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**RESEARCH ARTICLE**

# Nutritional and metabolic regulation of the metabolite dimethylguanidino valeric acid: an early marker of cardiometabolic disease

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**Wali JA, Koay YC, Chami J, Wood C, Corcilius L, Payne RJ, Rodionov RN, Birkenfeld AL, Samocha-Bonet D, Simpson SJ, O'Sullivan JF.** Nutritional and metabolic regulation of the metabolite dimethylguanidino valeric acid: an early marker of cardiometabolic disease. *Am J Physiol Endocrinol Metab* 319: E509–E518, 2020. First published July 14, 2020; doi:10.1152/ajpendo.00207.2020.—Dimethylguanidino valeric acid (DMGV) is a marker of fatty liver disease, incident coronary artery disease, cardiovascular mortality, and incident diabetes. Recently, it was reported that circulating DMGV levels correlated positively with consumption of sugary beverages and negatively with intake of fruits and vegetables in three Swedish community-based cohorts. Here, we validate these results in the Framingham Heart Study Third Generation Cohort. Furthermore, in mice, diets rich in sucrose or fat significantly increased plasma DMGV concentrations. DMGV is the product of metabolism of asymmetric dimethylarginine (ADMA) by the hepatic enzyme AGXT2. ADMA can also be metabolized to citrulline by the cytoplasmic enzyme DDAH1. We report that a high-sucrose diet induced conversion of ADMA exclusively into DMGV (supporting the relationship with sugary beverage intake in humans), while a high-fat diet promoted conversion of ADMA to both DMGV and citrulline. On the contrary, replacing dietary native starch with high-fiber-resistant starch increased ADMA concentrations and induced its conversion to citrulline, without altering DMGV concentrations. In a cohort of obese nondiabetic adults, circulating DMGV concentrations increased and ADMA levels decreased in those with either liver or muscle insulin resistance. This was similar to changes in DMGV and ADMA concentrations found in mice fed a high-sucrose diet. Sucrose is a disaccharide of glucose and fructose. Compared with glucose, incubation of hepatocytes with fructose significantly increased DMGV production. Overall, we provide a comprehensive picture of the dietary determinants of DMGV levels and association with insulin resistance.

DMGV; insulin resistance; liver; metabolism; nutrition

## INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) is the commonest liver disease in the Western world, affecting one in three people in the general population (4, 38, 42). NAFLD is a central driver of type 2 diabetes (T2D); however, not all patients with NAFLD progress to T2D (2, 29, 32, 37). A noninvasive biomarker that could determine which patients with steatosis develop metabolic complications such as type 2 diabetes (T2D) would facilitate early commencement of treatment to optimize patient outcomes. Using integrated nontargeted metabolomics and genomics, O'Sullivan et al. reported that plasma DMGV was an independent biomarker of fatty liver in the Framingham Heart Study Third Generation Cohort (30). Concentrations of DMGV increased according to severity of NAFLD and correlated strongly with hepatocyte ballooning (30). In three separate human cohorts of different ethnicity, DMGV independently predicted T2D more than 12 yr before diagnosis (30). Furthermore, the concentrations of DMGV decreased in parallel with metabolic improvements after Roux-en-Y gastric bypass surgery (30).

DMGV is the product of transamination of asymmetric dimethylarginine (ADMA) by the enzyme alanine-glyoxylate aminotransferase-2 (AGXT2) (8, 9). Another substrate of AGXT2 is β-aminoisobutyric acid (BAIBA), a myokine metabolite that increases in the circulation after exercise and is inversely associated with cardiometabolic disease risk (35, 41). BAIBA competes with ADMA as an AGXT2 substrate and reduced concentrations of BAIBA would allow greater conver-

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sion of ADMA to DMGV (17, 30). ADMA can also be metabolized by the cytoplasmic enzyme dimethylarginine dimethylaminohydrolase-1 (DDAH1) into citrulline (43). Interestingly, DDAH1 is suppressed in fatty liver; *Ddah1*$^{-/-}$ mice on a high-fat diet had more liver fat, higher expression of genes involved in de novo lipogenesis, and more inflammation than wild-type mice on the same diet (8, 9, 21). This suggests that the conversion of ADMA to citrulline via DDAH1 may protect from metabolic impairment, while decreased DDAH1 activity would leave more ADMA to flux through AGXT2 to DMGV (8, 9, 21).

More recently, a study from Sweden showed that baseline DMGV concentrations were associated with T2D as well as incident coronary artery disease and cardiovascular mortality (31). Consumption of sugary drinks was associated with elevated concentrations of DMGV, but intake of vegetables, fruits, and whole grains as well as exercise was associated with lower DMGV concentrations (31). Another study revealed high baseline concentrations of DMGV predicted reduced responsiveness to the metabolic benefits of endurance exercise training (34). DMGV was also shown to be a marker of poor metabolic outcomes in response to chronic exercise in healthy male adults (19).

Motivated by the above, we explored further the relationship between dietary macronutrients and plasma concentrations of DMGV and related metabolites (ADMA, citrulline, and BAIBA) in a large human community-based cohort; a cohort carefully phenotyped by hyperinsulinemic-euglycemic clamp, murine models, and hepatocytes.

## MATERIALS AND METHODS

*Mice and diets.* Animal care and study protocols were approved by the Institutional Animal Ethics Committee at the University of Sydney (Sydney, NSW, Australia). C57BL/6J male mice (4 wk old) were purchased from Animal Resources Centre (Western Australia) and were housed four per cage ($n$ = 20 mice/diet) at 24°C – 26°C and 44% – 46% humidity under 12:12_h light-dark cycle settings (6 AM to 6 PM light cycle) with ad libitum access to food and water.

