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Review

GC–MS and GC–MS/MS measurement of malondialdehyde (MDA) in clinical studies: Pre-analytical and clinical considerations

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ABSTRACT

Malondialdehyde (MDA; 1,3-propanedial, OHC-CH_2-CHO) is one of the most frequently measured biomarkers of oxidative stress in plasma and serum. L-Arginine (Arg) is the substrate of nitric oxide synthases (NOS), which convert L-arginine to nitric oxide (NO) and L-citrulline. The Arg/NO pathway comprises several members, including the endogenous NOS-activity inhibitor asymmetric dimethylarginine (ADMA) and its major metabolite dimethyl amine (DMA), and nitrite and nitrate, the major NO metabolites. Reliable measurement of MDA and members of the Arg/NO pathway in plasma, serum, urine and in other biological samples, such as saliva and cerebrospinal fluid, is highly challenging both for analytical and pre-analytical reasons. In our group, we use validated gas chromatography-mass spectrometry (GC–MS) and gas chromatography-tandem mass spectrometry (GC–MS/MS) methods for the quantitative determination in clinical studies of MDA as a biomarker of oxidative stress, and various Arg/NO metabolites that describe the status of this pathway. Here, the importance of pre-analytical issues, which has emerged from the use of GC–MS and GC–MS/MS in clinico-pharmacological studies, is discussed. Paradigmatically, two studies on the long-term oral administration of L-arginine dihydrochloride to patients suffering from peripheral arterial occlusive disease (PAOD) or coronary artery disease (CAD) were considered. Pre-analytical issues that were addressed include blood sampling, plasma or serum storage, study design (notably in long-term studies), and the alternative of measuring MDA in human urine.

Introduction

Oxidative stress and malondialdehyde

Reactive oxygen species (ROS), such as the superoxide radical monoanion ($O_2^{\bullet-}$) and the non-radical peroxide dianion (O_2^{2-}) can be produced both by enzymatic and non-enzymatic chemical reduction of the electrically uncharged diradical molecule, molecular oxygen ($O_2^{\bullet\bullet}$). ROS are highly reactive and attack various classes of biomolecules, notably of lipids, in their vicinity. This complex phenomenon is widely known as "oxidative stress" or "oxidant stress". Malondialdehyde (MDA) is a relatively stable product of lipid peroxidation. Major sources of MDA are polyunsaturated fatty acids (PUFAs), such as arachidonic acid (AA) (Scheme 1).

Searching PubMed (http://www.ncbi.nlm.nih.gov/pubmed; 17 November 2022) using the search term "oxidative stress" resulted in 293,578 articles, or 36,754 articles using the search term "oxidative stress malondialdehyde", and 3,996 articles using the term "oxidative

Abbreviations: AA, arachidonic acid; ADMA, asymmetric dimethylarginine; Arg, L-arginine; BHT, butylated hydroxytoluene; BMD, Becker muscular dystrophy; CAD, coronary artery disease; CID, collision-induced dissociation; COX, cyclooxygenase; CV, coefficient of variation; DDAH, dimethylarginine dimethylaminohydrolase; DMA, dimethyl amine; EBC, exhaled breath condensate. ECNICI, electron-capture negative-ion chemical ionization; GC–MS, gas chromatography-mass spectrometry; GC–MS/MS, gas chromatography-tandem mass spectrometry; GSH, glutathione; GSSG, glutathione disulfide; HbO_2, oxyhemoglobin; HNE, 4-hydroxy-2-nonenal; HPLC, high-performance liquid chromatography; LC-MS/MS, liquid chromatography-tandem mass spectrometry; M, molecular mass; MDA, malondialdehyde; MMA, monomethylarginine; MS, mass spectrometry; MW, molecular weight; *m/z*, mass-to charge ratio; NO, nitric oxide; NOS, nitric oxide synthase; PAOD, peripheral arterial occlusive disease; PFB, pentafluorobenzyl; PFB-Br, pentafluorobenzyl bromide; PFP, pentafluoropropionyl; PFPA, pentafluoropropionic anhydride; PG, prostaglandin; PRMT, protein arginine methyltransferase; PUFAs, polyunsaturated fatty acids; Q, quadrupole; QC, quality control; ROS, reactive oxygen species; RSD, relative standard deviation; RTR, renal transplant recipients; SD, standard deviation; SEM, standard error of the mean; SIM, selected-ion monitoring; SRM, selected-reaction monitoring; T2DM, type 2 diabetes mellitus; TBA, thiobarbituric acid; TBARS, thiobarbituric acid-reactive substances; Tx, thromboxane; TxA_2, thromboxane A_2; TxB_2, thromboxane B_2.

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stress isoprostane"; isoprostanes are a class of oxidative stress biomarkers that are isomeric to prostaglandins (PG). The particular reaction of ROS with lipids is generally known as "lipid peroxidation". MDA is a generally accepted and widely used biomarker of oxidative stress, notably of lipid peroxidation [1]. Oxidative stress is considered to be central to human life.

Chemistry and origin of biological malondialdehyde

Malondialdehyde (MDA) also known as malonaldehyde (1,3-propanedial, OHC-CH_2-CHO; $C_3H_4O_2$; MW, 72.06; CAS 542-78-9; PubChem, 10964) is a solid compound. The most characteristic features of MDA are the two carbonyl functions and its CH-acidity in aqueous solution (pK_a, 4.46 [2,3]). These properties have been utilized for the measurement of MDA in biological samples [1,4].

Biological MDA in lipids, including unsaturated fatty acids, such as AA, was first detected using a thiobarbituric acid (TBA) reagent [1,4,5]. Formation of MDA in large amounts was observed by incubating platelets with AA. Acetylsalicylic acid and indomethacin inhibited the formation of MDA, suggesting involvement of cyclooxygenase (COX) [6,7]. In humans, ingestion of aspirin (acetylsalicylic acid) inhibited the formation of thromboxane B_2 (TxB_2), the stable metabolite of thromboxane A_2 (TxA_2), as well as the formation of MDA [8]. Further experiments have shown that in human platelets, TxA_2 synthesis is associated with formation of glutathione disulfide (GSSG), MDA and 12-hydroxy-5,8,10-heptadecatrienoic acid [8–11]. Recombinant COX-1 and COX-2 were found to oxidize AA to MDA, 12-hydroxy-5,8,10-heptadecatrienoic acid and 15(*S*)-8-*iso*-prostaglandin $F_{2\alpha}$ (15(*S*)-8-*iso*-$PGF_{2\alpha}$; for simplicity 8-*iso*-$PGF_{2\alpha}$) in addition to other PGs and TxA_2 [12]. Interestingly, glutathione (GSH) was found to promote concomitant COX-catalyzed conversion of AA to MDA and 8-*iso*-$PGF_{2\alpha}$ [12]. These aspects of MDA, and of 4-hydroxy-2-nonenal (HNE), have been reviewed previously in greater detail [1,13,14]. In plasma and urine, MDA occurs both in a free form and conjugated to biomolecules, including certain amino acids, notably lysine and serine [15–18].

The L-arginine/nitric oxide pathway

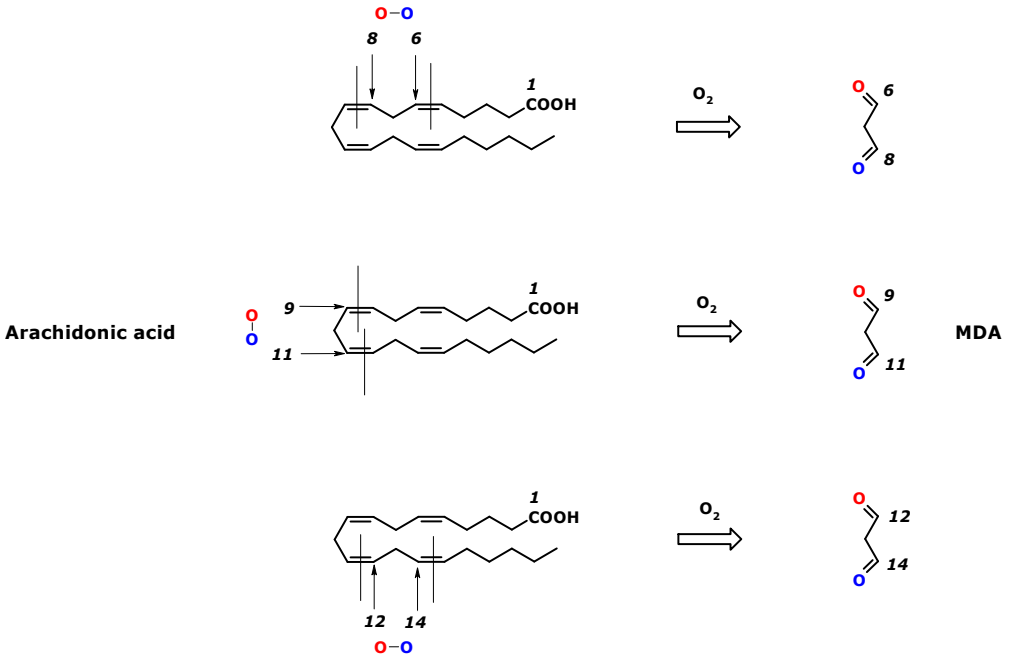
L-Arginine (Arg) is the substrate for the nitric oxide synthase (NOS) family. NOS isoforms convert Arg to nitric oxide (NO) and L-citrulline (Scheme 2). NO is a signaling gaseous molecule. It is a potent vasodilator and inhibitor of platelet aggregation. In biological fluids, such as blood, NO is practically not detectable. NO is oxidized to nitrite and nitrate. In plasma, serum, urine and other biological samples, the minor and major NO metabolites, i.e., nitrite and nitrate, respectively, serve as surrogates for local and whole body synthesis of NO [19]. The NOS activity is regulated by mono- and di-methylated Arg analogs, notably monomethylarginine (MMA) and asymmetric dimethylarginine (ADMA). MMA and ADMA are endogenous metabolites of Arg; they are formed by post-translational methylation of Arg residues in numerous proteins and subsequent regular proteolysis (Scheme 2). MMA and ADMA circulate in blood and are excreted in the urine, both unchanged and after hydrolysis by dimethylarginine diaminohydrolase (DDAH) to monomethylamine and dimethylamine (DMA), respectively. The status of the Arg/NO pathway in health and disease and in various conditions, such as physical exercise and pharmacological treatment can be characterized by measuring representative members in biological samples. They include nitrite and nitrate (measures of NOS activity), Arg (measure of substrate bioavailability), ADMA (measure of inhibition state of NOS activity), and DMA (measure of ADMA metabolism) [19,20].

