HHS Public Access

Author manuscript

Environ Res. Author manuscript; available in PMC 2017 November 23.

Published in final edited form as:

Environ Res. 2016 July ; 148: 1–6. doi:10.1016/j.envres.2016.03.015.

Acrolein Metabolites, Diabetes and Insulin Resistance

Aliya G. Feroe[1], **Roberta Attanasio**[2], and **Franco Scinicariello**[3,*]

[1]Department of Biology, Bowdoin College, Brunswick, Maine, USA

[2]Department of Biology, Georgia State University, Atlanta, Georgia, USA

[3]Division of Toxicology and Human Health Sciences, Agency for Toxic Substances and Disease Registry (ATSDR), Atlanta, Georgia, USA

Abstract

Acrolein is a dietary and environmental pollutant that has been associated *in vitro* to dysregulate glucose transport. We investigated the association of urinary acrolein metabolites N-acetyl-S-(3-hydroxypropyl)-L-cysteine (3-HPMA) and N-acetyl-S-(carboxyethyl)-L-cysteine (CEMA) and their molar sum (Σacrolein) with diabetes using data from investigated 2027 adults who participated in the 2005–2006 National Health and Nutrition Examination Survey (NHANES). After excluding participants taking insulin or other diabetes medication we, further, investigated the association of the compounds with insulin resistance (n=850), as a categorical outcome expressed by the homeostatic model assessment (HOMA-IR>2.6). As secondary analyses, we investigated the association of the compounds with HOMA-IR, HOMA-β, fasting insulin and fasting plasma glucose. The analyses were performed using urinary creatinine as independent variable in the models, and, as sensitivity analyses, the compounds were used as creatinine corrected variables. Diabetes as well as insulin resistance (defined as HOMA-IR>2.6) were positively associated with the 3-HPMA, CEMA and ΣAcrolein with evidence of a dose-response relationship ($p < 0.05$). The highest 3rd and 4th quartiles of CEMA compared to the lowest quartile were significantly associated with higher HOMA-IR, HOMA-β and fasting insulin with a dose-response relationship. The highest 3rd quartile of 3-HPMA and ΣAcrolein were positively and significantly associated with HOMA-IR, HOMA-β and fasting insulin. These results suggest a need of further studies to fully understand the implications of acrolein with type 2 diabetes and insulin

Keywords

N-acetyl-S-(carboxyethyl)-L-cysteine; N-acetyl-S-(3-hydroxypropyl)-L-cysteine; NHANES; HOMA

INTRODUCTION

Acrolein, an α,β-unsaturated aldehyde, is a common dietary and environmental pollutant and is released in the environment from combustion of petroleum fuels, biodiesel, plastic,

Corresponding author: Franco Scinicariello, MD, MPH, Centers for Disease Control and Prevention, Agency for Toxic Substances and Disease Registry, 4770 Buford Hwy, MS F57, Atlanta, GA 30341., Phone: (770)-488-3331, Fax: (770)-488-4178, fes6@cdc.gov.

paper and wood, and is a major component of tobacco smoke (ATSDR 2007). Dietary sources of acrolein are generated during the heating of vegetable oils and animal fats and acrolein is also present in beverages such as coffee and alcohol (Alwis et al. 2015; ATSDR 2007). Furthermore, acrolein is endogenously generated during lipid peroxidation, amine oxidase-mediated metabolism of polyamines, and myeloperoxidase (Moghe et al. 2015). Acrolein is highly water soluble, enabling it to rapidly enter body tissues and form conjugates with cellular glutathione (GSH). Upon conjugation, acrolein is further metabolized into N-acetyl-S-(carboxyethyl)-L-cysteine (CEMA) or catalyzed in N-acetyl-S-(3-hydroxypropyl)-L-cysteine (3-HPMA), the main urinary metabolite of acrolein (Abraham et al. 2011; Moghe et al. 2015; Stevens et al. 2008). Acrolein is a respiratory toxicant and has been associated with a number of health complications, including pulmonary edema, increased bronchial responsiveness (ATSDR 2007) and may have a role in chronic obstructive pulmonary disease (Bein and Leikauf 2011). It has also been associated with atherosclerosis (Park and Taniguchi 2008) and increased risk of cardiovascular disease (DeJarnett et al. 2014), Alzheimer's disease (Dang et al. 2010) and multiple sclerosis (Tully and Shi 2013). Furthermore acrolein protein adducts have been associated with diabetic complications such as diabetic nephropathy (Suzuki and Miyata 1999) and diabetic retinopathy (Grigsby et al. 2012). Two studies report an association between acrolein with type 2 diabetes (Daimon et al. 2003) or type 1 diabetes (Tsukahara et al. 2003). Recently, acrolein has been associated with dysregulation of glucose transport in human endothelial cells (O'Toole et al. 2014). The incidence of diabetes mellitus, particularly type 2 diabetes, is increasing worldwide (Danaei et al. 2011). In the United States, diabetes is estimated to affect 8.3% of the total population and 11.3% of the adult population 20 years and older. Along with age, family history, genetic variants, obesity, physical inactivity and smoking, environmental pollutants have also been associated with type 2 diabetes (Kuo et al. 2013). Due to the molecular evidence of acrolein effects on dysregulation of glucose transport, the objective of this study was to investigate whether urinary acrolein metabolites, 3-HPMA and CEMA, are associated with type 2 diabetes and insulin resistance in adults (20 years and older) using 2005–2006 National Health and Nutrition Examination Survey (NHANES) data. We hypothesized that acrolein metabolites were positively associated with diabetes and insulin resistance.

