

Occupational Exposures to Vapors and Gases, Liver Attenuation and Insulin Resistance

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ABSTRACT

Occupational exposures to vapors and gases, especially organic solvents, have been associated with liver toxicity. At high levels of exposure, solvents—characteristically chlorinated solvents—can cause overt hepatotoxicity, but at lower concentrations they have been associated with fatty liver disease. Accumulation of fat in the liver reflects metabolic changes, similar to those seen in insulin resistance and type 2 diabetes mellitus. Organic solvents are widely used in a variety of occupational settings. Few community-based studies have assessed occupational exposures and their associations with fatty liver disease or insulin resistance.

This is the first large study to date to examine whether occupational exposure to solvents is associated with changes in liver fat or with insulin resistance.

We evaluated relationships between occupational exposures, fatty liver disease, and insulin resistance in working participants aged 45-64 in the Multi-Ethnic Study of Atherosclerosis (MESA). Occupational classes were characterized as having a Vapor and Gas (VG) exposure that was low, medium, or high through the use of a job-exposure matrix (JEM), and we used this measure as a surrogate for occupational solvent exposure. We collapsed the low and medium categories in the JEM into one category so our analysis was between low and high VG exposure groups. We further isolated a subgroup, which was thought to have a high likelihood of solvent vapor exposure, from the high VG exposure group and compared this subgroup to the rest of the study's participants. Extent of fatty liver changes was measured by CT scan assessment of liver attenuation. Insulin resistance was assessed by the Homeostatic Model Assessment of Insulin Resistance (HOMA-IR) via serum measurements of fasting insulin and glucose. We restricted our primary analyses to participants who drank less than 14 alcoholic beverages per week, and adjusted for age, sex, diabetes and adiposity.

A small, but not statistically significant, decrease in hepatic attenuation (consistent with increased fat accumulation in the liver) was seen with increasing likelihood of VG or solvent vapor exposure. Unexpectedly we observed a trend toward decreased insulin resistance as the likelihood of VG or solvent vapor exposure increased. As in other studies, decreased liver attenuation was strongly associated with obesity, diabetes and with alcohol consumption.

A. INTRODUCTION:

Fatty Liver Disease

Fatty Liver Disease (FLD) is a medical syndrome characterized by accumulation of fat within hepatocytes of the liver, typically in a macrovesicular pattern (Brunt 2010). While sometimes it is asymptomatic and does not progress, it is often considered an early marker of metabolic changes and can be associated with liver dysfunction and progressive liver disease (Wong 2010). Because fatty changes in the liver is common among heavy drinkers (steatotic changes are present in as many as 90 percent of heavy alcohol consumers)(O'Shea 2010), and is associated with alcoholic hepatitis, FLD is often subdivided into alcohol-related and non-alcoholic liver disease.

Nonalcoholic fatty liver disease (NAFLD) is defined as the existence of hepatic steatosis, an accumulation of fat in the liver, without the presence of alcohol consumption as the causative factor (Clark 2002). NAFLD is frequently seen in the context of obesity. Obesity produces changes in the metabolism of the liver, such as hepatic insulin resistance and altered lipid synthesis (Bryne 2013). The deranged metabolism is associated with the accumulation of fatty acids and triglycerides within the liver. Diabetes mellitus and viral hepatitis are also associated with NAFLD. Factors such as age, race, and gender may modify the risk of accumulation of fat in the liver (Clark 2002, Wang 2013).

NAFLD is a broad term encompassing those with relatively benign hepatic steatosis, which is considered largely reversible, as well as those with an associated inflammatory process. The later condition is known as nonalcoholic steatohepatitis (NASH). NASH may eventually produce fibrotic changes in the liver and even progress to hepatic cirrhosis.

The prevalence of hepatic steatosis in the American population has been reported as 11 percent (Younossi 2011). Prevalence varies in different areas of the country. One study from Dallas, Texas, revealed that a third of the study's participants had the disorder (Browning 2004). The Dallas study, which included heavy drinkers in its population, seemed to show an effect of ethnicity on the development of FLD. Whites were more likely to have FLD than African-Americans. Moreover, the majority (79%) of those with FLD in the Dallas study had a normal level of alanine aminotransferase (an enzyme used to measure liver function). Lab tests therefore do not appear to be an effective screening method for FLD. The disorder is a problem outside the USA as well, as a study has shown that 29 percent of Japanese adults have hepatic steatosis (Jimba 2005).

The gold standard for the diagnosis of FLD and the more serious NASH is still the liver biopsy (Schwenzer 2009, Lee 2010). This is an invasive procedure with the potential for serious complications such as bleeding and infection. However, noninvasive imaging modalities such as ultrasound or computed tomography (CT) scan can also be used to evaluate patients for the presence of NAFLD.

Early studies using CT images to estimate liver fat were correlated through the use of rabbit livers (Ducommun 1979, Kawata 1984). The field advanced to the estimation of FLD in human

subjects. Park and colleagues correlated the results of hepatic attenuation (the radiodensity of the liver) on CT with liver biopsies which were done on 154 living potential liver donors. They found that CT scans were accurate in a qualitative way for detecting the presence of > 30% liver fat (100% specificity and 82% sensitivity) but not were quantitatively useful (Park 2006).

One year later, using 88 patients who had undergone a liver resection for metastatic disease, Kodama and colleagues studied the liver attenuations by CT of normal areas of liver along with the corresponding liver biopsy results. With significant associations between fat content on biopsy and liver attenuation on CT, they were able to form predictive models for estimating a liver's fat content from its attenuation on CT. They showed that a liver fat content of 30% occurs at about 40 Hounsfield units, a measure of attenuation in CT imaging, and 50% liver fat equates to approximately 25 Hounsfield units (HU). They suggested using the spleen as an organ with which to compare the liver. If the attenuation of the liver was less than the spleen (the Liver/Spleen ratio was < 1), the liver was thought to be fatty infiltrated. They also found contrast-unenhanced CT scans had a much better predictive value for estimating liver fat than did the contrast-enhanced scans (Kodama 2007).

Recently, Zeb and colleagues reported on the reliability of CT in screening for NAFLD (Zeb 2012) in the Multi-ethnic Study of Atherosclerosis, in which liver images had been obtained as part of efforts to characterize calcification in coronary arteries. The authors first removed heavy drinkers from their study. NAFLD was defined as a Liver/Spleen ratio of < 1, which corresponded in this study to a liver attenuation of < 51 in Hounsfield units (HU). By this definition, the prevalence of NAFLD in this study was 17.2 percent. The measurements for liver attenuation were highly reproducible (intra-reader and inter-reader correlations of 0.99 and 0.96, respectively). The study concluded that nonenhanced CT scans were a reliable method to detect NAFLD without resorting to biopsy.

Organic Solvents and the Liver

Organic solvents are used in a variety of occupational settings such as painting and dry-cleaning. Organic solvent exposure is known to produce toxic effects on multiple organ systems, including the liver, the nervous system, the reproductive system, and the kidneys (Landou 2007). This paper focuses on their effect on the liver. Organic solvents are known hepatotoxins. Abnormalities in liver function have been detected in workers exposed to the solvents of the painting industry (Chen 1991). At relatively high exposure levels, organic solvents can produce acute toxic hepatitis and, in some cases, fulminant hepatic necrosis (Malaguarnera 2012).

A major role of the liver is to metabolize toxicants. Absorbed solvents via the skin or lungs may eventually undergo metabolism in the liver. While the relationship between high level exposures to organic solvents, especially chlorinated solvents, is well established, these exposures are less common now. The potential role of ongoing exposure to lower concentrations of solvents that are currently in use is less clear. Further, the effect on a worker's liver of lower level of exposure may not be readily apparent, nor occur to providers caring for such patients when other causes such as alcohol and obesity are so prevalent. Nevertheless, chronic occupational exposure to organic solvents at a lower level is thought to cause a low-grade hepatotoxicity, with FLD as an

important pre-clinical observation, though the evidence for the effects of solvents at a lower level of exposure is much less extensive (Brodkin 1995; Lundqvist 1999; Døssing 1983).

Insulin Resistance

Insulin resistance is the hallmark of Type II diabetes. It is also associated with obesity, the metabolic syndrome and the polycystic ovary syndrome. Chronic hepatitis C infection has been implicated as well (Miyajima 2013). A few studies have suggested that organic solvents may be associated with increased fasting glucose and with insulin resistance (Kaukiainen 2004; Won 2011), possibly as a result of liver damage (Mokuda 1995).

