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Higher Activity of Polymorphic NAD(P)H:quinone Oxidoreductase in Liver Cytosols from Blacks Compared to Whites

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Abstract

In human liver, the two-electron reduction of quinone compounds such as menadione is catalyzed by cytosolic carbonyl reductase (CBR) and NAD(P)H:quinone oxidoreductase (NQO1) activities. We assessed the relative contributions of CBR and NQO1 activities to the total menadione reducing capacity in liver cytosols from black (n = 31) and white donors (n = 63). Maximal menadione reductase activities did not differ between black (13.0 ± 5.0 nmol/min.mg), and white donors (11.4 ± 6.6 nmol/min.mg; $p = 0.208$). In addition, both groups presented similar levels of CBR activities (CBR_{blacks} = 10.9 ± 4.1 nmol/min.mg versus CBR_{whites} = 10.5 ± 5.8 nmol/min.mg; $p = 0.708$). In contrast, blacks showed higher NQO1 activities (two-fold) than whites (NQO1_{blacks} = 2.1 ± 3.0 nmol/min.mg versus NQO1_{whites} = 0.9 ± 1.6 nmol/min.mg, $p < 0.01$). To further explore this disparity, we tested whether NQO1 activity was associated with the common *NQO1**2 genetic polymorphism by using paired DNA samples for genotyping. Cytosolic NQO1 activities differed significantly by *NQO1* genotype status in whites (NQO1_{whites}[*NQO1**1/*1] = 1.3 ± 1.7 nmol/min.mg versus NQO1_{whites}[*NQO1**1/*2 + *NQO1**2/*2] = 0.5 ± 0.7 nmol/min.mg, $p < 0.01$), but not in blacks (NQO1_{blacks}[*NQO1**1/*1] = 2.6 ± 3.4 nmol/min.mg versus NQO1_{blacks}[*NQO1**1/*2] = 1.1 ± 1.2 nmol/min.mg, $p = 0.134$). Our findings pinpoint the presence of significant interethnic differences in polymorphic hepatic NQO1 activity.

Keywords

Carbonyl reductase; NAD(P)H:quinone oxidoreductase; Ethnicity; Genotype; Liver; Menadione; Quinones

1. Introduction

Hepatic cytosolic carbonyl reductase (CBR) and NAD(P)H:quinone oxidoreductase (NQO1) catalyze the reduction by two-electron transfer of a wide range of quinone compounds using NADP(H) as cofactor. The resulting hydroquinone derivatives are rapidly converted to glucuronyl or sulfate conjugates which prevents subsequent reoxidation to quinones (Lind *et al.*, 1982). Thus, the formation of hydroquinones is considered a detoxification step because of the many deleterious effects of the parent quinones. For example, quinone compounds

can generate reactive oxygen species through reactions of redox cycling in aerobic conditions.

In human liver, the prototypical quinone substrate menadione (vitamin K3) is reduced to menadiol by CBR and NQO1 cytosolic activities (Riley and Workman, 1992; Forrest and Gonzalez, 2000). A prominent feature of NQO1 is its sensitivity to inhibition by the anticoagulant dicoumarol in the micromolar range. Dicoumarol is a specific competitive inhibitor that competes with the NADP(H) cofactor for binding to NQO1 (Hosoda *et al.*, 1974; Asher *et al.*, 2004). On the other hand, biochemical studies on human CBR showed that cytosolic CBR activity is not inhibited by dicoumarol concentrations in the micromolar range (Wermuth, 1981; Wermuth *et al.*, 1986; Rosemond and Walsh, 2004).