Commencing at 8 wk of age, mice were fed ad libitum isocaloric (~14.5 kJ/g) experimental diets (Specialty Feeds, Western Australia) for 18–19 wk. These diets were based on the AIN93G standard semipure rodent diets and were kept isocaloric by adjusting their cellulose content (33). The dietary interventions included the following comparisons: *1*) low- vs high-sucrose diet, *2*) 5% vs. 15% protein diet, and *3*) native starch vs. high-fiber-resistant starch diet (Table 1). Resistant starch was "gel-crisp" high-amylose RS type-2 (CRISP FILM Starch from Ingredion, IL). In addition, we conducted a study where mice were either fed control diet (AIN93G chow), a high-fat diet (HFD), or a high-fat, high-sucrose diet (HFHSD) for 1 or 20 wk (Table 1). All sampling occurred between 10 AM and 12 PM.

*Framingham Cohort.* The Framingham Heart Study (FHS) is a large and ongoing cohort study of cardiovascular health (11). Plasma samples from a randomly selected subset of the FHS Third Generation Cohort of this study were analyzed using hydrophilic interaction liquid chromatography followed by mass spectrometry. These participants also answered questionnaires on food intake, from which researchers could derive the average daily intake of various nutrients. DMGV abundance could thus be correlated with the intake of various foods and nutrients (11, 24).

*Human hyperinsulinemic-euglycemic clamps.* A cohort of Australian adults ($n$ = 64) with obesity [body mass index (BMI) of >30 kg/m$^2$] were studied at the Clinical Research Facility at the Garvan Institute of Medical Research (Sydney, Australia) using two-step hyperinsulinemic-euglycemic- clamps, as has been described in detail previously (5). Briefly, a 6-h, two-step hyperinsulinemic-euglycemic clamp procedure, incorporating deuterated glucose (Cambridge Isotope Laboratory, Tewksbury, MA) was applied. Liver insulin resistance was determined from the degree of suppression of endogenous glucose production (EGP) in response to a low-dose insulin (15 mU/m$^2$) infusion, while muscle insulin resistance was derived from the glucose infusion rate (GIR) during the high-dose insulin (80 mU/m$^2$) infusion (5). The study was approved by St. Vincent's Hospital Human Research Ethics Committee (Sydney, NSW, Australia).

*Metabolomics.* Plasma concentrations of metabolites were determined using published methodology incorporating the hydrophilic interaction liquid chromatography-tandem mass spectrometry (LC-MS/MS) method and amide chromatographic LC-MS/MS methods (20). DMGV was not commercially available and was synthesized according to the protocol reported by Klein et al. (18). Settings for multiple reaction monitoring (MRM) transitions and collision energies using triple-quadrupole mass spectrometry (QqQ-MS) were obtained for DMGV (synthesized), ADMA (Sigma-Aldrich), L-citrulline (Sigma-Aldrich), and β-aminoisobutyric acid (BAIBA) (Sigma-Aldrich) using direct infusion of analytical reference standards. MRM scan in positive ion mode was used to monitor ion transitions for DMGV (*m/z* 202.0 → 70.3), ADMA (*m/z* 203.1 → 70.3), L-citrulline (*m/z* 176.1 → 113.1, Sigma-Aldrich), and BAIBA (*m/z* 104.0 → 85.9, Sigma-Aldrich), and the MRM transitions were scheduled based on chromatographic retention time resolved on a hydrophilic interaction chromatographic 150 × 2.1 mm Atlantis HILIC column (Waters). Plasma extraction was done in plasma samples using previously published methods (20). Data were normalized relative to pooled samples that were analyzed in the sample queue after every five experimental samples, and normalized abundance was calculated for

Table 1. *Composition of experimental diets*

| Diets | Energy Density, kJ/g | %Energy from Protein | %Energy from Fat | %Energy from Carbohydrate |
|---|---|---|---|---|
| Low-sucrose diet | 14.5 | 5 (100% casein) | 20 (100% soy oil) | 75 (20% sucrose + 80% native starch) |
| High-sucrose diet | 14.5 | 5 (100% casein) | 20 (100% soy oil) | 75 (80% sucrose + 20% native starch) |
| 5% protein diet | 14.5 | 5 (100% casein) | 20 (100% soy oil) | 75 (20% sucrose + 80% native starch) |
| 15% protein diet | 14.5 | 15 (100% casein) | 20 (100% soy oil) | 65 (20% sucrose + 80% native starch) |
| Native starch diet | 14.5 | 10 (100% casein) | 20 (100% soy oil) | 70 (35% sucrose + 65% native starch) |
| Resistant starch diet | 14.5 | 10 (100% casein) | 20 (100% soy oil) | 70 (35% sucrose + 65% resistant starch) |
| AIN93G chow diet | 14.5 | 19 (100% casein) | 18 (100% soy oil) | 63 (16% sucrose + 21% dextrinized starch + 63% native starch) |
| High-fat diet | 18.0 | 19 (100% casein) | 45 (32% soy oil + 68% lard) | 36 (16% sucrose + 21% dextrinized starch + 63% native starch) |
| High-fat, high-sucrose diet | 18.0 | 19 (100% casein) | 45 (32% soy oil + 68% lard) | 36 (50% sucrose + 13% dextrinized starch + 37% native starch) |

Source of macronutrients is given in parentheses.


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each metabolite. Thus we measured the abundance of metabolites relative to the pooled sample (relative abundance) in our samples. Plasma from one mouse per cage was randomly selected giving four to five mice per diet.

*Hepatocyte culture.* Mouse hepatocyte cells (AML12) were cultured in glucose-free Dulbecco's modified Eagle's medium containing 1% FCS, 0.2% BSA and 1% penicillin and streptomycin (Sigma-Aldrich). Glucose (11 mM) or fructose (11 mM) either alone or in conjunction with 300 μM oleate were added to the media for 24 h before intracellular metabolite extraction.

*Statistical analyses.* Data are expressed as the means ± SE, and $P < 0.05$ was considered statistically significant. Statistical analyses of animal data were done in GraphPad PRISM software (San Diego, CA). For intake data from the FHS cohort, individual nutrient intake values were continuous, while specific food intake values were categorical ("Never," "1 per week," etc.). Spearman correlation coefficients were calculated between DMGV abundance and nutrient values, as nutrient levels were not generally normally distributed. Following correlation analysis, DMGV abundance was normalized using a Box-Cox transformation ($\lambda = 0.317$) and fiber intake was normalized using a $\log_2$ transformation. These normalized values were used to fit linear models of DMGV abundance, explained by soda and fiber, controlling for age, BMI, sex, smoking status, and blood pressure. Further details of statistical tests used are provided in relevant figure legends.