Accumulation of fat in the liver reflects metabolic changes, similar to those seen in insulin resistance and in type 2 diabetes mellitus. The nature of the metabolic changes of NAFLD has become a source of debate in the literature. Some authors favor insulin resistance as a necessary first step leading to its development (Chitturi 2002) while others consider a primary increase in the production of triglycerides within the liver to be the cause. There may in fact be several pathways. One recent study noted NAFLD to be associated with dyslipidemia, independent from insulin resistance (DeFilippis 2013).

Goals and Objectives

Few community-based studies have assessed occupational exposures and their associations with fatty liver disease or insulin resistance. In fact very little is known about liver damage from low level solvent exposure. Additional information is thus sorely needed concerning the effect of chronic occupational solvent exposure on the liver.

The study utilized participants in the Multi-Ethnic Study of Atherosclerosis (MESA). It examines the associations between occupational exposure to vapors and gases (VG), liver attenuation (measured by CT in HU) and insulin resistance. Organic solvents are known to enter the body through the lungs as a vapor as well as through the skin as a liquid. Across the many occupations considered in this study, the likelihood of exposure to VG will be used as a surrogate for likelihood of organic solvent exposure, and liver attenuation will be used as a marker of liver fat. Insulin resistance will be measured by the Homeostatic Model Assessment of Insulin Resistance or HOMA-IR (Wallace 2004).

We hypothesize that increasing the likelihood of occupational exposure to VG will be associated with a decrease in liver attenuation among currently employed individuals. Furthermore we expect that increasing the likelihood of exposure to VG at work will be associated with increased insulin resistance.

B. METHODS:

Subjects

MESA is a cohort study that was designed to study cardiovascular disease. Those with known heart disease were excluded. The MESA participants were between 45-84 years of age. They

represent four racial/ethnic categories (White, Chinese, African American, Hispanic) from New York, NY, Baltimore, MD, Forsyth County, NC, St. Paul, MN, Chicago, IL, and Los Angeles, CA. Almost all of the participants had initial cardiac CT scans which included images of the liver. It was therefore possible to obtain liver attenuation data (in Hounsfield units) on the MESA subjects. At their initial evaluation (Exam 1) each subject provided a body mass index measurement, a blood pressure measurements, a fasting glucose level, a fasting insulin level, and a lipid panel. They also answered an extensive questionnaire covering income level, education level, occupation type, job title, smoking status, and current medications as well as the necessary demographic information. Investigators recruited 6814 participants, who were initially examined between July 2000 and Aug 2002, for longitudinal follow-up. This analysis is a cross-sectional study of information from that initial examination and interview..

We restricted our analysis to MESA participants who were employed full-time at the time of the initial examination. MESA participants placed themselves in one of nine categories concerning their employment. Only those who responded that they were currently employed full time were included. Retired, part-time, and sick workers on medical leave were excluded, as were self-described homemakers.

Job Exposure Matrix

The job-exposure matrix (JEM) is a tool to assess potential exposures in occupational studies. It associates jobs, or their occupational codes, with likely exposures based on either measurements models, or expert opinions. Jobs can then be grouped into different levels of exposure. The matrix considers categories of workers and assigns “exposure” to each category rather than assessing the actual measured exposure of an individual or a group.

The MESA Study was the subject of efforts from the National Institute for Occupational Safety and Health (NIOSH) through the MESA Occupation ancillary study. No direct occupational exposure measurements were made in the cohort. Exposure assignment through the MESA JEM was based on standardized coding of occupational classification and subsequent assignment of occupational classification into exposure likelihood categories using expert opinion with some assistance from O*NET. The O*Net has been touted as a primary method for estimating workplace exposures but its scientific validity in that role is still undetermined (Cifuentes 2010). The authors provide evidence however that O*NET can be of help in the process of job exposure characterization. Using a JEM to characterize likelihood of exposure is not new in occupational epidemiology (Van Tongeren 2013). The JEM performs better than self reported exposures (Le Moual 2000).

Since the MESA is large, it's not feasible to assess all of the participant's actual worksite exposures. The JEM provides a way to estimate the likelihood of certain exposures for the various occupational codes associated with the MESA subjects. The validity of any JEM can certainly be criticized, but industrial hygienists have been shown to be good estimators of exposure on at least a qualitative basis (Kromhout 1987). This study employed hygienists to create the matrix, and furthermore we later collapsed the JEM provided by said hygienists into a more general (low versus high likelihood of exposure) form.

Based on text descriptions of the current jobs listed for the 6814 MESA participants, two trained reviewers from the National Institute for Occupational Safety and Health (NIOSH) gave almost every participant in Exam 1 a three-digit occupational code taken from the Census 2000 Industry and Occupation Codes (US Census Bureau). Any disputes were settled by a third coder. Only 17 subjects (<0.5%) were not able to be coded. Later three industrial hygienists, also from NIOSH, reviewed these occupational codes to create, by expert consensus, a job exposure matrix for vapors, gases, dusts and fumes (VGDF). If needed the hygienists referred to O*NET for additional information about the jobs codes. Only four of 509 occupational codes could not be categorized with regard to likelihood of exposures to the three separate categories, vapors and gases (VG), dusts, and fumes. The unclassified categories were too nonspecific to establish an estimate of exposure likelihood (e.g., "military member, not otherwise specified"). As a result 64 MESA participants could not be characterized with regard to likelihood of occupational exposure.

In the NIOSH JEM, vapors and gases (VG) were grouped as one category. Dust exposure and fume exposure two additional categories, not used in this analysis. Likely exposure in each category was rated as low, medium or high. With permission from NIOSH, the part of their JEM concerning potential exposure to VG was used for this study.

After considering the NIOSH JEM, it was decided to divide the JEM into only two groups for the purposes of our analysis, creating a binary exposure. We collapsed the two lower exposure categories. Group one (V/G1) for our initial analysis was composed of the low and medium likelihood of VG exposure from the NIOSH JEM. Per expert occupational health scientist faculty reviewers at the University of Washington (Joel Kaufman and Noah Seixas), both of these categories contained jobs with a low likelihood of exposure to solvent vapor. Group two (V/G2) for our analysis contained the high likelihood of VG exposure, the third category from the NIOSH JEM, which was considered by the health scientist faculty to contain the highest likelihood of solvent vapor exposure.

In order to further assess the effect of solvent exposure on NAFLD and on insulin resistance, a subgroup of V/G2, those occupations within this group with a likely high solvent vapor exposure (as opposed to non-solvent vapors and gases), was identified. This was done because our hypothesis was more narrowly related to exposure to solvent vapors rather than all VG exposures. A VG exposure includes solvent vapors but it includes other exposures as well. Again the experts (Joel Kaufman and Noah Seixas) in the DEOHS department at University of Washington reviewed the 34 jobs contained in the high likelihood of exposure to VG category (V/G2). Thirteen of these jobs were deemed to present a high likelihood of solvent vapor exposure. This subgroup was compared to all of the other jobs in the NIOSH JEM, to V/G1 and the non-solvent exposed component of V/G2.

The jobs with low or medium likelihood for VG exposure from the NIOSH JEM, jobs which were unlikely to be high in their potential exposure to solvent vapors, were thus combined with the 21 jobs remaining in V/G2 to become the low likelihood of exposure to solvent vapor category (LS) for our second analysis. The 13 jobs with high likelihood of exposure to solvent vapor became the second category (HS) for this analysis. The job titles in V/G2 as well as the job titles for the subset of V/G2 that formed the HS group are listed in Appendix 1.

Liver Attenuation

At entrance into the study, each MESA subject underwent 2 consecutive nonenhanced cardiac CT scans during a single session. Either a four detector row or a electron beam scanner was used (Zeb 2012). Participants were scanned from the carina to the heart apex. The scans for most adequately visualized the liver for liver attenuation measurements. Of the 6814 subjects, only 199 did not have adequate liver information to assess liver attenuation (2.9%).

The two scans were compared and the one with better liver visualization was sent to be assessed by two readers (radiologists) who measured liver and spleen attenuation in HU for all the scans. Attenuation was measured at regions of interest (ROI), which had to be > 100 square mm in size. One ROI was located in the spleen, two ROI were found in the liver's right lobe, and one ROI was found in the left lobe. The two ROI in the right liver lobe were found to best for reliability so their scores in HU were added together and then divided by two to find the mean liver attenuation. All the liver attenuation values used in this study were taken in this manner from the right liver. Zeb and colleagues have previously reported on this data (Zeb 2012) and their results concerning FLD in the MESA subjects conform with the results of other similar studies. They noted that liver attenuation of 40 HU (the marker in the literature for 30% liver fat) occurred at a Liver/Spleen ratio of 0.8.