Methods

Study population—The 2005–2006 NHANES, conducted by the U.S. National Center for Health Statistics (NCHS; Centers for Disease Control and Prevention, Atlanta, GA) is a cross-sectional, nationally representative survey of the non-institutionalized civilian population of the United States (Johnson et al. 2013). The survey employs a multistage stratified probability sample based on selected counties, blocks, households, and individuals within households. Certain subgroups of the population, such as Mexican American individuals, black non-Hispanic individuals, and older adults were oversampled to improve the estimate precision for these groups. The NCHS Research Ethics Review Board approved the NHANES 2005–2006 study protocols and all participants provided written informed consent. For our analysis, the study population is limited to individuals who were aged 20 years or older from whom urinary acrolein metabolites 3-HPMA and CEMA measurements were available. Pregnant women (n=147), women who were breastfeeding (n=23), and

participants missing other co-variables of interest were excluded leaving a total of 2027 eligible participants.

Exposure Measurements—The concentrations of the urinary acrolein metabolites—3-HPMA and CEMA—were determined using ultra performance liquid chromatography coupled with electro spray tandem mass spectrometry (UPLC-ESI/MSMS) (Alwis et al. 2012), by the Division of Laboratory Sciences (DLS), National Center for Environmental Health (NCEH), CDC. Levels below the limit of detection were entered as the limit of detection divided by the square root of two (Johnson et al. 2013). Moreover, an internal acrolein dose variable (ΣAcrolein) was created based on the sum of the molar 3-HPMA and CEMA. Urinary 3-HPMA and CEMA and ΣAcrolein were categorized as weighted quartiles based on the distribution of the urinary acrolein metabolite levels among the study population.

Outcome of Interest

Diabetes Case Definition—Diabetes was defined as glycated hemoglobin (A1C) ≥6.5% or fasting plasma glucose (FPG) ≥ 126 mg/dl, or self-reported current use of insulin or diabetes medication. Information on participants' current use of insulin and/or oral anti-diabetes medications were obtained during the household interview. Glycated hemoglobin was measured using ion exchange chromatography. Fasting plasma glucose was measured using the hexokinase enzymatic method.

Insulin resistance and HOMA, HOMA-B, Fasting Insulin, and Fasting Plasma Glucose—Markers of diabetes risk were determined in a subsample of 850 individuals not taking insulin or medication for diabetes. Insulin resistance was assessed using HOMA-IR (homeostatic model assessment). HOMA-IR is epidemiologically practical, widely used, and correlates acceptably (R = 0.73–0.88) with the hyperinsulinemic-euglycemic clamp test, which is generally considered to be the gold standard (Matthews et al. 1985; Wallace et al. 2004). HOMA-IR was calculated as fasting plasma glucose (mmol/L) × fasting insulin (uU, mL)/22.5 and insulin resistance was defined as HOMA-IR >2.6 (Ascaso et al. 2003, Loprinzi et al. 2014; Velagaleti et al. 2010 Zhao et al. 2014). Although our primary study outcome was insulin resistance, we also examined continuous HOMA-IR, beta-cell function (HOMA-β), fasting insulin, and fasting plasma glucose in secondary analyses. HOMA-β was calculated based on the formula: HOMA-β = [(20* fasting insulin)/(fasting plasma glucose − 3.5)] (Matthews et al. 1985).

Statistical Methods—To account for the complex, multistage sampling design of NHANES, we performed all analyses using the appropriate sample weights, strata, and cluster variables. All analyses were performed using the weights from the volatile organic compounds metabolites subsample as recommended by NCHS (Johnson et al. 2013.). SAS 9.3 (SAS Institute, Cary, NC) was used for all statistical analyses and SAS-Callable SUDAAN 10 (Research Triangle Institute, Research Triangle Park, NC) was used to account for the NHANES complex sample design. P-values were presented at the significance level of 0.05. Multivariable logistic regression was used to calculate adjusted odds ratios (ORs)

for diabetes and insulin resistance (HOMA-IR >2.6) by comparing participants in the highest urinary acrolein metabolites compared to their referent lowest quartile.

We ran three models: model 1 was adjusted for urinary creatinine and age; model 2 was further adjusted for demographic and socio behavioral variables, such as sex, race/ethnicity (non-Hispanic white, non-Hispanic black, Mexican American, and Other), education (less than high-school, high school graduate, some college, and above), alcohol consumption self-reported smoking status (current, former, or never smoker), serum cotinine (a biomarker of exposure to environmental tobacco smoke was natural log-transformed) and fasting time; and model 3 was further adjusted for confounding factors such as body weight status (underweight/normal, overweight and obese), and moderate and vigorous recreational activities.