A search in PubMed (http://www.ncbi.nlm.nih.gov/pubmed; 17 November 2022) using the search term "nitric oxide" resulted in 189,136 articles, or 36,371 articles using the search term "nitric oxide arginine", and 7,559 articles using the term "nitric oxide nitrite nitrate". These numbers indicate that the Arg/NO pathway is of particular research interest.

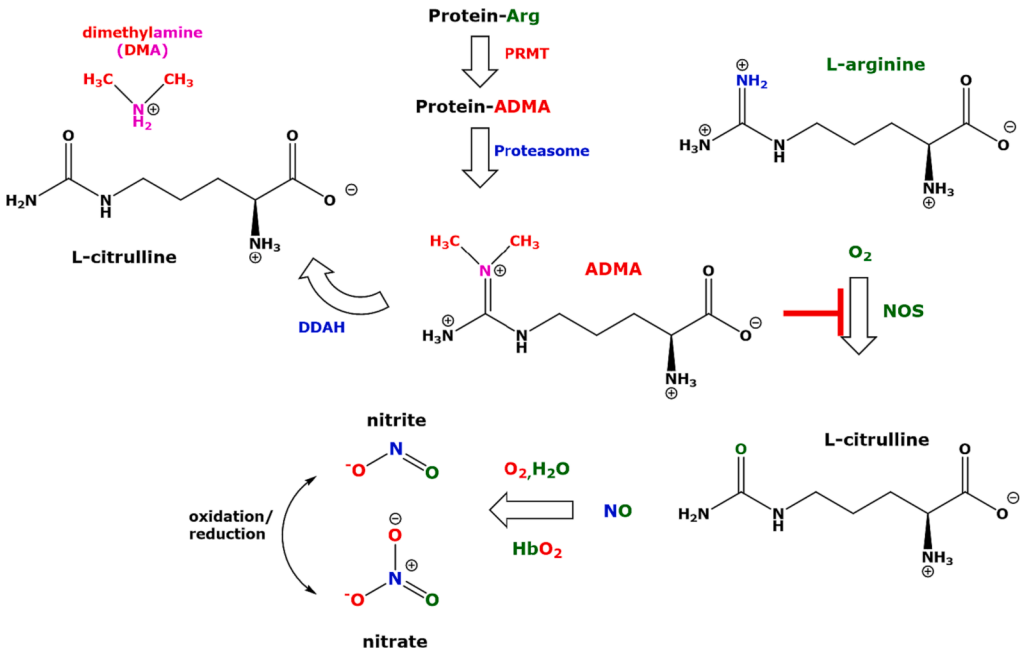
Analytical chemistry of malondialdehyde – general aspects

For recent reviews on MDA analysis in biological samples, see Refs. [1,21]. The most frequently used analytical methods for MDA are based on its reaction with thiobarbituric acid (TBA). The so-called thiobarbituric acid-reactive substances (TBARS) assay has been used for the assessment of lipid peroxidation [22] by spectrophotometry [23] or fluorimetry [24]. Because of a lack of specificity of these batch assays, they were improved by coupling with high-performance liquid chromatography (HPLC) [25–28].

In liquid chromatography tandem mass spectrometry (LC-MS/MS), 3-nitrophenyl hydrazine was used for the derivatization of MDA and its measurement in human plasma [29]. HPLC, LC-MS/MS, gas



Scheme 1. Simplified schematic of malondialdehyde (MDA) formation from the polyunsaturated arachidonic acid. In theory, peroxidation of arachidonic acid to form MDA can occur on three methylene groups between non-conjugated double bonds as indicated for arachidonic acid. Arrows indicate the C atoms, which are attacked by molecular oxygen, an $O_2^{\bullet\bullet}$-species. Vertical lines through the double bonds indicate the double bonds, which are broken/opened by this attack. Adapted from Ref. [1].



Scheme 2. Simplified schematic of a small part of the L-arginine/nitric oxide pathway. Free L-arginine is converted by nitric oxide synthase (NOS) to L-citrulline and nitric oxide (NO) using molecular oxygen (O_2) and various cofactors (not shown). NO is autoxidized to nitrite; oxyhemoglobin (HbO_2) oxidizes NO to nitrate. Nitrite and nitrate are interconvertible through redox reactions. NOS activity is inhibited by asymmetric dimethylarginine (ADMA), which is generated by asymmetric dimethylation of arginine residues in proteins by protein arginine methyltransferase (PRMT) and subsequent proteolysis. ADMA is hydrolyzed to L-citrulline and dimethylamine (DMA) by dimethylarginine diaminohydrolase (DDAH).

chromatography-mass spectrometry (GC–MS) and gas chromatography-tandem mass spectrometry (GC–MS/MS) methods for MDA are specific and more sensitive than batch TBARS assays. They are useful both for free and adducted MDA [30,31].

A unique GC–MS-based analytical method for MDA utilizes the C-H acidity of the methylene H atoms in aqueous solution [2,3]. Pentafluorobenzyl (PFB) bromide (PFB-Br) alkylates MDA on its central C atom to form the MDA-$(PFB)_2$ derivative, thereby leaving both aldehydic groups intact [32]. GC–MS and GC–MS/MS are useful for the quantitative measurement of free MDA in various biological samples [12,32,33].

Mass spectrometry in clinical chemistry and laboratories

Mass spectrometry (MS)-based analytical methods have found little application in classical clinical chemistry laboratories thus far, although these techniques are widely used in clinical studies since several decades. MS-based techniques, especially LC-MS/MS, are increasingly implemented in clinical laboratories [34]. This is also reflected by a recent search in the PubMed (http://www.ncbi.nlm.nih.gov/pubmed; 17 November 2022) which resulted in 3,231 articles using the search term "clinical chemistry gc-ms" (since 1971) and 4,645 articles using the search term "clinical chemistry lc-ms" (since 1982).

GC–MS and GC–MS/MS in clinical studies

In our group, we routinely use GC–MS and GC–MS/MS methods to measure various classes of analytes in biological samples. For the measurement of MDA and for several members of the Arg/NO pathway in clinical studies, fully validated stable-isotope dilution GC–MS and GC–MS/MS methods were developed and used (Scheme 3). The most widely used derivatization reagent in these methods is 2,3,4,5,6-pentafluorobenzyl bromide (PFB-Br) [35]. PFB-Br is used in our group for the derivatization and measurement of several classes of endogenous metabolites including MDA [32], nitrite and nitrate [36], creatinine [37], carboxylic acids including prostaglandins and thromboxane [38], and biogenic amines including histamine [39]. Amino acids including Arg and its metabolites ADMA are measured by GC–MS and GC–MS/MS after derivatization with 2 M HCl in methanol to prepare their methyl esters followed by pentafluoropropionic anhydride (PFPA) to form their pentafluoropropionic (PFP) derivatives [40]. In situ prepared trideuteromethyl ester of amino acids are used as internal standards [41]. Urinary and plasma DMA are analyzed by GC–MS after extractive derivatization with pentafluorobenzoyl chloride [42,43].

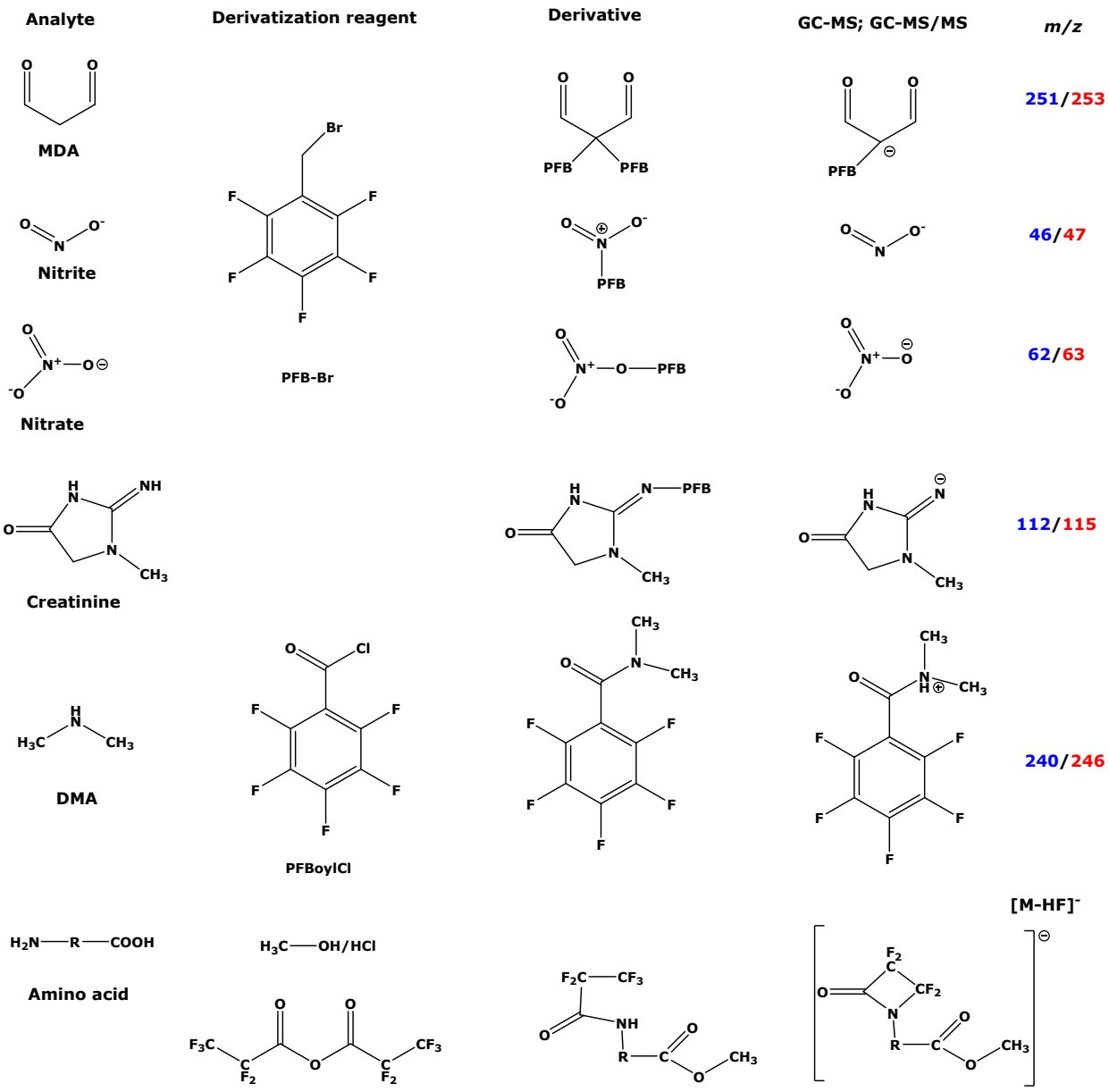
GC–MS and GC–MS/MS measurement of biological malondialdehyde as pentafluorobenzyl derivative

We developed GC–MS and GC–MS/MS methods which are based on the derivatization of MDA with PFB-Br. It utilizes the C-H-acidity (pK_a, 4.46) of the methylene group of MDA [2,3]. The carbanions of MDA react with PFB-Br to form a dipentafluorobenzyl derivative (Scheme 4). This derivatization reaction occurs in aqueous acetone at 50 °C and can take place in all biological samples and tissue suspensions in buffers (aqueous phase-acetone, 1:4, v/v). [1,3-$^2H_2$]MDA (d_2-MDA) is used as the internal standard for biological MDA (d_0-MDA). MDA labelled with ^2H on position C2 is not useful as internal standard in this method because of $^2H/^1H$ exchange.