Insulin Resistance

Homeostatic Model Assessment of Insulin Resistance was developed in 1985 as a way of estimating insulin resistance by utilizing the subject's fasting glucose and fasting insulin levels. Insulin resistance can also be measured with curve fitting models and dynamic data. HOMA-IR is structural model based on physiology which is easy to calculate. It correlates well with the more sophisticated glucose clamp technique (Bonora 2000). It has been used in hundreds of epidemiological studies and its validity is now widely accepted (Wallace 2004). Using this model, insulin resistance is calculated by multiplying the blood glucose level (mg/dL) by the insulin level (microIU/mL) and dividing by 405. Care should be taken in that glucose is reported in some studies in mmol/liter, which would require a different formula. We were interested to see if insulin resistance increased with an increase in likely exposure to VG for those within this study, and our results are not necessarily calibrated with other studies.

Exclusions

We excluded heavy alcohol consumers, defined as > 14 drinks per week for men and > 7 drinks per week for women. We also excluded participants who self-reported infection with hepatitis B or hepatitis C.

Statistical analysis

After combining the work histories and the liver attenuation data, a descriptive analysis examined the relationship between each of the outcomes (liver attenuation and HOMA-IR) and the covariates, as well as the relationships between the exposures and covariates, using tables and linear regression.

Multivariable linear regression then examined 1) the relationship between hepatic attenuation (the dependent variable, in Hounsfield units) and the occupational exposure groups and 2) the relationship between HOMA-IR index and the VG exposure groups. The two levels of higher exposure were compared to the low level VG exposure group which became the reference for each analysis. Two models were to be considered. Model 1 adjusts solely for age, gender, and race/ethnicity. Model 2 adjusts for a larger number of potentially confounding or precision variables which include body mass index (BMI), diabetes status, dyslipidemia status, serum triglyceride level, systolic blood pressure (SBP), smoking status, income level, education level, age, gender, and race/ethnicity.

Finally, the evidence of effect modification by race/ethnicity, gender, diabetes status, and BMI was assessed through stratification.

Covariates

Age for the MESA subjects is provided as a continuous variable, in years. Gender is categorical, male or female. Race/ethnicity is categorical and given as White, Chinese, African-American, and Hispanic, based on self report. BMI is calculated with the standard method - [weight(kg)] divided by [height(m) squared]. MESA subjects were placed in one of 4 categories for diabetes status: normal fasting glucose (no diabetes), impaired fasting glucose, treated diabetic, untreated diabetic. Their categorization was based on their fasting glucose and their reported use of insulin or oral hypoglycemics. For this study, however, the subjects were placed into only 2 categories: normal fasting glucose and impaired fasting glucose (composing the latter three MESA categories). Participants were considered to have dyslipidemia if low density lipoprotein level was > 130 , if their high density lipoprotein was < 40 or if the subject reported taking a medication for a lipid disorder. Fasting triglycerides were measured in mg/dL and considered as a continuous variable. Resting systolic blood pressure (SBP) was measured three times using a Dinamap Pro 100 device (General Electric) and then calculated as the average of the 2nd and 3rd measurements. SBP was measured as a continuous variable in mmHg. Current smokers were defined as those who reported smoking in the past 30 days. Education level was divided into: 1) High school graduate or less, 2) Technical school graduate and/or some college, and 3) College graduate. Yearly income level was divided into: 1) Less than \$50,000, 2) \$50-99,000, and 3) Greater than \$99,000. Education level and income level provide measures of socioeconomic status for the analysis.

C. RESULTS:

Of the 6814 MESA participants examined at the initial examination, 2605 were currently employed. Removing those who did report heavy drinking reduced the study group from 2605 to

2451. Only one subject in this reduced group failed to answer the questions concerning hepatitis B and C. After those who answered that they had hepatitis B or C were removed, the final study group contained 2408 subjects.

A potential problem arose in that 345 of the 2605 did not answer either of two questions regarding alcohol consumption (current alcohol yes/no, number of drinks per week). Of the 804 who did not complete the question regarding drinks per week, 452 indicated that they were not current drinkers; these were included in analysis as non-drinkers. Four stated they were current drinkers but did not quantify the number per week, leaving 349 participants who are difficult to classify with regard to alcohol intake.

It was decided to keep the 349 in the study with the assumption they were not heavy drinkers. However, due to the potential for misclassification, a sensitivity analysis was conducted with these subjects making the alternate assumption, that they were heavy drinkers.

Of the 2408, 2340 had liver CT scans that were acceptable to quantify liver attenuation, and 2392 had submitted blood tests sufficient to calculate insulin resistance. All responded to the income level question, and most of the 2408 (n=2077) responded to the education level question.

Descriptive Analysis

The differences between our initial two exposure groups, low likelihood of VG exposure (V/G1) and high likelihood of VG exposure (V/G2), were considered (Table 1). Then the differences in the groups for our second analysis, low likelihood of solvent exposure (LS) and high likelihood of solvent exposure (HS) were evaluated (Table 2). The age of the workers in the two analysis groups were very similar. The V/G1 exposure group was predominately female whereas only 24% of the V/G2 was female. The White group held 39% of the V/G1 jobs but only 23% of the V/G2 jobs. Whites had 39% of the LS occupations and 13% of the HS jobs. In contrast, Hispanics held 20% of LS jobs and 57% of HS occupations. The higher exposed groups were mostly male and had a smaller proportion of White workers.

Regarding income level, those in the highest income category composed 21% of V/G1 and 10% of V/G2. Those in the lowest income category were 42% of V/G1 and 59% of V/G2. In V/G1, fifty three percent had a college degree whereas in V/G2 only 27% had earned a college degree. The jobs with a higher potential for exposure to VG therefore tended to be held by less educated people with a lower income.

		TABLE 2			
EXPOSURE LEVEL	(Potential)	LOW SOLVENT VAPOR (N= 2368)		HIGH SOLVENT VAPOR (N= 40)	
AGE (YRS)		55 (SD 6.8)		55 (SD 8.2)	
GENDER (#)					
Female		1118	47%	8	20%
Male		1250	53%	32	80%
RACE (#)					
White		916	39%	5	13%
Asian		274	12%	5	13%
African-American		685	29%	7	18%
Hispanic		493	20%	23	57%
BMI Group					
<25		612	26%	9	23%
25-29		934	39%	21	52%
30-39		711	30%	10	25%
>39		111	5%	0	0%
FASTING GLUCOSE					
< 100 mg/dL		1857	79%	30	75%
>99 mg/dL (Plus all DM)		500	21%	10	25%
DYSLIPIDEMIA					
No		1031	44%	12	30%
Yes		1337	56%	28	70%
SMOKED IN LAST 30 DAYS					
No 84%		2040	86%	32	80%
Yes 16%		328	14%	8	20%
INCOME					
< 50 K		1003	42%	23	58%
50 - 99 K		861	36%	13	32%
100 K or more		504	21%	4	10%
EDUCATION					
High school or less		244	12%	15	47%
Tech school /some college		730	36%	9	28%
College graduate		1071	52%	8	25%
AVG SYS BLOOD PRESSURE (mmHg)		120.7 (SD 18.6)		124.3 (SD 19.8)	
AVG TRIGLYCERIDES (mg/dL)		129.9 (SD 86.7)		129.4 (SD 70.5)	
SD = Standard deviation					

Liver attenuation in the V/G groups

The V/G1 and V/G2 exposure groups were evaluated (Figure 1). Next they were stratified by BMI (Figure 2). No significant trend within each of the four BMI categories was detected.