## RESULTS

We conducted studies in mice where we carefully manipulated the ratios of the macronutrients and the dietary fiber content (Table 1). This allowed the examination of the impact of a nutrient in a controlled setting that is only possible in animal studies. The metabolic consequences of these dietary interventions have been described elsewhere (20, 40, 46). Briefly, compared with chow-fed mice, mice fed a high-fat diet or a high-fat, high-sucrose diet had significantly greater body weights as expected (13, 45). Mice fed a 15% protein diet had greater body weights than those maintained on a 5% protein diet (46). In contrast, replacing native starch in the diet with resistant starch led to lower body weights (20). Similarly, mice maintained on a high-sucrose diet had slightly lower body weights than those on a low-sucrose diet, which is consistent with the findings of other studies where high amounts of sugars were fed to mice as a solid food (44, 47).

*High-sucrose intake increases circulating DMGV concentrations in mice.* We first studied the nutrients that were previously significantly associated with DMGV concentrations, i.e., sucrose (e.g., from soda drinks) and dietary fiber (31). Strikingly, we saw a ~3.5-fold increase in plasma DMGV concentrations in mice consuming a high-sucrose diet (Fig. 1*A*). ADMA was readily detectable in mice fed a low-sucrose diet, but it was undetectable in mice fed a high-sucrose diet. This indicates a substantially increased conversion of ADMA into DMGV in the liver in response to sucrose feeding. This is likely facilitated by reduced BAIBA concentrations (thereby providing less substrate competition with ADMA), while citrulline concentrations were unaltered (Fig. 1*A*), which is unsurprising given the multiple sources of citrulline (27), and therefore, its levels do not always represent activity of DDAH1.

*High-fiber intake increases conversion of ADMA to citrulline in mice.* In the next mouse study, 65% of the carbohydrate energy was either sourced from native-wheat starch or high-fiber resistant starch. Compared with native starch, resistant starch intake led to an increase in ADMA concentration and a trend toward increased citrulline concentrations, indicating increased ADMA to citrulline conversion via DDAH1 (Fig. 1*B*). However, there was no difference in plasma DMGV or BAIBA concentrations (Fig. 1*B*). There are several types of dietary fiber [e.g., inulin, pectin, gums, resistant starch, etc. (22)], and it is possible that the inverse association between DMGV and dietary fiber observed in FHS (Fig. 2, below) could be due to fiber types other than resistant starch.

*High-fat and high-fat, high-sucrose diets increase circulating DMGV concentrations in mice.* HFD and HFHSD are commonly used in rodent research on obesity, hepatic steatosis, and insulin resistance (13, 45). We collected plasma samples after 1 and 20 wk of dietary intervention to measure the concentration of metabolites. Compared with chow diet, mice fed these obesogenic diets for 20 wk showed increased plasma DMGV concentrations (Fig. 1, *C* and *D*). The HFD and HFHSD did not alter plasma ADMA or BAIBA concentrations but produced a small increase in citrulline concentrations. This suggests an increased metabolism of ADMA via both AGXT2 and DDAH1 enzymes on HFD and HFHSD (Fig. 1, *C* and *D*).

It has previously been shown in mice that cardiometabolic health and life span are adversely affected by high-protein intake (40). We tested if the dietary protein content affects plasma DMGV concentration. Mice were fed a very low-protein diet (5% protein) or a relatively high-protein diet (15%

Fig. 1. Impact of consuming diets rich in sucrose; resistant starch; high-fat diet (HFD); high-fat, high-sucrose diet (HFHSD); and diets with increasing amount of protein on plasma dimethylguanidino valeric acid (DMGV) concentration. *A*: consumption of high-sucrose diet results in elevated DMGV levels in the plasma of mice. Shown is metabolic pathway resulting in the production of DMGV and plasma levels of DMGV, asymmetric dimethylarginine (ADMA), β-aminoisobutyric acid (BAIBA), and citrulline in mice maintained on a high- or low-sucrose diet for 18–19 wk. AU, arbitrary units. All data are presented as means ± SE and were analyzed by an unpaired *t* test between indicated groups ($n = 4$–5 mice per group). $^*P < 0.05$ and $^{***}P < 0.001$ between indicated groups. *B*: consumption of resistant starch does not alter the plasma levels of DMGV in mice. Shown is the metabolic pathway resulting in the production of DMGV and plasma levels of DMGV, ADMA, BAIBA, and citrulline in mice maintained on a diet rich in high-fiber-resistant starch or native starch for 18–19 wk. All data are presented as means ± SE and were analyzed by an unpaired *t* test between indicated groups ($n = 5$ mice per group). $^*P < 0.05$ between indicated groups. *C*: consumption of a high-fat diet (HFD) results in elevated DMGV levels in the plasma of mice. Shown is metabolic pathway resulting in the production of DMGV and plasma levels of DMGV, ADMA, BAIBA, and citrulline in mice maintained on an HFD for 1 or 20 wk. All data are presented as means ± SE and were analyzed by one-way ANOVA ($n = 9$ mice per group). $^*P < 0.05$ between indicated groups; ns, not significant. *D*: consumption of a high-fat, high-sucrose diet (HFHSD) results in elevated DMGV levels in the plasma of mice. Shown is metabolic pathway resulting in the production of DMGV and plasma levels of DMGV, ADMA, BAIBA, and citrulline in mice maintained on a HFHSD for 1 or 20 wk. All data are presented as means ± SE and were analyzed by one-way ANOVA ($n = 9$ mice per group). $^*P < 0.05$ between indicated groups; ns, not significant. *E*: consumption of increasing amounts of protein results in a trend toward elevated DMGV levels in the plasma of mice. Shown is the metabolic pathway resulting in the production of DMGV and plasma levels of DMGV, ADMA, BAIBA, and citrulline in mice maintained on a diet containing either 5% or 15% energy from protein for 18–19 wk. All data are presented as means ± SE and were analyzed by an unpaired *t* test between indicated groups ($n = 5$ mice per group).


