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Study of Cytochrome P450 2E1 and its Allele Variants in Liver Injury of Nondiabetic, Nonalcoholic Steatohepatitis Obese Women

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ABSTRACT

CYP2E1 enzyme is related to nonalcoholic steatohepatitis (NASH) due to its ability for reactive oxygen species production, which can be influenced by polymorphisms in the gene. The aim of this study was to investigate hepatic levels, activity, and polymorphisms of the CYP2E1 gene to correlate it with clinical and histological features in 48 female obese NASH patients. Subjects were divided into three groups: (i) normal; (ii) steatosis; and (iii) steatohepatitis. CYP2E1 protein level was assayed in microsomes from liver biopsies, and *in vivo* chlorzoxazone hydroxylation was determined by HPLC. Genomic DNA was isolated for genotype analysis through PCR. The results showed that liver CYP2E1 content was significantly higher in the steatohepatitis (45%; p=0.024) and steatosis (22%; p=0.032) group compared with normal group. Chlorzoxazone hydroxylase activity showed significant enhancement in the steatohepatitis group (15%, p=0.027) compared with the normal group. c2 rare allele of *Rsa1/Pst1* polymorphisms but no C allele of *Dra1* polymorphism was positively associated with CHZ hydroxylation, which in turn is correlated with liver CYP2E1 content (r=0.59; p=0.026). In conclusion, c2 allele is positively associated with liver injury in NASH. This allele may determine a higher transcriptional activity of the gene, with consequent enhancement in pro-oxidant activity of CYP2E1 thus affording liver toxicity.

Key terms: steatohepatitis, NASH, CYP2E1, obesity, genotype, chlorzoxazone.

INTRODUCTION

NASH is a progressive liver pathology associated to etiologic factors such as obesity, hyperlipidemia, insulin resistance, and type-II diabetes mellitus, with obesity being the most important (James and Day, 1998; Matteoni et al., 1999; Angulo et al., 1999; Ratziu et al., 2000). This disorder is observed in patients with no history of significant alcohol consumption, however histologically it resembles alcohol-induced liver injury (Ludwig et al., 1980). Fatty liver, the earliest and most prevalent stage of NASH, is thought to sensitize the liver to additional necroinflammatory insults, thus promoting disease progression to steatohepatitis and cirrhosis (Matteoni et al., 1999; Angulo et al., 1999). A number of metabolic parameters are related to NASH. These include imbalances in amino acid, glucose, and antiketogenic and ketogenic hormones in portal blood, mitochondrial dysfunction (Matteoni et al., 1999), pro-

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inflammatory cytokine release (Ratziu et al., 2000), oxidative stress, and CYP2E1 induction (Ludwig et al., 1980; Weltman et al., 1998; Videla et al., 2004a). CYP2E1 has a significant role in the biotransformation of ethanol and other xenobiotics, the gluconeogenesis from ketones, and fatty acid oxidation, being a major microsomal source of ROS (González, 2005). This enzyme is induced by pesticides, thyroid hormones, alcohol and ketones, the latter usually found in obesity, insulin resistance, and diabetes (Pagano et al., 2002; Fernández et al., 2003; Wang et al., 2003), thus contributing to liver pathologies such as alcoholic liver disease and non-alcoholic steatohepatitis (Lieber, 2004; Ishii, 2004).

From the pathogenic point of view, CYP2E1-derived ROS could represent a crucial factor in determining liver injury, particularly when antioxidant defenses are depleted (Videla et al., 2004a), thus contributing to the enhancement in lipid peroxidation, protein carbonylation, and DNA oxidation observed in NASH patients (Robertson et al., 2001). In addition, higher CYP2E1 activity is seen in a variety of conditions such as fasting, obesity, diabetes, and high-fat/low-carbohydrate diets (Yoo et al., 1991; Robertson et al., 2001; Pagano et al., 2002; Wang et al., 2003; Lieber, 2004, Ishii, 2004). The relationship between CYP2E1 and NASH was originally suggested in a rat nutritional model of NASH (Weltman et al., 1996) and was later confirmed in human NASH (Weltman et al., 1998), with CYP2E1 up-regulation being observed in clinical settings that are associated with steatohepatitis, namely, diabetes mellitus and obesity (Lucas et al., 1998). Furthermore, hepatic CYP2E1 protein expression and activity are higher in livers from obese patients with steatohepatitis than in those with uncomplicated steatosis (Chalasani et al., 2003) and correlated positively with the severity of liver damage (Videla et al., 2004a; Leclercq, 2004; Orellana et al., 2006), suggesting that CYP2E1 induction is related to progression of NASH.

The human CYP2E1 hepatic mRNA, protein, and enzyme activity exhibit an important interindividual variability (Lucas et al., 1993). It has been proposed that genetic polymorphisms of the gene encoding CYP2E1 could contribute to such variability and to the development of pathologies such as hepato-cellular carcinoma (Yu et al., 1995) and alcoholic liver disease (Tsutsumi et al., 1994). In addition, profound ethnic differences in CYP2E1 allele frequencies have previously been reported among Asians, Caucasians and Africans, which may explain ethnic differences in related metabolic activity and disease (Stephens et al., 1994).