Under very similar derivatization conditions, inorganic nitrite and inorganic nitrate react with PFB-Br to form PFB-NO_2 and PFB-ONO_2, respectively [36] (Scheme 5). Commercially available nitrite and nitrate labelled with ^{15}N are used as internal standards for biological nitrite and nitrate, respectively. Commercially available nitrite and nitrate labelled with ^{18}O are less useful as internal standards because of $^{18}O/^{16}O$ exchange.

Fig. 1 shows GC–MS spectra of synthetic d_0-MDA-$(PFB)_2$ and d_2-MDA-$(PFB)_2$ in the electron capture negative-ion chemical ionization (ECNICI) mode by scanning the first quadrupole (Q1). The most intense corresponding mass fragments are m/z 251 and m/z 253 due to loss of a PFB radical (181 Th) from the derivatives.

Product ion mass spectra of the MDA derivatives were generated consecutively by selecting on the first quadrupole Q1 m/z 251 for d_0-MDA-$(PFB)_2$ and m/z 253 for d_2-MDA-$(PFB)_2$. These ions were subjected to collision-induced dissociation (CID) in the second quadrupole Q_2 of the mass spectrometer using argon as the collision gas. The generated product ions were selected by scanning the third quadrupole Q_3 of the GC–MS/MS apparatus and reconstructed to obtain the GC–MS/MS mass spectra (not shown). The most characteristic product ions were m/z 175 for d_0-MDA-$(PFB)_2$ and m/z 177 for d_2-MDA-$(PFB)_2$, indicating that derivatization, ECNICI and CID did not affect the aldehyde groups [32].



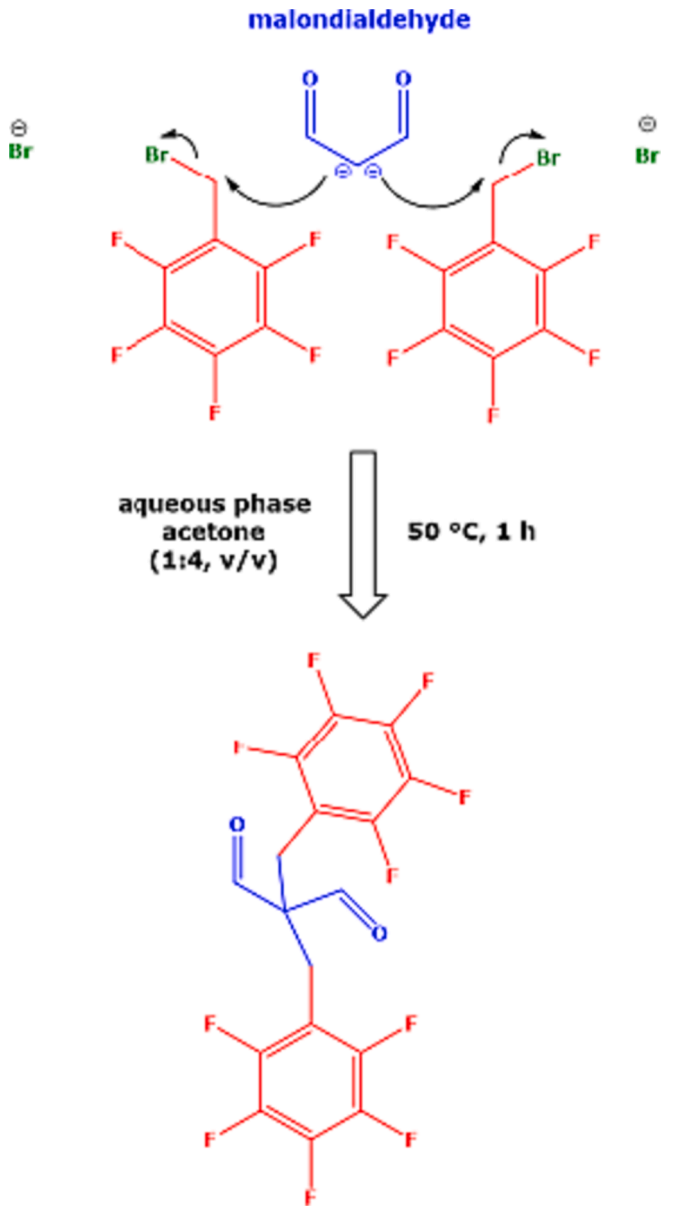
Scheme 3. Schematic of some analytes, derivatization reagents, derivatives and ions from negative-ion chemical ionization or positive-ion chemical ionization in GC–MS and GC–MS/MS methods used in the author's group. MDA, malondialdehyde; DMA, dimethylamine; PFB, pentafluorobenzyl; PFP, pentafluoropropionyl: M, molecular mass; *m/z*, mass-to-charge ions of unlabeled analytes (in blue) and stable-isotope labelled analytes (in red) serving as the internal standards. Most widely used stable isotopes are ^{2}H (natural abundance, 0.02 %) and ^{15}N (natural abundance, 0.4 %).

Quantitative GC–MS analyses of MDA are performed by selected-ion monitoring (SIM) of *m/z* 251 for d_0-MDA-$(PFB)_2$ and *m/z* 253 for d_2-MDA-$(PFB)_2$. When using a single quadrupole GC–MS apparatus, the quadrupole is alternately scanned between two pairs of voltages to let pass the ions *m/z* 251 for d_0-MDA-$(PFB)_2$ and *m/z* 253 for d_2-MDA-$(PFB)_2$. Quantitative GC–MS/MS analyses of MDA are performed by selected-reaction monitoring (SRM) of the mass transitions *m/z* 251 to *m/z* 175 for d_0-MDA-$(PFB)_2$ and *m/z* 253 to *m/z* 177 for d_2-MDA-$(PFB)_2$. Representative GC–MS and GC–MS/MS chromatograms from analyses of MDA in human plasma are shown in Fig. 2.

Fig. 2A shows that there are many ions with *m/z* 251 and *m/z* 253, but only the baseline-separated ions eluting at 10.57 min and 10.58 min are due to d_0-MDA-$(PFB)_2$ and d_2-MDA-$(PFB)_2$, respectively. Fig. 2B shows only two peaks from the mass transitions *m/z* 251 to *m/z* 175 and *m/z* 253 to *m/z* 177 eluting each at 10.58 min. The chromatograms of Fig. 2 impressively demonstrate the superiority of GC–MS/MS over GC–MS: it minimizes potential interferences by unknown substances. Yet, comparison of GC–MS (SIM mode) and GC–MS/MS (SRM mode) indicate that they deliver closely comparable MDA concentrations in human biological samples in a wide concentration range [32] (Fig. 3). These methods have provided important information on the analytical chemistry and biology of MDA in clinical studies (see next Section).

Aim of the present work

The high reliability of the measurement of biological MDA and several members of the Arg/NO pathway by GC–MS and GC–MS/MS enables a critical evaluation and identification of potential sources of pre-analytical shortcomings that may have been overseen in the past in published clinical, pharmacological, epidemiological and sport-medicinal studies. This is the primary goal of the present investigation. The present work also attempts to evaluate whether the study

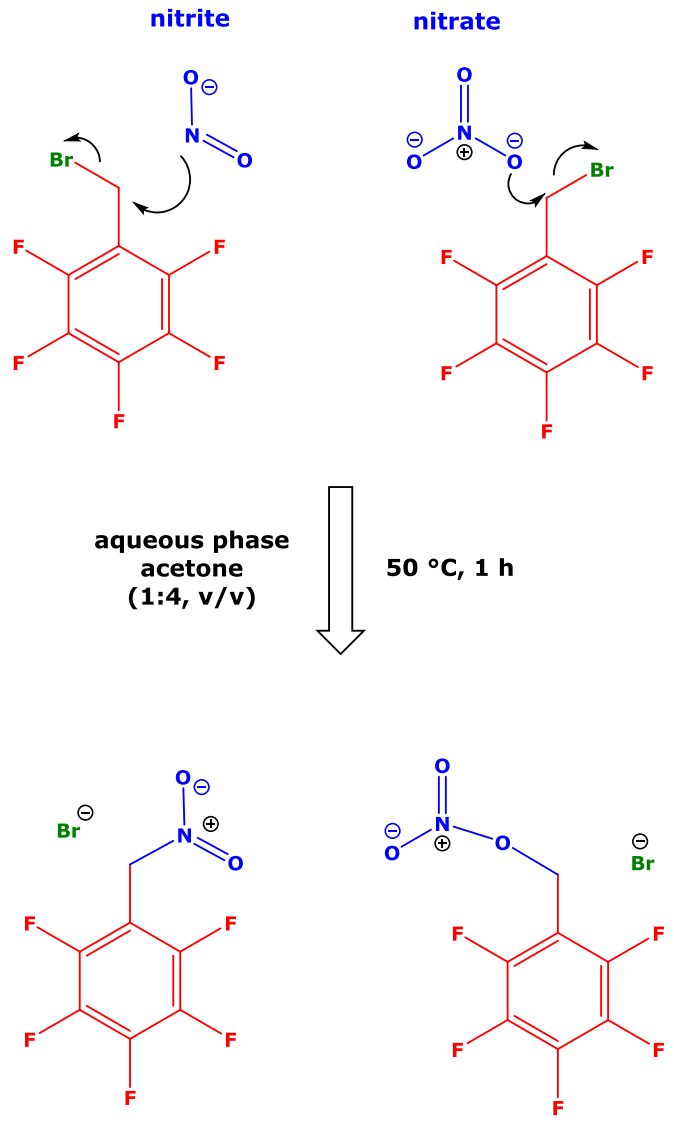


Scheme 4. Simplified schematic of the derivatization of malondialdehyde (MDA) with pentafluorobenzyl (PFB) bromide in aqueous acetone to form the 2,2-dipentafluorobenzyl derivative (MDA-$(PFB)_2$). A volume ratio of 1:4 for biological sample (e.g., 100 μL)-to-acetone (e.g., 400 μL) is required for a homogenous phase and solubilization of PFB-Br (e.g., 10 μL pure PFB-Br) [32].

design may represent an issue for consideration in planning future studies to minimize distortion of in vivo reality.

