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Precise glucose measurement in sodium fluoride-citrate plasma affects estimates of prevalence in diabetes and prediabetes

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Abstract

Objectives: Estimates of glucose concentrations vary among types of blood samples, which impact on the assessment of diabetes prevalence. Guidelines recommend a conversion factor to calculate plasma glucose from measurements of glucose in whole blood. The American Diabetes Association recommends the use of blood drawing tubes containing sodium fluoride (NaF) and citrate, which have not yet been evaluated regarding possible differences

in glucose concentration and conversion factors. Thus, we compared glucose measurements in NaF-citrate plasma and venous whole blood and estimated the impact of differences on diabetes and prediabetes prevalence.

Methods: Glucose differences were calculated by Bland-Altman analysis with pairwise comparison of glucose measurements from whole blood and NaF-citrate plasma (n=578) in clinical studies of the German Diabetes Center. Subsequently, we computed the impact of the glucose difference on diabetes and prediabetes prevalence in the population-based National Health and Nutrition Examination Survey (NHANES).

Results: Even upon conversion of whole blood to plasma glucose concentrations using the recommended conversion factor, mean glucose concentration difference remained 4.72 % higher in NaF-citrate plasma. Applying the higher glucose estimates, increases the population-based diabetes and prediabetes prevalence by 13.67 and 33.97 % or more than 7.2 and 13 million people in NHANES, respectively. Additional economic burden could be about 20 \$ billion per year due to undiagnosed diabetes.

Conclusions: The recommended conversion factor is not valid for NaF-citrate plasma. Systematic bias of glucose measurements due to sampling type leads to clinically relevant higher estimates of diabetes and prediabetes prevalence.

Keywords: blood glucose; diabetes prevalence; diagnostic thresholds; glucose metabolism

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Introduction

According to international guidelines, measurement of glucose in venous plasma shall be used as the reference for circulating glucose concentrations [1, 2]. However, venous whole blood measurement is generally used for point-of-care or bedside glucose monitoring, when rapid availability of results is required in clinical or experimental settings [3–5]. The American Diabetes Association (ADA) and American Association for Clinical Chemistry (AACC) have recently

recommended sodium fluoride-citrate (NaF-citrate) tubes as the reference for blood collection to measure venous plasma glucose as studies have shown that these tubes prevent decrease of glucose over time compared to other plasma preparations, e. g. fluoride-heparin, NaF-ethylenediaminetetraacetic acid (EDTA), or serum [6–9]. The conversion factor of 1.11 for comparison of glucose concentrations between plasma and whole blood is based on an almost 20-year old recommendation [10]. This conversion factor is also used by bedside devices for glucose monitoring [11]. Compared to other glucose-stabilizing agents, NaF-citrate better stabilizes glucose prior to measurement, but no systematic analysis of the relationship between measurements from whole blood and NaF-citrate plasma has been reported and the conversion factor to whole blood glucose concentrations is not known so far.

In the absence of a suitable conversion factor, the diagnosis of glucose metabolism disorders could differ [8, 12–14], resulting in inaccuracies in the epidemiological estimation of the prevalence and incidence of diabetes and prediabetes, i.e. impaired fasting glucose and/or impaired glucose tolerance [15]. Thus, undiagnosed diabetes or underestimated glucose concentrations can lead to avoidable increases in the socioeconomic burden of diabetes due to delayed prevention and treatment [16]. Accurate glucose quantification is therefore essential, also in the light of recently proposed prediabetes and diabetes endotypes and their possible impact on future precision diabetology [17–19].

To this end, we first compared glucose concentrations as assessed from NaF-citrate plasma, whole blood or plasma-converted whole blood (glucose concentration in whole blood multiplied by 1.11). Second, we estimated the impact of glucose concentrations as assessed from different sample types on diabetes and prediabetes prevalence.