Several polymorphic sites are found in the CYP2E1 gene. Dra1 polymorphism in the intron 6 of the gene deletes a restriction site for the enzyme Dra1 due to a transversion from adenine to thymine in the base 7,668, which is called "rare allele C" or CYP2E1*6 (Hu et al., 1997). The final effect of this change on CYP2E1 activity has not been determined. In addition, Rsa1 polymorphism consists in a transition from cytosine to thymine upstream of the gene in the regulatory region: 1,019. Furthermore, Pst1 polymorphism is produced by transversion from guanine to cytosine in the position 1,259, very close to Rsa1 polymorphism (Watanabe et al., 1994). These latter two polymorphisms are found in linkage disequilibrium and their rare allele is called c2 or CYP2E1*5B (Quiñones et al., 1999). The functional relevance of the Rsa1 polymorphism is due to its location in the binding site of the hepatic nuclear factor 1 (HNF-1), a liver-specific transcription factor that enhances the transcriptional activity of the human CYP2E1 promoter (Hayashi et al., 1991; Watanabe et al., 1994; Quiñones et al., 1999). Unlike alcoholic fatty liver disease (ASH), the metabolic features associated with NASH, such as fat accumulation, insulin resistance, and increased ketone bodies, would favor a higher transcriptional activity of the CYP2E1 gene (Yun et al., 1992; Zangar and Novak, 1997; Woodcroft et al., 2002). Based on these antecedents, the aim of this study was to investigate the role of functionally relevant CYP2E1 polymorphisms in NASH. For this purpose, the content and activity of CYP2E1 and the allele variants for the Dra1, Rsa1, and Pst1 polymorphisms were

analyzed in relation to the clinical, histological, and pathophysiological aspects of NASH.

METHODS

Patients and laboratory research

Forty-eight voluntary women subjected to gastroplasty or gastrectomy with a gastro-jejunal anastomosis were included in this study. A complete clinical history, including data on nutrition and alcohol consumption together with anthropometric measurements were obtained. Laboratory tests included liver enzymes, bilirubin, albumin, hepatitis B and C serology, and autoantibodies (anti-nuclear, antimitochondrial, and anti-smooth muscle antibodies). In addition, serum levels of ferritin, transferrin saturation, ceruloplasmin, cholesterol, triacylglycerols, low-density lipoprotein, high-density lipoprotein, and glucose were also determined. The index of insulin resistance was evaluated by Homeostasis Model Assessment (HOMA), calculated as (fasting insulin x fasting glucose)/22.5. Patients with significant alcohol consumption (more than 40 g of alcohol per week), previous use of steatogenic medications or any blood tests suggesting other specific liver diseases (e.g., viral hepatitis, autoimmune liver disease, primary biliary cirrhosis) or diabetes were excluded. Selected patients were subjected to a diet of 25 kcal/kg (30% lipids, 15% proteins), for at least 2 days prior to surgery. Liver biopsies of approx. 2 cm^3 were taken during the surgery for histological diagnosis. A half of each liver samples were fixed in 10% formaldehyde and paraffin-embedded, and sections were stained with either haematoxylin/eosin or Van Gieson's stains. Sections of each liver biopsy were blindly observed and evaluated for histological abnormalities by means of a previously defined code (Videla et al., 2004b). Obese patients were divided into three groups: (a) normal group (normal liver histology); (b) patients with steatosis (5-100% macrovesicular steatosis); and (c) patients with steatohepatitis (steatosis and lobular inflammation with hepatocyte ballooning, with or without fibrosis). The second half of the liver samples were stored at -80ºC for about 48 h until used for microsome preparation for immmunoblot.

The Ethics Committee of the Clinical Hospital of the Universidad de Chile approved the study protocol, according to the 1964 (Revised in October 2000) Declaration of Helsinki. Informed consent for using part of the blood and liver samples was obtained from all patients.

Immunoblotting

Liver microsomes were prepared by ultracentrifugation (Orellana et al., 2006) from at least 1 g of liver biopsies, and total microsomal protein content was measured according to Lowry et al. (1951), using bovine serum albumin (BSA) as standard. The liver CYP2E1 content was determined by Western blot. A polyclonal antibody to hepatic cytochrome P450 2E1 (Daiichi Pure Chemicals Co. Ltd., Tokyo, Japan) was used to detect the enzyme in 20 μg of microsomal protein per lane in Western Blot experiments (Towbin et al., 1979). Alkaline phosphatase-linked secondary antibody was used, and the bands were stained with Nitroblue tetrazolium (NBT)/ 5-bromo-4-cloro-3-indolylphosphate (BCIP) solution. Quantification of CYP2E1 was performed using acetone-treated rat liver microsomes containing cytochrome P450 2E1 (3 pmol/blot) as standard (Daiichi Pure Chemicals Co. Ltd.).

