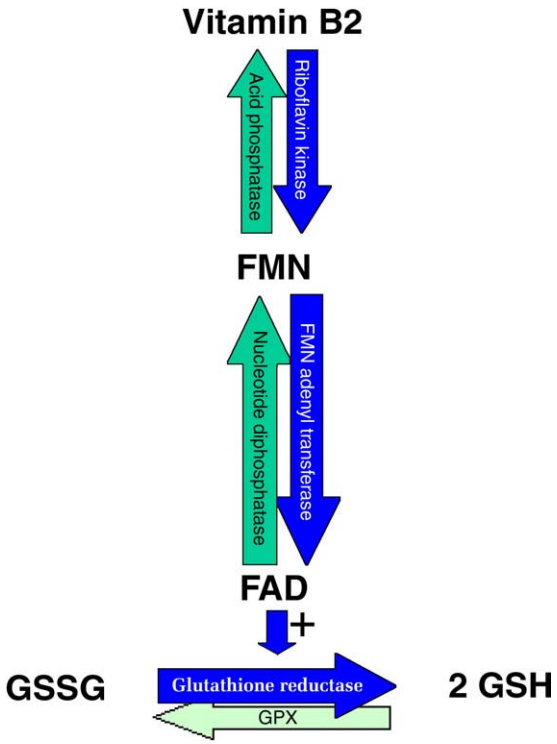

Fig. 3. A model of the possible interaction of ACP1 and GR based on the suggested mechanism by various previous authors [2,6,12,13,15] showing that GR activity might indirectly be influenced by ACP1 activity and nutritional riboflavin uptake.

In normal-weight women, however, this observation could not be made, as the difference between groups in mean GR activity ceased to give a significant value (P = .872) (Fig. 1).

The frequencies of each ACP1 genotype per BMI group in accordance to whether patients were classified as hypertensive or not were calculated (Table 2). Overall, the frequency of genotype AA was found to be significantly (P = .010) lower in the overweight and obese when hypertension was present. However, the drop in frequency of ACP1 genotype AA when comparing hypertensive and normotensive women did not give significant results in the overweight (P = .201) alone, although Fig. 2 might suggest otherwise. However, in the obese, the frequency of genotype AA was significantly (P = .028) lower in the hypertensive than in the normotensive women.

Inverse correlation between ACP1 activity and GR activity has been found in the hypertensive and obese (P = .022) but not in any other group.

Glutathione reductase activity was found to inversely correlate to blood levels of total cholesterol (P = .018) using the Spearman correlation test. Levels of GR activity were significantly (P = .003) higher in women with total cholesterol levels of less than 200 mg/dL when compared with women with levels exceeding that value.

Likewise, GR was shown to inversely correlate to LDL cholesterol levels using the Spearman correlation coefficient (P = .039). Values of GR activity were highest in the women presenting LDL cholesterol levels of less than 130 mg/dL, second highest in those with values between 130 and 159.99 mg/dL, and lowest in women having blood LDL cholesterol levels of more than 160 mg/dL.

No statistically significant correlation between HDL cholesterol levels and GR levels was found; however, a nonsignificant trend was observed for GR activity to be higher in individuals with HDL cholesterol levels of less than 40 mg/dL.

4. Discussion

In previous works, low-activity ACP1 phenotype has often been associated to an increased likelihood of obesity and to extreme body mass deviation in the already obese, as well as to a positive family history of obesity and a maximal rate of intrauterine growth [3,6,12,24]. It has been suggested that ACP1 influences BMI through its phosphotyrosine phosphatase action [3] via the modulation of glycolytic rate, insulin, and growth factor action [1-4]. Alternatively, it has also been suggested that ACP1 affects energy metabolism directly through controlling the availability of flavin mononucleotide, riboflavin, and FAD, acting as a flavin mononucleotide phosphatase [2,6,12,13,15]. However, in this study, a direct relationship between BMI and ACP1 genotype or activity could not be observed. Neither was ACP1 genotype or activity found to influence HOMA-IR. Instead, ACP1 genotype was found to correlate to ACP1 and GR activity as well as to LDL cholesterol levels.