To account for variation in the dilution of spot urinary samples, urinary creatinine was entered into the models as an independent variable, as suggested by previous studies (Barr et al. 2005). Serum cotinine was measured by an isotope-dilution-high-performance liquid chromatography/atmospheric pressure chemical ionization tandem mass spectrometry method (Bernert et al. 1997). Information about age (years), sex, race/ethnicity, and education were obtained from the household interview. Age was categorized as quartiles based on the weighted distribution of age among the study population. Race/ethnicity was divided into four categories: non-Hispanic White, non-Hispanic Black, Mexican American and Other (Other Hispanic and other race). Body weight status was classified as normal/underweight, overweight, and obese with body mass index (BMI) measures of <25, 25 – <30, and ≥30, respectively. Alcohol consumption and self-reported smoking status (current smoker, former smoker, or never a smoker) were obtained from the physical examination and associated questionnaire. Participants that reported smoking at least 100 cigarettes in their lifetime, and reported at the time of the household interview smoking every day or some days were defined as current smokers. Participants that reported smoking at least 100 cigarettes in their lifetime, and did not smoke at the time of the interview were defined as former smokers. Participants who reported having smoked less than 100 cigarettes in their lifetime were defined as never smoker. Fasting time in hours was used as a continuous measure because of the small proportion of participants who fasted less than the 8 hours as by the American Diabetes Association (2005) criteria for the diagnosis of diabetes. Information on recreational physical activity came from the NHANES questionnaire; participants were asked whether they engaged in regular moderate and/or vigorous recreational activities (categorized as yes or no). To avoid information bias due to self-reported cigarette smoking, we, also, used both self-reported cigarette use and serum cotinine cutoff to define smoking status (Pirkle et al. 2006): smokers included self-reported current smokers and those with serum cotinine levels > 10 ng/mL, and non-smokers included self-reported former and never smokers and those with serum cotinine levels ≤ 10. ng/mL, However, analyses performed with smoking status categorized as smokers and non-smokers, instead of self-reported cigarette smoking and serum cotinine as distinct independent variables, did not change the statistically significance of the reported associations (data not shown).

We used separate linear regression models to estimate associations between HOMA-IR, HOMA-β, fasting insulin and fasting plasma glucose (as dependent variables) and individual or molar sum of acrolein metabolites categorized by quartiles. HOMA-IR, HOMA-β, fasting insulin and fasting plasma glucose were natural log-transformed for analyses because the distribution of these variables were skewed left. These linear regressions were performed using all covariates used in the final model 3 of the logistic regression. Sensitivity analyses were conducted using the urinary acrolein metabolites standardized by urinary creatinine. Statistical tests for linear trends were conducted by modeling quartiles as an ordinal variable using integer values.

RESULTS

The geometric mean (GM) age of the participants was approximately 44 years. Among the participants 9.62% had diabetes, approximately 72.6 % were Non-Hispanic whites and 50.2 % were females. Approximately 58%, 28%, and 51% of the people reported that they had attended some college, never used alcohol, and never smoked, respectively and about 68% were either overweight or obese. The geometric mean (GM) (± SE) of the acrolein metabolites 3-HPMA and CEMA were 325.35 ± 14.74 μg/L and 95.66± 3.46 μg/, respectively. The GM of the molar sum of acrolein (ΣAcrolein) was 2.22 ± 0.04 μmol/L (Table 1).

Association of acrolein metabolites and diabetes—In multivariable logistic regression analyses (adjusted age and urinary creatinine), there were statistically significant associations between the metabolites 3-HPMA, CEMA and ΣAcrolein with diabetes prevalence (Model 1). Further adjustments with demographic, education and alcohol consumption and cigarette smoking variables (Model 2) and body weight status, caloric intake and recreational physical activity (Model 3) increased the magnitude of the associations. Diabetes was significantly associated with increasing 3-HPMA (OR, 95% CI vs. lowest quartile: 1.58, 1.13 – 2.20, 2nd quartile; 2.79, 1.67 – 4.66, 3rd quartile, and 3.15, 2.01 – 4.96, 4th quartile) (Table 2). Individuals in the 2nd, 3rd and 4th CEMA quartiles were more likely to have diabetes (2.16, 95%CI: 1.14 – 4.10; 2.64, 95%CI: 1.26 – 5.55; and 2.39, 95%CI: 1.25, 4.54, respectively) compared to the referent lowest CEMA quartile (Table 2). Analyses using the molar sum of acrolein metabolites also show that individuals in the 2nd, 3rd and 4th ΣAcrolein quartiles were more likely to have diabetes (1.72, 95%CI: 1.05 – 2.83; 2.77, 95%CI: 1.48 – 5.19; and 3.36, 95%CI: 1.79 – 6.30, respectively) compared to the lowest referent ΣAcrolein quartil,. There was evidence of a dose-response relationship based on the statically significance of the p-value for trend ($P<0.05$) in all models.

Sensitivity analyses using weighted quartiles based on the distribution of the urinary acrolein metabolites normalized by urine creatinine confirmed the statically significant association of the acrolein metabolites with diabetes (Supplemental Table 1).

Association of acrolein metabolites and insulin resistance—Table 3 presents the adjusted prevalence odds ratios for insulin resistance (HOMA-IR>2.6) in subsample of individuals not taking insulin or medication for diabetes (n=850). There were statistically significant associations between ΣAcrolein and the metabolites 3-HPMA and CEMA with

insulin resistance with evidence of dose-response relationship (p-value for trend <0.05) Sensitivity analyses using urinary acrolein metabolites normalized by urine creatinine confirmed the statically significant association between the acrolein metabolites with insulin resistance (Supplemental Table 1).