In vivo CYP2E1 activity assay

CYP2E1 activity was measured using CHZ as an *in vivo* probe in patients prior to surgery. This muscle relaxant has been proposed as a specific probe for CYP2E1 activity (Lucas et al., 1993a). Subjects were asked to avoid certain foods (grapefruit, vegetables from the mustard-green family, and beverages containing xanthine and alcohol) for at least 72 hours. Any medications were discontinued one week before this test to reduce the chances of drug interference. Blood samples were collected for 2 hours after administering a 500 mg

dose of CHZ orally; blood samples from each group of patients were received in plastic tubes with EDTA, centrifuged immediately and the serum frozen at -20 ºC until used. The serum concentrations of CHZ and 6-hydroxy-CHZ were separated and determined by reverse-phase high-performance liquid chromatography (HPLC), after de-conjugation with 2,000 units of β-glucuronidase at 37°C for 1 hour by using a LiChrospher® 100 RP-18 (5μm) column and acetonitrile/acetic acid, 30/70 (v/v) at a flow of 1 ml/min, measuring the absorbance at 285 nm. The CYP2E1 activity was expressed as 6-OH-CHZ/CHZ ratio.

Blood samples and DNA extraction

Blood samples were collected on EDTA from all women and used to obtain genomic DNA from peripheral leukocytes following the method of Miller et al. (1988). The genomic DNA was examined for purity at 260/280 nm absorption and re-purified with phenol/chloroform protocol as required. DNA was stored at -30ºC until used.

Genotyping methods

After DNA extraction, polymerase chain reaction (PCR) based restriction fragment length polymorphism (RFLP) was used to examine the polymorphism of interest. All samples were submitted to separate amplifications followed by digestion with appropriate restriction enzymes. *Dra1* alleles detection was carried out using the primers described by Hirvonen et al. (1993), yielding a 373 bp fragment used to amplify between 7421 and 7793 nucleotides inside intron 6 of the gene. For the *Rsa1* and *Pst1* polymorphisms, primers yielding a 413 bp fragment were used to amplify between -1372 and -960 nucleotides upstream of the gene to detect both sites (*Rsa1*/*Pst1*) (Hayashi et al., 1991). The PCR products were subjected to digestion with *Dra1*, *Rsa1* and *Pst1* restriction enzymes at 37ºC for 1h (GIBCO BRL, Life Technologies, Inc., Gaithersburg, MD, USA). The samples were then analyzed by agarose 3% gel electrophoresis (Bio-Rad Lab., Richmond, CA, USA).

Statistical analysis

Interval variables are expressed as means ± SEM, with the indicated number of samples unable to be processed (missing data). For categorical variables, frequencies and percentages were used. The differences were considered statistically significant at $p<0.05$. To compare interval variables and categorical variables among different groups we employed Student *t* test and chi^2, respectively. Odds ratios (OR) and 95% Confident Interval (CI95%) were used to compare the magnitude of fatty liver disease risk between the different group.

RESULTS

Table I shows the general clinical and biochemical characteristics of the normal group and patients with steatosis and steatohepatitis. Age, HDL, LDL, fasting glucose, AST, γGT, bilirubin, and albumin are comparable among groups. However, significant differences are observed between normal and steatohepatitis groups in body mass index (BMI), triacylglycerols, fasting insulin, HOMA, and ALT, and between normal and steatosis groups in cholesterol and HOMA. Examples for genotypic analysis of *Dra1*, *Rsa1,* and *Pst1* polymorphism are shown in Figure 1 (A and B), where patterns for obese women with different genotypes are observed after PCR amplification and restriction enzyme digestion. Table II shows the distribution of different *Dra1* and *Rsa1*/*Pst1* genotypes in the studied groups, where C* correspond to a group of CD and CC genotypes and c2* correspond to a group of c1c2 and c2c2 (alleles with the nucleotide exchanges). The allele frequencies for C (fc) were 0.115, 0.139, and 0.235 for normal, steatosis, and steatohepatitis groups, respectively, whereas those for c2 (fc2) were 0.115, 0.055 and 0.235, respectively. The risk of steatosis and steatohepatitis in women carriers of C*-susceptibility allele variants was 71% greater compared with the normal group. In the case of c2*, the risk was close to null value for steatosis. On the other hand, the risk of steatohepatitis in women

carriers of c2*-susceptibility allele was 75% greater compared with the normal group.

The immunodetection of the CYP2E1 enzyme in microsomes obtained from liver biopsies of women with normal liver histology, steatosis, or steatohepatitis was standardized using rat CYP2E1 (Fig. 2 A). The results obtained through band densitometry analysis of Western blots from patients with wild-type alleles (c1c1 and DD) show statistically significant increases in the amount of CYP2E1 in steatosis and steatohepatitis in relation to the normal group, 22% and 45% respectively (Fig. 2 B).

In order to analyze whether the observed increase in the immunodetected amount of CYP2E1 is reflected in the enzyme activity, we assayed the in vivo CYP2E1-dependent hydroxylation of CHZ in the studied groups, expressed as the ratio between the resulting product 6-OH-CHZ and the substrate CHZ. The results showed comparable values in the steatosis and normal groups, whereas those in the steatohepatitis group were significantly higher than the normal group (35%; p=0.027) (Fig. 3). Furthermore, *in vivo* CHZ hydroxylation and liver CYP2E1 content are significantly correlated (r=0.59; p=0.026).

