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Elevated Methylmalonic Acid, a Marker of Functional Vitamin B12 Deficiency, Is an Independent Predictor of Cardiovascular Risk in Beninese Taxi-Motorbike Drivers

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Abstract

Background: Taxi-motorbike drivers (TMDs) in Benin constitute an occupational group with significantly elevated cardiovascular disease risk. However, the relationships between nutritional status, metabolic biomarkers, and predicted cardiovascular risk in this population remain poorly characterized. Notably, functional vitamin B12 deficiency indicated by elevated methylmalonic acid (MMA) levels, rather than plasma vitamin B12 concentrations alone, may represent an important modifiable factor contributing to cardiometabolic dysregulation in this high-risk cohort. This study aimed to evaluate the association between MMA levels and predicted cardiovascular risk, as measured by the Framingham Risk Score (FRS) among TMDs in Cotonou, Benin. **Methods:** A cross-sectional cohort of 176 TMDs (mean age 39.3 ± 7.7 years) underwent clinical and biochemical assessments including blood pressure, glucose, lipids, uric acid, homocysteine, folate, vitamin B12, and MMA. Participants were stratified by MMA tertiles to evaluate relationships with cardiometabolic factors. The FRS was categorized into low-risk (<10%) and elevated-risk (≥10%) groups to enhance analytical clarity and clinical interpretability. Multivariable logistic regression models adjusted for demographic, metabolic, renal, and nutritional confounders investigated the association between MMA and elevated FRS. **Re-**

sults: The cohort exhibited elevated homocysteine levels, with a mean of 29.4 μmol/L, and had an average FRS of 7.4%. MMA levels were elevated (mean: 183.2 nmol/L) despite normal plasma vitamin B12 (mean:424.1 pmol/L). Stratification by MMA tertiles showed a significant graded increase in FRS (T1: 6.0%, T3: 8.6%, $p = 0.003$) and corresponding inverse vitamin B12 levels (T1: 456.7 pmol/L, T3: 349.7 pmol/L, $p < 0.001$). No significant differences were observed in other cardiometabolic parameters across MMA tertiles. In fully adjusted models, participants in the highest MMA tertile (>197 nmol/L) had nearly sevenfold greater odds of elevated FRS compared to the lowest tertile (aOR = 6.99; 95% CI: 1.20 - 30.67; $p = 0.030$). **Conclusions:** This study identifies a strong and independent association between elevated MMA levels and increased predicted cardiovascular risk among TMDs in Benin. This finding highlights elevated MMA as a significant and independent contributor to cardiovascular risk in this population and supports its potential as a valuable biomarker for improving risk assessment in underserved, high-risk groups like TMDs. Further investigation into vitamin B12 supplementation as a possible intervention is warranted.

Keywords

Cardiovascular Disease, Framingham Risk Score, Methylmalonic Acid, Taxi-Motorbike Drivers, Vitamin B12

1. Introduction

Cardiovascular disease (CVD) is a leading cause of morbidity and mortality globally, affecting both urban and occupationally exposed populations in low-resource settings [1] [2]. Effective risk stratification is critical for early intervention and prevention of CVD. The Framingham Risk Score (FRS) remains a widely used tool for estimating 10-year CVD risk, integrating traditional factors such as age, blood pressure, and lipid profiles [3]. However, its reliance on these conventional markers may overlook metabolic nuances, particularly in younger and high-risk populations with specific occupational exposures [4].

In this context, the triglyceride-glucose (TyG) index has emerged as a robust, low-cost surrogate marker of insulin resistance (IR), subclinical metabolic dysfunction, and CVD [5] [6]. Our recent study among taxi-motorbike drivers (TMDs) in Cotonou—a group with high exposure to traffic-related air pollution, sedentary behavior, and psychosocial stress—demonstrated that the TyG index strongly predicted elevated FRS, highlighting its utility in identifying individuals with heightened cardiometabolic risk in resource-limited settings [4] [7]. Furthermore, we identified that elevated vitamin B12 was modestly protective against an increased TyG index, highlighting the importance of micronutrient status in modulating IR and subsequent CVD risk. However, vitamin B12 status is multifaceted, and circulating levels alone may not fully capture its metabolic effects. Methylmalonic acid (MMA), a metabolic derivative in the propionate pathway, accumulates when

vitamin B12-dependent conversion to succinyl-CoA is impaired. Thus, elevated MMA is a highly specific and functional marker of intracellular vitamin B12 deficiency, often preceding changes in blood vitamin B12 levels [8].

Elevated MMA levels have been consistently associated with adverse cardiovascular outcomes across diverse populations. Evidence from recent studies demonstrates that serum MMA predicts increased risk of CVD in the general population [9], acute myocardial infarction, and mortality in patients with suspected or established coronary heart disease (CHD) [10]. Furthermore, elevated MMA—but not circulating or dietary vitamin B12—was associated with increased risk of cardiovascular mortality specifically in patients with CHD [11] and chronic kidney disease [12]. Importantly, this association extends to general population cohorts, where elevated MMA predicts increased all-cause mortality [13].