Application of GC–MS and GC–MS/MS methods in two clinical studies

The peripheral arterial occlusive disease (PAOD) and the coronary artery disease (CAD) studies have been published previously in detail [32,44–47]. The data reported in these publications and in the present work were taken in part from these publications and in part from the PhD theses by Jessica Lachmuth [48] and Sabine Rothmann [49]. A detailed description of these studies including patients' characterization, drug supplementation, sampling, GC–MS and GC–MS/MS, results (Tables S1–S8) and statistical analyses is provided in the Supplement to this work. These two clinical studies were paradigmatically used in the present work to discuss pre-analytical and analytical factors in the



Scheme 5. Simplified schematic of the derivatization of nitrite and nitrate with pentafluorobenzyl (PFB) bromide in aqueous acetone to form the nitro-pentafluorobenzyl derivative (PFB-NO_2) and nitric ester-pentafluorobenzyl derivative (PFB-ONO_2). A volume ratio of 1:4 for biological sample (e.g., 100 μL)-to-acetone (e.g., 400 μL) is required for a homogenous phase and solubilization of PFB-Br (e.g., 10 μL pure PFB-Br) [36].

GC–MS-based analysis primarily of biological MDA.

Discussion of pre-analytical factors in clinical studies

General aspects

With respect to the various aspects of clinical trials, the reader may find information at https://en.wikipedia.org/wiki/Clinical_trial (uploaded on 9 November 2022) and in the references cited therein. Clinical trials are designed to answer specific questions for instance about biomedical interventions by drugs or dietary supplements. Clinical trials are conducted upon approval by health authority/ethics committee. Clinical trials can vary in size with respect to the number of treated subjects, the involved research centers and the study duration. Research subjects are recruited according to inclusion and exclusion criteria. The clinical study design aims to ensure the scientific validity and reproducibility of the results. In the so-called interventional study, the investigators give the research subjects a certain dose drug (for

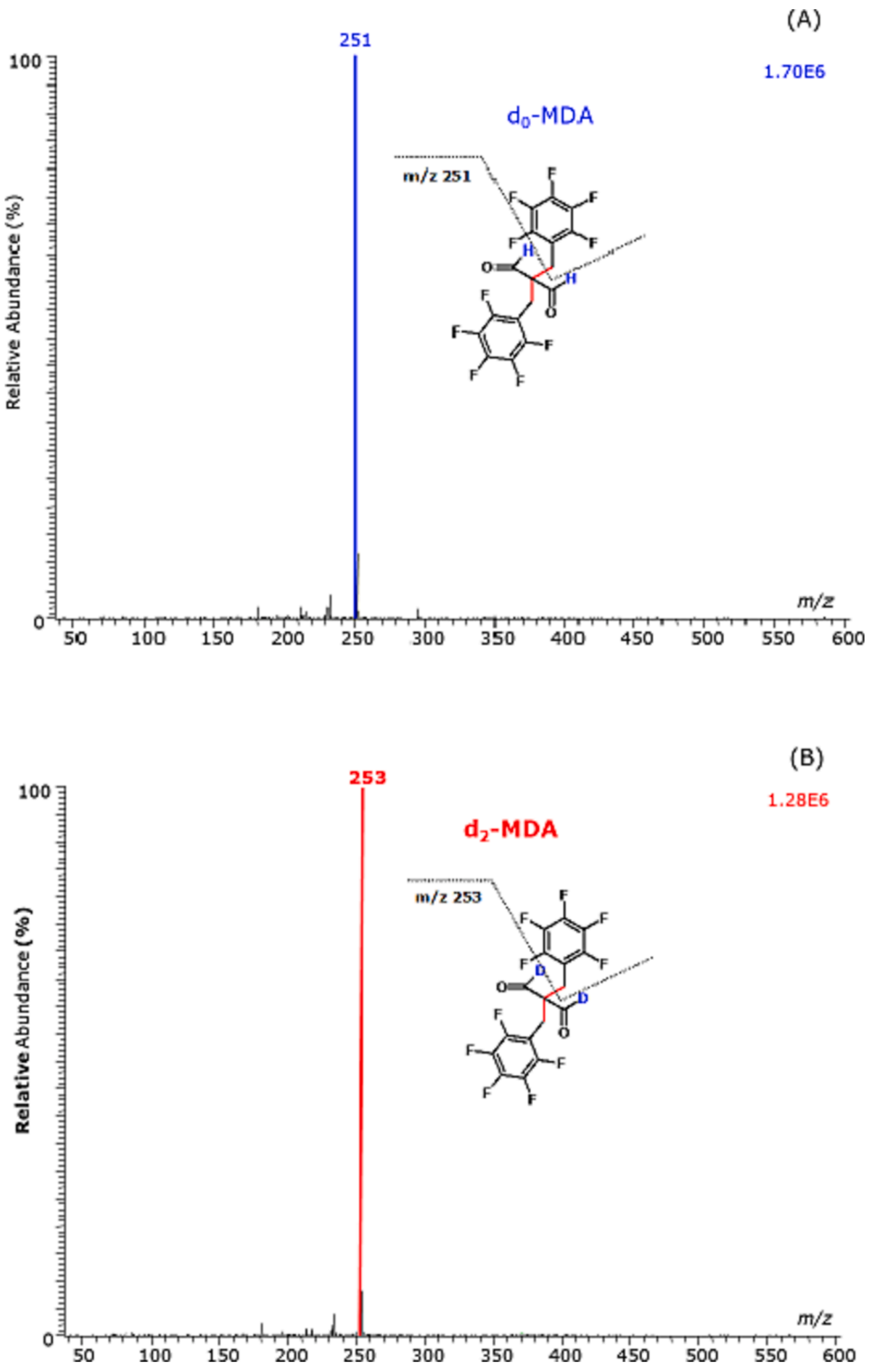


Fig. 1. GC–MS mass spectra in the electron capture negative-ion chemical ionization (ECNICI) mode of (A) unlabeled MDA (d_0-MDA) and of (B) [1,3-$^2H_2$] MDA (d_2-MDA) as their di-pentafluorobenzyl derivatives. d_0-MDA-$(PFB)_2$ produces the anion with the mass-to-charge (m/z) 251. d_2-MDA-$(PFB)_2$ produces the anion with m/z 253. The GC–MS mass spectra were generated by scanning the first quadrupole (Q1) in the m/z range 50–600. The triple stage quadrupole (TSQ) GC–MS/MS instrument model TSQ 7000 (ThermoFisher) was used. Methane was used as the reactant gas. Adopted from Ref. [32].

instance L-arginine) or placebo (for instance mannitol) for a previously defined period (for instance 12 or 24 weeks). Prior to start the treatment, i.e., at baseline, participants are investigated clinically to gain the biomedical measures such as blood pressure. In addition, blood, urine and possibly other biological samples are collected for the measurement of biochemical parameters. At the end of the study, researchers perform clinical investigations and collect biological samples for measuring biochemical parameters. After completion of the study, investigators compare by using proper statistical methods the clinical and biochemical outcomes from treated subjects with those receiving the placebo. The investigators assess how the subjects' health changed in the groups due to the treatment.

Clinical trials need to be carefully prepared both with respect to biomedical and biochemical aspects. The focus of the present work are biochemical aspects. Measurement of many different biochemical parameters may require different specific procedures.

A more general issue is the time plan of the laboratory measurements. Should the biological samples be analyzed immediately after

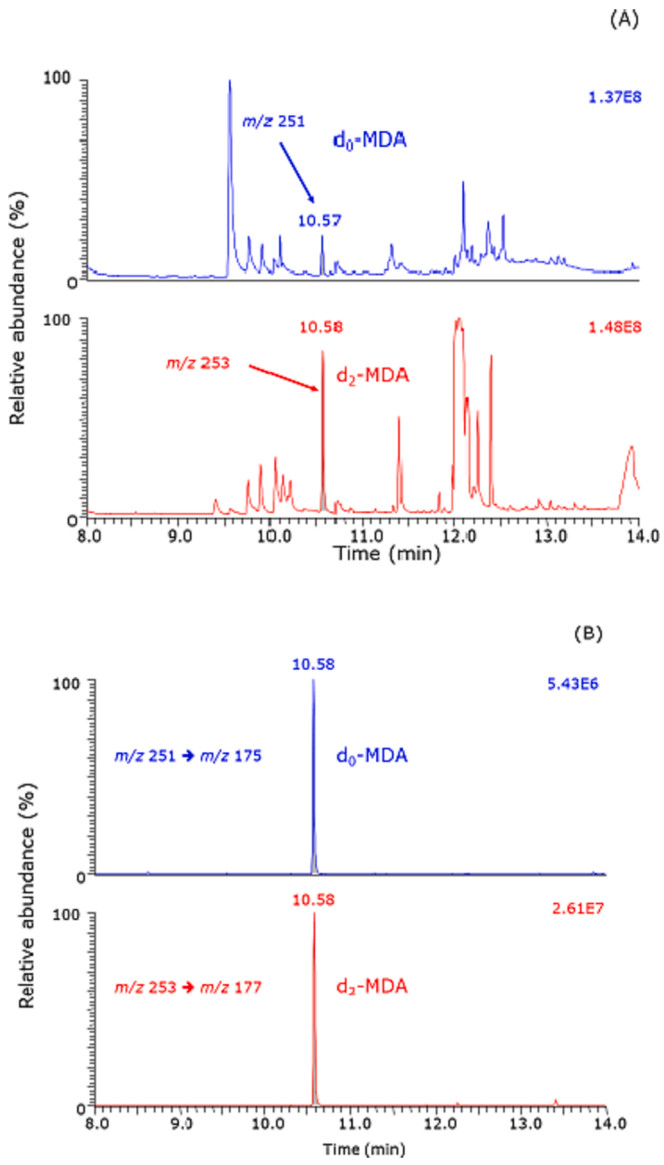


Fig. 2. Typical GC–MS chromatograms from quantitative analyses of unlabeled MDA (d_0-MDA) in a 100-μL human plasma aliquot by GC–MS (A) and GC–MS/MS (B). d_2-MDA was used as the internal standard. In GC–MS, selected-ion monitoring (SIM) of m/z 251 and m/z 253 was performed. In GC–MS/MS, selected-reaction monitoring (SRM) of the transitions m/z 251 to m/z 175 and m/z 253 to m/z 177 was performed. Adapted from Ref. [32].

their collection at baseline and at the end of the study? In this case, biochemical parameters would be analyzed at least two times, i.e., at the beginning (baseline) and at the end of the study. Would then the analytical results measured at two different time points be reliably comparable? How to proceed further, if the concentrations of biochemical parameters in the verum and placebo differ statistically? Should the study be stopped?