The analysis of the relationship between *in vivo* catalytic activity of CYP2E1, assayed as CHZ hydroxylase, and genotypes is shown in Figure 4. The results show that the increase in CYP2E1 activity is statistically significant for c2* comprising c1c2 and c2c2 genotypes (Fig. 4 A), whereas no significant association is observed between CHZ hydroxylation for C* comprising CD and CC genotypes (Fig. 4 B).

TABLE 1

Clinical and biochemical characteristics of obese patients with non-alcoholic fatty liver disease with different degrees of liver injury

Parameters	Liver histology				
	Normal (Controls) (n=13)	Steatosis (n=18)	p Value (n=17)	Steatohepatitis	p Value
Age (years)	40 ± 3.3	43 ± 4.3	0.609	41 ± 3.5	0.841
BMI (kg/m^2)	37 ± 1.3	36 ± 2.1	0.715	50 ± 2.3	<0.001*
Cholesterol (mg/dL)	151 ± 12.0	189 ± 12.0	0.038*	183 ± 11.2	0.064
Triacylglycerols (mg/dL)	107 ± 15.0	134 ± 27.2	0.441	189 ± 33	0.050*
LDL (mg/dL)	112 ± 9.5	126 ± 10.5	0.351	102 ± 6.9	0.390
HDL (mg/dL)	51 ± 4.5	43 ± 3.5	0.165	42 ± 3.3	0.110
Fasting glucose (mmol/L)	5.3 ± 0.5	5.9 ± 0.4	0.352	5.6 ± 0.9	0.791
Fasting insulin (μU/ml)	14.1 ± 2.3	25 ± 4.5	0.064	37 ± 7.3	0.013*
HOMA	3.3 ± 0.7	6.5 ± 0.8	0.008*	8.7 ± 0.8	<0.001*
AST (IU/L)	26 ± 3.0	36 ± 7	0.257	31 ± 3.5	0.306
ALT (IU/L)	32 ± 3.2	50 ± 11	0.186	48 ± 5.5	0.028*
γ-GT (IU/L)	39 ± 6.5	45 ± 7.9	0.584	37 ± 3.2	0.769
Bilirubin (mg/dL)	0.6 ± 0.1	0.5 ± 0.1	0.497	0.6 ± 0.1	1.000
Albumin (g/dL)	4.3 ± 0.2	4.3 ± 0.1	1.000	4.1 ± 0.1	0.345

Values represent means ± SEM for the number (n) of subjects indicated.. Abbreviations: BMI, body mass index; LDL, low density lipoprotein; HDL, high density lipoprotein; AST, aspartate aminotransferase; ALT, alanine aminotransferase; γ-GT, γ-glutamyltranspeptidase;. HOMA, homeostasis model assessment.
* Significant p values in relation to controls.

TABLE 2

CYP2E1 genotype distribution and allele frequencies (*f*) according to hepatic injury in obese patients with non-alcoholic fatty liver disease

	Liver histology						
	Normal n(%)	Steatosis n(%)	ORa	CI95%	Steatohepatitis n(%)	ORa	CI95%
Dra1							
DD	10(21)	13(27)	Ref	Ref	10(21)	Ref	Ref
CD	3(6)	5(10)	1.71	0.27-10.65	6(13)	2.0	0.30-14.15
CC	0	0	-	-	1(2)	-	-
C*	3(6)	5(10)	1.71	0.27-10.65	7(15)	1.71	0.30-9.59
*f*c	0.115	0.139			0.235		
Rsa1/Pst1							
c1c1	10(21)	16(33)	Ref	Ref	10(21)	Ref	Ref
c1c2	3(6)	2(4)	0.98	0.07-14.18	6(13)	2.0	0.30-14.15
c2c2	0	0	-	-	1(2)	-	-
c2*	3(6)	2(4)	0.98	0.07-14.18	7(15)	1.75	0.10-3.17
*f*c2	0.115	0.055			0.235		

Abbreviations: f, frequency of the indicated alleles; Ref, Reference allele (wild type); ORa, Age adjusted Odd ratios; CI95%, 95% Confident interval.

DISCUSSION

The pathogenic mechanisms underlying the development of NASH are not completely understood, particularly how apparently diverse etiologies such as alcohol consumption and obesity can give rise to similar histological features (Lieber, 2004; Ishii, 2004). Contrarily to NASH, several studies related to CYP2E1 polymorphisms and alcoholic fatty liver disease (ASH) are available, although they are highly contradictory. In fact, in the Japanese population, Tsutsumi et al. showed an association between c2 allele and ASH (Lucas et al., 1993b), while Maezawa et al (1994) reported an association with c1 allele. In the North American population, Carr et al. found no association between *Rsa1* polymorphism and ASH (Carr et al., 1995), whereas Pirmohamed et al. showed a significant correlation between c2 allele and ASH [Pirmohamed et al., 1995). These observed differences could be due to: (i) clinical differences among patients; (ii) ethnic variations in the studied populations; and (iii) the mechanism for the increase of CYP2E1 activity involved. In ASH, up-regulation of hepatic CYP2E1 activity is exerted at the post-translational level due to ethanol effect on protein stabilization (Carroccio et al., 1994), therefore no association with polymorphisms should be expected. Conversely, metabolic conditions associated with NASH could act at genetic level modifying the transcriptional activity of the CYP2E1 gene, which in turn, may also be influenced by polymorphisms.