Materials and methods

Volunteers

To compare glucose concentrations obtained from different types of sample material, we analyzed samples collected during ongoing studies at the Clinical Research Center, of the German Diabetes Center (DDZ, Düsseldorf, Germany). All studies are approved by the Ethics Committee of the Medical Faculty of the Heinrich-Heine-University Düsseldorf. Individuals for comparative analysis were enrolled in the ongoing prospective German Diabetes Study (GDS), ST-Elevation Myocardial Infarction (STEMI) Diabetes and STEMI (DISTEMI) Study, Intermittent Fasting to improve Insulin Secretion (IFIS) study and Nonalcoholic Steatohepatitis (NASH) patient's itinerary (NASH-PI) at the German

Diabetes Center (DDZ) in Düsseldorf (Germany). Ethics reference numbers: 4508 (GDS), 5961R (DISTEMI) and 2021-1432 (IFIS) and 2022-1815 (NASH-PI), respectively and registered at Clinicaltrials.gov (identifier number: NCT01055093 (GDS), NCT05046483 (DISTEMI), and NCT04607096 (IFIS), respectively).

In order to estimate the prevalence of diabetes in the US-population, we used data from the National Health and Nutrition Examination Survey (NHANES, NCT00005154) 2017–2018 for diabetes and 2017–2020 for prediabetes prevalence computations, which are publicly available (<https://www.cdc.gov/nchs/nhanes/index.htm>). For this purpose, we considered the analysis of individuals of this cohort for whom measurements of fasting glucose (from NaF-plasma without citrate), diabetes diagnosis and glycosylated hemoglobin (HbA_{1c}) were available. All studies are conducted according to the Declaration of Helsinki (2013 version). Written informed consent was obtained from all participants prior to inclusion.

Glucose measurements

We performed 578 measurements in NaF-citrate plasma or whole blood obtained from 79 volunteers (from GDS, DISTEMI and IFIS studies) in the 10 h fasted state and/or during an oral glucose tolerance test (OGTT). During the OGTT, up to 9 glucose measurements were performed between time points –1 and 180 min. Volunteers were stratified by glycemic status and classified as having normal glucose tolerance (controls, n=21), prediabetes (n=31) or overt type 2 diabetes (n=27) based on their clinical diagnosis. Prediabetes was defined according to ADA guidelines by fasting plasma glucose ranging between 5.6 and 6.9 mmol/L and/or HbA_{1c} between 39 and 47 mmol/mol [20].

For the all DDZ studies, participants were asked to discontinue glucose-lowering medications, to refrain from strenuous exercise (>30 min) and from alcohol intake as well as to adhere to a balanced iso-caloric diet for three days prior to each study visit. All participants underwent an OGTT with a 75 g glucose (AccuCheck Dextro O.G-T., Roche, Basel, Switzerland) load or measurements of fasting plasma glucose as described elsewhere [21] but using NaF-citrate containing tubes or whole blood instead of fluoride-heparin. Glucose was immediately quantified in whole blood at bedside by Biosen C-line (EKF Diagnostics, Cardiff, United Kingdom) without further conversion of glucose concentration by the conversion factor using a chip-based enzymatic-amperometric oxidase method. Venous blood was transferred to a capillary suitable for whole blood glucose measurements by Biosen C-line, which was calibrated prior to each measurement as described by the manufacturer. For glucose measurement from NaF-citrate plasma, blood was collected by using special tubes (VACUETTE® FC Mix, #454510 (GREINER BIO-ONE, Frickenhausen, Germany)), which were then inverted 2–3 times and directly placed on a tube roller for at least 5 min. Subsequently, tubes were centrifuged for 10 min at 1622×g and room temperature within 30 min. Plasma was then transferred to fresh cups and glucose was quantified on a Cobas c311 (Roche, Basle, Switzerland) using the hexokinase method. Daily calibration and quality control measurements of plasma glucose were conducted as described by the manufacturer. Moreover, reliability and validity of plasma glucose measurements is regularly confirmed by ring trials (Referenzinstitut für Bioanalytik, Bonn, Germany).