Association of acrolein and metabolites and HOMA-IR, HOMA-β, fasting insulin and fasting plasma glucose—Although our primary study outcome was insulin resistance, we also examined HOMA-IR, beta cell function (HOMA-β), fasting insulin, and fasting plasma glucose in secondary analyses. Table 4 presents the β values changes of the multivariate analyses in natural log-transformed HOMA-IR as the outcome of interest comparing the three highest quartiles of the compounds to their respective referent lowest quartile. The highest 3rd and 4th quartiles of CEMA compared to the lowest quartile were associated with higher HOMA-IR, HOMA-β, and fasting insulin with a dose-response relationship (Table 4). Although there was an increase of HOMA-IR, HOMA-β, and fasting insulin in all 3-HPMA quartiles compared to the lowest corresponding referent quartile, the statistically significantly associations were present only in the third quartile, and in the second quartile for insulin only (Table 4). Similar associations were, also, found when ΣAcrolein was used as independent variable (Table 4). Sensitivity analyses using urinary acrolein metabolites normalized by urine creatinine showed similar results (Supplemental Table 2).

DISCUSSION

To our knowledge this is the first study that investigates an association between urinary acrolein metabolites CEMA and 3-HPMA with type 2 diabetes and insulin resistance. In a representative sample of U.S. adults, increasing levels of acrolein metabolites were positively associated with diabetes and insulin resistance after adjustment for diabetes risk factors. Also, in secondary analyses, we found a statistically significant association of acrolein metabolite CEMA with HOMA-IR, HOMA-β, and fasting insulin. Furthermore, we found a non-monotonic association of urinary 3-HPMA with HOMA-IR, HOMA-β, and fasting insulin.

Although acrolein has been associated with diabetes complications, such as diabetic nephropathy (Suzuki et al. 1999) and diabetic retinopathy (Grigsby et al. 2012), only one study reported an association of acrolein with type 2 diabetes (Daimon et al. 2003). Daimon et al. (2003) reported higher urinary levels of acrolein adduct, measured by an enzyme-linked immunosorbent assay method using anti-acrolein adduct antibodies in patients with diabetes mellitus (n=100) compared to the control of no diabetes (n=50). Although our findings of an association of acrolein with type 2 diabetes is consistent with the study of Daimon et al. (2003), the novelty of this study is the association of acrolein metabolites with insulin resistance and HOMA-IR in individuals not taking insulin or medication for diabetes.

There are several mechanisms of acrolein toxicity, both directly and indirectly, including induction of oxidative stress and inflammation (Moghe et al. 2015) which are important factors in the insurgency of insulin resistance (Keane et al. 2015). Acrolein is primarily metabolized though GHS, and therefore it contributes directly to cellular oxidative stress

through depletion of glutathione (Kehrer and Biswal. 2000). Moreover, acrolein is an endogenous product, as well as an originator of lipid peroxidation reactions (Uchida et al 1998). Increased lipid peroxidation is associated with diabetes and diabetes complications (Davi et al. 2005). Therefore, acrolein may contribute to insulin resistance through its oxidative and inflammatory toxicities.

Our finding of increased HOMA-IR, with increased insulin and beta cell function may well be explained biologically through the induction of the *let-7* family by acrolein. Recently, small non-coding single stranded RNAs, or micro RNA (miRNA), that negatively regulate or repress target gene expression, have been associated with glucose metabolism. Zhu et al. (2011) using transgenic mouse showed that *let-7* expression of *let-7* resulted in suppression of several genes in the insulin signaling/PI3K/Akt and mTOR pathways and overexpression of *let-7* resulted "in peripheral glucose intolerance and compensatory overproduction of insulin from islet β cells". Recently, O'Toole et al. (2014) reported that exposure of human umbilical vein endothelial cells to acrolein induced insulin resistance through activation of the microRNA (miRNA) *let-7a*. Upregulation of *let-7a* reduced Akt phosphorylation in response to insulin. *Ex vivo* experiments with aortas from mice exposed to acrolein also resulted in upregulation of *let-7a* with a decrease in insulin-stimulated Akt phosphorylation. (O'Toole et al. 2014).

This study used the NHANES 2005–2006 dataset, a large, national survey whose findings are generalizable to the U.S. adult non-institutionalized population. However, there are several limitation to this study, such as the assessment of certain diabetes risk factors in the NHANES surveys relied on self-reporting, which may be subject to recall bias. The cross-sectional design is unable to assess questions of causality, although it can be beneficial in refining and supporting hypotheses. Moreover, Medina-Navarro et al. (2004) reported *in vitro* production of acrolein, likely as fatty acid product, generated during the glucose-autoxidation process, thus suggesting that acrolein may be produced during hyperglycemic states. Therefore, the potential for reverse causation as explanation of our findings may not be excluded.

CONCLUSION

In conclusion, we find that urinary acrolein metabolites were associated with diabetes and insulin resistance. Although acrolein exposure is widespread there is a scarcity of information about the effects the chemical on human health. Our findings bring new insight of a potential human health effect to acrolein exposure. These results underscore the importance of reducing environmental sources of acrolein exposure in the U.S. population by reducing exposure to tobacco smoke, to smoke from cooking oil and grease or burning wood products, as well as to exhaust from diesel or gasoline vehicles. However, further studies, such as longitudinal studies are needed to fully understand the implications of our findings.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

DISCLAIMER: The findings and conclusion in this report are those of the author and do not necessarily represent the views of CDC/ATSDR.