Data presented in this work for *in vivo* CYP2E1 activity, immunodetected amounts of liver biopsy CYP2E1, and genetic polymorphisms, were obtained from non-diabetic, morbidly obese patients, excluding diabetic women and men, as previous studies show controversial results in relation to the effect of glucose on hepatic CYP2E1 amount and activity (Wang et al., 2003; Woodcroft et al., 2002) and sex as a factor in CYP2E1 levels (Dekant et al., 1995).

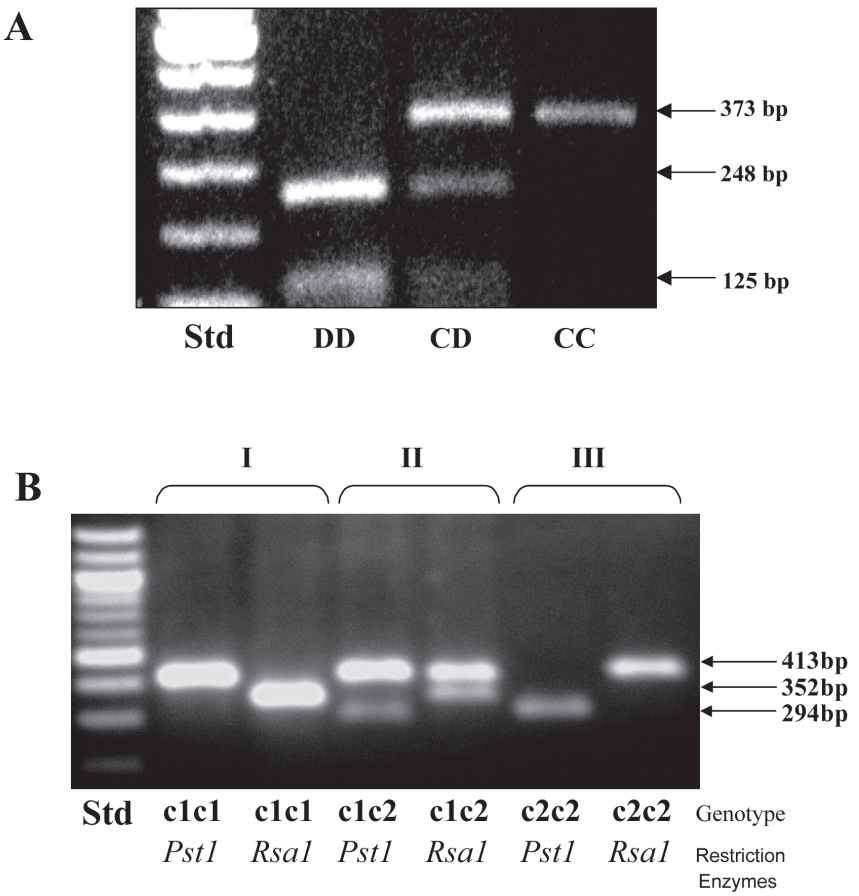

Figure 1: Representative profiles of RFLP-PCR analysis of CYP2E1 for *Dra1* polymorphism (A) and *Rsa1* and *Pst1* polymorphisms (B) in obese patients. In A, patients exhibit DD, CD, and CC genotypes. The 248 bp and 125 bp fragments obtained after *Dra1* digestion represent wild alleles (D) and the 373 bp fragment corresponds to the rare allele (C). In B, the 352 bp fragment found after *Rsa1* digestion represents the wild allele (c1) and that of 413 bp, the rare allele (c2). The 294 bp fragment obtained after *Pst1* digestion corresponds to the rare allele (c2) and that of 413 bp represents the wild-type allele (c1).

Patients studied show liver function tests within normal ranges, in agreement with previous observations (García-Monzon et al., 2000; Mofrad et al., 2003). In this clinical setting, obese patients with steatosis exhibited hepatic CYP2E1 levels 22% higher than those with normal liver histology, which is not reflected in significant changes in the *in vivo* CYP2E1 activity assessed by the CHZ hydroxylation assay. In patients with NASH, however, liver CYP2E1 content is 45% higher than normal values in concomitance with the 15% enhancement of *in vivo* CYP2E1 activity, suggesting the attainment of a functionally significant CYP2E1 induction in these patients. Data reported indicate that *in vivo* CHZ hydroxylation constitutes a non-invasive and reliable indicator of CYP2E1 activity under conditions of enhanced gene expression. Enhancement in liver CYP2E1 content and activity with severe morphological alterations assessed by liver histology is observed in patients with insulin resistance, independently on CYP2E1 genotype. These observations suggest that CYP2E1 induction is a major factor for liver injury in obese patients with NASH, probably due to substantial attenuation of the repressive effect of