The relative contributions of CBR and NQO1 activities to the total quinone reducing capacity was estimated by Wermuth *et al.* in a set of eight human livers. The authors concluded that hepatic CBR accounted for the bulk (55–70%) of the total quinone reductase activity (Wermuth *et al.*, 1986; Ross and Siegel, 2004). In support of this observation, low levels of NQO1 protein were detected by immunoblot analysis of five liver cytosolic samples (Siegel and Ross, 2000). To the best of our knowledge, the range of interindividual variability and the relative contributions of hepatic CBR and NQO1 cytosolic activities have not been evaluated in groups with relatively robust sample sizes. This is somewhat surprising given the prominent role of CBR and NQO1 during the biotransformation of several drug substrates and environmental toxins (Rosemond and Walsh, 2004). In addition, it is also now recognized that in some cases, the ethnic background of an individual may be a useful surrogate to assist during the identification of environmental and/or genetic factors potentially associated with distinctive phenotypic traits. There are many relevant examples of substantial disparities in the metabolism of xenobiotics among individuals with different ethnic background (Burchard *et al.*, 2003; Evans and Relling, 2004; Daar and Singer, 2005). In consequence, our first aim was to evaluate the extent of interindividual variability on CBR and NQO1 activities in samples from black and white subjects. Therefore, we measured CBR and NQO1 activities in liver cytosolic fractions from black ($n = 31$) and white ($n = 63$) liver donors by using the substrate menadione and the specific NQO1 inhibitor dicoumarol.

There is a well-characterized genetic polymorphism in *NQO1* (*NQO1**2, rs1800566) that results in a proline-to-serine change at position 187 in the aminoacid chain of NQO1. The resulting NQO1 protein variant (NQO1_{S187}) is rapidly degraded by the ubiquitin proteasomal system. Protein turnover studies by Siegel *et al.* demonstrated that the half-life of variant NQO1_{S187} was approximately 1.2 h, whereas the half life of wild type NQO1 (NQO1_{P187}) was more than 18 h (Siegel *et al.*, 2001). The distribution of the *NQO1**2 polymorphism has been characterized in different ethnic groups. The variant *NQO1**2 allele is relatively common among North American blacks and whites, and the allele frequencies are similar in both ethnic groups ($q = 0.19$ for blacks, and $q = 0.16 - 0.20$ for whites) (Gaedigk *et al.*, 1998; Blanco *et al.*, 2002). The phenotypic effect of the *NQO1**2 polymorphism has been characterized in some normal human tissues, tumors and cell lines (Ross *et al.*, 1994; Siegel *et al.*, 1999; Ross and Siegel, 2004). For example, NQO1 activity was significantly lower in peritoneal tumor samples from subjects with heterozygous *NQO1**1/*2 genotypes than in those tumors from patients with homozygous *NQO1**1/*1 genotype (Fleming *et al.*, 2002). Interestingly, the impact of the *NQO1**2 polymorphism on hepatic NQO1 activity has not been reported. Therefore, our second aim was to investigate whether the common *NQO1**2 genetic polymorphism impacts on cytosolic NQO1 activities by using paired DNA samples for genotype analysis. Together, our findings provide the necessary platform to further evaluate the impact of polymorphic *NQO1* on the metabolism

of different xenobiotic substrates, and document a previously unrecognized difference in the levels of hepatic NQO1 activities between blacks and whites.

2. Materials and Methods

2.1 Human liver cytosols and DNA samples

The Institutional Review Board of the State University of New York at Buffalo approved this research. Information on the donors' ethnicity assumed that subjects fall into discrete ethnic categories (black or white). In most cases, no information was available regarding the exact geographical origin of the donors, and no information was available on the ethnicity of the donor's parents. Human liver tissue from black (n = 31) and white donors (n = 63) was processed at St. Jude Children's Research Hospital, and was provided by the Liver Tissue Procurement and Distribution System (NIH Contract #N01-DK-9-2310), and by the Cooperative Human Tissue Network, respectively. Liver tissue samples were processed following standardized procedures to obtain cytosolic fractions and DNA.