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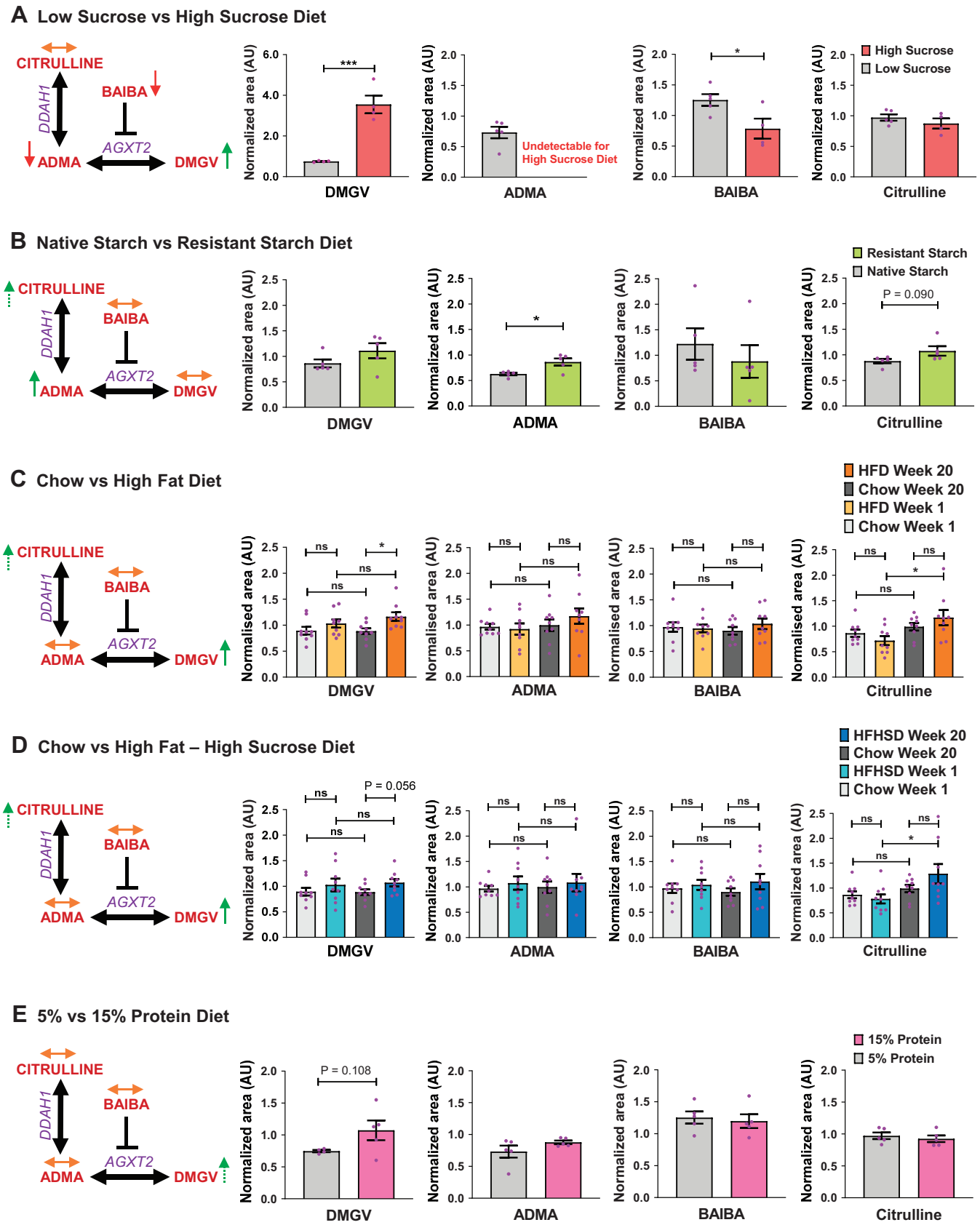



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protein) for 18–19 wk (for comparison, the AIN93G chow diet provides ~19% energy from protein) (33). Increasing dietary protein from 5% to 15% resulted in a trend toward increased DMGV concentrations but did not affect plasma ADMA or citrulline concentration (Fig. 1*E*). It is possible that increasing dietary protein content beyond 15% could lead to statistically significant increases in plasma DMGV.

*Circulating DMGV levels in humans correlate with consumption of soda drinks and dietary fiber.* To investigate the association between nutrient consumption and plasma DMGV in humans, we analyzed the dietary data in the FHS Third Generation cohort. This included macronutrients (protein, fat, and carbohydrate), sugary drinks ("soda"), and dietary fiber (Fig. 2*A*). Important characteristics of study participants are