Comparisons of glucose from whole blood and fluoride-heparin plasma to elucidate the conversion factor of 1.11, were conducted from

data of the prospective German Diabetes Study (GDS, n=1821 persons, until 2019), which provided simultaneous fasting glucose concentrations in whole blood (Biosen C-line) and fluoride-heparin plasma (product number #41.1394.005, Sarstedt, Nümbrecht, Germany). This resulted in 1821 comparisons between glucose concentrations in whole blood and fluoride-heparin plasma. Of note, these GDS participants did not belong to the initial cohort for comparison of whole blood and NaF-citrate glucose.

Analysis of glucose differences between NaF-citrate plasma and other sample types was performed in another 14 overnight fasted individuals from the NASH-PI study, who provided glucose concentrations from different sample types. Glucose concentrations were measured in NaF-citrate plasma and compared to glucose concentrations assessed by Cobas c311 from fluoride-heparin, K₂-EDTA (BD Vacutainer®, #368841, Becton & Dickinson, Plymouth, United Kingdom), fluoride-EDTA plasma (S-Monovette, #05.1073, Sarstedt, Nümbrecht, Germany) or serum tubes (BD Vacutainer®, #366882, Becton & Dickinson, Plymouth, United Kingdom).

Laboratory measurements

Routine laboratory variables and hematocrit was measured as previously described [17, 21]. If applicable routine laboratory variables were converted as recommended by International System of Units.

Statistical analysis

Data are presented as mean $\pm$ 95 % confidence interval (CI) for normally distributed data and median (25th; 75th percentiles) for non-normally distributed data. Analyses were performed using GraphPad Prism (v. 9.0.2, San Diego, CA, USA). Statistical tests used and data presented are indicated in corresponding figure legends. A p-value (p) < 0.05 was judged as significant.

Bland-Altman analysis

The difference between NaF-citrate and whole blood glucose concentration was calculated by Bland-Altman analysis before and after conversion of whole blood glucose concentrations with the recommended conversion factor (concentration [glucose in whole blood] $\times$ 1.11=plasma-converted whole blood), with subsequent simple linear regression to reveal changes over the whole range of glucose in blood.

Assessment of diabetes and prediabetes prevalence in NHANES

To assess the impact of potential bias in glucose measurements on the classification of diabetes and prediabetes, we conducted the analyses on the NHANES 2017–2018 dataset for diabetes and NHANES 2017–2020 dataset for prediabetes, which provide glucose measurements obtained using NaF (without citrate) tubes. To further estimate the clinical relevance of the potential bias in glucose measurements, we then incorporated the measurement error of 4.18 % for people with diabetes and 5.13 % for people with prediabetes (Supplemental Figure 1A–C) into the observed glucose concentration for each adult individual (those aged 18 and above). In order to evaluate the overall impact of the glucose concentrations bias, we also included people who were diagnosed with diabetes or prediabetes

based on HbA_{1c} or with a previous diabetes diagnosis. These data were extracted from the National Diabetes Statistics Report 2020, appendix A, Table 5, definition 3, which is publicly available at the homepage of the Centers for Disease Control and Prevention.

To obtain accurate population estimates and ensure the validity of our analysis, we utilized specific survey weights in our prevalence estimation, using the survey weight package (version 4.1-1). Furthermore, we calculated the confidence intervals for the prevalence estimates using the survey R package, which incorporates recalibrated survey weights. Finally, we assessed the increase in the prevalence of diabetes and prediabetes due to this glucose concentration bias. These calculations were based on the differences in glucose concentrations and the US population data from the United States Census Bureau as of April 1, 2020 (with a total population of 331,449,281 people, <https://www.census.gov/data/tables/time-series/demo/popest/2010s-national-total.html>).

Results

Cohort characteristics

Participants were stratified by anthropometric and routine laboratory variables according to subgroups: glucose tolerant controls, prediabetes and type 2 diabetes (Table 1).