This relationship is thought to reflect the downstream effects of B12 insufficiency on mitochondrial energy metabolism, oxidative stress, and cellular lipid processing—key processes that underline CVD pathophysiology [14].

Despite its recognized role as a superior functional biomarker of vitamin B12 status and its proposed involvement in mechanisms leading to cardiovascular injury, the association between MMA and established CVD risk prediction tools has not been thoroughly investigated, particularly in high-risk occupational populations within resource-constrained settings. Building on our earlier observation of a relationship between plasma vitamin B12 levels and the TyG index in TMDs, an important question arises: could this association be more accurately characterized by using MMA, which reflects functional rather than just total vitamin B12 status? Additionally, it remains unclear whether MMA correlates with the calculated 10-year cardiovascular risk estimated by FRS in this group. Given that TMDs face a convergence of traditional CVD risk factors alongside occupational and potential nutritional stressors, investigating the role of MMA could yield vital insights for enhancing early risk identification and the development of targeted interventions in this vulnerable population.

Therefore, leveraging our previous findings, this study aims to investigate whether MMA, as a functional marker of vitamin B12 status, provides a more robust assessment of the relationship between vitamin B12 and predicted cardiovascular risk—as estimated by the FRS—in a cohort of TMDs in Cotonou, Benin. We hypothesized that elevated MMA levels would be associated with predicted 10-year CVD risk, independent of traditional risk factors and plasma vitamin B12 levels. This work seeks to refine risk stratification strategies by integrating novel metabolic and functional nutritional biomarkers, ultimately informing targeted interventions for high-risk occupational groups in low- and middle-income countries.

2. Methods

2.1. Study Design and Population

This analytical cross-sectional investigation was conducted within a cohort of taxi-motorbike drivers (TMDs) in Cotonou, Benin. The study included actively

employed male TMDs aged ≥18 years who met the eligibility criteria of current professional activity, absence of known cardiovascular disease, and no conditions affecting vitamin B12 or homocysteine metabolism (e.g., renal impairment, thyroid disorders), and who participated in a comprehensive health assessment campaign, as described elsewhere [7] [15] [16]. Current smokers were excluded to minimize confounding effects of tobacco use on cardiometabolic parameters. A total of 176 TMDs, with a mean professional driving duration of 11 years and complete laboratory measurements, were included in the final analysis. Although formal sample size calculation was not performed a priori, the final sample size provided sufficient statistical power for multivariate analyses, as demonstrated by the significant effects observed for the primary outcomes. The study protocol received approval from the Benin Environmental Agency, and all participants provided written informed consent before enrollment.

2.2. Data Collection and Laboratory Measurements

Trained research personnel collected data through structured interviews and clinical measurements. Data included demographic characteristics, health behaviors, medical history, occupational details, and anthropometric measurements (height and weight used to calculate body mass index). Blood pressure was measured twice using calibrated sphygmomanometers after five minutes of rest, with the average value recorded for analysis.

Fasting venous blood samples were collected following an overnight fast of at least 8 hours and processed within two hours of collection. Plasma was separated by centrifugation and stored at −20˚C until analysis. Biochemical parameters including fasting glucose, lipid profiles, and renal function markers were analyzed using standardized automated clinical analyzers, as detailed elsewhere [15]. Vitamin B12 and folate levels were quantified using the SimulTRAC-SNB radio-isotope kit [17]. Methylmalonic acid (MMA) and homocysteine concentrations were determined using liquid chromatography-tandem mass spectrometry (LC-MS/MS), ensuring high analytical specificity and sensitivity. The average intra- and inter-assay coefficients of variation were both less than 6%. All laboratory analyses were performed at the NGERE Research Unit ("Nutrition-Génétique-Exposition aux risques environnementaux"), Faculty of Medicine, Nancy, France, following strict quality control protocols and manufacturer specifications.

2.3. Cardiovascular Risk Assessment

The Framingham Risk Score (FRS) was calculated to estimate 10-year cardiovascular disease risk using established algorithms incorporating age, systolic blood pressure, total cholesterol, HDL cholesterol, and smoking status [18]. Given the exclusion of smokers and diabetic participants from the study cohort, corresponding adjustments were made in risk calculation. Participants were classified according to established risk categories: low risk (<10%), intermediate risk (10% - 20%), and high risk (>20%). For analytical purposes, intermediate and high-risk categories were combined (FRS ≥10%) to identify participants with substantially ele-

vated cardiovascular risk.

2.4. Statistical Analysis

Continuous variables were expressed as mean ± standard deviation (SD) or median with interquartile range (IQR). Categorical data were summarized as frequencies and percentages. To explore the influence of MMA on cardiometabolic factors, participants were stratified into tertiles based on the sample distribution of MMA levels. The tertile cut-offs were defined as follows: tertile 1 (T1) comprised participants with MMA levels below the 33rd percentile (<142 nmol/L); tertile 2 (T2) included those with levels between the 33rd and 66th percentiles (142 - 197 nmol/L); and tertile 3 (T3) included those with levels above the 66th percentile (>197 nmol/L).