Figure 1

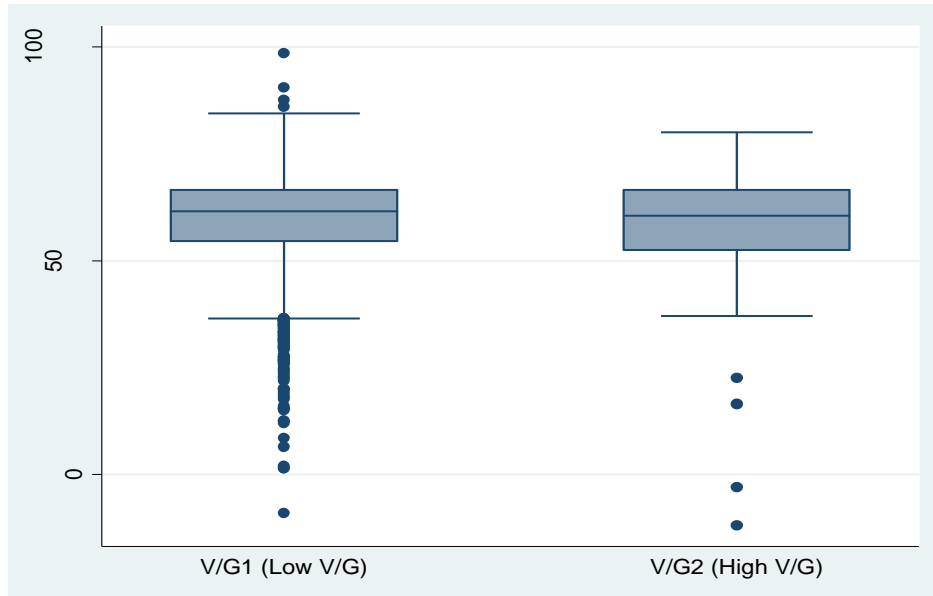
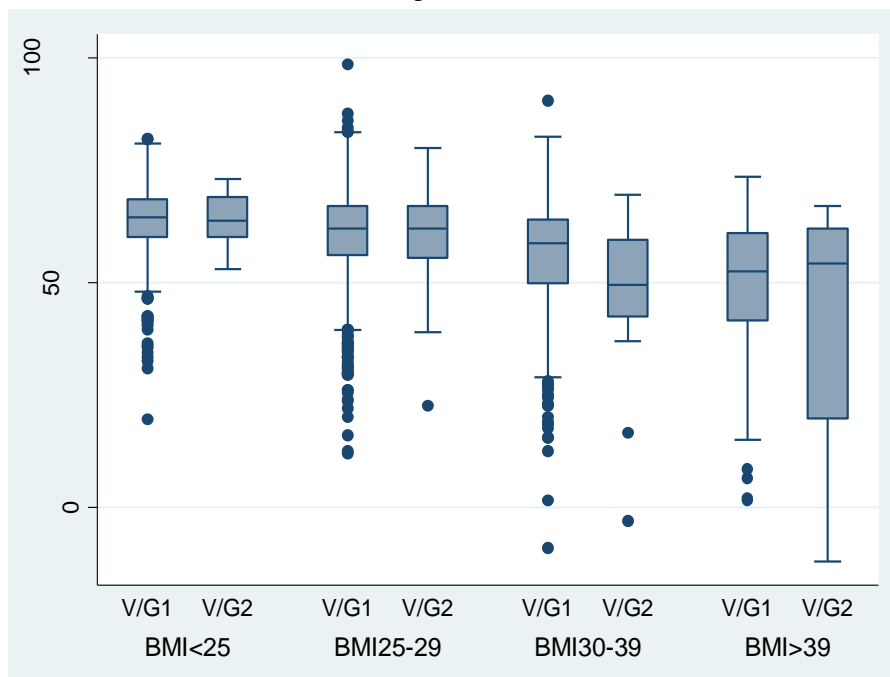
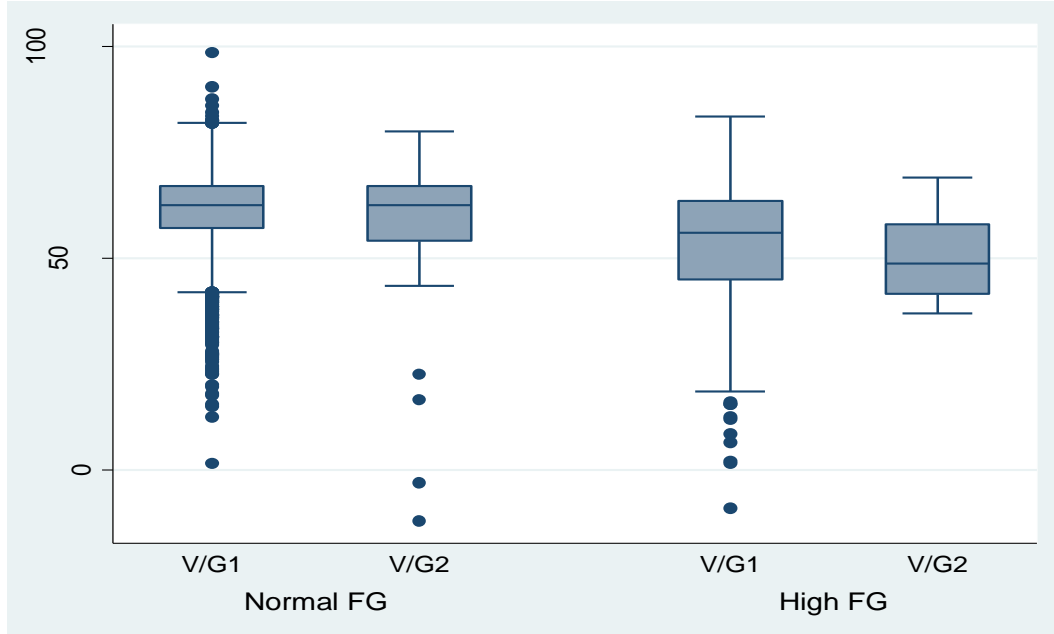


Figure 2



The same approach was taken with respect to diabetes status (Figure 3). Again no definite trend was visible in the two V/G exposure groups, although liver attenuation appeared somewhat lower among the higher exposure likelihood category of those with impaired glucose tolerance.

Figure 3



FG = Fasting glucose

Lastly, the effect of gender and race were examined in the two V/G exposure groups (Figure 4 and Figure 5). No significant trend was seen.

Figure 4

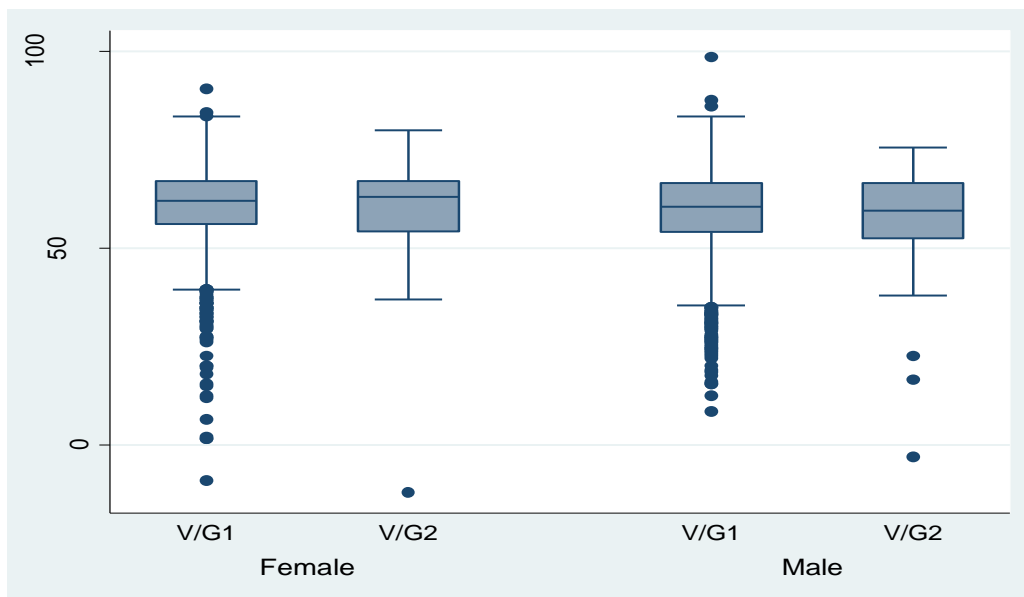
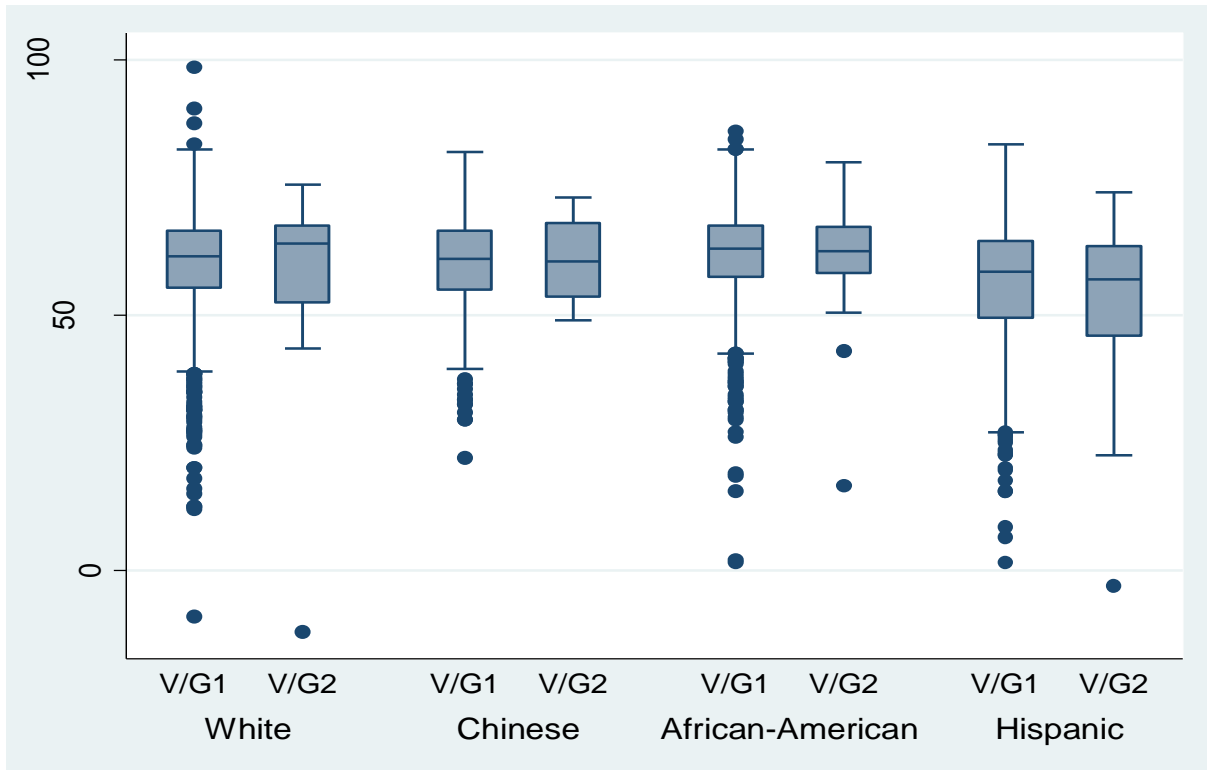