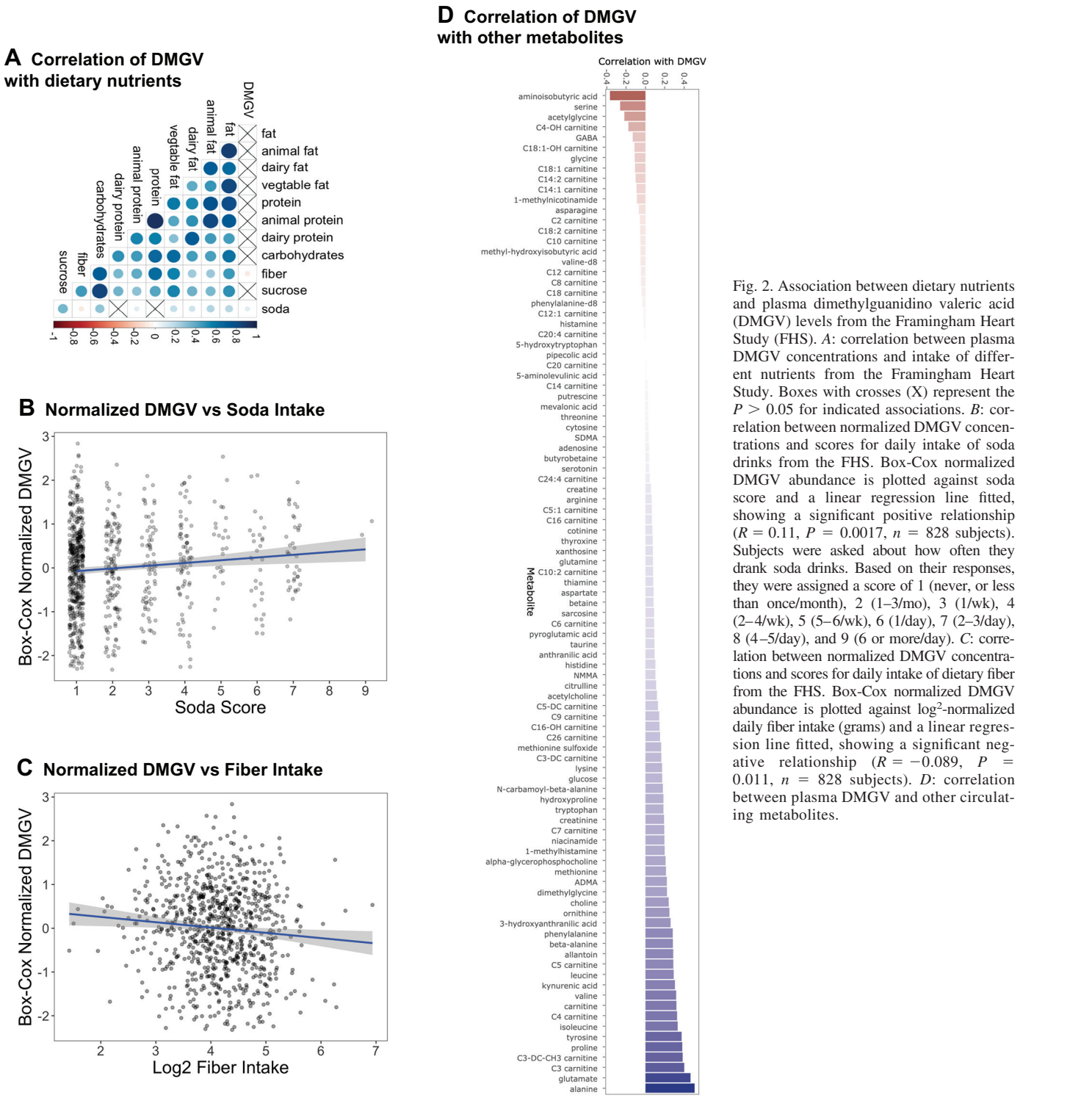


Fig. 2. Association between dietary nutrients and plasma dimethylguanidino valeric acid (DMGV) levels from the Framingham Heart Study (FHS). *A*: correlation between plasma DMGV concentrations and intake of different nutrients from the Framingham Heart Study. Boxes with crosses (X) represent the $P > 0.05$ for indicated associations. *B*: correlation between normalized DMGV concentrations and scores for daily intake of soda drinks from the FHS. Box-Cox normalized DMGV abundance is plotted against soda score and a linear regression line fitted, showing a significant positive relationship ($R = 0.11$, $P = 0.0017$, $n = 828$ subjects). Subjects were asked about how often they drank soda drinks. Based on their responses, they were assigned a score of 1 (never, or less than once/month), 2 (1–3/mo), 3 (1/wk), 4 (2–4/wk), 5 (5–6/wk), 6 (1/day), 7 (2–3/day), 8 (4–5/day), and 9 (6 or more/day). *C*: correlation between normalized DMGV concentrations and scores for daily intake of dietary fiber from the FHS. Box-Cox normalized DMGV abundance is plotted against $\log^2$-normalized daily fiber intake (grams) and a linear regression line fitted, showing a significant negative relationship ($R = -0.089$, $P = 0.011$, $n = 828$ subjects). *D*: correlation between plasma DMGV and other circulating metabolites.


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Table 2. *Characteristics of participants in the FHS Third Generation cohort randomly selected for metabolomic analyses*

| Sample size | 828 |
|---|---|
| Age | 43 yr (SD = 9.06) |
| Sex | 46.4% men; 53.6% women |
| Weight | 171.5 lb (SD = 40.76) |
| Height | 67.11 in. (SD = 3.79) |
| BMI | 26.64 (SD = 5.31) |

BMI, body mass index; FHS, Framingham Heart Study.

given in Table 2. Consistent with data from the Swedish cohorts (31), we observed a significant positive correlation between the consumption of soda drinks and circulating DMGV concentration ($\rho = 0.084$, $P = 0.016$, $n = 828$ subjects) (Fig. 2, *A* and *B*, and Table 3). In contrast, increased intake of dietary fiber was associated with reduced concentrations of DMGV ($\rho = -0.088$, $P = 0.012$, $n = 828$ subjects) (Fig. 2, *A* and *C*, and Table 4). Linear models of normalized DMGV abundance also showed a significant positive association with soda ($P = 0.0017$), and a significant negative association with fiber ($P = 0.011$). After adjusting the linear models for age, sex, BMI, systolic and diastolic blood pressure, and cigarettes per day, the association between DMGV and sugary drinks remained highly significant (*P* value changed from 0.0017 to 0.0020), but the effect of fiber became nonsignificant (*P* value changed from 0.011 to 0.42) (Table 3 and 4). Moreover, circulating DMGV concentrations correlated strongly with other substrates of AGXT2 including alanine, ADMA, and BAIBA (Fig. 2*D*). However, we did not observe an association between DMGV and other nutrients analyzed. Analyzing data by the source of protein or fat (animal vs. plant) did not yield significant results.