Following this stratification, differences in cardiometabolic and nutritional parameters across MMA tertiles were evaluated with Kruskal-Wallis tests and chi-square tests for categorical variables.

To ensure our sample size of 176 participants was adequate for multivariable analysis, we performed a post-hoc power calculation, which confirmed >80% power to detect associations of moderate magnitude given an alpha of 0.05 and the observed effect size. The association between MMA levels and 10-year cardiovascular risk was then examined through a series of multivariable logistic regression models, which controlled sequentially for confounders: Model 1 adjusted for age, BMI, and blood pressure; Model 2 added renal function markers (creatinine, uric acid); and Model 3 further added nutritional biomarkers (vitamin B12, folate, homocysteine). Results are presented as adjusted odds ratios (aOR) with 95% confidence intervals (CI), with statistical significance set at a two-tailed p-value <0.05. All analyses were performed using IBM SPSS Statistics version 27.

3. Results

3.1. Characteristics of the Study Population

Table 1 shows the cardiometabolic and nutritional profile of the entire study cohort (mean age 39.3 ± 7.7 years). The study cohort exhibited a mean systolic pressure of 134.2 mmHg and a mean diastolic pressure of 84.8 mmHg. Mean fasting glucose, uric acid, homocysteine, and creatinine levels were 4.3 mmol/L, 58.7 mg/L, 29.4 µmol/L and 11.5 mg/L, respectively. Nutritional assessments showed median folate and vitamin B12 levels within normal limits (6.5 nM and 388 pmol/L, respectively), while MMA values were elevated (median: 162 nmol/L). Cardiovascular risk estimation yielded a mean FRS of 7.4%, with TyG index averaging 7.8 (Table 1).

3.2. Characteristics of Taxi-Motorbike Drivers Stratified by Methylmalonic Acid Tertiles

Stratifying participants by MMA tertiles showed a clear trend of increasing cardiovascular risk and worsening functional vitamin B12 status across the groups. Participants in the highest MMA tertile (T3: >197 nmol/L) were significantly older

(40.4 years) compared to those in the lowest tertile (T1: <142 nmol/L; 37.0 years, p = 0.015) (Table 2). Most notably, the 10-year FRS demonstrated a significant graded increase across MMA tertiles (T1: 6.0%; T2: 7.6%; T3: 8.6%; p = 0.003), indicating a strong positive association between functional vitamin B12 status and predicted cardiovascular risk. Concurrently, plasma vitamin B12 levels were markedly lower in the highest MMA tertile (349.7 pmol/L) compared to the lower tertiles (T1: 456.7; T2: 461.6 pmol/L; p < 0.001), confirming MMA's role as a functional indicator of vitamin B12 status despite apparently adequate plasma B12 levels in the lower tertiles. Overall, our data showed a trend of increasing homocysteine with higher MMA tertiles, although this did not reach statistical significance. Additionally, no significant differences were observed across MMA tertiles for other cardiometabolic parameters, including BMI, blood pressure, glucose, creatinine, uric acid, folate, and TyG index (all p > 0.05) (Table 2).

Table 1. Cardiometabolic and nutritional profiles among taxi-motorbike drivers in Cotonou.

	Mean (SD)	Median	IQR
Age (years)	39.3 (7.7)	39	34.0 - 44.0
BMI (Kg/m²)	23.8 (3.9)	23.1	21.0 - 25.7
SBP (mmHg)	134.2 (16.5)	134.2	120.0 - 140.0
DBP (mmHg)	84.8 (11.50)	85	80.0 - 90.0
Glucose (mmol/L)	4.3 (0.7)	4.3	3.9 - 4.6
Creatinine (mg/L)	11.5 (1.3)	11.6	10.5 - 12.3
Uric acid (mg/L)	58.7 (11.2)	59	50.0 - 66.0
Folate nM	7.1 (2.8)	6.5	5.4 - 8.2
Vitamin B12 (pmol/L)	424.1 (164.9)	388	317.0 - 507.0
Homocysteine (µmol/L)	29.4 (18.5)	23.7	17.0 - 36.0
MMA (nmol/L)	183.2 (82.5)	162	131.0 - 213.8
10-year CVD, FRS (%)	7.4 (4.8)	5.6	3.9 - 9.4
TyG	7.8 (0.5)	7.8	7.5 - 8.1
	n/N (%)	**95% CI**	
Hypertension	85/176 (48,3)	40.8 - 55.8	
Alcohol use	71/176 (40.3)	33.1 - 47.9	

Continuous variables were presented as mean ± SD or median with interquartile range (IQR), categorial variables were presented as n (%). BMI: body mass index; CI: confidence interval, CVD: cardiovascular disease; FRS: Framingham risk score; MMA: methylmalonic acid; SBP: Systolic blood pressure; DBP: Diastolic blood pressure; TyG: Triglyceride-Glucose index.