Alternatively, should the biological samples be analyzed simultaneously once at the end of study? In this case, the biological samples would differ in age, and questions may arise. Do the concentrations of labile analytes decrease stronger in the older samples compared to the younger samples? 2) Do the concentrations of analytes increase stronger in the older samples compared to the younger samples because of artificial formation during storage?

Should such aspects be included in the study design of clinical trials?

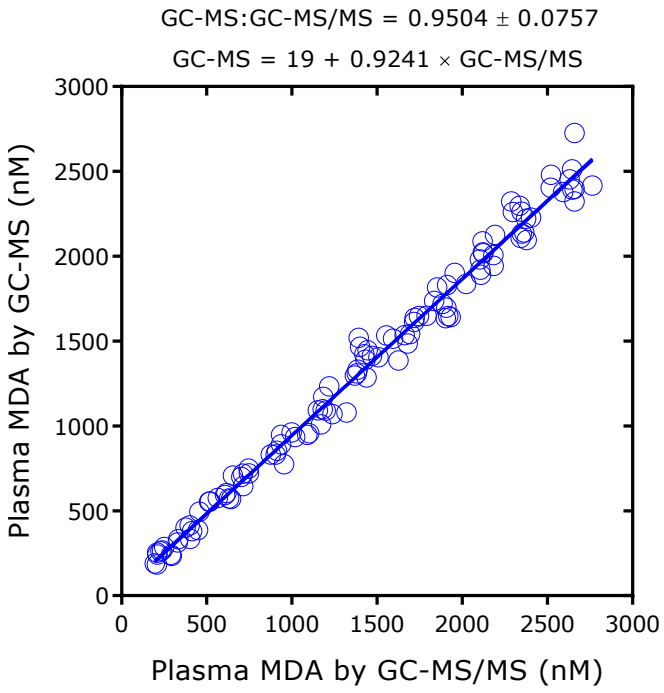


Fig. 3. Comparison of plasma MDA concentrations measured in 110 human samples by GC–MS (SIM of m/z 251 and m/z 253) and GC–MS/MS (SRM of the transitions m/z 251 to m/z 175 and m/z 253 to m/z 177). Deming regression analysis between GC–MS and GC–MS/MS concentrations for plasma MDA was performed. d_2-MDA was used as the internal standard at 1000 nM. Adapted from Ref. [32].

Specific aspects of MDA analysis

Plasma, serum and tissue are the most frequently analyzed biological samples for MDA. Blood sampling including anti-coagulation and hemolysis, conditions for sample storage including temperature and time, and artificial formation of MDA, notably in long-term clinical studies, are well-recognized pre-analytical factors that may compromise measurement of MDA. A more recently recognized, in the past rarely considered MDA-specific issue concerns the study-design, even in placebo-controlled studies [1,50]. These factors are discussed separately below.

Blood sampling – type of anti-coagulation and hemolysis

Anti-coagulation and hemolysis are two major well-recognized pre-analytical factors for many endogenous substances and drugs in plasma and serum [51–56]. Commercially available vacutainers used to draw blood may be contaminated to varying degrees with the analytes to be measured. The content of MDA in monovettes we used in blood sampling in clinical studies including the PAOD and CAD studies was measured to be 5–10 nM, i.e., neglectable, except for the citrate monovettes (Fig. 4). Not contaminating MDA but rather coagulation/anti-coagulation-specific factors are responsible for differences in reported plasma and serum samples. Blood-sampling induced hemolysis, is likely to include *ex vivo* peroxidation of free and esterified PUFAs from red blood cells and COX-1-induced peroxidation of arachidonic acid in platelets. At baseline, we found an inverse correlation between plasma MDA and plasma potassium concentrations of healthy young men, without visible hemolysis [57]. Considerable differences for MDA concentrations were measured in freshly prepared serum and plasma (EDTA, heparin) from blood of a healthy subject. The lowest MDA concentrations were measured in the serum samples (Fig. 4) [32].

The MDA concentration measured by GC–MS/MS in plasma samples of healthy young volunteers who ingested placebo, aspirin (acetylsalicylic acid, ASA) or paracetamol was found to correlate with the

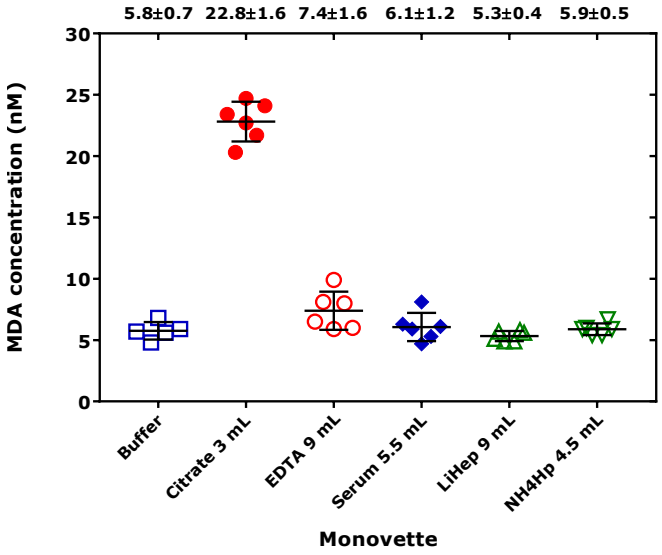


Fig. 4. Concentration of malondialdehyde (MDA) in the indicated monovettes (Sarstedt, Germany) filled with aqueous buffer of pH 7.4. MDA was measured by GC–MS/MS. The Figure was constructed using data from a previously reported study [32].

oxyhemoglobin (HbO_2) plasma concentration, albeit to a varying extent. The lowest variation in HbO_2 and MDA concentrations was seen in the volunteers treated with paracetamol (acetaminophen) (Fig. 5). The highest plasma HbO_2 levels were measured in plasma of volunteers who ingested acetylsalicylic acid. Fig. 5 suggests that estimation of hemolysis-induced formation of MDA would very difficult. During the observation period of 4 h, no appreciable changes in plasma MDA concentration was seen in the volunteers including those who ingested aspirin (Fig. 5 and Ref. [32]). This is in line with the observation that in humans MDA and TxA_2 inhibition by ingested aspirin (600 mg) requires considerable time to occur (Ref. [8]).

Minimization of *ex vivo* coagulation/anti-coagulation-dependent formation of MDA can by achieved by using COX inhibitors such as acetylsalicylic acid or indomethacin to block enzymatic formation of MDA [6,7,58]. The use of butylated hydroxytoluene (BHT) at high concentrations has also been described especially in TBA assays [59]. In our studies, we do not use COX inhibitors or BHT in plasma or urine to suppress MDA formation.

A difficulty remains, nevertheless. Optimum measurement of endogenous analytes such as MDA [1], nitrite and nitrate [19] or DMA [43] in serum or plasma may require different types of anti-coagulation and the commercially available vacutainers/monovettes may be differently contaminated with the analytes. Circulating DMA concentration in healthy young women was determined to be 1.4 ± 0.2 μM in serum, 1.7 ± 0.12 μM in lithium heparin plasma, and 9.8 ± 1.4 μM in EDTA plasma [43]. DMA was identified as an abundant contaminant in EDTA vacutainer tubes (9.3 ± 1.9 nmol/monovettes), with serum and lithium heparin vacutainers being contaminated with considerably smaller amounts of DMA (0.4 ± 0.01 and 0.9 ± 0.01 nmol/monovettes, respectively) [43]. Thus, serum would be recommended as the most appropriate matrix for measuring DMA in human blood. Analyte-specific blood sampling could be a solution, but would complicate clinical studies.

Sample storage and time point of analysis

Formation of MDA during storage of human plasma in the absence and in presence of externally added anti-oxidants has been reported [44,47,59,60,61]. Artefactual formation of free non-conjugated MDA in collected urine samples seems not be relevant when measured by GC–MS or GC–MS/MS [44,47]. In many clinical studies, urine is collected by spontaneous micturition without information of the urine volume and the period of collection.

Many analytes measured in blood, plasma, serum and urine are also present in the laboratory air and may represent potential contributors to those being analyzed in study samples. There is evidence that atmospheric nitrogen oxides (NO_x) including nitrite and nitrate can entry into biological samples being analyzed by physical adsorption during sample working up [62]. Atmospheric NO_x are also absorbed by the derivatization reagent PFB-Br, and the use of commercially available 1-mL containing flasks of PFB-Br is recommended [62]. MDA is also present in the exhaled air [63] and in sputum [64] and could contribute to MDA in the study samples. Conditions known to influence the extent of contribution of environmental NO_x are kind and temperature of the biological sample, as well as the time of exposition of the sample to the laboratory air [62]. We are not aware whether these issues also apply to MDA. Precautions should be taken to minimize such effects, especially during sample aliquoting and spiking with a stable-isotope labeled internal standard such as d_2-MDA for subsequent GC–MS analysis [32]. During aliquoting and spiking of thawed (preferentially in ice bath) biological samples, they should be treated individually or in very small groups, and be kept immediately closed to minimize external MDA entry into the samples. Such a procedure is labor-intensive and costs time but saves analytical reliability.