Figure 5



Liver attenuation in the Solvent Vapor groups

The LS and HS exposure groups were examined (Figure 6) and then looked at them stratified by BMI (Figure 7). No significant trend within each of the four BMI categories was detected; no high-solvent exposure individuals were in the highest BMI category..

Figure 6

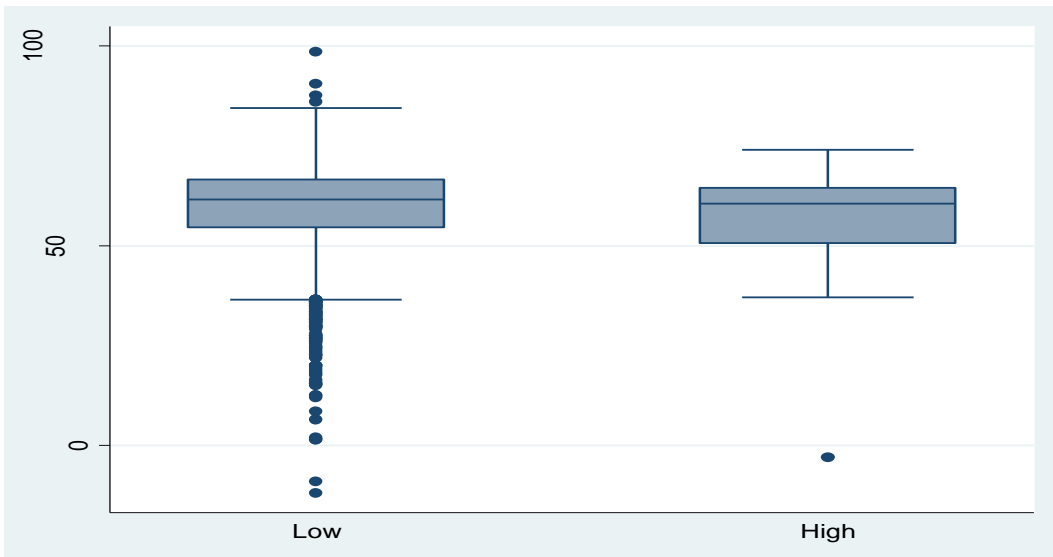
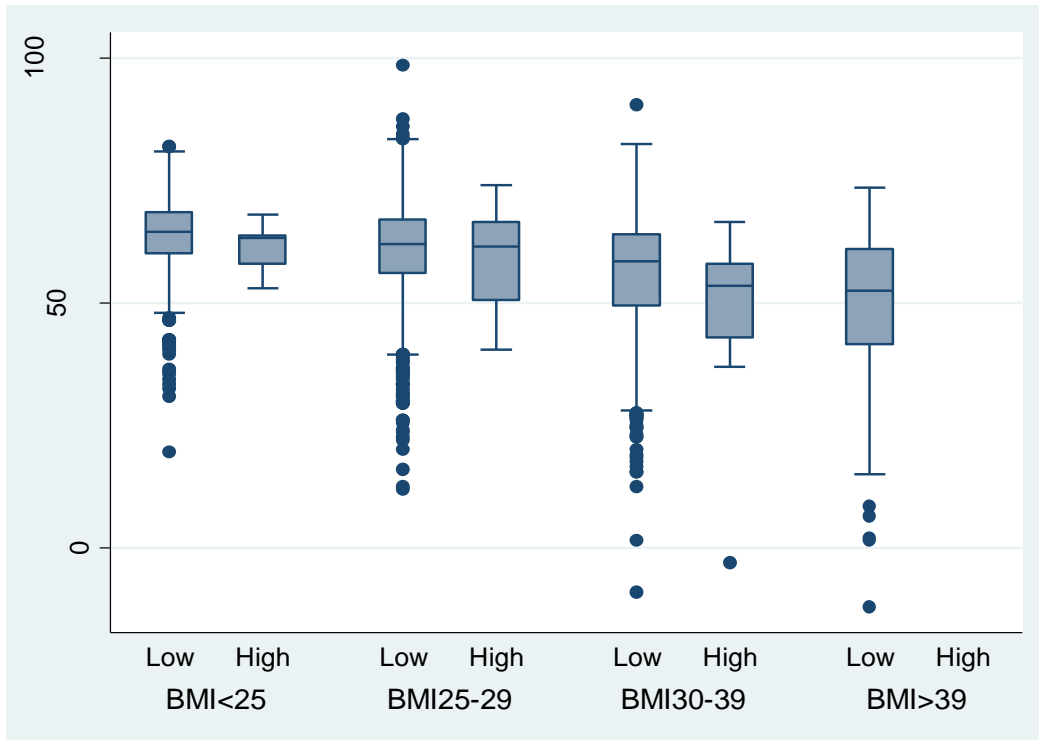
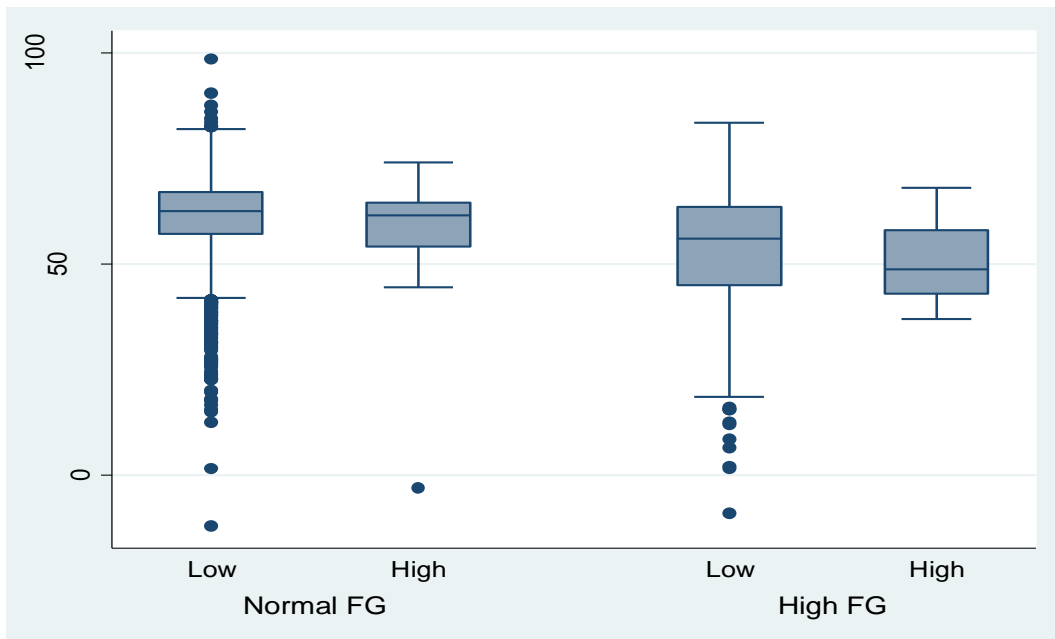


Figure 7



The same approach was taken with respect to diabetes status (Figure 8). Again no significant trend was visible after stratifying by diabetes status.

Figure 8



FG = Fasting glucose

Lastly, the effect of gender and race were examined in the LS and HS groups (Figure 9 and Figure 10). No significant trend was seen.