Table 2. Distribution of cardiometabolic risk factors across methylmalonic acid tertiles.

	Tertile 1 (MMA < 142 nM)	Tertile 2 (142 < MMA < 197 nM)	Tertile 3 (MMA > 197 nM)	P value
n	60	60	56	
Age (years)	37.0 (35.0 - 39.0)	40.4(38.5 - 42.4)	40.1 (37.9 - 42.2)	0.015
BMI (Kg/m²)	23.4 (22.6 - 24.3)	23.7 (22.4 - 24.9)	24.3 (23.2 - 25.4)	0.289
SBP (mmHg)	132.2 (127.7 - 136.8)	135.5 (130.3 - 140.6)	135.4 (132.0 - 138.8)	0.293
DBP (mmHg)	82.8 (79.8 - 85.8)	86.2 (82.7 - 89.7)	85.6 (82.7 - 88.4)	0.193
Glucose (mmol/L)	4.2 (4.1 - 4.4)	4.4 (4.2 - 4.6)	4.3 (4.1 - 4.5)	0.812
Creatinine (mg/L)	11.4 (11.0 - 11.7)	11.7 (11.4 - 12.0)	11.5 (11.1 - 11.8)	0.216
Uric acid (mg/L)	59.4 (56.4 - 62.3)	58.6 (55.2 - 61.9)	59.0 (56.4 - 61.7)	0.893
Folate_nM	6.4 (5.9 - 6 - 9)	7.2 (6.6 - 7.9)	7.1 (6.4 - 7.9)	0.289
Vitamin B12 (pmol/L)	456.7 (409.3 - 504.2)	461.6 (416.5 - 506.8)	349.7 (317.6 - 381.8)	<0.001
Homocysteine (µmol/L)	25.6 (21.8 - 29.3)	29.6 (24.0 - 35.2)	31.7 (26.6 - 36.8)	0.194
MMA (nmol/L)	114.8 (109.3 - 120.3)	168.6 (164.1 - 173.1)	274.5 (251.4 - 297.6)	<0.001
10-year CVD, FRS (%)	6.0 (4.9 - 7.0)	7.6 (6.3 - 8.8)	8.6 (7.1 - 10.0)	0.003
TyG	7.8 (7.7 - 7.9)	7.8 (7.7 - 7.9)	7.9 (7.7 - 8.0)	0.801
	n (%)	n (%)	n (%)	
Hypertension	28 (46.7)	27 (45.0)	27 (48.2)	0.911
Alcohol use	23 (38.3)	27 (45.0)	17 (30.5)	0.694

Continuous variables were presented as mean (95% CI), categorial variables were presented as n (%). BMI: body mass index; CI: Confidence interval; CVD: cardiovascular disease; FRS: Framingham risk score; MMA: methylmalonic acid; SBP: Systolic blood pressure; DBP: Diastolic blood pressure; TyG: Triglyceride-Glucose index. P values were from Kruskal-Wallis and chi-square tests.

3.3. Strong and Independent Association of Elevated MMA with High FRS

The association of MMA with the predicted 10-year CVD risk (as quantified by the FRS) was evaluated across three increasingly adjusted multivariable logistic regression models. In all models, the reference group was the lowest MMA tertile (T1: 142 nmmol/L). In Model 1, adjusted for age, BMI, systolic and diastolic blood pressure, participants in the highest MMA tertile (T3: >197 nmol/L) had a significantly greater odds of elevated FRS compared to T1 (adjusted odds ratio [aOR]: 5.29, 95% CI: 1.12 - 14.92, p = 0.035), while the association for T2 was not significant (Table 3). Model 2, which additionally controlled for creatinine and uric acid, showed that belonging to T3 remained significantly associated with higher FRS (aOR: 5.97, 95% CI: 1.20 - 19.66, p = 0.029), and T2 again showed no significant association.

In the fully adjusted model, which accounts for potential confounding by re-

lated nutritional biomarkers, the association reached its greatest magnitude and remained statistically significant. Participants in the highest MMA tertile had 6.99 times higher odds (aOR = 6.99; 95% CI: 1.20 - 30.67; p = 0.030) of elevated CVD risk, while the second tertile consistently showed no association (Table 3).

Table 3. Association between methylmalonic acid tertiles and 10-year cardiovascular disease risk.

	MMA tertiles	aOR	95% CI	p value
Model 1	**T2 vs. T1**	2.10	0.51 - 8.62	0.305
	T3 vs. T1	5.29	1.12 - 14.92	0.035
Model 2	**T2 vs. T1**	1.61	0.37 - 7.06	0.521
	T3 vs. T1	5.97	1.20 - 19.66	0.029
Model 3	**T2 vs. T1**	1.80	0.37 - 8.64	0.465
	T3 vs. T1	6.99	1.20 - 30.67	0.030

aOR: Adjusted odd ratios; CI: Confidence interval; MMA: Methylmalonic acid. The relationship between 10-year CVD risk and MMA was assessed by multivariable logistic regression, adjusted in three models: model 1 adjusted for age, BMI, SBP, and DBP; model 2 added renal functional markers (creatinine and uric acid); and model 3 further added nutritional biomarkers (vitamin B12, folate, homocysteine).