2.2 Menadione reductase, CBR and NQO1 activities in liver cytosols

Maximal cytosolic menadione reductase activities were measured by recording the rate of oxidation of the NADP(H) cofactor at 340 nm using menadione (200 μ M) as substrate (NADP(H) molar absorption coefficient, 6220 $M^{-1}cm^{-1}$) (Wermuth, 1981). Maximal CBR and NQO1 cytosolic activities were determined by using the specific NQO1 inhibitor dicoumarol (5 μ M) in the presence of the substrate menadione and the NADP(H) cofactor. CBR activity was estimated as the fraction of total menadione reductase activity that is not inhibited by dicoumarol (NQO1) (Benson *et al.*, 1980; Wermuth *et al.*, 1986). Different dicoumarol concentrations (range: 1 – 20 μ M) were tested for NQO1 inhibition in the presence of 200 μ M menadione by using pooled cytosols and individual samples, respectively. Consistent with previous reports, maximal and reproducible NQO1 inhibition was achieved with 5 μ M dicoumarol (Wermuth *et al.*, 1986; Preusch *et al.*, 1991; Bello *et al.*, 2004). For further validation, reverse inhibition experiments were performed on a fraction of the samples by using the specific CBR inhibitor rutin (150 μ M), menadione and NADP(H) (Wermuth *et al.*, 1986; Forrest and Gonzalez, 2000). The analysis in parallel of 30 cytosols (15 blacks, 15 whites) showed that comparable levels of CBR and NQO1 activities were obtained with either rutin (CBR inhibitor) or dicoumarol (NQO1 inhibitor).

Measurements for each sample were performed in duplicate using a Cary Varian Bio 300 UV-visible spectrophotometer equipped with thermal control and proprietary software for enzyme kinetic data analysis. Experiments were performed to determine conditions that ensure maximal velocities and linear relationships with respect to incubation times and cytosolic protein concentrations. The intraday CV for maximal menadione reductase activity was 1.4% (n = 10 determinations), and the inter-day CV was 2.2% (n = 15 determinations), respectively. The lower limit of detection was 0.1 nmol/min.mg.

Typical incubation mixtures (final volume: 1.0 ml) contained potassium phosphate buffer (0.1 M, pH 7.4), 200 μ M NADP(H) (Sigma Aldrich, St. Louis, MO), 200 μ M menadione (Sigma Aldrich), and 5 μ M dicoumarol (if applicable). Complete mixtures were equilibrated for 2 min at 37°C after the addition of cytosols. The rates of NADP(H) oxidation were recorded for 4 min at an acquisition speed of 10 readings/s (2400 readings). Enzymatic velocities were automatically calculated by linear regression of the $\Delta_{Abs}/\Delta_{time}$ points and expressed as nmol/min.mg. Regression coefficients $r \geq 0.95$ were obtained for all enzymatic measurements. Cytosolic protein concentrations were determined with an assay based on Bradford's technique using bovine serum albumin as standard (BioRad Assay, Hercules, CA).

2.3 NQO1 genotyping

The *NQO1**2 polymorphism (rs1800566) was examined by PCR-RFLP analysis as described (Wiemels *et al.*, 1999; Blanco *et al.*, 2002). Briefly, Each 50- μ l PCR contained 20 ng of genomic DNA, nuclease-free water, 50 pmol of each primer (forward: 5'-CCTCTCTGTGCTTTCTGTATCC-3', and reverse: 5'-GATGGACTTGCCCAAGTGATG-3'), 2.5 units of Amplitaq Gold DNA polymerase (Perkin-Elmer Life and Analytical Sciences, Boston, MA), 250 μ M each dNTP (Invitrogen, Carlsbad, CA), and Gene Amp PCR buffer (Perkin-Elmer). PCR amplifications were performed in a MJ Research PTC 200 Thermal cycler. PCR products (299 bp) were digested with *Hinf*I (Invitrogen) and analyzed by electrophoretic separation on 3% agarose gels (BioWhittaker, Rockland, ME). Samples with homozygous *NQO1**1/*1 (C/C) genotypes presented two bands (85 bp and 214 bp), heterozygous *NQO1**1/*2 (C/T) samples presented four bands (63 bp, 85 bp, 151 bp, and 214 bp) and homozygous *NQO1**2/*2 (T/T) samples presented three bands (63 bp, 85 bp, and 151 bp), respectively. Adequate negative and positive controls were included in all PCR runs. Randomly selected PCR products were sequenced with both forward and reverse PCR primers for quality control. The resultant trace files were assembled and analyzed using the public SNP server at the National Cancer Institute (<http://lpgws.nci.nih.gov/perl/snp/snp.cgi.pl>). Perfect concordance was observed between *NQO1* genotypes determined by PCR-RFLP and direct sequencing, respectively.