*Hepatic and muscle insulin resistance in humans is associated with increased circulating DMGV levels.* In humans, plasma DMGV concentrations correlate strongly with the extent of liver steatosis (30), but its relationship with insulin resistance in muscle and liver remains unknown. We measured plasma DMGV concentrations in a cohort of obese nondiabetic individuals studied using two-step hyperinsulinemic-euglycemic clamps (5). This allowed identification of the following distinct phenotypic groups: *i*: insulin sensitive in both muscle and liver (MSLS); *ii*: insulin resistant in liver (MSLR); and *iii*: insulin resistant in muscle (MRLS). We found that DMGV levels were significantly higher while ADMA and citrulline levels were lower in the presence of either hepatic (MSLR group) or skeletal muscle (MRLS group) insulin resistance (vs. MSLS group) (Fig. 3*A*, *i–v*). The trends for plasma BAIBA concentrations were similar to ADMA and citrulline results (Fig. 3*Aiv*). This suggests that plasma DMGV is not only sensitive to hepatic fat and hepatic insulin resistance but is also perturbed by metabolic impairment in other insulin sensitive tissues.

*Exposure of hepatocytes to fructose and oleate increases DMGV synthesis.* Sucrose (a disaccharide of glucose and fructose) and high-fructose corn syrup (a ~1:1 mixture of monosaccharide glucose and fructose) are the most commonly used caloric sweeteners in sugary drinks (3, 25). Increased intake of fructose induces de novo lipogenesis in the liver and leads to hepatic steatosis as wells as insulin resistance (12, 26, 39). We cultured AML12 mouse hepatocytes with glucose or

Table 3. *Soda: regression table showing coefficients and standard errors for successive linear models of Box-Cox normalized DMGV abundance*

| | Dependent Variable | | | | |
|---|---|---|---|---|---|
| | DMGV | | | | |
| | (*1*) | (*2*) | (*3*) | (*4*) | (*5*) |
| Soda | 0.062*** | 0.064*** | 0.063*** | 0.056*** | 0.055*** |
| | (0.020) | (0.020) | (0.018) | (0.018) | (0.018) |
| Age | | 0.024*** | 0.015*** | 0.012*** | 0.011*** |
| | | (0.004) | (0.003) | (0.004) | (0.004) |
| Sex | | −0.257*** | −0.072 | −0.025 | −0.020 |
| | | (0.070) | (0.063) | (0.064) | (0.064) |
| BMI | | | 0.088*** | 0.083*** | 0.082*** |
| | | | (0.006) | (0.006) | (0.006) |
| Systolic BP, mmHg | | | | −0.0002 | −0.001 |
| | | | | (0.003) | (0.003) |
| Diastolic BP, mmHg | | | | 0.012*** | 0.013*** |
| | | | | (0.004) | (0.004) |
| Cigarettes/day | | | | | 0.005* |
| | | | | | (0.003) |
| Constant | −0.137** | −1.028*** | −3.090*** | −3.703*** | −3.687*** |
| | (0.056) | (0.187) | (0.213) | (0.291) | (0.292) |
| Observations | 828 | 828 | 828 | 827 | 824 |
| $R^2$ | 0.012 | 0.076 | 0.281 | 0.291 | 0.295 |
| Adjusted $R^2$ | 0.011 | 0.073 | 0.278 | 0.286 | 0.289 |
| Residual SE | 0.995 | 0.963 | 0.850 | 0.845 | 0.844 |
| | (df = 826) | (df = 824) | (df = 823) | (df = 820) | (df = 816) |
| *F* statistic | 9.871*** | 22.722*** | 80.511*** | 56.171*** | 48.855*** |
| | (df = 1; 826) | (df = 3; 824) | (df = 4; 823) | (df = 6; 820) | (df = 7; 816) |

There was a significant positive linear relationship between soda scores and dimethylguanidino valeric acid (DMGV). The association was then tested with the stepwise addition of the following clinically significant covariates as indicated: age, sex, body mass index (BMI), systolic and diastolic blood pressure (BP), and cigarettes per day. The association between soda and DMGV remained highly significant. *$P < 0.1$, **$P < 0.05$, ***$P < 0.01$.


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Table 4. *Fiber: regression table showing coefficients and standard errors for successive linear models of Box-Cox normalized DMGV abundance*

| | Dependent Variable | | | | |
|---|---|---|---|---|---|
| | DMGV | | | | |
| | (*1*) | (*2*) | (*3*) | (*4*) | (*5*) |
| Fiber | −0.121** | −0.116** | −0.063 | −0.046 | −0.034 |
| | (0.048) | (0.046) | (0.041) | (0.041) | (0.042) |
| Age | | 0.022*** | 0.013*** | 0.010*** | 0.009** |
| | | (0.004) | (0.003) | (0.003) | (0.004) |
| Sex | | −0.312*** | −0.129** | −0.072 | −0.067 |
| | | (0.067) | (0.061) | (0.062) | (0.063) |
| BMI | | | 0.088*** | 0.082*** | 0.081*** |
| | | | (0.006) | (0.006) | (0.006) |
| Systolic BP, mmHg | | | | −0.0003 | −0.001 |
| | | | | (0.003) | (0.003) |
| Diastolic BP, mmHg | | | | 0.013*** | 0.014*** |
| | | | | (0.004) | (0.004) |
| Cigarettes/day | | | | | 0.004 |
| | | | | | (0.003) |
| Constant | −0.501** | −0.288 | −2.553*** | −3.317*** | −3.359*** |
| | (0.201) | (0.254) | (0.270) | (0.349) | (0.349) |
| Observations | 828 | 828 | 828 | 827 | 824 |
| $R^2$ | 0.008 | 0.072 | 0.272 | 0.284 | 0.288 |
| Adjusted $R^2$ | 0.007 | 0.069 | 0.269 | 0.279 | 0.282 |
| Residual SE | 0.997 | 0.965 | 0.855 | 0.849 | 0.849 |
| | (df = 826) | (df = 824) | (df = 823) | (df = 820) | (df = 816) |
| *F* statistic | 6.436** | 21.396*** | 77.004*** | 54.198*** | 47.068*** |
| | (df = 1; 826) | (df = 3; 824) | (df = 4; 823) | (df = 6; 820) | (df = 7; 816) |

There was a significant negative linear relationship between log²-normalized fiber intake and dimethylguanidino valeric acid (DMGV). The association was then tested with the stepwise addition of the following clinically significant covariates as indicated: age, sex, body mass index (BMI), systolic and diastolic blood pressure (BP), and cigarettes per day. After adjusting for age and sex, the inverse relationship between fiber and DMGV remained significant, but in the presence of the other covariates the association became nonsignificant. $^{**}P < 0.05$, $^{***}P < 0.01$.

fructose alone or in combination with the fatty acid oleate. Compared with glucose treatment, incubation of hepatocytes with fructose or fructose + oleate resulted in increased production of DMGV. These treatments did not alter ADMA concentrations (Fig. 3*B*). This result indicates that the fructose component of sucrose could be responsible for significant increase in DMGV production in liver.