Determination of glucose differences

To determine the difference of glucose concentrations between NaF-citrate and whole blood, we compared 578 paired samples obtained at fasting or during OGTT. Using Bland-Altman analysis, glucose concentrations in NaF-citrate plasma were 15.12 % (95 % CI=5.14–25.90 %) or 1.11 mmol/L (95 % CI=–0.02–2.24 mmol/L) higher than those measured in whole blood (Figure 1A). After conversion of whole blood to plasma concentrations (plasma-converted whole blood) using the conversion factor ($\times$ 1.11), the mean difference remained 4.72 % (95 % CI=–5.34–14.77 %) equaling 0.35 mmol/L (95 % CI=–0.38–1.08 mmol/L) (Figure 1B). The difference decreased slightly in the higher glucose concentration range (slope=–0.1277, p=0.0301) as calculated by linear regression of single data points from Bland-Altman analysis. We further checked whether the glucose difference alters during OGTT. The difference of the converted glucose concentrations ranged from 3.82 % at fasting (time=–1 min) to 6.91 % at the end of the OGTT (time=180 min, Figure 1C). However, glucose differences did not indicate alterations during OGTT (one-way ANOVA, p=0.2478).

The glucose difference between NaF-citrate and plasma-converted whole blood was 4.60 % (95 % CI=–7.68–16.88 %) or 0.29 mmol/L (95 % CI=–0.50–1.09 mmol/L), 5.13 % (95 % CI=–3.45–13.71 %) or 0.37 mmol/L (95 % CI=–0.22–0.96 mmol/L) and 4.18 % (95 % CI=–2.18–10.54 %) or 0.45 mmol/L (95 %

Table 1: Cohort characteristics.

Variable	Controls	Prediabetes	Type 2 diabetes
n (male)	21 (11)	31 (22)	27 (19)
Age, years	59.7 (54.0–65.7)	61.0 (56.1–67.2)	59.0 (54.00–67.5)
Body weight, kg	80.1 (73.7–85.0)	84.4 (73.5–97.5)	91.6 (77.1–102.8)
Body mass index, kg/m ²	24.8 (23.1–26.9)	27.5 (23.8–31.2)	30.4 (27.0–33.8)
Fasting glucose, mmol/L	5.3 (5.1–5.6)	5.7 (5.6–5.9)	8.6 (6.9–9.6)
HbA _{1c} , mmol/mol	35.9 (34.4–37.7)	40.3 (37.7–42.1)	51.5 (44.3–53.0)
Serum creatinine, $\mu\text{mol/L}$	82.97 (71.16–89.73)	81.30 (72.49–89.28)	76.02 (64.53–81.33)
eGFR, mL min ⁻¹ 1.73 m ⁻²	80.46 (71.30–90.92)	80.49 (72.40–91.42)	87.32 (74.09–97.50)
Cystatin C, mg/L ^a	1.00 (0.88–1.08)	0.99 (0.87–1.12)	1.03 (0.87–1.16)
Triglycerides, mmol/L	1.18 (0.72–1.44)	1.05 (0.68–1.25)	1.54 (1.11–1.88)
HDL cholesterol, mmol/L	1.49 (1.28–1.63)	1.31 (1.04–1.48)	1.28 (0.98–1.43)
Uric acid, $\mu\text{mol/L}$	330 (265–387)	329 (292–399)	291 (262–333)
RBC count, 10 ¹² /L	4.62 (4.38–4.86)	4.66 (4.34–4.92)	4.68 (4.38–5.03)
Hematocrit, %	41.06 (38.85–43.00)	40.96 (39.20–42.80)	40.44 (38.40–43.30)
Hemoglobin, g/L	141 (131–149)	138 (130–145)	137 (125–150)

Cohort characteristics of volunteers. ^aFor cystatin C n=21 (controls), n=27 (prediabetes) and n=14 (type 2 diabetes). Numbers in brackets indicate 25 and 75 % percentile, respectively. eGFR, estimated glomerular filtration rate; HDL, high-density lipoprotein; RBC, red blood cell.