IRB approval: CDC/ATSDR has determined that our research did not meet the criteria for human research as per federal regulation and therefore did not require review.

Competing Financial Interests: The authors declare they have no actual or potential competing financial interests.

References

Abraham K, Andres S, Palavinskas R, Berg K, Appel KE, Lampen A. Toxicology and risk assessment of acrolein in food. Molecular nutrition & food research. 2011; 55(9):1277–90. [PubMed: 21898908]

Alwis KU, Blount BC, Britt AS, Patel D, Ashley DL. Simultaneous analysis of 28 urinary VOC metabolites using ultra high performance liquid chromatography coupled with electrospray ionization tandem mass spectrometry (UPLC-ESI/MSMS). Anal Chim Acta. 2012; 750:152–60. [PubMed: 23062436]

Alwis KU, de Castro BR, Morrow JC, Blount BC. Acrolein Exposure in U.S. Tobacco Smokers and Non-Tobacco Users: NHANES 2005–2006. Environ Health Perspect. 2015; 123:1302–1308. [PubMed: 26024353]

American Diabetes Association. Classification and diagnosis of diabetes. Sec. 2. In Standards of Medical Care in Diabetes—2015. Diabetes Care. 2015; 38(Suppl 1):S8–S16.

Ascaso JF, Pardo S, Real JT, Lorente RI, Priego A, Carmena R. Diagnosing insulin resistance by simple quantitative methods in subjects with normal glucose metabolism. Diabetes Care. 2003; 26(12):3320–5. [PubMed: 14633821]

ATSDR. Agency for Toxic Substances and Disease Registry. Toxicological Profile for Acrolein. Atlanta, GA: US Department of Health and Human Services, Public Health Service; 2007.

Barr DB, Wilder LC, Caudill SP, Gonzalez AJ, Needham LL, Pirkle JL. Urinary creatinine concentrations in the U.S. population: implications for urinary biologic monitoring measurements. Environ Health Perspect. 2005; 113(2):192–200. [PubMed: 15687057]

Bein K, Leikauf GD. Acrolein - a pulmonary hazard. Molecular nutrition & food research. 2011; 55(9):1342–60. [PubMed: 21994168]

Bernert JT Jr, Turner WE, Pirkle JL, Sosnoff CS, Akins JR, Waldrep MK, et al. Development and validation of sensitive method for determination of serum cotinine in smokers and nonsmokers by liquid chromatography/atmospheric pressure ionization tandem mass spectrometry. Clin Chem. 1997; 43(12):2281–91. [PubMed: 9439445]

Daimon M, Sugiyama K, Kameda W, Saitoh T, Oizumi T, Hirata A, et al. Increased urinary levels of pentosidine, pyrraline and acrolein adduct in type 2 diabetes. Endocrine journal. 2003; 50(1):61–7. [PubMed: 12733710]

Danaei G, Finucane MM, Lu Y, Singh GM, Cowan MJ, Paciorek CJ, et al. National, regional, and global trends in fasting plasma glucose and diabetes prevalence since 1980: systematic analysis of health examination surveys and epidemiological studies with 370 country-years and 2.7 million participants. Lancet. 2011; 378(9785):31–40. [PubMed: 21705069]

Dang TN, Arseneault M, Murthy V, Ramassamy C. Potential role of acrolein in neurodegeneration and in Alzheimer's disease. Curr Mol Pharmacol. 2010; 3(2):66–78. [PubMed: 20302565]

Davi G, Falco A, Patrono C. Lipid peroxidation in diabetes mellitus. Antioxidants & redox signaling. 2005; 7(1–2):256–68. [PubMed: 15650413]

DeJarnett N, Conklin DJ, Riggs DW, Myers JA, O'Toole TE, Hamzeh I, et al. Acrolein exposure is associated with increased cardiovascular disease risk. Journal of the American Heart Association. 2014; 3(4)

Grigsby J, Betts B, Vidro-Kotchan E, Culbert R, Tsin A. A possible role of acrolein in diabetic retinopathy: involvement of a VEGF/TGFbeta signaling pathway of the retinal pigment epithelium in hyperglycemia. Current eye research. 2012; 37(11):1045–53. [PubMed: 22906079]

Johnson CL, Paulose-Ram R, Ogden CL, Carroll MD, Kruszon-Moran D, Dohrmann SM, et al. National health and nutrition examination survey: analytic guidelines, 1999–2010. Vital Health Stat 2. 2013; (161):1–24.