insulin on CYP2E1 expression (Fig. 5), which is normally exerted at transcriptional and post-transcriptional levels (Woodcroft et al., 2002). In addition, specific relevant polymorphic sites in the CYP2E1 gene may represent contributory factors for CYP2E1 induction (Fig. 5), as the ethanol effect in ASH (23, 38, 40). In fact, CYP2E1 variant allele c2 shows a statistically significant association with the *in vivo* CHZ hydroxylase activity, which in turn is related to liver injury in obese women. *In vitro* studies have shown that rare c2 allele is associated with higher transcriptional activity protein expression and enzyme activity than the wild-type c1 allele (Hayashi et al., 1991; Watanabe et al., 1994).

Recent works suggest that the main factor responsible for increase of CYP2E1 expression is insulin rather than ketone bodies, both in diabetic or fasting mammals (Woodcroft et al., 2002) and in rat hepatocytes with metabolic syndrome associated with NASH (Pagano et al., 2002). Through kinases IP3, P70 S6 and Src, this hormone would exert a suppressive effect on the CYP2E1 expression by the concerted action of enhanced CYP2E1 mRNA turn-over and inhibition of

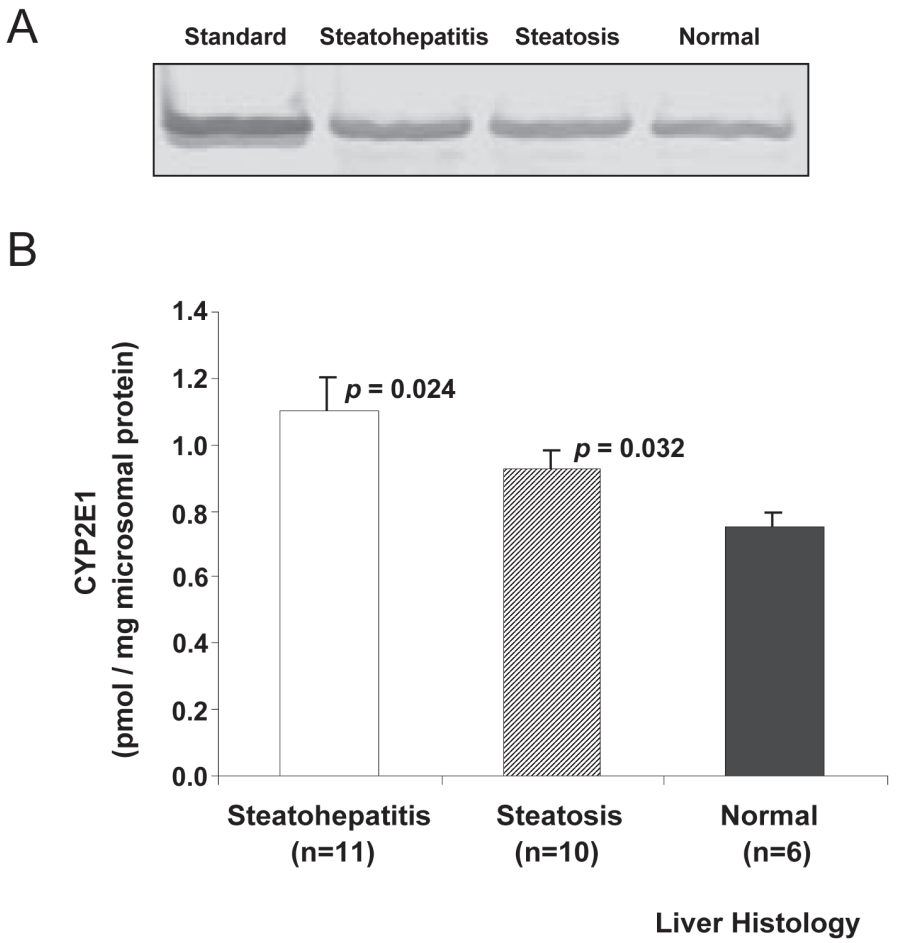

Figure 2: (A) Representative SDS-PAGE of microsomal protein (20 mg per well) of liver biopsy samples from obese women with normal liver histology and patients with steatosis or steatohepatitis, using 3pmol/well of standard rat CYP2E1 (MW= 56 Kda) for quantification of the CYP2E1 amount. (B) Respective densitometric quantification expressed as means ± SEM for the indicated number in parenthesis of patients (missing data = 11); p-values are calculated in relation to the normal group (normal liver histology).