## DISCUSSION

The metabolite DMGV is emerging as a valuable marker of cardiometabolic disease. Its concentration in plasma correlates strongly with liver fat (30) and incident coronary artery disease/cardiovascular mortality (31) and predicts future T2D (30, 31). Increased intake of sugary drinks is associated with higher plasma concentrations of DMGV while reduced concentrations of DMGV are seen in subjects following diets rich in vegetables, after Roux-en-Y gastric weight loss surgery, and post exercise intervention (30, 31, 34). In this study, we showed that increased intake of sugary drinks and dietary fiber is associated with elevated and lower concentrations of DMGV, respectively, in community-dwelling individuals. Our dietary interventions in mice showed that diets rich in sucrose or fat or their combination increase DMGV concentrations. In the case of a very high-sucrose diet, there was a dramatic increase in AGXT2-mediated conversion of ADMA to DMGV, but metabolism of ADMA into citrulline by DDAH1 remained unaltered. After HFD, there was a less pronounced yet significant increase in conversion of ADMA into both DMGV and citrulline. In contrast, replacing native starch with high-fiber-resistant starch led to increased ADMA concentrations and a trend toward increased conversion of ADMA into citrulline, but DMGV concentrations remained unaffected. This may be con-

Fig. 3. Insulin resistance and exposure of hepatocytes to fructose increases dimethylguanidino valeric acid (DMGV). *A*: insulin resistance in liver and muscle results in increased plasma DMGV and decreased plasma asymmetric dimethylarginine (ADMA) levels. *i*: Metabolic pathway resulting in the production of DMGV and related metabolites [ADMA, β-aminoisobutyric acid (BAIBA), and citrulline] in nondiabetic obese subjects with insulin resistance in liver or muscle. *ii*, *iii*, *iv*, and *v*: Plasma DMGV (*ii*), ADMA (*iii*), BAIBA (*iv*), and citrulline (*v*) concentrations in obese nondiabetic subjects that were either insulin sensitive in both muscle and liver (MSLS) ($n = 12$) or insulin resistant in liver (MSLR) ($n = 8$) or insulin resistant in muscle (MRLS) ($n = 9$). AU, arbitrary units. All data are presented as means ± SE and were analyzed by an unpaired *t* test between indicated groups. $^{*}P < 0.05$ and $^{**}P < 0.01$ between indicated groups. Data analysis by ANOVA-Tukey's post hoc test yielded the following *P* values: DMGV [MSLS vs. MSLR = 0.31; MSLS vs. MRLS = 0.05], ADMA [MSLS vs. MSLR = 0.002; MSLS vs. MRLS = 0.003], BAIBA [ MSLS vs. MSLR = 0.39; MSLS vs MRLS = 0.09], and citrulline [MSLS vs. MSLR = 0.08; MSLS vs. MRLS = 0.06]. *B*: exposure of hepatocytes to fructose results in increased DMGV production. AML12 hepatocytes were treated with glucose (11 mM) or fructose (11 mM) in isolation or in combination with 300 μM oleate for 24 h. Metabolites were measured in hepatocytes after these treatments. All data are presented as means ± SE and were analyzed by one-way ANOVA ($n = 4$ independent experiments). $^{*}P < 0.05$ between indicated groups. *C*: summary of results: impact of sucrose, fat, protein, and resistant starch intake on circulating concentrations of DMGV, ADMA, BAIBA, and citrulline. Dotted arrows represent trends that are statistically not significant. R. Starch, resistant starch.