Figure 9

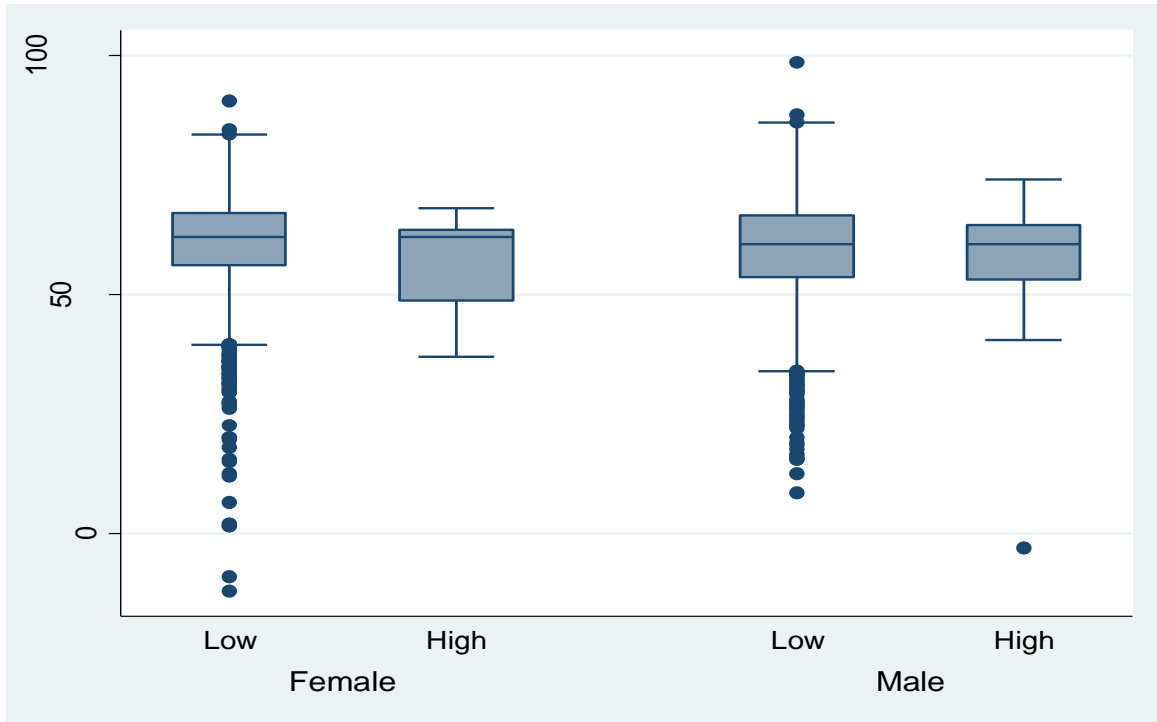
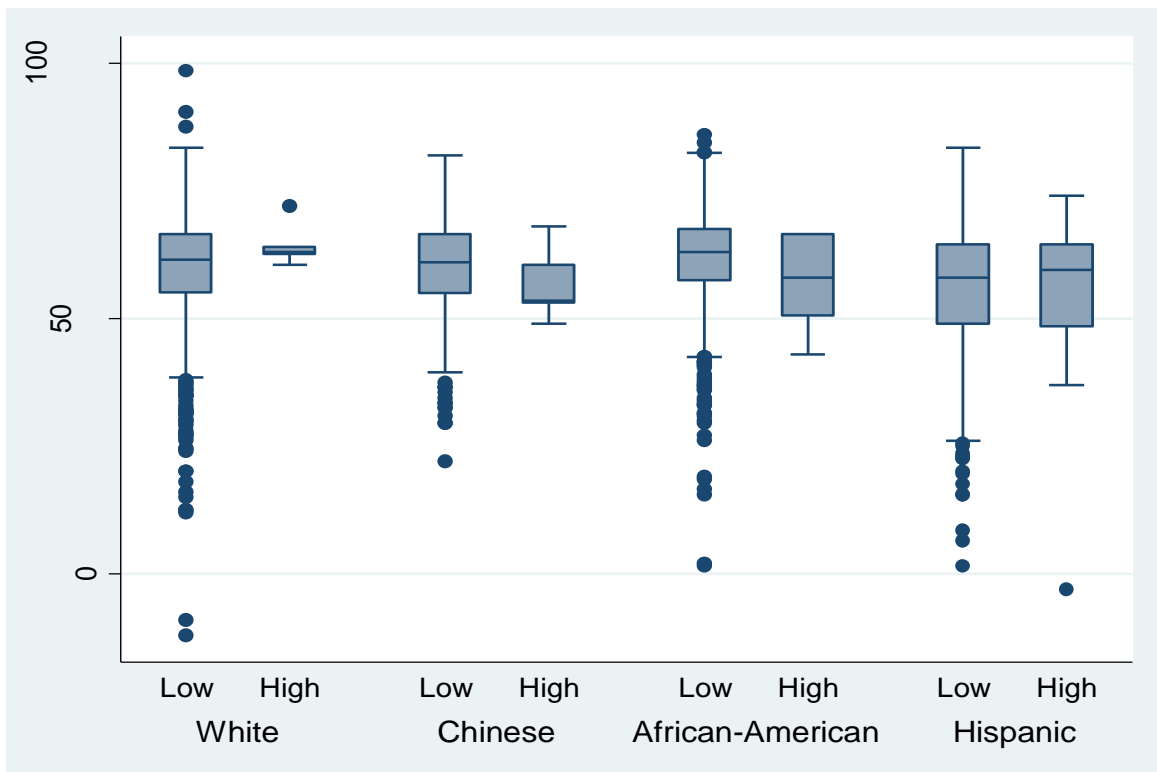