2.4 Statistical analysis

Values from cytosolic enzymatic activities were used to compute descriptive statistics for each group (e.g. means, standard deviations, ranges and percentiles). Cytosolic enzymatic activities from each group were used to generate histograms and Q-Q plots to evaluate normal frequency distributions. Unpaired Student's *t* tests were used to compare population means of data sets normally distributed. The Mann-Whitney test was used to compare population means of data sets with non-normal distributions and relatively small sample sizes ($n = 20$). The Mann-Whitney test with normal approximation for large data sets was used to compare population means of data sets with non-normal distributions and sample sizes with $n = 20$ (Zar, 1984; Glantz, 1993; Jones, 2002). In all cases, differences were considered to be significant at $p < 0.05$. Computations were performed with Microsoft Excel 2000 version 9.0 (Microsoft), SYSTAT II version 11.00.01, and GraphPad Prism version 4.03 (GraphPad Software Inc, San Diego, CA).

3. Results

3.1 Menadione reductase, CBR and NQO1 activities in liver cytosols from black and white donors

First, we sought to evaluate maximal menadione reductase activities in liver cytosolic fractions from blacks ($n = 31$) and whites ($n = 63$). Demographic, and additional relevant information on the donor history is listed in table 1. Extensive literature searches showed that none of the drugs listed are known inducers of human CBR and NQO1 activities. Both groups of donors were comparable in terms of age (mean_{whites} = 46 years, mean_{blacks} = 34 years), and sex (males_{whites} = 64%, females_{whites} = 36%, and males_{blacks} = 58%, females_{blacks} = 42%). There was substantial variability in menadione reductase activities within each group. The largest range of menadione reductase activity was found among whites (range: <0.1 nmol/min.mg – 29.0 nmol/min.mg) as compared to blacks (range: 4.2 – 24.7 nmol/min.mg). Statistical comparisons demonstrated that cytosolic menadione reductase activities were similar between black and white donors (blacks = 13.0 ± 5.0 nmol/min.mg versus whites = 11.4 ± 6.6 nmol/min.mg; Student's *t* test, $p = 0.208$. Figure 1).

We used the specific NQO1 inhibitor dicoumarol in the presence of menadione to evaluate the relative contributions of cytosolic CBR and NQO1 activities in black and white donors. Correlation analyses showed no significant associations between NQO1 and CBR activities in samples from whites (Pearson's correlation coefficient, $r = 0.07$) and blacks (Pearson's correlation coefficient, $r = 0.0005$), respectively. The levels of expression of some drug-metabolizing enzymes experience marked changes during development in infants and children (Kearns *et al.*, 2003). In this study, a small number of samples were obtained from pediatric donors (<12 yr of age; $n = 3$ whites, and $n = 5$ blacks). Exploratory analyses showed no significant differences in the levels of menadione reductase, CBR and NQO1 activities when pediatric versus non-pediatric donors were compared in samples from whites and blacks, respectively (data not shown). Smoking status (yes/no) was available for a subset of 77 samples (82%). Statistical comparisons showed no significant differences in cytosolic menadione reductase, CBR and NQO1 activities when smokers versus non-smokers were compared in samples from both ethnic groups (data not shown).

We observed variable levels of CBR activities in whites (range: $<0.1 - 28.0$ nmol/min.mg), and blacks (range: $4.1 - 21.5$ nmol/min.mg). Statistical comparisons showed that maximal CBR activities from blacks and whites did not differ significantly ($CBR_{\text{blacks}} = 10.9 \pm 4.1$ nmol/min.mg versus $CBR_{\text{whites}} = 10.5 \pm 5.8$ nmol/min.mg; Student's *t* test, $p = 0.708$. Figure 2, panel A). In contrast, blacks presented significantly higher NQO1 activities than whites ($NQO1_{\text{blacks}} = 2.1 \pm 3.0$ nmol/min.mg versus $NQO1_{\text{whites}} = 0.9 \pm 1.6$ nmol/min.mg; Mann-Whitney with normal approximation, $p < 0.01$ Figure 2, panel B). The percentages of samples with non-detectable NQO1 activity (<0.1 nmol/min.mg) were 33% (whites) and 13% (blacks). On average, NQO1 activity accounted for 15% and 7% of the total cytosolic menadione reductase activity in blacks and whites, respectively. However, the relative contributions of NQO1 activity differed widely among individuals. For example, NQO1 activity accounted for up to 66% of the total menadione reductase activity in one sample. The ranges of NQO1 relative contributions were $<0.1 - 32\%$ for whites, and $<0.1 - 66\%$ for blacks (Figure 3).