A model of possible interaction between ACP1 and GR via ACP1's flavin mononucleotide phosphatase action is shown in Fig. 3. Here, FAD acts as coenzyme to GR, activating it, and is reversibly reduced to $FADH_2$ in the process [25]. This model could suggest that low ACP1 activities, as found in genotype AA, should show the highest GR activities by providing the most FAD. High ACP1 activities, as found in genotypes CC, AC, and BC, might however lead to a possible decrease in GR activity due to a lack in coenzyme. Nutritional riboflavin uptake might bias the effect ACP1 possibly has on GR because it provides a more direct source of FAD and thus probably has a greater effect on GR activity than ACP1 genotype or activity. Thus, it cannot be expected for GR and ACP1 activity to inversely correlate in the non–riboflavin deficient. In accordance with this, both enzymes were not found to correlate in the overall population of this study, suggesting sufficient levels of riboflavin. However, ACP1 genotype was found to have a significant effect on mean GR activity (P < .001); and ACP1 genotype AA was found to have the highest mean GR activity.

In literature, levels of GR activity have been found to be significantly lower in hypertensive patients than in the respective control groups [16,17]. These findings could be confirmed in this study (P = .001). A fact not taken into account in previous studies, however, when evaluating GR

activities with respect to hypertension was BMI. Fig. 1 shows the levels of mean GR activity in hypertensive and normotensive patients who were obese, overweight, or of normal weight, respectively. Although the level of GR activity rose in correlation to BMI in the normotensive (P = .006), it did not vary to a statistically significant degree in the hypertensive (P = .573). This is surprising because obesity has previously been reported to be closely associated to increased levels of oxidative stress and oxidative damage [26,27]. Evidence suggests that this may be a result of constant overeating as opposed to an effect of obesity itself. It has been shown that the production of reactive oxygen species (ROS) increases drastically after glucose, cream, and protein uptake as well as after a mixed meal in obese and nonobese populations [27-29]. Indexes of oxidative damage have been found to drastically decrease after 1 week of 1000-kcal/d dietary restriction as well as after a 48-hour fast in obese and normal subjects, respectively [27], making the hypothesis that the observed changes may be a result of weight loss improbable. As the glutathione system acts as ROS scavenger, one would thus expect to observe increasing values of GR activity as BMI increases, regardless of the presence of hypertension.

However, inadequate antioxidant enzyme response has been proposed to contribute to hypertension [16,17]. We support this hypothesis, suggesting that, as ROS production increases with the frequency of overeating in patients with higher BMI, antioxidant enzyme response to ROS might decompensate, favoring oxidative damage and possibly leading to hypertension. In a scenario of heightened oxidative stress, FAD demand by activated GR might exceed availability because dietary uptake of riboflavin might not be sufficient to counterbalance FAD loss, thus eventually leading to a decreased GR activity due to lack of its cofactor. Interestingly, Dandona et al [27] concluded in their work that a significant reduction in ROS production, oxidative stress, and oxidative damage can be achieved by dietary restriction and weight loss alone, without the need for antioxidant administration. Nevertheless, they found that, after ingestion of a mixed meal, in contrast to ingestion of purified nutrients such as glucose or cream, no single peak increase in ROS could be detected, an observation that was hypothetically explained by the presence of innate antioxidants in a mixed meal [27,30].

Negative correlation between ACP1 activity and GR activity has been reported previously when GR activity assay was performed in the absence of FAD [13]. The correlation disappeared when GR assay was carried out after preincubation with FAD [13]. Bearing in mind Fig. 3 and that the effect of nutritional riboflavin uptake might possibly outweigh the effect of ACP1 enzymatic activity on GR, we interpret these findings as an indication of riboflavin deficiency in the population studied. Thus, we propose a negative correlation of ACP1 activity and GR activity in the riboflavin deficient. Considering hypertension to be at least in part a result of inadequate antioxidant response and antioxidant stress to be highest in the obese, it is feasible that obese women presenting hypertension should be biochemically riboflavin deficient. In this study, negative correlation between ACP1 enzymatic activity and GR activity (P = .022) was found in the obese and hypertensive, but not in any other group, supporting this hypothesis (Fig. 4).

The observation that hypertensive obese women may be riboflavin deficient is of interest, as it may open the door to a possible treatment or at least to a possible reduction in the risk of hypertension for obese women through the correction of any such deficiency. Indeed, clinical data have been presented by Merchant et al [31] showing a significant reduction in the incidence of hypertension in HIV-positive pregnant women taking multivitamins including, among others, 20 mg of riboflavin. In their work, the hypothesis that the decrease in blood pressure observed after the administration of the multivitamin may be due to a reduction of