4. Discussion

This study provides novel evidence that elevated MMA is a strong and independent predictor of increased 10-year CVD risk among TMDs in Cotonou, Benin. This offers important new insights for cardiovascular risk stratification in this understudied high-risk occupational cohort. The cohort exhibited widespread hypertension, elevated homocysteine, and MMA levels, while mean glucose, folate, and vitamin B12 values appeared normal. Notably, stratification by MMA revealed that TMDs in the highest MMA tertile were significantly older and had markedly lower plasma vitamin B12 concentrations, confirming MMA's superiority as a functional marker over total plasma vitamin B12.

This study's key finding is a strong, independent association between elevated MMA and increased 10-year cardiovascular risk, demonstrating a clear dose-response relationship. Even after adjusting for traditional risk factors, renal function, and nutritional biomarkers (vitamin B12, folate, and homocysteine), the highest MMA tertile was associated with a nearly sevenfold increase in risk (aOR = 6.99). The fact that the association strengthened with successive adjustment suggests MMA captures a unique pathophysiological pathway.

4.1. Methylmalonic Acid as an Integrative Biomarker of Nutritional and Metabolic Cardiovascular Risk

MMA has gained increasing recognition as a promising biomarker in CVD risk prediction, supported by growing evidence from recent research studies demon-

strating its strong and independent associations with adverse cardiovascular outcomes. Elevated MMA levels, which indicate functional vitamin B12 deficiency and mitochondrial dysfunction, have been consistently linked with higher incidence and risk of various cardiovascular events including acute myocardial injury [19], heart failure [9] [20], coronary artery disease [21], and overall cardiovascular mortality in coronary heart disease [11] and chronic kidney disease [12]. Large-scale population studies, including analyses of National Health and Nutrition Examination Survey (NHANES) cohorts, report that higher serum MMA is independently linked with greater odds of prevalent CVD. For instance, one NHANES-based study of over 5000 adults found that each unit increase in log-transformed MMA corresponded to approximately a threefold increase in adjusted CVD odds (OR ~3.08) [9]. Further, investigations have explored MMA's mediation role linking oxidative balance scores and dietary factors to CVD risk, reinforcing MMA as a functional biomarker that integrates nutritional and metabolic dimensions important for cardiovascular health [22]. Collectively, these findings support MMA as a valuable candidate biomarker for the early identification and stratification of individuals at elevated cardiovascular risk, particularly in populations vulnerable to functional vitamin B12 deficiency and metabolic dysregulation.

4.2. Methylmalonic Acid Reveals Distinct Mechanisms in Cardiovascular Risk Beyond Homocysteine Pathways

Notably, the lack of association between MMA and homocysteine levels challenges the prevailing paradigm that homocysteine-mediated pathways solely account for the cardiovascular risk linked to vitamin B12 deficiency. This finding suggests that MMA reflects a separate pathological mechanism, potentially involving mitochondrial dysfunction, impaired energy metabolism, or direct toxic effects on the vascular endothelium [9] [14]. The finding that circulating vitamin B12 levels alone does not show this association underscores the clinical relevance of measuring functional vitamin B12 status rather than relying solely on circulating levels.

MMA accumulation has been shown to directly inhibit mitochondrial electron transport chain complexes, particularly succinate dehydrogenase, which leads to increased production of reactive oxygen species, oxidative stress, and subsequent endothelial injury [14] [23] [24]. Moreover, vitamin B12 deficiency impairs mitochondrial β-oxidation of odd-chain fatty acids because of disrupted MMA metabolism, resulting in the buildup of toxic fatty acid derivatives. This lipotoxicity may contribute to insulin resistance and promote atherosclerotic processes [25]. A systematic review of cohort studies further supports this mechanistic relationship between MMA and CVD. Its key findings showed that low vitamin B12 levels were associated with a significantly higher risk of incident cerebral ischemia, an effect that persisted even after controlling for homocysteine, suggesting that the adverse vascular effects are only partially mediated by homocysteine and likely involve

MMA-related mechanisms [26]. Taken together, these findings demonstrate that elevated MMA levels are mechanistically and clinically linked to the development of vascular dysfunction and increased risk of CVD.