3.2 NQO1 phenotype-genotype associations in black and white donors

We analyzed *NQO1**2 genotype distributions in both groups by using paired DNA samples to further explore the basis of variable hepatic NQO1 activity. Siegel *et al.* showed that subjects with the homozygous *NQO1**2/*2 genotype had no detectable NQO1 protein in saliva, while subjects with heterozygous *NQO1**1/*2 genotype had lower levels of NQO1 as compared to individuals with homozygous *NQO1**1/*1 genotype (Siegel *et al.*, 1999). In addition, studies with cell culture models and tumor samples described correlations between *NQO1**2 genotype status and NQO1 enzymatic activity (Traver *et al.*, 1997; Fleming *et al.*, 2002; Ross and Siegel, 2004). Thus, we compared NQO1 cytosolic activities after stratifying by *NQO1* genotype. Allele frequencies were: $p = 0.84$, $q = 0.16$ for blacks, and $p = 0.78$, $q = 0.22$, for whites. In whites the genotype distribution was as follows: 59% were homozygous for the C allele, 38% were heterozygous C/T, and 3% were homozygous for the T allele. In blacks, 68% presented the homozygous C/C genotype, and 32% were heterozygous C/T. *NQO1* genotype distributions were in Hardy-Weinberg equilibrium in both groups (Chi square test; whites, $p = 0.527$, and blacks, $p = 0.620$). In blacks, statistical comparisons failed to show significant differences between cytosolic NQO1 activities in samples from donors with homozygous *NQO1**1/*1 genotype as compared to samples from donors with heterozygous *NQO1**1/*2 genotype ($NQO1_{\text{blacks}[NQO1*1/*1]} = 2.6 \pm 3.4$ nmol/min.mg versus $NQO1_{\text{blacks}[NQO1*1/*2]} = 1.1 \pm 1.2$ nmol/min.mg, Mann-Whitney test, $p = 0.134$. Figure 4, panel A). On the other hand, NQO1 activities differed significantly by *NQO1* genotype status in the group of white donors ($NQO1_{\text{whites}[NQO1*1/*1]} = 1.3 \pm 1.7$ nmol/

min.mg versus $NQO1_{\text{whites}}[NQO1*1/*2 + NQO1*2*2] = 0.5 \pm 0.7$ nmol/min.mg, Mann-Whitney test with normal approximation, $p < 0.01$. Figure 4, panel B).

4. Discussion

The two-electron reduction of carbonyl moieties is a crucial step in the metabolism of many quinone drugs and environmental toxins (Rosemond and Walsh, 2004). CBR and NQO1 are the main sources of cytosolic quinone reductase activity in human liver (Wermuth *et al.*, 1986; Jarabak J. and Harvey R. G., 1993). However, there is a paucity of reports describing the contributions of CBR and NQO1 in relatively large groups of samples from individuals with different ethnic backgrounds. Therefore, the first aim of the present study was to determine the relative contributions of CBR and NQO1 activities in liver cytosols from black and white donors using the prototypical quinone substrate menadione and the specific NQO1 inhibitor dicoumarol. Dicoumarol has been extensively used to investigate the consequences of lack of NQO1 function in studies on sub-cellular fractions and intact cells (Benson *et al.*, 1980; Preusch *et al.*, 1991; Ross *et al.*, 2000; Fleming *et al.*, 2002; Bello *et al.*, 2004). Dicoumarol (range: 1 – 25 μ M) did not inhibit the menadione reductase activity of recombinant human CBR1 in the presence of NADP(H) cofactor (Gonzalez Covarrubias and Blanco, unpublished observations).