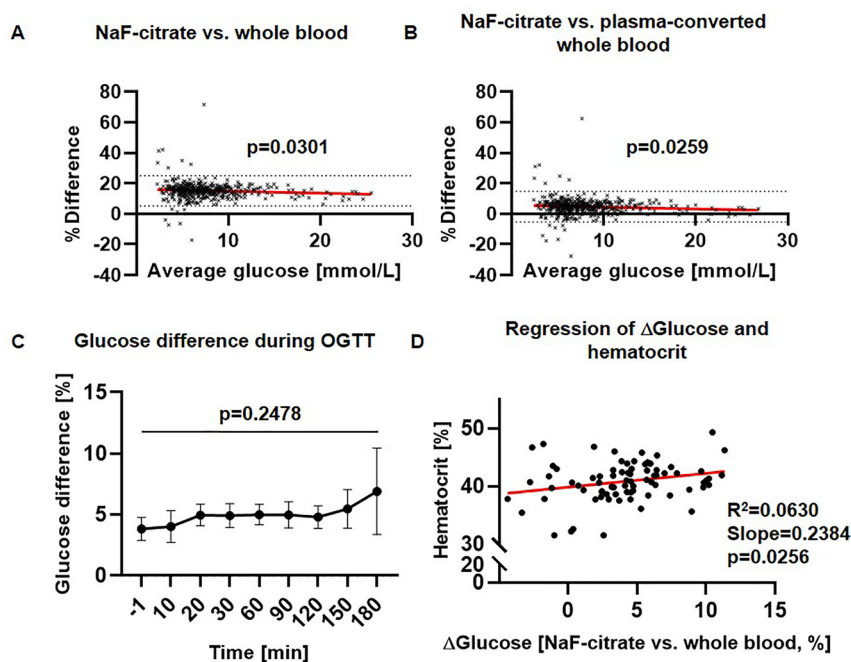


Figure 1: Analysis of glucose difference and its correlation to hematocrit. (A) Glucose concentrations of n=578 paired measures from NaF-citrate plasma or whole blood were compared by Bland-Altman analysis. %Difference represents glucose concentration of NaF-citrate plasma – glucose concentration in whole blood in percent. Average glucose indicates mean of NaF-citrate plasma and whole blood BG levels. Crosses indicate single data points. Dotted lines represent lower and upper boundaries of 95 % CI for glucose difference. (B) Glucose concentrations from whole blood were multiplied by 1.11 (plasma-converted whole blood) and again compared to NaF-citrate plasma glucose concentrations using Bland-Altman analysis. Red lines indicate the slopes for linear regression of single data points from Bland-Altman analyses to estimate alterations of glucose differences over the whole glucose range. Dotted lines represent lower and upper boundaries of 95 % CI for glucose difference. (C) Glucose difference during OGTT. Glucose difference was calculated for each indicated time point as percentage of the difference after conversion of whole blood glucose concentrations with the conversion factor compared to NaF-citrate plasma. Error bars indicate 95 % CI, n=59–66. Alterations of glucose differences over time were calculated by one-way ANOVA with Tukey's correction for multiple comparison. (D) Black dots represent individual data points. Simple linear regression analysis of the difference between plasma and whole blood (Δ Glucose) glucose at time point –1 min of the OGTT and hematocrit, n=78.

CI=−0.33–1.23 mmol/L) for glucose tolerant, prediabetes and type 2 diabetes humans, respectively (Supplemental Figure 1A–C). There was no difference in the bias of glucose measurements across the groups (one-way ANOVA, $p=0.322$). Moreover, slopes for linear regression of Bland-Altman analysis for these groups were not different (one-way ANOVA, $p=0.4803$). Of note, we assessed the conversion factor between whole blood and fluoride-heparin plasma. Indeed, glucose concentrations were 11.1 % higher in fluoride-heparin plasma compared to whole blood ($\beta=11.10$, $n=1821$, $p<0.001$, Supplemental Table 1). Vice versa, dividing fluoride heparin plasma glucose concentrations by 1.11 (conversion factor) yielded comparable glucose concentrations between fluoride-heparin plasma and whole blood ($p=0.433$).