4.3. Methylmalonic Acid and TyG Index as Distinct Biomarkers in Cardiovascular Risk Assessment

The observed association between MMA and FRS—but not with the TyG index—suggests that MMA may influence cardiovascular risk through pathways distinct from adiposity-driven insulin resistance in this cohort of TMDs. The FRS incorporates age, blood pressure, and lipid parameters, all of which can be affected by mechanisms related to vitamin B12 deficiency and MMA accumulation, such as endothelial dysfunction, hyperhomocysteinemia-induced oxidative stress, and chronic inflammation. In contrast, the TyG index primarily reflects adiposity-driven insulin resistance and dyslipidemia, which directly influence metabolic components of the FRS, such as triglyceride and glucose levels [27]. Elevated MMA levels reflect functional vitamin B12 deficiency, which may contribute to cardiovascular risk through non-metabolic mechanisms such as endothelial dysfunction, oxidative stress, and hyperhomocysteinemia—factors that also contribute to the FRS via blood pressure and vascular aging components. Thus, while both biomarkers are associated with the FRS, they likely operate through distinct mechanistic pathways. The TyG index captures metabolic syndrome-related risk [27], whereas MMA captures micronutrient deficiency-related vascular stress [9]. Their independent associations with the FRS underscore the multifactorial nature of cardiovascular risk in high-risk occupational populations, where occupational stressors, nutritional factors, and metabolic health collectively shape risk profiles. This reinforces the utility of combining complementary biomarkers—each reflecting different pathophysiological processes—to achieve a more comprehensive risk assessment.

4.4. Strengths and Limitations

The strengths of this study include its focus on a high-risk, understudied occupational population, the use of a validated functional marker of vitamin B12 status, and comprehensive adjustment for potential confounders across multiple models. The consistent dose-response relationship across increasing MMA tertiles strengthens the causal inference. However, several limitations should be considered. While the exclusion of smokers and individuals with diabetes strengthened the internal validity of our findings by removing major confounders, it limits the generalizability of our results to the overall population of TMDs and introduces a notable selection bias. This is particularly relevant for tobacco use, which is a well-established, major cardiovascular risk factor. Excluding smokers likely resulted in a study cohort with a lower baseline risk profile and potentially different risk factor interrelationships than the broader population of TMDs. Consequently, the relationship between MMA and predicted cardiovascular risk observed in this subset

of TMDs may not fully capture the combined or interactive effects of smoking and MMA-related vascular pathology that would be present in a real-world setting. Therefore, future studies should validate MMA and its optimal cut-off value in more heterogeneous samples of TMDs that include smokers and individuals with impaired glucose metabolism. Additional limitations include the cross-sectional design, which precludes establishing temporality between MMA elevation and cardiovascular risk progression. Although sufficient for our primary analyses, the limited sample size may have reduced our power to detect more subtle associations. Furthermore, residual confounding from unmeasured factors—such as dietary habits, physical activity levels, genetic variation affecting vitamin B12 absorption, and traffic-related air pollution exposures—may have influenced our findings. These limitations should be considered when interpreting the results. Lastly, the use of predicted rather than actual cardiovascular events as outcome measures constitutes an intermediate surrogate endpoint, underscoring the need for longitudinal studies to validate these findings and better understand MMA's role in cardiovascular risk among occupational cohorts, especially considering the exclusion of tobacco users.

4.5. Public Health Implications

These findings carry significant implications for cardiovascular risk assessment in resource-limited settings. MMA emerges as a sensitive biomarker of functional vitamin B12 deficiency that is strongly associated with elevated predicted cardiovascular risk in urban exposed TMDs. Incorporating MMA measurement could complement conventional risk assessment tools by identifying high-risk individuals who might otherwise remain undetected. In occupational groups such as TMDs, where healthcare access is limited and work constraints hinder preventive care, a simple blood test for MMA could enable more precise stratification and facilitate timely, targeted interventions.

From a policy perspective, these results suggest that nutritional interventions addressing functional vitamin B12 deficiency might represent a cost-effective strategy for cardiovascular risk reduction in similar populations. Future studies should evaluate whether MMA-guided supplementation and intervention strategies can effectively mitigate cardiovascular risk, particularly in occupational groups with high metabolic demands.

5. Conclusions

This study demonstrates that elevated MMA is strongly and independently associated with increased predicted cardiovascular risk among TMDs in Cotonou. This relationship remains significant after adjusting for traditional risk factors and related nutritional biomarkers, indicating underlying pathophysiological mechanisms beyond homocysteine-mediated pathways.

These findings highlight the potential utility of MMA as a novel biomarker for cardiovascular risk stratification in high-risk occupational populations and un-

derscore the need for further research on targeted nutritional interventions to address functional vitamin deficiencies in high-risk or resource-limited settings.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

[1] Jaganathan, S., Jaacks, L.M., Magsumbol, M., Walia, G.K., Sieber, N.L., Shivasankar, R., *et al.* (2019) Association of Long-Term Exposure to Fine Particulate Matter and Cardio-Metabolic Diseases in Low- and Middle-Income Countries: A Systematic Review. *International Journal of Environmental Research and Public Health*, **16**, Article 2541. https://doi.org/10.3390/ijerph16142541