Hepatic CBR activity accounted for the bulk of the total menadione reducing capacity in liver cytosols from whites (average = 93%), and blacks (average = 85%). CBR activity varied widely in samples from both ethnic groups. Interestingly, our data indicate that CBR activity does not differ in liver cytosols from black and white donors (Figure 2, panel A). CBR activity plays an important role in the metabolism of many clinically relevant drugs such as the anticancer anthracycline doxorubicin and the antipsychotic haloperidol (Someya *et al.*, 1992; Kudo and Ishizaki, 1999; Forrest and Gonzalez, 2000). For example, hepatic CBR activity accounts for approximately 40% of the clearance of haloperidol by catalyzing the reduction of haloperidol (HP) to reduced haloperidol (RHP). Lam *et al.* reported similar plasma RHP/HP ratios in black ($n = 39$) and white ($n = 66$) schizophrenic patients (Lam *et al.*, 1995). Thus, it is possible that similar RHP/HP plasma ratios may in part result from the comparable levels of hepatic CBR activities among blacks and whites.

Our study documents the presence of significant differences in hepatic cytosolic NQO1 activities from whites and blacks (Figure 2, panel B). On average, samples from blacks presented 2.3-fold higher NQO1 activity than samples from whites. It is possible that complex interplays between genetic and epigenetic factors relatively exclusive for a particular ethnic group may be contributing to the differences in levels of NQO1 cytosolic activities between blacks and whites. Kalinowski *et al.* reported higher levels of NAD(P)H-oxidase and endothelial NO synthase (eNOS) in endothelial cells from blacks as compared to cells isolated from white donors. In turn, endothelial cells from blacks generated significantly more superoxide (O_2^-), which may help to explain the differences in racial predisposition to the endothelium dysfunction during vascular distress (Kalinowski *et al.*, 2004). The free radical superoxide contributes to oxidative stress, and it is dismutated to hydrogen peroxide, a much less harmful product, by the family of superoxide dismutase (SOD) enzymes. Recently, Zitouni *et al.* described higher SOD activities in diabetic black patients with African ancestry as compared to white patients from the UK (Zitouni *et al.*, 2005). Our results appear to be in line with these observations because a major function of NQO1 is to decrease the formation of oxidative species, and variable NQO1 levels impact on the intracellular redox balance (Talalay and Dinkova-Kostova, 2004). Therefore, our findings on hepatic NQO1 activities may provide further support to the contemporary notion that redox cellular balance may operate differently between blacks and whites (Cardillo *et al.*, 1999; Perregaux *et al.*, 2000; Kalinowski *et al.*, 2004).

We also detected a significant association between cytosolic NQO1 activities and *NQO1* genotypes in liver samples from whites. The average NQO1 activity in the group of samples with homozygous *NQO1**1/*1 genotype was 2.6-fold higher than the activity for the group with heterozygous *NQO1**1/*2 plus homozygous *NQO1**2/*2 genotypes, respectively. The fold difference in NQO1 activity after stratification by *NQO1* genotype was similar in blacks (2.4-fold), but comparisons between genotype categories did not reach statistical significance. Therefore, additional research is needed to confirm the potential trend towards an *NQO1* genotype-phenotype association in black donors. Together, our observations on human liver samples further extend and confirm the effect of *NQO1**2 genotype with respect to NQO1 phenotype that has been reported for other human tissues (Traver *et al.*, 1992; Siegel *et al.*, 1999; Ross and Siegel, 2004). In consequence, further *NQO1* genotype-phenotype association studies in human liver samples with different NQO1 substrates are warranted.

In conclusion, we pinpointed the presence of significant differences in the levels of cytosolic NQO1 activities from white and black liver donors. Therefore, it will be of paramount interest to investigate whether interethnic differences in hepatic NQO1 activities also exist for substrates of clinical and toxicological relevance such as the antitumor antibiotic mitomycin C and naturally occurring naphthoquinones (Munday, 2004; Talalay and Dinkova-Kostova, 2004).

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Figure 1.

Maximal menadione reductase activities in liver cytosols from blacks and whites. The lower and upper boundaries of each box define the 25th and 75th percentile, respectively. The whiskers indicate the lowest and the highest value. The thin line inside each box indicates the median, and the bold line shows the population mean. Differences between the means from both groups were assessed by the Student's t test.

Figure 2.

CBR (panel A) and NQO1 (panel B) activities in liver cytosols from whites and blacks. Bold lines indicate the mean of each group. Differences between the means were assessed by the Student's t test (panel A), and by the Mann-Whitney test with normal approximation (panel B).