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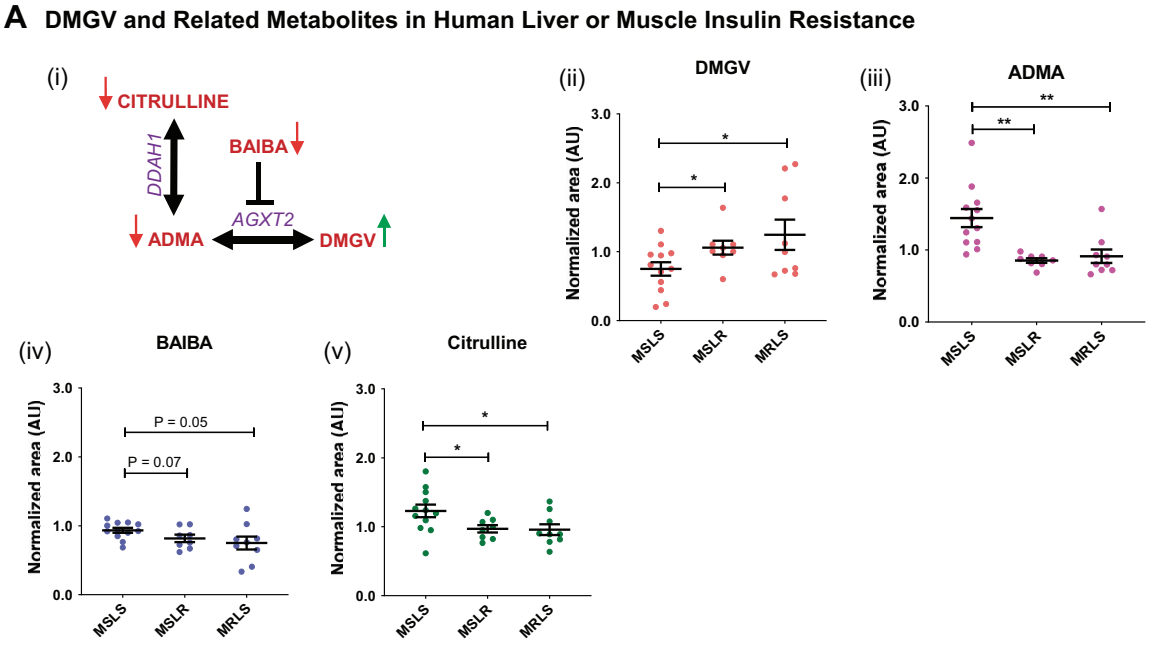
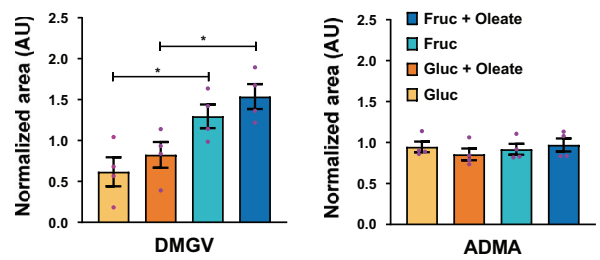
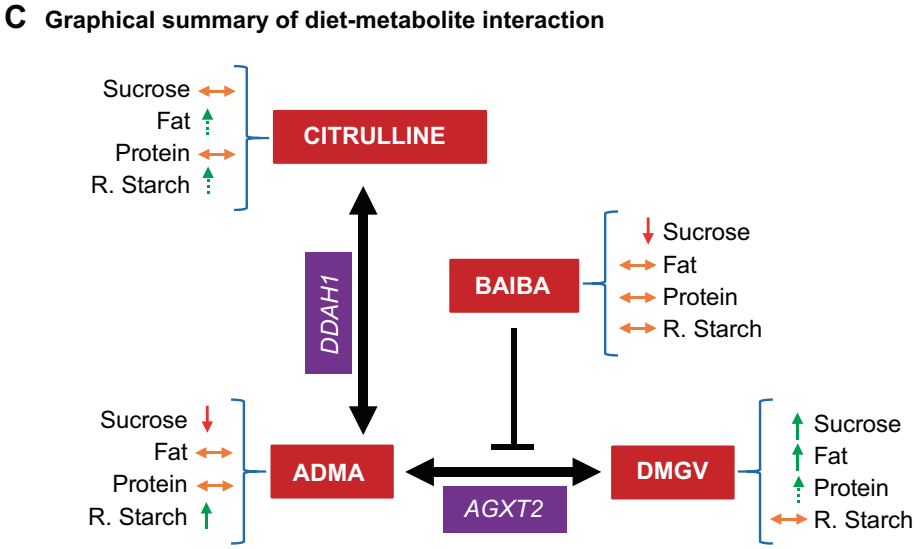
sistent with the association of citrulline with enterocyte mass and intestinal function (6, 10) [high fiber intake increases cecum size and colonic cell proliferation in rodents (16, 48)]. Overall, our results show that ADMA is metabolized into DMGV in the context of increased components of an unhealthy diet (Fig. 3*C*).

Interestingly, we found that DMGV concentrations increased and ADMA concentrations decreased in subjects with





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hepatic or muscle insulin resistance, and the DMGV/ADMA results mirrored the findings in mice on a very high-sucrose diet. It is therefore unsurprising that DMGV correlated strongly with consumption of sugary drinks in humans (31). This extends the clinical value of DMGV from liver biology to other organ/global metabolic health, and the skeletal muscle result may underscore the DMGV response to exercise, which is intriguing as AGXT2 is not expressed in skeletal muscle (14). The fructose component of the sucrose strongly stimulates de novo lipogenesis in the liver that promotes visceral adiposity and insulin resistance (1, 26, 39). Fructose consumption, particularly in beverages, has been linked to T2D and cardiovascular disease (23, 28). We found that fructose also increased DMGV production in hepatocytes. Future studies must examine the mechanisms that link DMGV with ectopic lipid deposition and insulin resistance in muscle and liver.

A limitation of our work is that we measured the concentration of metabolites in circulation, which does not always reflect the activity of enzymes involved (DDAH1 and AGXT2). The steady-state levels of biomolecules in biological samples reflect the equilibrium between their production, catabolism, transport, and excretion. ADMA is metabolized by both DDAH and AGXT2 enzymes, and its circulating concentration is therefore a less sensitive parameter for estimation of AGXT2 activity in vivo (15, 36). Similarly, citrulline is a less specific readout of DDAH1 activity as it is also produced in the urea cycle and by nitric oxide synthases (7). In comparison, DMGV is exclusively produced by AGXT2 and its concentrations are a more sensitive measure of AGXT2 activity (15, 36). This might explain why the changes in DMGV are not always associated with expected complementary changes in plasma ADMA and citrulline levels. In addition, while AGXT2 is the only enzyme described in the literature that could generate DMGV (15, 36), it is possible that other metabolic pathways also exist that could produce DMGV, and this should be investigated in future studies.

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## DISCLAIMERS

This manuscript was not prepared in collaboration with investigators of the Framingham Heart Study and does not necessarily reflect the opinions or views of the Framingham Heart Study, Boston University, or National Heart, Lung, and Blood Institute.

## DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

## AUTHOR CONTRIBUTIONS

J.A.W., D.S.-B., S.J.S., and J.F.O. conceived and designed research; J.A.W., Y.C.K., C.W., L.C., R.J.P., and D.S.-B. performed experiments; J.A.W., Y.C.K., J.C., C.W., L.C., R.J.P., R.N.R., and D.S.-B. analyzed data; J.A.W., Y.C.K., J.C., R.N.R., D.S.-B., S.J.S., and J.F.O. interpreted results of experiments; J.A.W., J.C., and C.W. prepared figures; J.A.W. drafted manuscript; J.A.W., Y.C.K., J.C., A.L.B., D.S.-B., S.J.S., and J.F.O. edited and revised manuscript; J.A.W., Y.C.K., J.C., C.W., L.C., R.J.P., R.N.R., A.L.B., D.S.-B., S.J.S., and J.F.O. approved final version of manuscript.

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