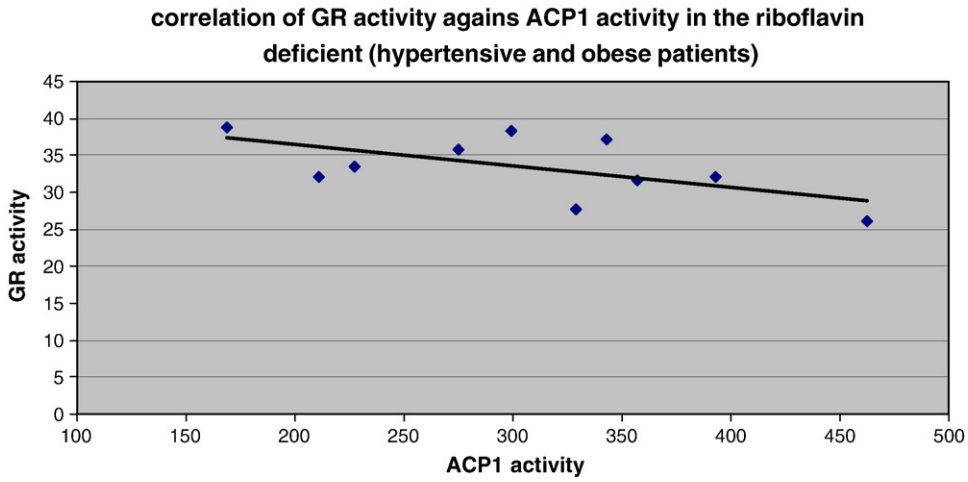

Fig. 4. Glutathione reductase activity and ACP1 activity showed inverse correlation in those women who were obese and hypertensive only (r = −0.626, P = .022). No direct or inverse correlation between ACP1 activity and GR activity could be observed in the overall population. However, mean GR activity was significantly different (P < .001) for each ACP1 genotype.

homocysteine levels through vitamin B6, vitamin B12, and folate action is discussed, as the other components of the multivitamin were not shown to have any effect on gestational hypertension. It is, however, also possible that the observed effect of that study may be due to riboflavin.

It seems probable that carriers of ACP1 genotype AA should show best endogenous protection against oxidative stress, as they were shown to have the highest levels of GR activity and high nutritional riboflavin uptake can only be expected to enhance that effect. Carriers of genotypes AC, BC, and CC can be expected to counterbalance worse endogenous protection against oxidative stress through high nutritional riboflavin uptake only. The findings of the study of Bottini et al [32] on the prevalence of ACP1 genotype and sex ratio in newborn infants of smoking mothers do appear to support this hypothesis.

Thus, carriers of ACP1 genotype AA might be expected to have a lower risk of developing obesity-related hypertension, as their system of GR-related antioxidant defense should be less likely to decompensate.

This hypothesis was tested by comparing the frequencies of ACP1 genotypes in the 3 weight groups for the cases of hypertension and normotension, respectively. Table 2 shows the results obtained. A tendency was shown for ACP1 genotype AA to be significantly less frequent in hypertensive patients ($P = .010$) when compared with normotensive patients (Fig. 2), supporting our hypothesis.

As our data suggest that a decreased GR activity may play a role in the development of hypertension, discussing its relationship to other factors of cardiovascular risk is of interest, albeit this subject has received little interest in recent international publications. Significantly reduced levels of GR activity have been found in grade I hypertensive patients [33], in non–insulin-dependent diabetes mellitus (NIDDM) type 2 patients with and without cardiovascular complications [34,35], especially in those of male sex [35], and in smokers [36,37]. By demonstrating an inverse correlation between HOMA-IR and GR activity ($r = -0.389$, $P < .001$), this study has provided data that support previously published findings of lower levels of GR activity in NIDDM type 2 patients [34,35]. However, the factor that most influenced HOMA-IR in this study was, not surprisingly, BMI (HOMA-IR and BMI showed a correlation coefficient of $r = 0.376$, $P < .001$). We suggest that the observed strong relationship between GR activity and HOMA was due to the increasing GR activity with increasing BMI in the non–riboflavin-deficient, nonhypertensive subjects, rather than being a result of any biochemical influence of these compounds on each other. This supposition is supported by the fact that no statistically significant correlation could be found between HOMA-IR and GR activity in hypertensive patients of any weight because of the already decreased GR activity in this group. Thus, the observed decrease in GR activity in NIDDM type 2 patients may be an expression of the presence of a metabolic syndrome including hyperlipidemia and hypertension, rather than due to the diabetes alone. Fig. 5 shows the inverse correlation of GR activity and HOMA-IR in nonhypertensive patients in relation to ACP1 genotype. The graph clearly shows that mean HOMA-IR is lowest in carriers of ACP1 genotype AA.