Figure 3. Relative contributions of cytosolic CBR and NQO1 activities in white (panel A), and black (panel B) liver donors. Relative CBR and NQO1 contributions to the total menadione reducing capacity are expressed as percent fractions (%).

Figure 4.

Relationship between NQO1 phenotype and *NQO1* genotypes in blacks (panel A), and whites (panel B). Bold lines indicate the mean of each group. Differences between the means were assessed by the Mann-Whitney test (panel A), and by the Mann-Whitney test with normal approximation (panel B).

Table 1

Characteristics of liver donors

Sample	ID	Sex	Ethnicity	Age	Patient Disease ^a	Liver Disease	Smoker	Drug ^b
1	1	M	B	2	Normal	Normal	No	Ddvp
2	35	F	B	1	Biliary Atresia	Cirrhotic	No	Unknown
3	151	M	B	32	Normal	Normal	Unknown	Unknown
4	177	M	B	39	Normal	Normal	No	Ddvp
5	202	F	B	5	Normal	Normal	Unknown	Unknown
6	216	M	B	20	Normal	Normal	Yes	Dopamine
7	237	M	B	23	Normal	Normal	No	Unknown
8	245	M	B	19	Normal	Normal	No	Dopamine
9	247	M	B	46	Normal	Normal	No	Dopamine
10	248	M	B	41	Normal	Normal	No	Dex/HC
11	253	F	B	45	Normal	Normal	No	Dex/HC
12	359	M	B	6	Normal	Normal	No	Dopamine
13	362	F	B	41	Cardiovascular	Normal	No	Cimetidine/H2 blocker
14	435	F	B	58	Cardiovascular	Normal	No	None
15	480	F	B	51	Epilepsy	Normal	Yes	Ddvp
16	492	F	B	63	Diabetes	Normal	No	Dopamine
17	836	F	B	53	Unknown	Unknown	Unknown	Unknown
18	850	F	B	44	Unknown	Unknown	Unknown	Unknown
19	847	M	B	59	Unknown	Unknown	Unknown	Unknown
20	159	F	B	7	Unknown	Unknown	Unknown	Unknown
21	844	M	B	68	Unknown	Unknown	Unknown	Unknown
22	38	F	W	64	Cancer -NS-	NI adj tissue	Unknown	Unknown
23	122	M	W	67	Colorectal Cancer	NI adj tissue	Unknown	Unknown
24	152	F	W	40	Normal	Normal	Unknown	Unknown
25	162	M	W	18	Normal	Normal	Unknown	Ddvp
26	174	F	W	23	Normal	Normal	Yes	Dex/HC
27	175	M	W	32	Normal	Normal	No	Thyroid
28	218	M	W	19	Normal	Normal	No	Ddvp

Sample	ID	Sex	Ethnicity	Age	Patient Disease ^a	Liver Disease	Smoker	Drug ^b
29	318	F	W	2	Normal	Normal	No	Barb/toin
30	320	M	W	25	Normal	Normal	Yes	Cimetidine/H2 blocker
31	329	M	W	32	Unknown	Necrosis	No	Unknown
32	331	F	W	62	Normal	Normal	Yes	Dopamine
33	334	M	W	63	Normal	Normal	No	Dopamine
34	335	M	W	36	Normal	Normal	Yes	Barb/toin
35	340	M	W	52	Normal	Normal	No	Dopamine
36	346	M	W	24	Normal	Normal	No	Unlikely prob
37	347	F	W	4	Normal	Normal	No	Cimetidine/H2 blocker
38	348	M	W	43	Normal	Normal	No	Dopamine
39	351	M	W	49	Normal	Normal	Yes	Dopamine
40	353	F	W	68	Normal	Normal	No	Dex/HC
41	356	M	W	37	Normal	Normal	Yes	Dopamine
42	378	M	W	81	Normal	Normal	No	Dex/HC
43	390	F	W	59	Unknown	Normal	No	Unknown
44	394	F	W	52	Unknown	Unknown	No	Unknown
45	395	F	W	57	Unknown	Unknown	No	Unknown
46	397	M	W	52	Unknown	Unknown	No	Unknown
47	411	M	W	30	Normal	Normal	No	Unknown
48	430	M	W	66	Normal	Normal	No	Unknown
49	431	M	W	56	Unknown	Normal	No	Unknown
50	433	M	W	46	Cancer -NS-	Normal	Yes	Thyroid
51	434	M	W	64	Normal	Normal	Yes	Dex/HC
52	436	F	W	49	Psychiatric	Normal	No	Barb/toin
53	439	M	W	48	Normal	Normal	No	Thyroid
54	465	F	W	61	Normal	Normal	No	Ethanol
55	466	M	W	20	Normal	Normal	Yes	Dopamine
56	469	M	W	28	Normal	Normal	No	Dex/HC
57	470	F	W	68	Cardiovascular	Normal	Yes	Ddavn
58	475	F	W	70	Normal	Normal	No	Dopamine
59	476	F	W	66	Cardiovascular	Normal	Yes	Statin