Linear regression analysis showed a positive association of the difference between NaF-citrate and whole blood (Δ Glucose [NaF-citrate vs. plasma-converted whole blood, %]) with hematocrit (Figure 1D). This translates into a glucose reduction of 2.38 % by a 10 % increase in hematocrit. Multiple regression analysis corroborated our finding as only hematocrit and none of the other variables from cohort characteristics was associated with the difference between NaF-citrate and whole blood ($p=0.042$, Supplemental Table 2).

A further analysis compared glucose concentrations from NaF-citrate plasma with that from K₂-EDTA-, fluoride-heparin and fluoride-EDTA plasma as well as serum of another 14 persons including people with prediabetes ($n=4$) and type 2 diabetes ($n=10$). Glucose concentrations were 3.51–7.20 % higher in NaF-citrate plasma, resulting in differences ranging up to 0.58 mmol/L (Supplemental Table 3).

Impact of glucose difference on diabetes and prediabetes prevalence in the US-population

To evaluate the effect of differences in glucose measurement on the prevalence of diabetes, we analyzed data from the NHANES comprising 2,887 participants. In 2017–2018, 13.77 % of the NHANES participants had clinically diagnosed diabetes (Table 2). Considering the glucose difference from NaF-citrate and plasma-converted whole blood, the estimated new diabetes prevalence (type 1, type 2, and others) was 15.95 %. This reflects an increase of 13.67 % in diabetes prevalence, equivalent to an additional 7,225,594 people affected by diabetes in the US population. Using the upper boundary of the 95 % confidence interval for the difference in glucose concentration, diabetes prevalence may even increase to 26.07 % of the US population. In contrast, the lower boundary of the 95 % confidence interval would lead

to similar estimates of diabetes prevalence at 12.92 % compared to the initial diabetes prevalence of 13.77 %. For prediabetes, defined with borderline fasting glucose and HbA_{1c} (cf. methods), NHANES 2017–2020 original prevalence was 11.57 %, which in turn increases to 15.50 % or additional 13,025,956 people with prediabetes in respect to higher glucose values when NaF-citrate plasma is used. This reflects an increase of 33.97 % in prediabetes prevalence. Lower and upper boundary of the new prediabetes prevalence estimation were 9.60 and 21.73 % (Table 2).

Discussion

This study illustrates that measuring glucose concentrations from whole blood, even after conversion to plasma concentrations, leads to a marked difference compared to NaF-citrate plasma and subsequently to considerable bias for determination of the diabetes and prediabetes prevalence.

We found a residual glucose bias of ~5 % using NaF-citrate compared to plasma-converted whole blood, which was consistent across all degrees of glycemia. Kuwa et al. reported an increase of glucose concentrations by 11.3 % in venous plasma, not NaF-citrate plasma, when compared to whole blood, which is in line with the accepted conversion factor of 1.11 from whole blood to plasma [22]. The present study confirmed this conversion factor between whole blood and fluoride-heparin plasma. However, Kuwa et al. and the accepted conversion factor did not refer to citrate-containing blood collection tubes, which are recommended nowadays by the AACC, ADA and International Federation

Table 2: Estimation of diabetes prevalence.

Estimation of diabetes prevalence	Diabetes prevalence of NHANES cohort, % (95 % CI [% of cohort])	Prediabetes prevalence of NHANES cohort, % (95 % CI [% of cohort])
Original estimate	13.77 (12.01–15.53)	11.57 (9.72–13.42)
Mean corrected estimate	15.95 (14.04–17.83)	15.50 (13.39–17.62)
Lower bound of estimate	12.92 (11.24–14.59)	9.60 (7.92–11.28)
Upper bound of estimate	26.07 (23.65–28.49)	21.73 (19.26–24.21)

Estimates indicate computations of diabetes prevalence from NHANES data (year: 2017–2018, $n=2887$ diabetes $n=568$). Prediabetes prevalence computations were based on NHANES data from 2017 to 2020. For mean corrected estimate glucose difference was used as indicated in results section, for lower estimate glucose difference was −2.18 % and for upper estimate glucose difference was 10.54 % for people with diabetes and −3.45 and 13.71 % for people with prediabetes, respectively; 95 % CI, 95 % confidence interval.