When is the best time to analyze MDA in biological samples collected in long-term clinical studies, in which samples collected at the end of the study are much younger than those collected at baseline? The results of the PAOD and CAD studies indicate that the time point of GC–MS/MS measurement of MDA in plasma, but not urine, may be a major concern [1,32,44–49]. The most likely explanation for the observations is artificial formation of MDA in stored plasma, which is a lipid-rich biological sample. Whether MDA is continuously formed during storage at the frozen state or during the thawing process is unknown. It seems to be a diverging but saturable effect [50], yet abundant enough to cause

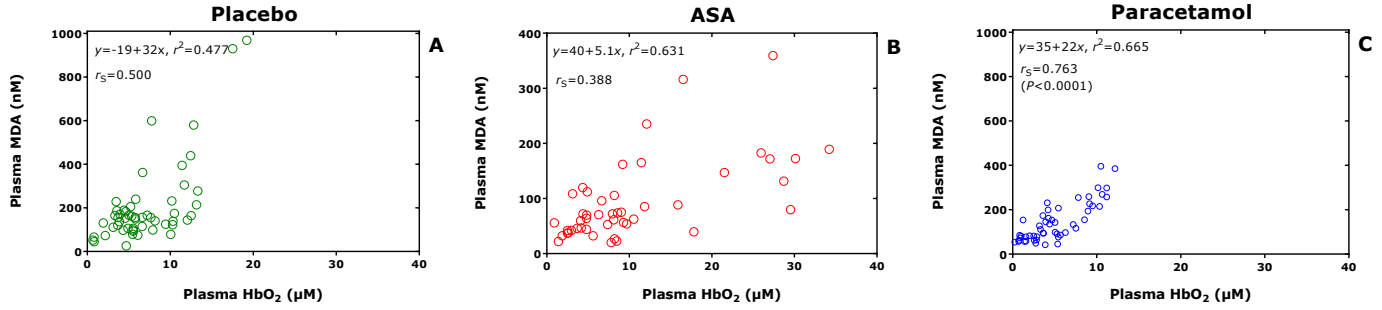


Fig. 5. Relationship between the plasma concentrations of MDA and oxyhemoglobin (HbO_2) in 10 healthy young volunteers who ingested tablets containing (A) 500-mg placebo, (B) 500 mg acetylsalicylic acid (ASA) or (C) 500 mg paracetamol (acetaminophen). MDA was measured by GC–MS/MS [32] and HbO_2 spectrophotometrically [33]. Blood samples were drawn at baseline and within a period of about 4 h after treatment. The Figures were constructed using data from a previously reported study [32].

statistically significant differences in the concentrations of MDA in plasma at baseline and at the end of the studies. That this effect occurred both in the placebo and in the verum groups of the PAOD and CAD studies lets conclude that higher MDA concentrations in the older plasma samples is not an effect of the administered drug, i.e., L-arginine, but higher artificial MDA formation.

Commonly, samples collected at baseline and at the end of clinical studies are analyzed at the end of the studies. We also follow this procedure in our clinical studies. This proceeding allows performing GC–MS and GC–MS/MS analyses under closely comparable experimental conditions for GC–MS and GC–MS/MS apparatus, laboratory and personnel for all study samples. The time needed to measure all study samples is by far much shorter than the length of the study. In the case of MDA measurement in plasma samples collected in long-term clinical studies, this strategy would most likely generate insignificant results.

A serious alternative would be to analyze MDA in plasma/serum samples at two time points: 1) after complete collection of the baseline samples; and 2) after complete collection of the samples at the end of the study. Care should be taken that samples are collected closely in time, both at baseline and at the end of the study. In this case, difficulties may arise from differences at the time points with respect to experimental conditions including GC–MS or GC–MS/MS apparatus (e.g., tuning, GC column changing), laboratory (e.g., preparation and standardization of the internal standard; different charges of PFB-Br) and possibly personnel. Care should also be taken with respect to these issues.

It is worthy of mention, that in the vast majority of the reported clinical studies involving MDA measurement in plasma or serum no information is report with respect to the storage conditions and the age of the samples during analysis. Yet, this information must be provided. Reporting detailed protocols including times and periods of sample collection, conditions of sample storage and final analysis ensures scientific visibility.

Study design issues

When is the best time point(s) for the analysis of MDA in collected plasma or serum samples especially in long-term clinical studies? Needs this issue to be included in the study design? The most commonly used practice is measurement of analytes in samples collected at baseline and after completion of the whole study. It is believed that this ensures very similar analytical conditions for sample work up and instrumental analysis. This is likely true for analytes that are stable under the sample storage conditions and are not artificially formed during storage.

In the two placebo-controlled CAD and PAOD studies, we investigated biological and biochemical effects of L-arginine supplementation (3 g/d for 3 or 6 months) or placebo supplementation (3 g/d mannitol for 3 or 6 months). In these studies, we measured MDA concentrations in plasma samples collected at baseline and after treatment (3 and 6 months, respectively) at the end of each study. Thus, baseline and after-treatment plasma samples differed by 3 and 6 months on the time point of analysis. In both studies, we observed considerably lower MDA concentrations in the plasma samples collected after 3 or 6 months than in the samples collected at the beginning [44,47]. In contrast, the MDA concentrations in the urine samples collected in parallel did not differ at baseline and after the end the studies. These observations, strongly suggest that storage time is a major concern in plasma but not in urine samples in long-term studies and needs to be considered in the study design. On the other hand, in short-term clinical studies, storage period of plasma samples for MDA measurement is not an issue [57]. Fig. 6 shows that the plasma MDA concentration did not change over time during exercise in young volunteers who ingested NaCl (placebo) or $NaNO_3$ (verum). It is noticeable that the concentration of MDA in the heparinized plasma samples of the healthy young volunteers is in the range of only 0.2–0.6 μM, i.e., considerably lower than in the elderly PAOD and CAD patients (see Table 1) [32,44–49].

Quality control

In the framework of clinical studies, we implemented quality control (QC) systems for endogenous analytes including metabolites of the Arg/NO pathway [19], MDA and 8-*iso*-$PGF_2\alpha$ [65]. Such QC systems are useful to determine the accuracy and the precision by which endogenous substances were measured in biological samples at the time points of analysis [19]. Yet, they are not useful to correct for artificial formation of analytes such as MDA during long storage periods.

An example for a QC system for MDA in human plasma is shown in Fig. 7. We used a pooled citrated plasma donated by a healthy young donor, which was frozen aliquoted (100 μL) at − 80 °C. Prior to freezing, MDA was measured in 18 aliquots. The baseline MDA concentration in these samples (QC 0) was determined to be 70.4 nM with a coefficient of variation (CV) of 7.7%. Study samples and QC samples ($n = 2$ to 6) were analyzed in parallel within 8 working days (QC 1 to 8). In the QC samples, the concentration of MDA was measured with a precision (CV) of 2.3 to 13.3% and with a bias of −3 to 29%. The MDA plasma concentration in the study samples [66] was determined to be (median with interquartile range) 150 [115–194] nM.

Reference values and intervals

Oxidative stress and lipid peroxidation are generally believed to be involved in numerous human diseases [67,68]. Yet, reliable quantitative measurement of MDA in plasma and serum samples is highly challenging. Despite the many attempts to define reference intervals and the plethora of reported data on MDA [69], no reference intervals were

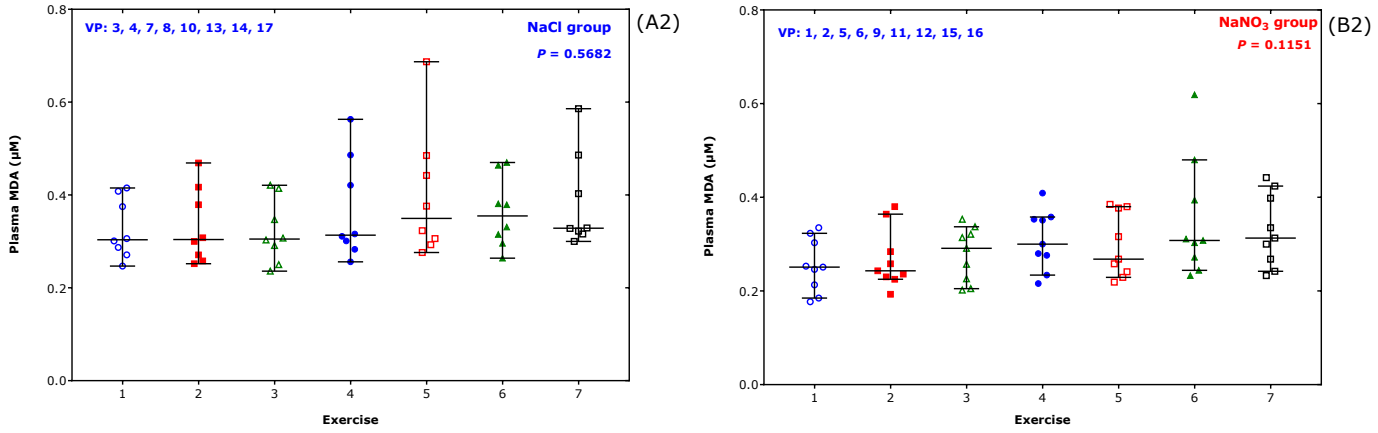


Fig. 6. Malondialdehyde (MDA) concentrations in heparinized plasma samples of the volunteering persons (VP) of the NaCl (A2) and $NaNO_3$ (B2) groups at the seven individual time points of exercise after supplementation (see Scheme 2). Data in (A2) and (B2) are shown as median with 95% confidence interval. Adapted from [57] (part of the supplementary Fig. S3 in [57]). Exercise numbers correspond to a period of about 60 min in total.

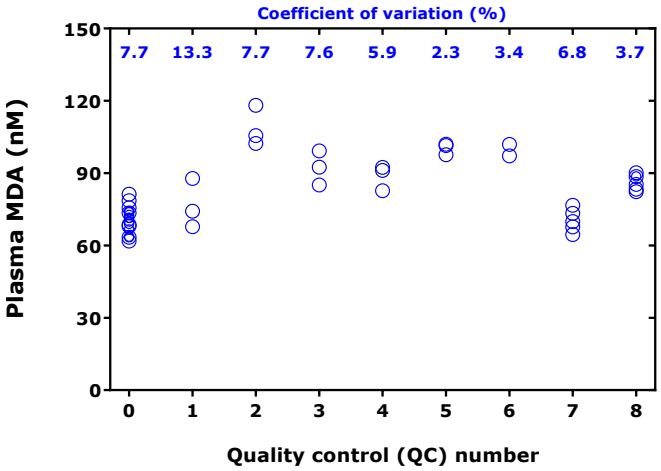


Fig. 7. Malondialdehyde (MDA) concentration in a pooled citrated human plasma sample serving as quality control (QC) prior to (QC 0; n = 18) and after (QC 1 to 8) starting analyzing study samples. QC samples were analyzed within 8 working days in parallel in eight runs with citrated human plasma samples (n = 237) collected in a clinical study [66]. QC samples and study samples were stored aliquoted (100 μL) at −80 °C. MDA was measured by GC–MS/MS using d_2-MDA (1000 nM) as the internal standard [32].

established for MDA plasma and serum or for MDA in exhaled breath condensate (EBC) [63].