Sample	ID	Sex	Ethnicity	Age	Patient Disease ^a	Liver Disease	Smoker	Drug ^b
60	477	F	W	38	Normal	Normal	No	Barb/toin
61	478	F	W	56	Normal	Normal	Yes	Thyroid
62	482	M	W	78	Cardiovascular	Fibrotic	No	Dex/HC
63	483	M	W	62	Unknown	Normal	No	Unknown
64	484	F	W	34	Normal	Normal	Yes	Thyroid
65	491	F	W	22	Normal	Normal	Yes	Dex/HC
66	856	M	W	67	Unknown	Unknown	Unknown	Unknown
67	849	F	W	46	Unknown	Unknown	Unknown	Unknown
68	865	M	W	34	Unknown	Unknown	Unknown	Unknown
69	837	F	W	40	Unknown	Unknown	Unknown	Unknown
70	161	M	B	41	Cancer -NS-	Cancer -NS-	Unknown	Unknown
71	440	M	B	29	Normal	Normal	No	None
72	445	M	B	46	Normal	Normal	Yes	Dex/HC, Erythro, Barb/toin, Ddvp, Ethanol
73	458	F	B	27	Unknown	Normal	Unknown	Unknown
74	479	F	B	57	Cardiovascular	Normal	Yes	Erythro
75	620	M	B	43	Normal	Normal	Yes	Dopamine
76	624	M	B	23	Normal	Normal	Yes	Dopamine, Cimetidine, Erythro, Dex/H, Ddvp
77	635	F	B	41	Normal	Normal	No	Unknown
78	659	M	B	52	Normal	Normal	No	Thyroid, Dex/HC, Ethanol, Dopamine
79	811	M	B	20	Normal	Normal	No	Unknown
80	322	M	W	3	Other	Necrosis	No	Dex/HC, Thyroid, Midazolam
81	323	M	W	43	Cancer -NS-	Normal	No	Dex/HC
82	328	M	W	50	Normal	Normal	Yes	Dex/HC, Cimetidine, Dopamine, Ddvp
83	343	M	W	35	Normal	Normal	No	Ddvp, Dopamine, Ethanol, Dex/HC
84	350	F	W	75	Cardiovascular	Normal	No	Dex/HC, Dopamine, Ddvp
85	355	M	W	40	Normal	Normal	No	Unknown
86	365	M	W	28	Normal	Normal	Yes	Dex/HC, Dopamine
87	369	F	W	66	Cardiovascular	Normal	Yes	Sex steroid, Thyroid, Ddvp, Dopamine, Ethanol
88	370	M	W	45	Diabetes	Normal	Yes	Dex/HC, Dopamine
89	372	M	W	37	Unknown	Normal	Yes	Ethanol
90	373	M	W	28	Normal	Normal	Yes	Dex/HC, Dopamine

Sample	ID	Sex	Ethnicity	Age	Patient Disease ^a	Liver Disease	Smoker	Drug ^b
91	374	M	W	72	Cardiovascular	Normal	No	Unknown
92	376	M	W	47	Normal	Normal	Yes	Dopamine
93	377	M	W	75	Diabetes	Normal	No	Dopamine
94	379	M	W	34	Normal	Normal	No	Cimetidine, Ddvp, Dopamine, Dex/HC

^aCancer -NS-; not specified

^bDex: dexamethasone; HC: hydrocortisone; Ddvp: vasopressin