[2] Xia, Y., Liu, Z., Hu, B., Rangarajan, S., Ah Tse, L., Li, Y., *et al.* (2023) Associations of Outdoor Fine Particulate Air Pollution and Cardiovascular Disease: Results from the Prospective Urban and Rural Epidemiology Study in China (Pure-China). *Environment International*, **174**, Article ID: 107829. https://doi.org/10.1016/j.envint.2023.107829

[3] Nyiambam, W., Sylverken, A., Owusu, I., Buabeng, K., Boateng, F. and Owusu-Dabo, E. (2020) Cardiovascular Disease Risk Assessment among Patients Attending Two Cardiac Clinics in the Ashanti Region of Ghana. *Ghana Medical Journal*, **54**, 140-145. https://doi.org/10.4314/gmj.v54i3.3

[4] Avogbe, P.H., Henry, A.Z.V. and Sanni, A. (2025) Metabolic and Nutritional Biomarkers for Cardiovascular Risk Stratification in Beninese Taxi-Motorbike Drivers: Interrelationships between Homocysteine, Folate, Vitamin B12, Tyg Index, and Framingham Risk Score. *Occupational Diseases and Environmental Medicine*, **13**, 223-240. https://doi.org/10.4236/odem.2025.134016

[5] Tao, L., Xu, J., Wang, T., Hua, F. and Li, J. (2022) Triglyceride-Glucose Index as a Marker in Cardiovascular Diseases: Landscape and Limitations. *Cardiovascular Diabetology*, **21**, Article No. 68. https://doi.org/10.1186/s12933-022-01511-x

[6] Chen, X., Wang, L., Jiang, Y., Tang, W., Xiao, J., Deng, W., *et al.* (2025) Interplay between Triglyceride-Glucose Index and Cardiovascular Events in Hypertensive Patients with Controlled Blood Pressure (SBP<140 mmHg): Insights from the SPRINT Trial. *Frontiers in Endocrinology*, **16**, Article 1508667. https://doi.org/10.3389/fendo.2025.1508667

[7] Henry, A.Z.V., Avogbe, P.H. and Sanni, A. (2025) The Triglyceride-Glucose Index: An Affordable Tool for Cardiovascular Risk Assessment in Taxi-Motorbike Drivers in Cotonou, Benin. *Journal of Biosciences and Medicines*, **13**, 308-320. https://doi.org/10.4236/jbm.2025.139026

[8] Vashi, P., Edwin, P., Popiel, B., Lammersfeld, C. and Gupta, D. (2016) Methylmalonic Acid and Homocysteine as Indicators of Vitamin B-12 Deficiency in Cancer. *PLOS ONE*, **11**, e0147843. https://doi.org/10.1371/journal.pone.0147843

[9] Wang, X., Li, W. and Xiang, M. (2022) Increased Serum Methylmalonic Acid Levels Were Associated with the Presence of Cardiovascular Diseases. *Frontiers in Cardiovascular Medicine*, **9**, Article 966543. https://doi.org/10.3389/fcvm.2022.966543

[10] Dhar, I., Lysne, V., Ulvik, A., Svingen, G.F.T., Pedersen, E.R., Bjørnestad, E.Ø., *et al.* (2023) Plasma Methylmalonic Acid Predicts Risk of Acute Myocardial Infarction and Mortality in Patients with Coronary Heart Disease: A Prospective 2-Cohort Study. *Journal of Internal Medicine*, **293**, 508-519. https://doi.org/10.1111/joim.13610

[11] Guo, J., Liu, X., Wang, Z., Lu, R., Liu, Y., Zhang, Y., *et al.* (2023) Methylmalonic Acid, Vitamin B12, and Mortality Risk in Patients with Preexisting Coronary Heart Disease: A Prospective Cohort Study. *Nutrition Journal*, **22**, Article No. 63. https://doi.org/10.1186/s12937-023-00900-6

[12] Wu, S., Chang, W., Xie, Z., Yao, B., Wang, X. and Yang, C. (2023) Association of Serum Vitamin B12 and Circulating Methylmalonic Acid Levels with All-Cause and Cardiovascular Disease Mortality among Individuals with Chronic Kidney Disease. *Nutrients*, **15**, Article 2980. https://doi.org/10.3390/nu15132980

[13] Riphagen, I.J., Minović, I., Groothof, D., Post, A., Eggersdorfer, M.L., Kootstra-Ros, J.E., *et al.* (2020) Methylmalonic Acid, Vitamin B12, Renal Function, and Risk of All-Cause Mortality in the General Population: Results from the Prospective Lifelines-MINUTHE Study. *BMC Medicine*, **18**, Article No. 380. https://doi.org/10.1186/s12916-020-01853-x

[14] Liu, Y., Wang, S., Zhang, X., Cai, H., Liu, J., Fang, S., *et al.* (2022) The Regulation and Characterization of Mitochondrial-derived Methylmalonic Acid in Mitochondrial Dysfunction and Oxidative Stress: From Basic Research to Clinical Practice. *Oxidative Medicine and Cellular Longevity*, **2022**, Article ID: 7043883. https://doi.org/10.1155/2022/7043883