Circadian rhythmicity

Similarly, circadian rhythmicity is also generally assumed to be associated with oxidative stress and to have implications for human health and disease. Yet, there discrepant reports [70–75]. Wilking and colleagues concluded in their review that „We believe that for a more efficacious management of diseases that have both circadian rhythm and oxidative stress components in their pathogenesis, targeting both systems in tandem would be far more successful" [70]. The most efficient and generally followed strategy to overcome variation of a potential circadian rhythmicity of MDA formation in clinical studies would be sampling of biological samples at fixed day or night times.

MDA in diseases

Table 1 summarizes reported MDA concentrations in human plasma, serum and red blood cells in health and disease. In freshly collected EBC and bronchoalveolar liquid (BAL) samples of healthy young humans, we have measured MDA concentrations of the order of 50 nM by GC–MS/MS (unpublished data). Discrepant data were reported with respect to the subjects age, gender, smoking and alcohol consumption (Table 1) [69,76–82].

MDA is considered to be associated with many diseases, including Alzheimer and Parkinson and diabetes [83,84]. Yet, there considerable discrepancies as well [68,85–89].

Oxidative stress is commonly associated with kidney disease. By means of GC–MS/MS [32] and a commercially available TBARS assay we found no transrenal MDA concentration differences after reperfusion [90]. Urinary MDA concentrations as measured by the TBARS assay were similar in kidney recipients and healthy controls [90]. Oxidative damage in clinical ischemia/reperfusion injury has been questioned [91,92].

A recent prospective cohort study showed that post-transplantation plasma MDA (as measured by a non-commercially fluorimetric TBARS assay after extraction with *n*-butanol) is associated with cardiovascular mortality in renal transplant recipients (RTR) [93]. In that study, the median circulating baseline MDA concentration was 5.4 μM in 604 RTR. It is noticeable that the association between plasma MDA concentration and the risk for cardiovascular mortality was stronger in RTR with relatively lower plasma ascorbic acid concentrations [93].

We measured by GC–MS the excretion rate of MDA in urine samples of healthy donors before (pre) and after donation (post) of a kidney and in RTR of previously described cohorts [93,94]. Median MDA excretion rate expressed as μmol/24 h or corrected by creatinine excretion decreased statistically significantly due to donation of a kidney by about 30 % (Fig. 8). Median MDA excretion in the urine of RTR was 1.6–1.9 times higher compared to that of the healthy donors prior to kidney donation. These results suggest that urinary MDA may be useful as a measure of oxidative stress. Whether urinary MDA indicates whole-body oxidative stress, or rather local oxidative stress in the kidney remains to be investigated.

MDA and anti-oxidants supplementation

Many clinical studies investigated the effects of anti-oxidant supplementation in health and disease [95–113]. Use anti-oxidants include

Table 1
Reported MDA concentrations in serum, plasma and red blood cells in health and disease.

Health/disease	Matrix	Anticoagulation	Method	MDA concentration	Reference
Health	Serum PlasmaPlasma	none HeparinEDTA	GC–MS/MS	0.42 μM (n = 18) 0.49 μM (n = 18) 2.76 μM (n = 18)	[32]
DiabetesHealth	PlasmaPlasma	Not reportedNot reported	Batch TBARSBHT (5 μM), butanol	0.44 μM (n = 20) 0.47 μM (n = 14)	[61]
Health	PlasmaSerum	EDTA Heparin Citratenone	HPLC-UV	0.26 μM (n = 13) 1.27 μM (n = 13) 0.89 μM (n = 13) 1.16 μM (n = 13)	[74]
Health	Plasma	EDTA	HPLC-UV	0.4–1.3 μM males (n = 107) 0.3–1.2 μM females (n = 106) 0.66 μM sm (n = 92) vs 0.60 μM nsm (n = 122)[a]	[74]
Diabetes (T2DM)Health	SerumRBC	Heparin (?)	TBARS	Serum: 0.4 μM healthy vs. 0.4 μM diabetic RBC: 0.4 μM healthy vs. 0.8 μM diabetic [b]	[87]
Diabetes (T2DM)Normal glucose tolerance	Plasma	unknown	Batch TBARS	1.0 μM (n = 93) 1.0 μM (n = 96)	[92]
Diabetes	Serum	None	HPLC-UV	0.75/1.05 μM healthy/diabetes 1st trimester 0.72/1.08 μM healthy/diabetes 2nd trimester 0.79/0.82 μM healthy/diabetes 3rd trimester	[83]

[a] Sm, smokers; nsm, nonsmokers.
[b] RBC, red blood cells.

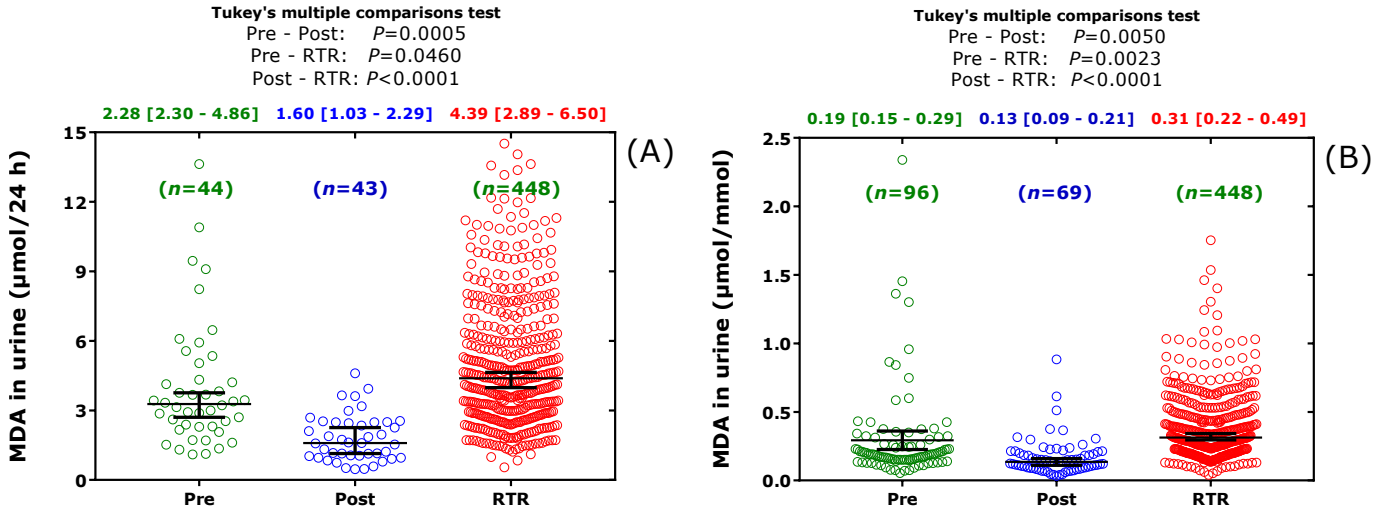


Fig. 8. Urinary excretion rate of malondialdehyde (MDA) by healthy kidney donors before (pre) and after (post) donation of a kidney and by renal transplant recipients (RTR). (A) in µmol MDA/24 h; (B) corrected for creatinine excretion (µmol MDA/mmol creatinine). MDA and creatinine were measured simultaneously in 100-µL urine aliquots by a GC–MS method [32]. Data on the top are MDA values given as median with interquartile range.

vitamin E (α-tocopherol), ω-3 fatty acids (EPA and DHA) and *N*-acetylcysteine (NAC). PUFAs supplementation seems to increase MDA formation, most likely due to the elevation of the circulating EPA concentration in the patients supplemented with ω-3 fatty acids rather than due to elevation of oxidative stress [102]. In the case of the circulating isoprostanes as biomarkers of oxidative stress, a normalization to their precursors in plasma/serum lipids have been discussed [103]. Yet, such a standardization is problematic mainly because of the definition of a proper lipid. This difficulty applies much more to MDA, which have many precursors. In contrast, normalization of 3-nitro-tyrosine to its precursor tyrosine has been established [104].

In a clinico-pharmacological study, Iranian rheumatoid patients received NAC (2 × 600 mg/day) or placebo in addition to the basic therapy [105]. The mean serum MDA concentration in the placebo group of the study was 2.2 µM at baseline and 2.2 µM after 12 weeks, indicating no effects of NAC on oxidative stress [105] (Fig. 9). In contrast, the mean serum MDA concentration in the NAC group was 4.2 µM at baseline, but only 1.5 µM after 12 weeks, suggesting a strong decrease of oxidative stress upon NAC treatment (Fig. 9). These serum MDA concentrations are within the wide-ranges reported in the literature for healthy and ill subjects using various methodologies, including assays based on TBARS assays and GC–MS [1,68]. However, the baseline serum MDA concentrations were almost two times higher in the NAC group compared to the placebo group [105]. The issues mentioned above for MDA also apply to nitrite + nitrate (NOx) measured in the study [105] (Fig. 9). It arises the question whether studies with greatly differing baseline values of MDA, NOx and other biochemical parameters in the placebo and verum groups should be terminated prior to start the planned supplementation if they are primary outcome parameters of the study [50] as originally designed [105].

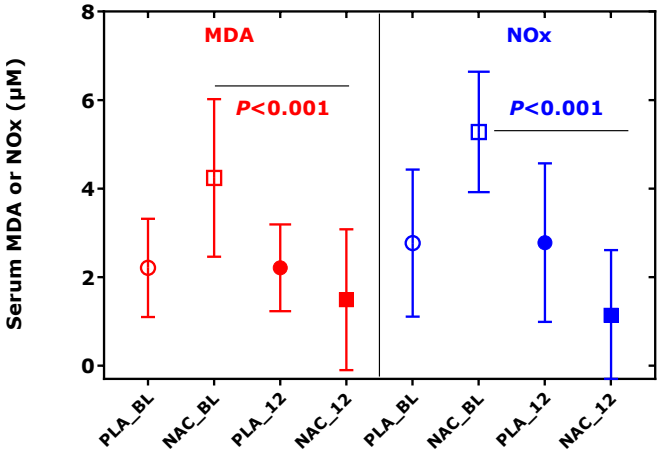


Fig. 9. Malondialdehyde (MDA) and nitrite + nitrate (NOx) concentrations (mean ± SD) in rheumatoid patients at baseline (BL) and after a 12-weeks (12) treatment with placebo (PLA) or N-acetylcysteine (NAC, 2 × 600 mg/day) [105]. The Figure was constructed by the best ability of the author of the present work using the data reported in previous clinical study by Esalatmanesh et al. [105]. In that study, MDA in serum was measured by TBARS assay, and the sum of nitrite and nitrate in the serum was determined by an assay based on the Griess reaction, both of commercial origin. For a more detailed discussion see Ref. [50].