Glutathione reductase also influences cardiovascular risk by influencing cholesterol levels. Its activity was reported to be inversely correlated to very low-density lipoprotein and

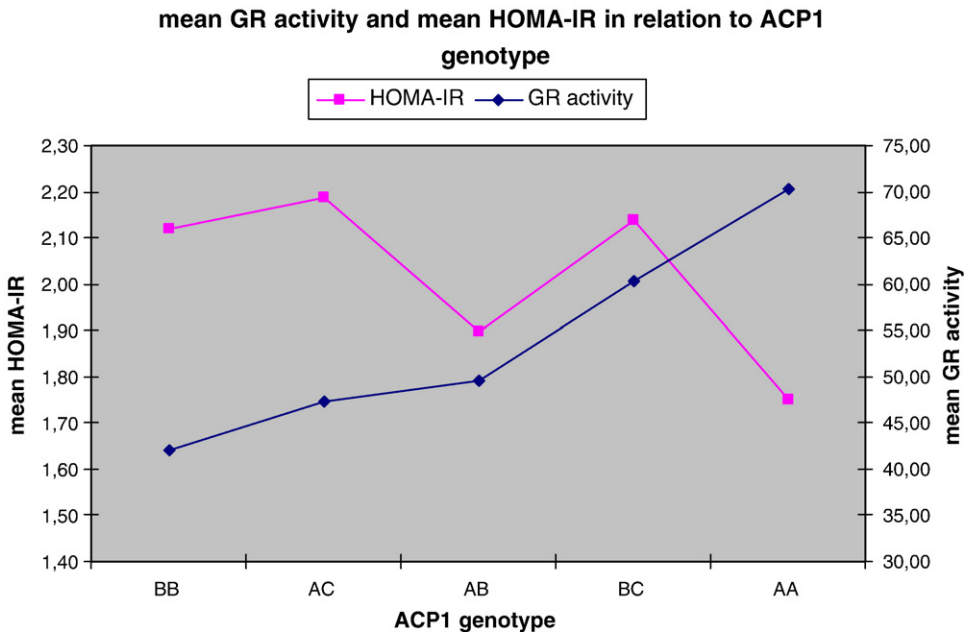

Fig. 5. The inverse relationship of mean GR activity and mean HOMA-IR in normotensive patients in relation to ACP1 genotype. Mean GR activity was significantly ($P < .001$) different for each ACP1 genotype. Differences in mean HOMA-IR were not significant between genotypes.

LDL cholesterol levels as well as to BMI in hypercholesterolemic children [38]. Serdar et al [39] discussed the progression of coronary artery disease in 1-, 2-, and 3-vessel disease, finding the odds ratio of lower GR and glutathione peroxidase levels to be increased in 3-vessel disease with respect to 1- and 2-vessel disease and control groups. These data may indicate that oxidative damage and inadequate antioxidative defenses may play a much more outstanding role in the development of cardiovascular pathologies than commonly assumed.

Cardiovascular risk is influenced by plasma lipid levels to no small degree. ACP1 allele A has previously been reported to exert a protective effect against hypertriglyceridemia and hypercholesteremia by Bottini et al [10], allegedly through its phosphotyrosine phosphatase action, dephosphorylating adipocyte lipid-binding protein. These findings could be confirmed by the present study in which carriers of the ACP1∗A allele were found to have the lowest levels of LDL cholesterol, increasing in the order AA < AB < AC < BB < BC. A nonsignificant trend was found showing total cholesterol levels to increase in the same order as LDL cholesterol levels, highlighting the possible protective effect of ACP1 allele A. With regard to HDL cholesterol, a nonsignificant trend was found presenting carriers of ACP1 allele C with the lowest levels of HDL cholesterol. No correlation could be found relating ACP1 genotype to triglyceride levels in this population.

Glutathione reductase was shown to inversely correlate to LDL cholesterol levels using the Spearman correlation coefficient ($P = .039$). Furthermore, GR activity was found to inversely correlate to blood levels of total cholesterol ($P = .018$), in agreement with previously published data [38]. Levels of GR activity were significantly ($P = .003$) higher in women with total cholesterol levels of less than 200 mg/dL when compared with women with levels exceeding that value. No statistically significant correlation between HDL cholesterol levels and GR levels was found; however, a nonsignificant trend was observed for GR activity to be higher in individuals with HDL cholesterol levels of less than 40 mg/dL.