for Clinical Chemistry and Laboratory Medicine [6, 13, 23]. Consequently, the current conversion factor is not sufficient for conversion of glucose concentrations from whole blood for comparison with NaF-citrate plasma. Other studies support our findings, by reporting also higher glucose concentrations in tubes containing NaF and citrate instead of NaF only [9, 12, 24]. Of note, the present study revealed a bias of 4.72 % which clearly exceeds the ADA-recommended bias of 2.2 % for comparing glucose measurements [13]. Importantly, the bias was consistent during both fasting and dynamic measurements such as an OGTT. Thus, this bias will impact on diabetes diagnosis under fasting and 2-h OGTT conditions. Moreover, the present study confirmed that NaF-citrate plasma is superior in stabilizing glucose compared to other relevant types of plasma [9, 12, 14]. Consequently, we assume that the increased bias depends on improved inhibition of glycolysis by NaF-citrate tubes [12] despite known hematocrit interference which is reflected in the conversion factor. Thus, we strongly question the current conversion factor for the new reference method, namely NaF-citrate plasma.

Also, the present study reports a positive correlation between the glucose bias and hematocrit, which is in line with one previous study proposing an inverse relationship between hematocrit and whole blood glucose concentrations with a decrease of 0.2 mmol/L glucose for a 10 % increase in hematocrit [14].

Previous reports calculated the bias for other blood sample materials and its impact on diabetes prevalence or diagnosis [15, 25, 26], but not for citrate-containing blood drawing tubes. By testing the impact of the citrate plasma glucose bias on the number of diabetes cases in NHANES, we found a 13.67 % higher diabetes prevalence, which yields an additional 7.2 million cases in the whole US-population when using NaF-citrate plasma. Taken into account the current diagnostic thresholds, this bias could therefore lead to a significantly higher number not only of undiagnosed diabetes, but also prediabetes. This may likely have significant consequences for the need of lifestyle interventions in prediabetes and combined lifestyle and drug treatments in people with type 2 diabetes, which could result in higher socioeconomic burden with substantial public health costs [16, 27]. Our findings underscore the significant impact of the glucose measurement bias introduced by the use of NaF-citrate tubes on diabetes prevalence. The estimated new diabetes and prediabetes prevalence was derived from NHANES, a population-based survey that follows a complex design, providing valid conclusions for the US population. The total costs of undiagnosed diabetes in the US amount to approximately 20 \$ billion per year. Early diagnosis can reduce these costs by preventing comorbidities when

calculated based on additional cost of undiagnosed diabetes (2864 \$ per person/year) and additional 7.2 million diabetes cases when using glucose concentrations from NaF-citrate for diagnosis [16, 28]. Dall et al. calculated costs of 18 \$ billion a year by undiagnosed diabetes including medical and nonmedical costs. Costs for undiagnosed prediabetes were 25 \$ billion per year in the US-population [29]. On the other hand, Li et al. have shown that many interventions to prevent diabetes are cost saving or very cost-effective [30]. Thus, early identification and diagnosis of (pre)diabetes by precise glucose measurements would help to reduce the economic burden of diabetes [31]. People with prediabetes may have higher chances for remission to normoglycemia than people with manifest type 2 diabetes. Our data suggest that prevalence of prediabetes increases about 2.5-fold compared to manifest diabetes prevalence after adjusting for glucose concentration difference. This underscores the need for precise glucose measurements, in particular diagnosing prediabetes as the high-risk condition for future overt diabetes. In contrast, one could also question the current diagnostic thresholds with NaF-citrate indicating higher glucose concentrations. However, establishing new thresholds for diagnosing diabetes and prediabetes would require large-scale longitudinal epidemiological studies. Furthermore, the recently proposed diabetes and prediabetes endotypes may challenge the current view on diagnostic criteria. These endotypes differ in the development of comorbidities, which may not solely depend on fasting plasma glucose [17, 18, 32].