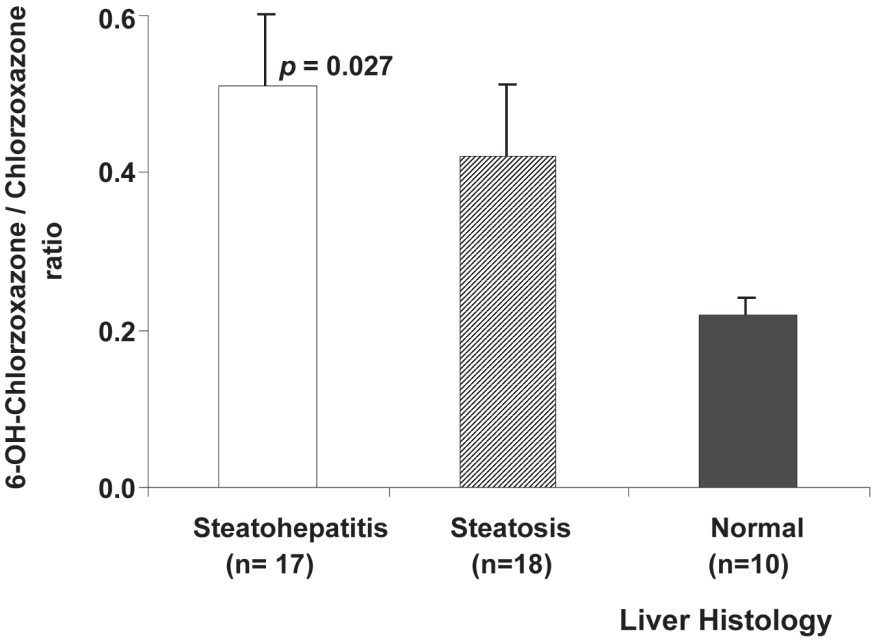

Figure 3: *In vivo* chlorzoxazone (CHZ) hydroxylase activity of CYP2E1 in obese patients with normal liver histology and patients with steatosis or steatohepatitis. Values represent the means ± SEM for the indicated number of patients (missing data = 3), expressed as the 6-hydroxy-CHZ (6-OH-CHZ)/CHZ ratios; p-values are calculated in relation to the normal group (normal liver histology).

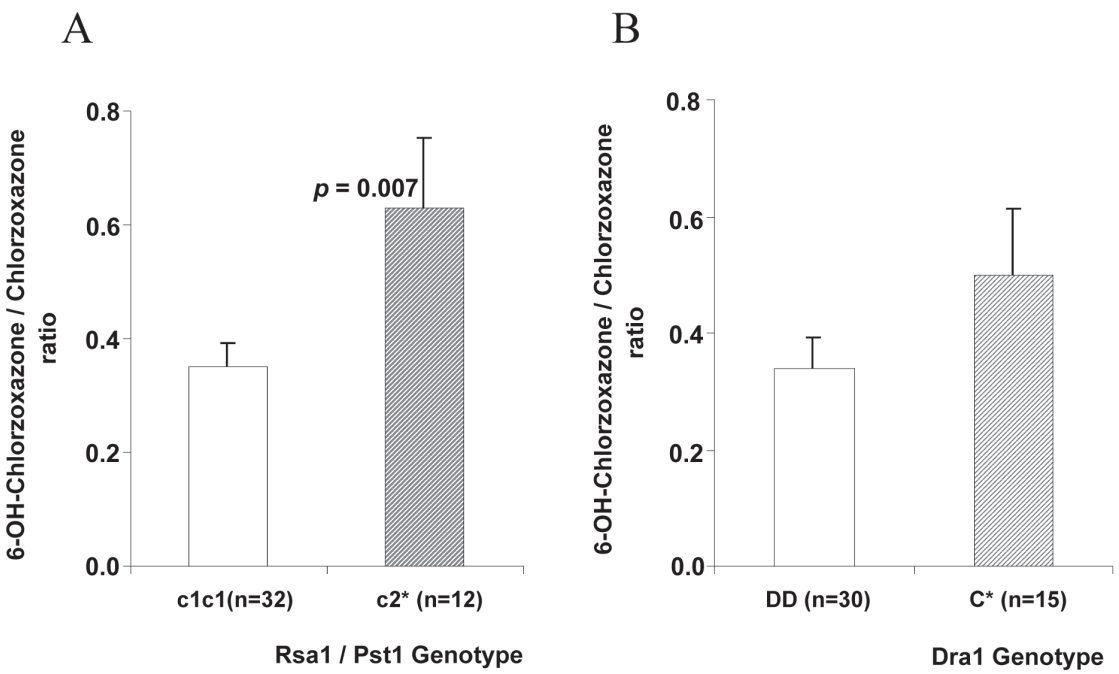

Figure 4: Analysis of *in vivo* chlorzoxazone hydroxylase activity in wild-type and rare genotypes of CYP2E1 in obese women (missing data = 3). (A) *Rsa1*/*Pst1* genotypes, in which c2* correspond to c1c2 or c2c2 genotypes. (B) *Dra1* genotype, in which C* corresponds to CD or CC genotypes. The p-values shown are obtained from comparison between rare and wild-type genotypes.