Keane KN, Cruzat VF, Carlessi R, de Bittencourt PI Jr, Newsholme P. Molecular Events Linking Oxidative Stress and Inflammation to Insulin Resistance and beta-Cell Dysfunction. Oxid Med Cell Longev. 2015; 2015:181643. [PubMed: 26257839]

Kehrer JP, Biswal SS. The molecular effects of acrolein. Toxicological sciences:an official journal of the Society of Toxicology. 2000; 57(1):6–15. [PubMed: 10966506]

Kuo CC, Moon K, Thayer KA, Navas-Acien A. Environmental chemicals and type 2 diabetes: an updated systematic review of the epidemiologic evidence. Curr Diab Rep. 2013; 13(6):831–49. [PubMed: 24114039]

Loprinzi PD, Abbott K. Physical activity and total serum bilirubin levels among insulin sensitive and insulin resistant U.S. adults. J Diabetes Metab Disord. 2014; 13:47. [PubMed: 24708607]

Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia. 1985; 28(7):412–9. [PubMed: 3899825]

Medina-Navarro R, Duran-Reyes G, Diaz-Flores M, Hicks JJ, Kumate J. Glucose-stimulated acrolein production from unsaturated fatty acids. Human & experimental toxicology. 2004; 23(2):101–5. [PubMed: 15070069]

Moghe A, Ghare S, Lamoreau B, Mohammad M, Barve S, McClain C, et al. Molecular mechanisms of acrolein toxicity: relevance to human disease. Toxicological sciences : an official journal of the Society of Toxicology. 2015; 143(2):242–55. [PubMed: 25628402]

O'Toole TE, Abplanalp W, Li X, Cooper N, Conklin DJ, Haberzettl P, et al. Acrolein decreases endothelial cell migration and insulin sensitivity through induction of let-7a. Toxicological sciences : an official journal of the Society of Toxicology. 2014; 140(2):271–82. [PubMed: 24812010]

Park YS, Taniguchi N. Acrolein induces inflammatory response underlying endothelial dysfunction: a risk factor for atherosclerosis. Ann N Y Acad Sci. 2008; 1126:185–9. [PubMed: 18448814]

Pirkle JL, Bernert JT, Caudill SP, Sosnoff CS, Pechacek TF. Trends in the exposure of nonsmokers in the U.S. population to secondhand smoke: 1988–2002. Environ Health Perspect. 2006; 114(6): 853–8. [PubMed: 16759984]

Stevens JF, Maier CS. Acrolein: sources, metabolism, and biomolecular interactions relevant to human health and disease. Molecular nutrition & food research. 2008; 52(1):7–25. [PubMed: 18203133]

Suzuki D, Miyata T. Carbonyl stress in the pathogenesis of diabetic nephropathy. Internal medicine (Tokyo, Japan). 1999; 38(4):309–14.

Tsukahara H, Sekine K, Uchiyama M, Kawakami H, Hata I, Todoroki Y, et al. Formation of advanced glycosylation end products and oxidative stress in young patients with type 1 diabetes. Pediatric research. 2003; 54(3):419–24. [PubMed: 12761359]

Tully M, Shi R. New insights in the pathogenesis of multiple sclerosis--role of acrolein in neuronal and myelin damage. Int J Mol Sci. 2013; 14(10):20037–47. [PubMed: 24113583]

Uchida K, Kanematsu M, Morimitsu Y, Osawa T, Noguchi N, Niki E. Acrolein is a product of lipid peroxidation reaction. Formation of free acrolein and its conjugate with lysine residues in oxidized low density lipoproteins. The Journal of biological chemistry. 1998; 273(26):16058–66. [PubMed: 9632657]

Velagaleti RS, Gona P, Chuang ML, Salton CJ, Fox CS, Blease SJ, et al. Relations of insulin resistance and glycemic abnormalities to cardiovascular magnetic resonance measures of cardiac structure and function: the Framingham Heart Study. Circ Cardiovasc Imaging. 2010; 3(3):257–63. [PubMed: 20208015]

Wallace TM, Levy JC, Matthews DR. Use and abuse of HOMA modeling. Diabetes Care. 2004; 27(6): 1487–95. [PubMed: 15161807]

Zhao D, Cho J, Kim MH, Friedman D, Guallar E. Diabetes, glucose metabolism, and glaucoma: the 2005–2008 National Health and Nutrition Examination Survey. PloS one. 2014; 9(11):e112460. [PubMed: 25393836]

Zhu H, Shyh-Chang N, Segre AV, Shinoda G, Shah SP, Einhorn WS, et al. The Lin28/let-7 axis regulates glucose metabolism. Cell. 2011; 147(1):81–94. [PubMed: 21962509]

Table 1

Sample size and weighted characteristics of adult participants (20 years of age and older) in NHANES 2005–2006

	n	Weighted GM (SE)
Urinary 3-HPMA (μg/L), GM (SE)	2027	324.63 (15.18)
Urinary CEMA (μg/L), GM (SE)	2027	95.71 (3.51)
ΣAcrolein (3-HPMA + CEMA) (μmol/L), GM (SE)	2027	1.96 (0.09)
Creatinine-adjusted 3-HPMA (μg/g of creatinine), GM (SE)	2027	339.69 (16.54)
Creatinine-adjusted CEMA (μg/g of creatinine), GM (SE)	2027	100.15 (3.65)
Creatinine-adjusted ΣAcrolein (μmol/g of creatinine), GM (SE)	2027	2.06 (0.09)
Age (Years), GM (SE)	2027	43.84 (0.72)
Urinary Creatinine (mg/dL), GM (SE)	2027	95.57 (1.95)
BMI (Kg/m2) GM (SE)	2027	27.93 (0.22)
Serum Cotinine (ng/mL), GM (SE)	2027	0.45 (0.08)
c-reactive protein (mg/dL), GM (SE)	2027	0.18 (0.01)
Hour of fasting, GM (SE)	2027	5.30 (0.16)
Fasting Plasma Glucose (mmol/L), GM (SE) *	968	5.65 (0.06)
Insulin (uU, mL), GM (SE) *	959	8.57 (0.25)
HOMA-IR, GM (SE) *	958	2.15 (0.08)
HOMA-β	958	84.92 (1.55)
Insulin Resistance (HOMA-IR>2.6) *		% SE
No, % (SE)	549	61.52 (2.17)
Yes, % (SE)	409	38.48 (2.17)
Sex		
Men, % (SE)	1030	49.49 (0.65)
Women, % (SE)	997	50.51 (0.65)
Race/Ethnicity		
Non-Hispanic White, % (SE)	999	72.03 (2.69)
Non-Hispanic Black, % (SE)	476	11.15 (2.04)
Mexican-American, % (SE)	405	7.77 (1.08)
Other, % (SE)	147	9.05 (1.28)
Body Weight		
Obese, % (SE)	709	34.23 (1.53)
Overweight, % (SE)	700	33.70 (1.34)
Underweight/Normal weight, % (SE)	618	32.07 (1.56)
Smoking Status		
Current Smokers, % (SE)	473	23.89 (1.60)
Former Smokers, % (SE)	513	25.13 (1.48)
Never Smokers, % (SE)	1041	50.99 (2.03)
Alcohol Consumption		
No Alcohol, % (SE)	707	29.11 (2.06)