The present study provides a comprehensive pairwise comparison based on high sample number ($n=578$) with robust statistical evaluation of glucose concentrations from different material. This led to identification of a systematic difference in glucose quantification. Moreover, we were able to stratify our glucose concentration comparison to subgroups with different glucose tolerance. Using data from NHANES enabled to draw population-wide conclusions on prediabetes and diabetes prevalence. Of course, it would be of interest to directly estimate the prediabetes and diabetes prevalence based on different fasting plasma glucose measurements. However, interpretation of the results is limited by wide 95%-confidence intervals. Lastly, a direct comparison of glucose concentrations assessed from citrate-only against fluoride-citrate tubes would be of interest as recommended in guidelines [33]. As these analyses were not possible in the current study, we provide comparison of glucose concentration from NaF-citrate to fluoride-heparin which are equivalent when fluoride-heparin tubes are directly put into ice water slurry after blood drawing as done in this study.

In conclusion, we highlight that the measurements of glucose concentrations from recommended NaF-citrate tubes reveal considerably higher glucose concentrations

compared to glucose assessment from whole blood after conversion with the recommended conversion factor and different type of plasma which biases prediabetes and diabetes prevalence. Guidelines should consider higher glucose concentrations from NaF-citrate tubes either by recommendation of adjusted conversion factors or adaption of diagnostic thresholds for glucose from NaF-citrate plasma.

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Research ethics: All studies are approved by the Ethics Committee of the Medical Faculty of the Heinrich-Heine-University of Düsseldorf. Individuals for comparative analysis were enrolled in the ongoing prospective German Diabetes Study (GDS), ST-Elevation Myocardial Infarction (STEMI) Diabetes and STEMI (DISTEMI) Study, Intermittent Fasting to improve Insulin Secretion (IFIS) study and Nonalcoholic Steatohepatitis (NASH) patient's itinerary (NASH-PI) at the German Diabetes Center (DDZ) in Düsseldorf (Germany). Ethics reference numbers: 4508 (GDS), 5961R (DISTEMI) and 2021-1432 (IFIS) and 2022-1815 (NASH-PI), respectively and registered at Clinicaltrials.gov (identifier number: NCT01055093 (GDS), NCT05046483 (DISTEMI), and NCT04607096 (IFIS) respectively). Data from the National Health and Nutrition Examination Survey (NHANES, NCT00005154) 2017–2018 for diabetes and 2017–2020 for prediabetes prevalence computations are publicly available (<https://www.cdc.gov/nchs/nhanes/index.htm>). All studies are conducted according to the Declaration of Helsinki (2013 version).

Informed consent: Written informed consent was obtained from all participants prior to inclusion.

Author contributions: G. H., S. T. and M. R. initiated and conceived the project. G. H. and S. T. collected, interpreted data and supervised blood glucose analysis. I. Y., C. M., M. B., O.-P. Z., and M. S. performed clinical examinations and researched the data. K. S. and M. M. R. conducted statistical analysis. V. B. and R. W. supervised studies and interpreted data. M. R. is PI of all clinical studies at DDZ, edited and reviewed the manuscript. All authors contributed to writing and editing of the manuscript. M. R. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Competing interests: M. R. reports lecture fees from Astra Zeneca and Novo Nordisk, contribution to advisory boards of Boehringer Ingelheim, Eli Lilly, Novo Nordisk, and Target RWE, M. R. has also received investigator-initiated support from Boehringer Ingelheim, Nutricia/Danone and Sanofi-Aventis, paid to the German Diabetes Center. R. W. reports lecture fees from Novo Nordisk and Sanofi. R.W. served on advisory boards for Akcea Therapeutics, Daiichi Sankyo, Sanofi and NovoNordisk. All other authors declare no conflict of interest.

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Data availability: The data that support the findings of this study are not openly available due to reasons of sensitivity and are available from the corresponding author upon reasonable request.

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