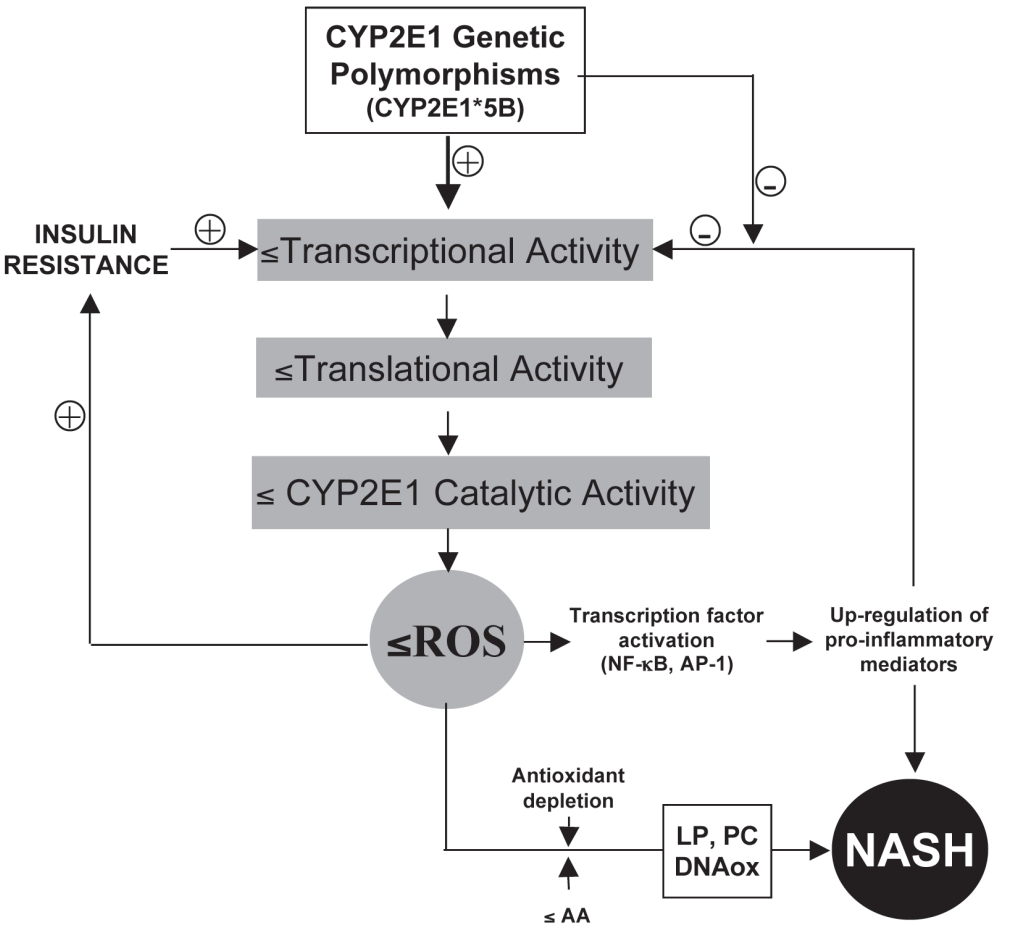

Figure 5: Factors that influence liver CYP2E1 expression, reactive O2 species (ROS) production, and development of nonalcoholic steatohepatitis (NASH) associated with obesity. Abbreviations: NF-κB, nuclear factor-κB; AP-1, activating protein-1; AA, arachidonic acid; LP, lipid peroxidation; PC, protein carbonylation; DNAox, DNA oxidation.

transcription of the gene. However, Schattenberg et al. (2005) postulated that overexpression of CYP2E1 leads to impairment of hepatic insulin signaling (down-regulation), potentially contributing to insulin resistance associated with NASH, an effect that may be due to the ability of CYP2E1 to induce oxidative stress (Fig. 5).

Together, these issues indicate that the relationship between NASH and insulin resistance is complex, therefore pointing to further studies of genetic expression factors on CYP2E1 in NASH, in addition to metabolic and environmental factors.

On the other hand, it has been shown that CYP2E1 genetic polymorphisms may avoid the down-regulation of transcriptional activity of this gene by proinflammatory cytokines, leading to more severe pathology due to an increase in the toxic potential of the enzyme (Mofrad et al., 2003). In this respect, changes in HNF1α binding to the CYP2E1 promoter region also have been suggested as potential suppression mechanisms of CYP2E1 expression (Roe et al., 2001; Hakkola et al., 2003), where *Rsa1* polymorphism could affect the negative feedback regulation mediated by cytokines. Our results show that CYP2E1*5B genetic polymorphism, which has been related to increase of CYP2E1 expression (Hayashi et al., 1991; Watanabe et al., 1994), influence the significant statistical association between CYP2E1 level and liver injury.

In conclusion, polymorphism of CYP2E1 and insulin resistance are associated with higher levels and activity of the enzyme in obese patients with NASH, which have been related to increased risk of liver damage through enhancement of ROS production (Fig. 5). Therefore, in NASH etiology the occurrence of genetic polymorphisms increasing CYP2E1 expression could represent susceptibility factors to acquire insulin resistance mediated by oxidative stress.

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