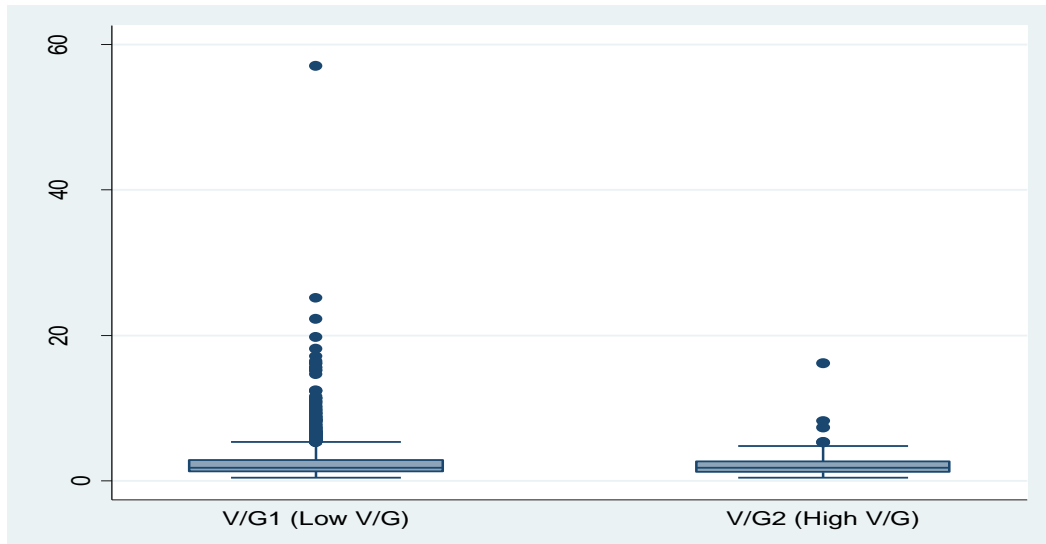
Figure 10



HOMA-IR

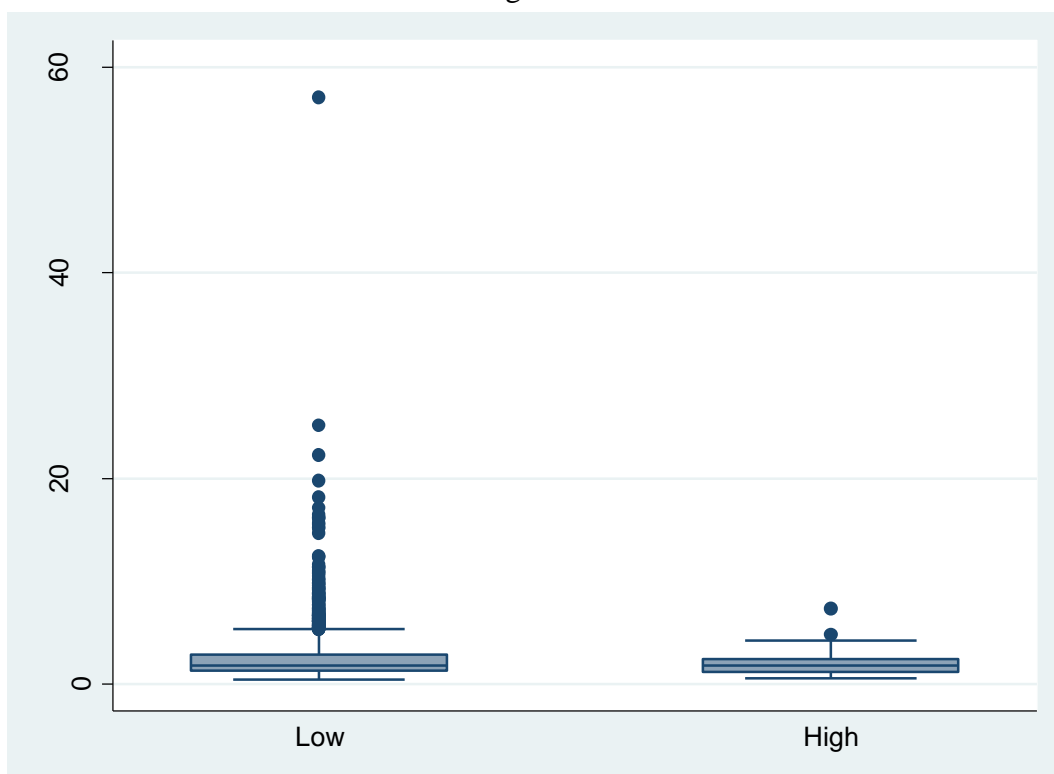
The calculated insulin resistance was examined for each V/G exposure groups (Figure 11). No trend was noticeable.

Figure 11



When the LS and HS exposure groups were evaluated, no trend was visible (Figure 12).

Figure 12



The averages and standard deviations for the outcomes of the two analyses are given in Tables 3 and Table 4. A decrease in liver attenuation is seen from low to high in likely VG exposure and from the LS group to HS group. Mean insulin resistance seems to decrease with a higher likelihood of exposure to VG and to solvent vapors.

TABLE 3		
POTENTIAL EXPOSURE LEVEL	MEAN LIVER ATTENUATION (HU)	MEAN HOMA-IR
LOW V/G	59.4 (SD 11.7)	2.49 (SD 2.4)
HIGH V/G	57.3 (SD 14.5)	2.29 (SD 2.0)

TABLE 4		
POTENTIAL EXPOSURE LEVEL	MEAN LIVER ATTENUATION (HU)	MEAN HOMA-IR
LOW SOLVENT VAPOR	59.4 (SD 11.8)	2.5 (SD 2.4)
HIGH SOLVENT VAPOR	56.6 (SD 13.3)	2.11 (SD 1.4)

Multivariable Regression Analysis of Liver attenuation and HOMA-IR

As described in the methods section, the V/G1 and V/G2 groups were assessed in the same manner as the LS and HS groups, using two models.

As in other studies, NAFLD, as approximated by decreased liver attenuation, was strongly associated with obesity, diabetes status, and increased triglycerides, as shown in Table 5 (from the V/G1 versus V/G2 analysis). SBP seemed to have a small effect as well. Ethnicity was a factor in liver attenuation. African-Americans seemed to be resistant to a decrease in liver attenuation as compared to Whites. These same associations were seen in both the V/G1 versus V/G2 analysis and in the LS versus HS analysis.

We observed a small, but not statistically significant, association of decreased liver attenuation with occupation in the category with higher likelihood of exposure to vapor and gases compared to the reference low exposure group (-1.4 HU, 95% confidence interval -3.8 to 1.0) in the fully adjusted model (Table 6). No association was found between liver attenuation and occupation in the group with the higher likelihood of solvent vapor exposure, compared to the reference low exposure group (-0.3 HU, 95% confidence interval -4.0 to 3.5) in the fully adjusted model (Table 7).

A small, but not significantly significant, decrease in insulin resistance was seen between occupation in a category with higher likelihood of exposure to vapors and gases (V/G2)

compared to the reference low exposure group [-0.298, 95% confidence interval -0.75 to 0.15] and for the category with higher likelihood of exposure to solvents (HS) compared to the reference low exposure group [-0.606, 95% confidence interval -1.31 to 0.097] in the fully adjusted models. See Table 6 and 7.

The two models for liver attenuation and for HOMA-IR were run with those who were difficult to classify as to their alcohol use (n=349) as if they were all nondrinkers (the above analysis) and as if they were all heavy drinkers (and excluded from this study). This sensitivity analysis revealed no significant difference in the coefficients or confidence intervals. The results of for the LS versus HS exposure groups, excluding those who were unclear as to their alcohol use, is given in Table 8.

On the assumption that higher alcohol may in some way be correlated with jobs in the V/G2 grouping, the models were also run with no MESA subjects being removed for any amount of alcohol use. Instead weekly alcohol use was added to the adjustments of model 2. This method did not result in a significant change in the coefficients or confidence intervals so the two models with the original adjustments were maintained.

TABLE 5					
	COVARIATES (for V/G1 and V/G2 groups)	MULTIPLE LINEAR REGRESSION COEFFICIENTS			
		MODEL 1	95% CI	MODEL 2	95% CI
LIVER ATTENUATION (HU)	Age	0.028	{-0.042 to 0.098}	0.078	{0.004 to 0.145}
	Male Gender	-0.78	{-1.74 to 0.181}	-0.328	{-1.27 to 0.685}
	Race/Ethnicity:				
	White	ref		ref	
	Chinese	0.155	{-1.46 to 1.77}	-1.58	{-3.18 to 0.098}
	African-American	1.93	{0.757 to 3.11}	2.67	{1.51 to 3.92}
	Hispanic	-3.72	{-5.01 to -2.44}	-1.64	{-3.12 -0.195}
	BMI:				
	<25			ref	
	25-29			-2.14	{-3.40 to -0.931}
	30-39			-6.22	{-7.64 to -4.86}
	>40			-13.5	{-15.9 to -10.9}
	Impaired glucose			-5.12	{-6.33 to -3.98}
	Dyslipidemia			-0.278	{-1.25 to 0.727}
	High triglycerides			-0.027	{-0.032 to -0.021}
	SBP			-0.031	{-0.059 to -0.005}
	Education Level:				
	High School graduate or less			ref	
	Tech school/Some College			-0.771	{-2.41 to 0.869}
	College graduate			-0.663	{-2.40 to 1.07}
	Income Level:				
<\$50,000			ref		
\$50-99,000			-0.395	{-1.52 to 0.726}	
>\$100,000			-0.627	{-1.97 to 0.721}	
Smoker			1.16	{-0.213 to 2.53}	
Ref = Reference group					
HU = Hounsfield units					
HOMA-IR = Homeostatic model assessment for insulin resistance					
Model 1 = VG exposure adjusted for age, race/ethnicity and gender					
Model 2 = VG exposure adjusted for age, race/ethnicity, gender, body mass index, fasting glucose, dyslipidemia status, triglyceride level, systolic blood pressure, smoking status, education level, and income level					

TABLE 6					
	EXPOSURE GROUPS (Jobs with likely exposure to:)	MULTIPLE LINEAR REGRESSION COEFFICIENTS			
		MODEL 1	95% CI	MODEL 2	95% CI
LIVER ATTENUATION (HU)	Low Vapors/Gases (V/G1)	Ref		Ref	
	High Vapors/Gases (V/G2)	-0.985	{-3.36 to 1.39}	-1.38	{-3.75 to 0.993}
HOMA-IR INDEX	Low Vapors/Gases (V/G1)	Ref		Ref	
	High Vapors/Gases (V/G2)	-0.402	{-0.876 to 0.071}	-0.298	{-0.745 to 0.149}

Ref = Reference group
 HU = Hounsfield units
 HOMA-IR = Homeostatic model assessment for insulin resistance
Model 1 = Adjusted for age, race/ethnicity and gender
Model 2 = Adjusted for age, race/ethnicity, gender, body mass index, fasting glucose, dyslipidemia status, triglyceride level, systolic blood pressure, smoking status, education level, and income level

TABLE 7					
	EXPOSURE GROUPS (Jobs with likely exposure to:)	MULTIPLE LINEAR REGRESSION COEFFICIENTS			
		MODEL 1	95% CI	MODEL 2	95% CI
LIVER ATTENUATION (HU)	Low Solvent Vapors (LS)	Ref		Ref	
	High Solvent Vapors (HS)	-1.05	{-4.76 to 2.66}	-0.255	{-4.0 to 3.49}
HOMA-IR INDEX	Low Solvent Vapors (LS)	Ref		Ref	
	High Solvent Vapors (HS)	-0.669	{-1.41 to 0.069}	-0.606	{-1.31 to 0.097}