[15] Avogbe, P.H. and Sanni, A. (2022) Hyperuricemia and Associated Cardiometabolic Factors: A Cross-Sectional Study on Taxi-Motorbike Drivers Working in Cotonou, Benin. *Avicenna Journal of Medical Biochemistry*, **10**, 46-51. https://doi.org/10.34172/ajmb.2022.06

[16] Avogbe, P.H. and Sanni, A. (2022) Low Plasma Potassium and High Iron Levels Increased the Risk of Dyslipidemia among Non-Diabetic Taxi-Motorbike Drivers Living and Working in Cotonou, Benin. *Open Journal of Endocrine and Metabolic Diseases*, **12**, 159-175. https://doi.org/10.4236/ojemd.2022.127012

[17] Fofou-Caillierez, M.B., Guéant-Rodriguez, R., Alberto, J., Chéry, C., Josse, T., Gérard, P., *et al.* (2019) Vitamin B-12 and Liver Activity and Expression of Methionine Synthase Are Decreased in Fetuses with Neural Tube Defects. *The American Journal of Clinical Nutrition*, **109**, 674-683. https://doi.org/10.1093/ajcn/nqy340

[18] D'Agostino, R.B., Vasan, R.S., Pencina, M.J., Wolf, P.A., Cobain, M., Massaro, J.M., *et al.* (2008) General Cardiovascular Risk Profile for Use in Primary Care. *Circulation*, **117**, 743-753. https://doi.org/10.1161/circulationaha.107.699579

[19] Tang, F., Yan, M., Wang, Z., Chen, Z., Liu, Y., E, M., *et al.* (2025) Mitochondrial Metabolite Methylmalonic Acid, Subclinical Myocardial Injury, and Its Incremental Predictive Value for Cardiovascular Mortality Risk. *Archives of Medical Research*, **56**, Article ID: 103226. https://doi.org/10.1016/j.arcmed.2025.103226

[20] Polytarchou, K., Dimitroglou, Y., Varvarousis, D., Christodoulis, N., Psachoulia, C., Pantziou, C., *et al.* (2020) Methylmalonic Acid and Vitamin B12 in Patients with

Heart Failure. *Hellenic Journal of Cardiology*, **61**, 330-337. https://doi.org/10.1016/j.hjc.2019.10.010

[21] Biselli, P.M., Guerzoni, A.R., de Godoy, M.F., Eberlin, M.N., Haddad, R., Carvalho, V.M., *et al.* (2009) Genetic Polymorphisms Involved in Folate Metabolism and Concentrations of Methylmalonic Acid and Folate on Plasma Homocysteine and Risk of Coronary Artery Disease. *Journal of Thrombosis and Thrombolysis*, **29**, 32-40. https://doi.org/10.1007/s11239-009-0321-7

[22] Yang, X., Zhang, Z., Ye, F., Liu, P., Peng, B. and Wang, T. (2024) Association between Oxidative Balance Score and Cardiovascular Diseases: Mediating Analysis of Methylmalonic Acid Based on the NHANES Database. *Frontiers in Nutrition*, **11**, Article 1476551. https://doi.org/10.3389/fnut.2024.1476551

[23] Stepien, K.M., Heaton, R., Rankin, S., Murphy, A., Bentley, J., Sexton, D., *et al.* (2017) Evidence of Oxidative Stress and Secondary Mitochondrial Dysfunction in Metabolic and Non-Metabolic Disorders. *Journal of Clinical Medicine*, **6**, Article 71. https://doi.org/10.3390/jcm6070071

[24] Guo, J., Wang, S., Wan, X., Liu, X., Wang, Z., Liang, C., *et al.* (2024) Mitochondria-derived Methylmalonic Acid Aggravates Ischemia-Reperfusion Injury by Activating Reactive Oxygen Species-Dependent Ferroptosis. *Cell Communication and Signaling*, **22**, Article No. 53. https://doi.org/10.1186/s12964-024-01479-z

[25] Tejero, J., Lazure, F. and Gomes, A.P. (2024) Methylmalonic Acid in Aging and Disease. *Trends in Endocrinology & Metabolism*, **35**, 188-200. https://doi.org/10.1016/j.tem.2023.11.001

[26] Rafnsson, S.B., Saravanan, P., Bhopal, R.S. and Yajnik, C.S. (2010) Is a Low Blood Level of Vitamin B12 a Cardiovascular and Diabetes Risk Factor? A Systematic Review of Cohort Studies. *European Journal of Nutrition*, **50**, 97-106. https://doi.org/10.1007/s00394-010-0119-6

[27] Kurniawan, L.B. (2024) Triglyceride-Glucose Index as a Biomarker of Insulin Resistance, Diabetes Mellitus, Metabolic Syndrome, and Cardiovascular Disease: A Review. *EJIFCC*, **35**, 44-51.