	n	Weighted GM (SE)
1–5 drinks per day, % (SE)	1184	63.94 (2.41)
>5 drinks per day, % (SE)	136	6.95 (0.69)
Education Level		
Less than High School, % (SE)	563	17.23 (1.50)
Completed High School, % (SE)	478	24.62 (1.13)
More than High School, % (SE)	986	58.15 (2.17)
Diabetes		
No, % (SE)	1750	90.38 (0.61)
Yes, % (SE)	277	9.62 (0.61)
Moderate/Vigorous Recreational Activities		
No, % (SE)	790	32.86 (2.11)
Yes, % (SE)	1237	67.14 (2.11)

* Values are based on the subsample of participants not taking insulin or medication for diabetes

Table 2

Adjusted* odd ratio (aOR) for diabetes for adult participants (20 years of age and older) in NHANES 2005–2006 (n=2027)

	Model 1 aOR (95% CI)	Model 2 aOR (95% CI)	Model 3 aOR (95% CI)
CEMA Q1 [n=425]	referent	referent	referent
CEMA Q2 [n=508]	2.51 (1.43, 4.38)	2.35 (1.30, 4.24)	2.16 (1.14, 4.10)
CEMA Q3 [n=534]	3.15 (1.55, 6.42)	3.07 (1.53, 6.16)	2.64 (1.26, 5.55)
CEMA Q4 [n=560]	2.60 (1.34, 5.07)	2.86 (1.49, 5.51)	2.39 (1.25, 4.54)
p-trend	0.01	0.01	0.04
3-HPMA Q1 [n=464]	referent	referent	referent
3-HPMA Q2 [n=525]	1.64 (1.21, 2.22)	1.56 (1.10, 2.21)	1.58 (1.13, 2.20)
3-HPMA Q3 [n=528]	2.42 (1.47, 4.00)	2.63 (1.66, 4.17)	2.79 (1.67, 4.66)
3-HPMA Q4 [n=510]	2.00 (1.21, 3.31)	3.09 (2.03, 4.70)	3.15 (2.01, 4.96)
p-trend	0.005	0.0000	0.0002
ΣAcrolein Q1 [n=457]	referent	referent	referent
ΣAcrolein Q2 [n=518]	1.85 (1.15, 2.95)	1.75 (1.12, 2.72)	1.72 (1.05, 2.83)
ΣAcrolein Q3 [n=543]	2.54 (1.42, 4.53)	2.69 (1.58, 4.58)	2.77 (1.48, 5.19)
ΣAcrolein Q4 [n=509]	2.16 (1.13, 4.12)	3.34 (1.85, 6.02)	3.36 (1.79, 6.30)
p-trend	0.03	0.0005	0.0017

Model 1: Adjusted for age and urinary creatinine.

Model 2: as Model 1 plus adjusted for Race/Ethnicity, sex, length of fasting, education attainment, alcohol consumption, self-reported cigarette smoking, and serum cotinine.

Model 3: as Model 2 plus adjusted for body weight status, and recreational activity.

Quartiles CEMA (μg/L): Q1: ≤ 47.49; Q2: 47.80 – 100.11; Q3: 100.12 – 196.98; Q4: >196.98. Quartiles 3-HPMA (μg/L): Q1: ≤ 144.44; Q2: 144.45 – 324.67; Q3: 324.68 – 707.35; Q4: >707.35. Quartiles ΣAcrolein (μmol/L): Q1: ≤ 0.92; Q2: 0.93 – 2.02; Q3: 2.03 – 4.15; Q4: >4.15.