To explain our findings, we hypothesize that a high GR activity, as found in patients with ACP1 genotype AA, may suppress cholesterol production in the endoplasmic reticulum by affecting hydroxymethylglutaryl–coenzyme A reductase activity indirectly. The reduction of glutathione disulfide (GSSG) to two molecules of glutathione (GSH) using GR requires reduced nicotinamide adenine dinucleotide phosphate ($NADPH/H^+$) as a proton donator producing $NADP^+$. The total amount of NADPH needed for this reaction is substantial, and up to 10% of total glucose uptake may be directed toward the pentose phosphate way to produce sufficient NADPH to maintain GR function [40]. However, 2 molecules of $NADPH/H^+$ are required in the reduction of hydroxymethylglutaryl–coenzyme A to mevalonic acid, the rate-limiting step in cholesterol synthesis. Thus, in high oxidative stress, NADPH may become little or even unavailable for the production of mevalonic acid, being used to a higher degree in the activated GR system. As total cholesterol levels may thus go down in any patient under high oxidative stress having high GR activities, the level of cholesterol available for the production of LDL lipoproteins in the liver may go down.

As described above, the maintenance of a high GR activity in oxidative stress is FAD and thus riboflavin and ACP1 dependent, possibly explaining the correlations found between ACP1 genotype and LDL cholesterol levels. Interestingly, should this hypothesis hold true, it would mean that high oxidative stress, as created by high glucose and cream uptake or by a mixed meal, would initially limit cholesterol production by indirect negative feedback through lack of $NADPH/H^+$. Should the GR system fail in high oxidative stress through lack of FAD, however, it may possibly lead to an increased production in cholesterol through an increased availability of $NADPH/H^+$ liberated from the GR system.

It should also be kept in mind that ROS are primarily produced by NADPH oxidase, an enzyme that was shown to be crucial in the pathogenesis of atherosclerosis and that uses NADPH for the production of superoxide radicals. p47phox is 1 of the 6 subunits of NADPH oxidase and is crucial for its function. It was shown to be induced by every meal [30]. Thus, the induction of NADPH oxidase by any meal may enhance the effect of a lack in NADPH initially inhibiting cholesterol synthesis in patients with an adequate antioxidant response through the glutathione system and sufficient supply of riboflavin. However, should the glutathione system fail through lack of riboflavin, as suggested above, there not only may be NADPH available for the production of LDL cholesterol, but also NADPH available for the production of additional ROS, producing oxidized LDL and favoring atherosclerosis. Interestingly, Yuvaraj et al [41] have recently published data showing the significant decrease in triglyceride and very low-density lipoprotein cholesterol in tamoxifen-induced lipid abnormalities after the coadministration of coenzyme Q10, riboflavin, and niacin.

The sum of our findings may indicate important implications of insufficient biochemical riboflavin levels in the development of cardiovascular disease through the possible influence on LDL cholesterol levels and the development of hypertension. However, little data have been published in international journals concerning riboflavin levels in cardiovascular risk groups and patients with diabetes.

To the best of our knowledge, no recent studies exist that specifically assess riboflavin status in human diabetic populations, warranting further investigation of the subject. However, riboflavin deficiency has been shown to be highly prevalent among diabetic rats and mice. Eighty-three percent of genetically diabetic KK mice were found to be deficient in riboflavin on a normal diet, showing significantly reduced GR activity [42], whereas 100% of streptozotocin-diabetic rats showed riboflavin deficiency and reduced GR activity [43].

It has been shown that riboflavin supplementation of 1.6 mg/d may significantly reduce levels of homocysteine in homozygote carriers of the 677C3T polymorphism of the

MTHFR gene by as much as 40% in those patients with lower riboflavin status at baseline and by 22% overall [44]. This may significantly reduce cardiovascular risk.

The impact riboflavin deficiency may have is shown by the fact that 27% of heart failure patients were found to be deficient in riboflavin compared with 2% in healthy controls. In those heart failure patients not taking multivitamin supplements, 42% were found to be deficient compared with 22% of heart failure patients on supplements. These findings could not be related to the presence of renal disease or the use of diuretics in the studied group [45]. The benefits of supplementing congestive heart failure patients with micronutrients including, among others, riboflavin, in addition to standard therapy with β-blockers and angiotensin-converting enzyme inhibitors, have been demonstrated. A significant reduction in left ventricular volume and a 5% improvement in ejection fraction as well as some small improvements in life-quality scores were observed after 9 months of treatment, although no improvements in New York Heart Association score or 6-minute walk time were observed [46].

5. Conclusion

We propose insufficient antioxidant response through biochemical riboflavin deficiency and low GR activity to be a possible risk factor for developing obesity-related hypertension as well as high plasma LDL cholesterol levels. It has been suggested by us that ACP1 genotype AA constitutes a protective factor not only against oxidative stress–related hypertension, but also against other cardiovascular risk factors such as plasma lipid levels and increased HOMA-IR. Likewise, we suggested that high nutritional riboflavin uptake becomes increasingly crucial as BMI increases, to maintain GR activity high and thus antioxidant defenses at their best, minimizing cardiovascular risk.

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