Ref = Reference group
 HU = Hounsfield units
 HOMA-IR = Homeostatic model assessment for insulin resistance
Model 1 = Adjusted for age, race/ethnicity and gender
Model 2 = Adjusted for age, race/ethnicity, gender, body mass index, fasting glucose, dyslipidemia status, triglyceride level, systolic blood pressure, smoking status, education level, and income level

TABLE 8: Sensitivity Analysis (Those with uncertain alcohol use excluded)					
	EXPOSURE GROUPS (Jobs with likely exposure to:)	MULTIPLE LINEAR REGRESSION COEFFICIENTS			
		MODEL 1	95% CI	MODEL 2	95% CI
LIVER ATTENUATION (HU)	Low Solvent Vapors (LS)	Ref		Ref	
	High Solvent Vapors (HS)	-0.617	{-4.75 to 3.51}	0.897	{-3.28 to 5.07}
HOMA-IR INDEX	Low Solvent Vapors (LS)	Ref		Ref	
	High Solvent Vapors (HS)	-0.735	{-1.59 to 0.120}	-0.692	{-1.51 to 0.132}
Ref = Reference group					
HU = Hounsfield units					
HOMA-IR = Homeostatic model assessment for insulin resistance					
Model 1 = Adjusted for age, race/ethnicity and gender					
Model 2 = Adjusted for age, race/ethnicity, gender, body mass index, fasting glucose, dyslipidemia status, triglyceride level, systolic blood pressure, smoking status, education level, and income level					

D. DISCUSSION:

This project represents the first population-based cohort to assess quantitatively-determined hepatic fat content in relation to occupational exposures. No strong or statistically significant associations were found, though the results suggest an association between occupational exposures to vapors and gases and increased liver fat, consistent with our hypothesis. In contrast, the findings suggest an association between increased occupational exposures to vapors and gases and decreased insulin resistance, which is counter to our hypothesis.

Model 1 and 2 of the multivariable linear regression support my hypothesis that increasing a worker's potential VG exposure would be associated with a decrease in his or her liver attenuation although the association is not strong. The association appeared weaker when separating out a group thought to have the highest likelihood of exposure to solvent vapors (HS).

The major weakness of this study is the potential for exposure misclassification due to the use of a relatively imprecise job-exposure matrix, and this may have contributed to the lack of strong associations. Different experts would potentially categorize the job exposures of the MESA subjects in slightly different ways, and individual workers within and between categories have very different exposures that are not well-characterized on the basis of job title alone. The more knowledge exists about the worker the easier it is to categorize him or her. The JEM for the MESA subjects, developed by NIOSH, relied on data that was somewhat limited as to the exact exposures of the subjects and, in addition, some "lumping" of jobs occurred.

For example, in the NIOSH JEM, physicians were placed with surgeons in the category with medium likelihood of exposure to VG. Of course physicians and surgeons could vary immensely as to their exposure to VG from a minimal level to a very high level of exposure. In addition people with a very specific occupational code could vary considerably in their exposure depending on where they are employed. Someone who is coded as a fire fighter may in fact

rarely fight fires. Misclassification is a therefore always a concern with job exposure matrices (Kauppinen 1992).

Our primary interest was in solvent exposure, which represents only a proportion of occupational vapor and gas exposures. Hence, we attempted to strengthen the association of VG exposure and liver attenuation first by altering the NIOSH JEM to create a binary exposure to VG. Expert reviewers a subset of occupational exposures, with likely high solvent vapor exposure, from the NIOSH grouping with highest likelihood of VG exposure. Neither of our binary exposure categorizations revealed a strong association, but the direction of the effect was consistent.

In the case of insulin resistance, an unexpected association, though not statistically significant, was observed. Our hypothesis was that increased likelihood of VG exposure would be associated with increased insulin resistance. Won and colleagues recently demonstrated increased insulin resistance with chronic exposure to monocyclic aromatic hydrocarbons (MAHs) such as the solvent toluene (Won 2011). However, our study suggests the opposite relationship. Those MESA subjects in the higher VG exposure category (a surrogate for a higher likelihood of solvent exposure) actually seemed to show a decrease in their insulin resistance. The same trend was noticeable when moving from the LS to HS exposure groups. We cannot determine whether our findings can be ascribed to a true association, exposure misclassification, or residual confounding. Further, it's possible that the more specific exposure to MAHs may yield different results than a less specific exposure to VG (our marker for a variety of solvent exposures).

Since insulin secretion by the pancreas pulsates, it would be best to obtain multiple fasting insulin and glucose measurements in order to find the average level for each. This is not commonly done in the literature, and it was not done in the MESA study. Finding and using an average insulin and glucose level for the HOMA-IR calculation might have improved our results.

The tendency toward decreased liver attenuation in the higher exposure groups (V/G2, HS) coupled with the trend toward decreased insulin resistance in these same groups would seem to make fatty liver disease (as represented by the decreased liver attenuation) independent of insulin resistance. This is far from conclusive however and additional work is needed.

Study Design

The study is unique in that it connects a JEM to a continuous variable from CT imaging - liver attenuation (in HU). The JEM is a valuable useful way to characterize the exposures in large categories of workers. MESA is large study and an appropriate place to apply this method. The study was strong in that the JEM categorized the VG exposure for nearly all of the occupational codes and the data for liver attenuation and HOMA-IR calculation was nearly complete. In addition, thorough demographic data on MESA subjects, including ethnicity/race, was available. The JEM was based on the subjects' current, not their usual, jobs. This is the preferred way for studying NAFLD, which is a reversible process.

Limitations

1) As stated above, a JEM can result in substantial exposure misclassification. can vary between qualified experts as to which exposure categories the subjects are placed. This may be why we were unable to show stronger associations.

- 2) As workers may have recently changed jobs, misclassification in the JEM may have occurred.
- 3) Incomplete knowledge of alcohol consumption is another limitation. Of the 2408 subjects recruited into this study, 345 (14%) did not indicate their level of alcohol consumption.
- 4) No liver biopsies were done and liver attenuation on CT provides only an estimate of liver fat.
- 5) As in all cross sectional studies, temporal relationships cannot be demonstrated.

F. CONCLUSIONS:

A small, but not statistically significant, decrease in hepatic attenuation (meaning increased NAFLD) was seen in the groups with a higher likelihood of occupational solvent vapor exposure (V/G2 and HS). As in other studies, decreased liver attenuation was strongly associated with an increase in BMI, diabetes status, alcohol consumption and with elevated triglycerides.

Unlike other studies, we did not observe an increase in insulin resistance with an increase in likely occupational exposure to vapors and gases (our marker for solvent exposure). In fact the opposite trend was seen. With increasing likelihood of VG exposure, we noted that insulin resistance seemed to decrease.

APPENDIX 1

JOBS IN THE HIGH EXPOSURE CATEGORIES			
High Vapor/Gas (V/G2)		High Solvent Vapor (HS)	
Automotive Service Technicians		Automotive Service Technicians	
Bus and Truck Mechanics		Bus and Truck Mechanics	
Carpet, Floor, and Tile Installers		Carpet, Floor, and Tile Installers	
Chemists and Materials Scientists		Chemists and Materials Scientists	
Metal Pickling Equipment Operators & Tenders		Metal Pickling Equipment Operators & Tenders	
Construction Laborers		Chemical Processing Machine Setters	
Chemical Processing Machine Setters		Furniture Finishers	
Chemical Engineers		Industrial and Refractory Machinery Mechanics	
Farmers and Ranchers		Laundry and Dry-Cleaning Workers	
Furnace, Kiln, Oven, Drier Workers		Painters, Construction and Maintenance	
Furniture Finishers		Painting Workers	
Fire Fighters		Pest Control Workers	
First-Line Supervisors of Fire Fighting		Roofers	
First-Line Supervisors of Landscaping			
Grounds Maintenance Workers			
Highway Maintenance Workers			
Hairdressers, Hairstylists			
Industrial Engineers			
Industrial Truck and Tractor Operators			
Industrial and Refractory Machinery Mechanics			
Laundry and Dry-Cleaning Workers			
Miscellaneous Agricultural Workers			
Miscellaneous Assemblers and Fabricators			
Miscellaneous Construction			
Other Extraction Workers			
Other Life, Physical, and Social Science			
Painters, Construction and Maintenance			
Painting Workers			
Pest Control Workers			
Pipelayers, Plumbers, Pipefitters			
Plasterers and Stucco Masons			
Roofers			
Structural Metal Fabricators and Fitters			
Water and Liquid Waste Treatment Plant			

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