Table 3

Adjusted* odd ratio (aOR) for Insulin Resistance (HOMA-IR>2.6) outcomes for adult participants (20 years of age and older) in NHANES 2005–2006 (n=850)

	Model 1 aOR (95% CI)	Model 2 aOR (95% CI)	Model 3 aOR (95% CI)
CEMA Q1 [n=153]	referent	referent	referent
CEMA Q2 [n=205]	1.59 (0.96, 2.62)	1.57 (0.96, 2.56)	1.88 (0.95, 3.72)
CEMA Q3 [n=245]	2.62 (1.54, 4.46)	3.04 (1.76, 5.25)	3.59 (1.90, 6.79)
CEMA Q4 [n=247]	2.42 (1.51, 3.89)	3.81 (1.99, 7.30)	4.18 (1.89, 9.22)
p-trend	0.005	0.002	0.004
3-HPMA Q1 [n=202]	referent	referent	referent
3-HPMA Q2 [n=239]	1.95 (1.15, 3.31)	1.92 (1.12, 3.29)	2.59 (1.45, 4.62)
3-HPMA Q3 [n=223]	2.67 (1.51, 4.70)	2.80 (1.54, 5.08)	4.87 (2.17, 10.94)
3-HPMA Q4 [n=186]	1.36 (0.85, 2.17)	1.91 (1.03, 3.55)	2.63 (1.23, 5.61)
p-trend	0.008	0.02	0.007
ΣAcrolein Q1 [n=187]	referent	referent	referent
ΣAcrolein Q2 [n=245]	1.54 (0.89, 2.66)	1.46 (0.81, 2.61)	1.65 (0.81, 3.38)
ΣAcrolein Q3 [n=226]	2.87 (1.69, 4.87)	3.10 (1.81, 5.30)	4.64 (2.40, 8.97)
ΣAcrolein Q4 [n=192]	1.40 (0.86, 2.27)	2.22 (1.19, 4.14)	2.88 (1.44, 5.75)
p-trend	0.005	0.001	0.0003

Model 1: Adjusted for age and urinary creatinine.

Model 2: as Model 1 plus adjusted for Race/Ethnicity, sex, length of fasting, education attainment, alcohol consumption, self-reported cigarette smoking, and serum cotinine.

Model 3: as Model 2 plus adjusted for body weight status, and recreational activity.

Quartiles CEMA (μg/L): Q1: ≤ 47.49; Q2: 47.80 – 100.11; Q3: 100.12 – 196.98; Q4: >196.98.. Quartiles 3-HPMA (μg/L): Q1: ≤ 144.44; Q2: 144.45 – 324.67; Q3: 324.68 – 707.35; Q4: >707.35. Quartiles ΣAcrolein (μmol/L): Q1: ≤ 0.92; Q2: 0.93 – 2.02; Q3: 2.03 – 4.15; Q4: >4.15.

Table 4

Adjusted beta –coefficient (95% CI) for diabetes markers in adult participants (20 years of age and older) in NHANES 2005–2006

	Ln - HOMA-IR	Ln –HOMA-β	Ln - Fasting Insulin	Ln - Fasting Plasma Glucose
N	850	850	851	860
CEMA Q1	0.0	0.0	0.0	0.0
CEMA Q2	0.10 (−0.08, 0.27)	0.09 (−0.05, 0.23)	0.09 (−0.07, 0.25)	0.00 (−0.02, 0.03)
CEMA Q3	0.16 (0.02, 0.31)	0.19 (0.09, 0.30)	0.17 (0.04, 0.30)	−0.01 (−0.03, 0.01)
CEMA Q4	0.29 (0.07, 0.50)	0.21 (0.08, 0.35)	0.26 (0.06, 0.45)	0.02 (−0.01, 0.04)
p-trend	0.03	0.005	0.03	0.11
3-HPMA Q1	0.0	0.0	0.0	0.0
3-HPMA Q2	0.17 (0.02, 0.32)	0.08 (−0.02, 0.19)	0.15 (0.01, 0.28)	0.02 (0.00, 0.04)
3-HPMA Q3	0.29 (0.05, 0.52)	0.27 (0.10, 0.43)	0.28 (0.07, 0.48)	0.01 (−0.02, 0.05)
3-HPMA Q4	0.21 (−0.04, 0.45)	0.15 (−0.09, 0.39)	0.18 (−0.06, 0.42)	0.01 (−0.02, 0.05)
p-trend	0.11	0.02	0.09	0.17
ΣAcrolein Q1	0.0	0.0	0.0	0.0
ΣAcrolein Q2	0.05 (−0.12, 0.22)	0.03 (−0.14, 0.20)	0.05 (−0.12, 0.21)	0.00 (−0.02, 0.03)
ΣAcrolein Q3	0.27 (0.09, 0.45)	0.29 (0.12, 0.47)	0.27 (0.10, 0.45)	−0.00 (−0.03, 0.03)
ΣAcrolein Q4	0.19 (−0.03, 0.42)	0.15 (−0.07, 0.37)	0.17 (−0.05, 0.40)	0.01 (−0.02, 0.03)
p-trend	0.002	0.0000	0.0005	0.84

*Adjusted for age, race/ethnicity, sex, length of fasting, education attainment, alcohol consumption, self-reported cigarette smoking, serum cotinine, body weight status recreational activity, and urinary creatinine.

Quartiles CEMA (μg/L): Q1: ≤ 47.49; Q2: 47.80 – 100.11; Q3: 100.12 – 196.98; Q4: >196.98.. Quartiles 3-HPMA (μg/L): Q1: ≤ 144.44; Q2: 144.45 – 324.67; Q3: 324.68 – 707.35; Q4: >707.35. Quartiles ΣAcrolein (μmol/L): Q1: ≤ 0.92; Q2: 0.93 – 2.02; Q3: 2.03 – 4.15; Q4: >4.15.