In case of supplementing chemically reactive anti-oxidants such as NAC, which are known to react with carbonylic compounds including MDA [106], their potential interference with MDA assays needs to be investigated pre-analytically at relevant concentrations for NAC and its major metabolites L-cysteine and GSH.

Metformin (a dimethylated biguanide) is a pleiotropic drug and to exert anti-oxidative effects [107]. Metformin (1 to 2 g/d for 12 weeks) has been tested in combination with astaxanthin (a carotenoid) in patients suffering from type 2 diabetes mellitus [108]. Plasma MDA was measured by a TBARS assay as reported in Ref. [107] to be of the order of 18 µM, which is an extraordinarily high concentration, and was found not to change [108].

In a clinico-pharmacokinetic study, we investigated the effects of metformin supplementation (3 × 500 mg/d) for six weeks to Becker muscular dystrophy (BMD) patients [109,110]. In that study, we also measured MDA in serum and urine samples by GC–MS [32]. Metformin supplementation decreased mean serum MDA concentration by 21% (from 0.99 µM at baseline to 0.78 µM after six weeks), while creatinine-corrected MDA urinary excretion was about 0.2 µmol MDA/mmol creatinine and did not change at all [111]. To our knowledge, urinary MDA is rarely measured and its clinical significance is unexplored. In the BMD study, MDA excretion correlated inversely with the creatinine excretion [111]. Previously, we found a close positive correlation between the urinary excretion rates of MDA and nitrite [112]. This observation and the association of circulating and urinary MDA have not been investigated in depth thus far. As free MDA (pK_a, 4.5) and nitrite (pK_a, 3.6) are of comparable size largely negatively charged in human urine (pH range, 5.5 to 7.8), renal transport systems for anions, possibly also involving carbonic anhydrases [113], are likely to be involved in

the excretion/reabsorption of MDA and nitrite in the kidneys (see Ref. [93]).

Summary and outlook

Mass spectrometry is widely applied in clinical research is increasingly implemented in clinical laboratories [34,114,115] because of its exclusive feature among analytical techniques to utilize a unique property of inorganic and organic material, i.e., its mass. Yet, good science needs good mass spectrometry [116] and deep knowledge of known and potential pitfalls in analytical processes and ways for their avoidance, especially when analyzing biological samples which are inherently complex and practically not suitable for direct analysis [50,56,104,116–120]. Crucial and at first glance paradoxical concerns refer to a series of pre-analytical factors that include sample collection and storage until sample work up. Non-identification and non-controlling of pre-analytical concerns are likely to distort the in vivo reality because of generation of highly questionable analytical results. Even storage of plasma samples at low temperatures for a considerable period for instance during an ongoing clinical study may result in artificial generation of analytes. This has been observed for MDA [61], the subject of the present work, in plasma samples, which are rich in lipids, potential origin of MDA. Artificial generation of MDA in urine has not been observed, suggesting urine as a serious alternative matrix to blood. Yet, plasma and serum are still the most frequently analyzed biological samples for MDA as a measure of lipid peroxidation [1]. In the present article, several pre-analytical issues affecting reliable quantitative determination of MDA by GC–MS after derivatization with pentafluorobenzyl bromide [32] within the frame work of clinical studies were discussed in detail, including examples from own research. They include blood sampling and anti-coagulation, sample aliquoting, storage, thawing spiking with the internal standard d_2-MDA, implementing a QC system for MDA and its measurement in human urine. Examples from reported clinical studies by other research groups were also presented and discussed. The greatest challenges in the analysis of MDA in human plasma and serum are still pre-analytical issues and largely independent of the analytical approach. Mass spectrometry in combination with chromatography (i.e., GC–MS, GC–MS/MS, and LC-MS/MS) is a guarantor of good analytics but cannot abstain from good pre-analytics. Clinical journals reporting results on oxidative studies and Arg/NO pathway should permit authors to describe in the work the analytical approaches used in the studies including the time points of sample collection, storage and analysis, for high transparency and comparability. The possibility that study design may improve analytical outcome was also addressed.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Ethical statement

This study did not involve material from humans or animals.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jmsacl.2023.08.001.

References

[1] D. Tsikas, Assessment of lipid peroxidation by measuring malondialdehyde (MDA) and relatives in biological samples: Analytical and biological challenges, Anal. Biochem. 524 (2017) 13–30, https://doi.org/10.1016/j.ab.2016.10.021.
[2] R. Hüttel, Ü. Malondialdehyd, 1. Mitteilung, Ber. Deutsch. Chem. Ges. 74 (1941) 1825–1829.
[3] M.M. Osman, The acidity of malondialdehyde and the stability of its complexes with nickel(II) and copper (II), Helvetica Chim. Acta 55 (1) (1972) 239–244.
[4] H. Esterbauer, R.J. Schaur, H. Zollner, Chemistry and biochemistry of 4-hydroxynonenal, malonaldehyde and related aldehydes, Free Radic. Biol. Med. 11 (1) (1991) 81–128.
[5] F. Bernheim, M.L. Bernheim, K.M. Wilbur, The reaction between thiobarbituric acid and the oxidation products of certain lipids, J. Biol. Chem. 174 (1948) 257–1164.
[6] J.B. Smith, C.M. Ingerman, M.J. Silver, Malondialdehyde formation as an indicator of prostaglandin production by human platelets, J. Lab. Clin. Med. 88 (1976) 167–172.
[7] H.J. Weiss, B. Lages, Platelet malondialdehyde production and aggregation responses induced by arachidonate, prostaglandin-G2, collagen, and epinephrine in 12 patients with storage pool deficiency, Blood 58 (1981) 27–33.
[8] A. Szczeklik, R.J. Gryglewski, L. Grodzińska, J. Musiał, M. Serwońska, E. Marcinkiewicz, Platelet aggregability, thromboxane A_2 and malondialdehyde formation following administration of aspirin to man, Thromb. Res. 15 (1979) 405–413.
[9] T. Tomita, K. Umegaki, E. Hayashi, Basis aggregation properties of washed rat platelets: correlation between aggregation, phospholipid degradation, malondialdehyde, and thromboxane formation, J. Pharmacol. Methods 10 (1983) 31–44.
[10] J.W. Burch, P.T. Services, Glutathione disulfide production during arachidonic acid oxygenation in human platelets, Prostaglandins 39 (2) (1990) 123–134.
[11] W.L. Smith, L.J. Marnett, D.L. DeWitt, Prostaglandin and thromboxane biosynthesis, Pharmac. Ther. 49 (3) (1991) 153–179.
[12] D. Tsikas, M.T. Suchy, J. Niemann, P. Tossios, Y. Schneider, S. Rothmann, F. M. Gutzki, J.C. Frölich, D.O. Stichtenoth, Glutathione promotes prostaglandin H synthase (cyclooxygenase)-dependent formation of malondialdehyde and 15(*S*)-8-*iso*-prostaglandin $F_{2\alpha}$, FEBS Lett. 586 (2012) 3723–3730.
[13] M. Perluigi, R. Coccia, D.A. Butterfield, 4-Hydroxy-2-nonenal, a reactive product of lipid peroxidation, and neurodegenerative diseases: a toxic combination illuminated by redox proteomics studies, Antioxid. Redox Signal. 17 (2012) 1590–1609.
[14] A. Ayala, M.F. Muñoz, S. Argüelles, Lipid peroxidation: production, metabolism, and signaling mechanisms of malondialdehyde and 4-hydroxy-2-nonenal, Oxid. Med. Cell. Longev. 2014 (2014), 360438.
[15] L.G. McGirr, M. Hadley, H.H. Draper, Identification of N-alpha-acetyl-epsilon-(2-propenal)lysine as a urinary metabolite of malondialdehyde, J. Biol. Chem. 260 (29) (1985) 15427–15431.
[16] H.H. Draper, M. Hadley, L. Lissemore, N.M. Liang, P.D. Cole, Identification of N-epsilon-(2-propenal)lysine as a major urinary metabolite of malondialdehyde, Lipids 23 (1988) 626–628.
[17] M. Hadley, H.H. Draper, Identification of *N*-(2-propenal)serine as a urinary metabolite of malondialdehyde, FASEB J. 2 (2) (1988) 138–140.
[18] M. Hadley, H.H. Draper, Identification of *N*-(2-propenal)ethanolamine as a urinary metabolite of malondialdehyde, Free Radic. Biol. Med. 6 (1) (1989) 49–52.
[19] D. Tsikas, A critical review and discussion of analytical methods in the L-arginine/nitric oxide area of basic and clinical research, Anal. Biochem. 379 (2008) 139–163, https://doi.org/10.1016/j.ab.2008.04.018.
[20] D. Tsikas, Urinary Dimethylamine (DMA) and Its Precursor Asymmetric Dimethylarginine (ADMA) in Clinical Medicine, in the Context of Nitric Oxide (NO) and Beyond, J. Clin. Med. 9 (6) (2020) 1843, https://doi.org/10.3390/jcm9061843.
[21] M. Khoubnasabjafari, K. Ansarin, A. Jouyban, Critical review of malondialdehyde analysis in biological samples, Curr. Pharmac. Anal. 12 (2016) 4–17.
[22] J.M.C. Gutteridge, B. Halliwell, The measurement and mechanism of lipid peroxidation in biological systems, Trends Biochem. Sci 15 (4) (1990) 129–135.
[23] R.O. Sinnhuber, T.C. Yu, TeC Yu, Characterization of the red pigment formed in the 2-thiobarbituric acid determination of oxidative rancidity, Food Res. 23 (1958) 626–633.
[24] K. Yagi, A simple fluorometric assay for lipoperoxide in blood plasma, Biochem. Med. 15 (2) (1976) 212–216.
[25] K. Yoden, T. Iio, Determination of thiobarbituric acid-reactive substances in oxidized lipids by high-performance liquid chromatography with a postcolumn reaction system, Anal. Biochem. 182 (1) (1989) 116–120.
[26] J. Lykkesfeldt, Determination of malondialdehyde as dithiobarbituric acid adduct in biological samples by HPLC with fluorescence detection: comparison with ultraviolet-visible spectrophotometry, Clin. Chem. 47 (2001) 1725–1727.
[27] R.P. Bird, S.S.O. Hung, M. Hadley, H.H. Draper, Determination of malonaldehyde in biological materials by high-pressure liquid chromatography, Anal. Biochem. 128 (1) (1983) 240–244.
[28] C.R. Wade, P.G. Jackson, A.M. Van Rij, Quantitation of malondialdehyde (MDA) in plasma, by ion-pairing reverse phase high performance liquid chromatography, Biochem. Med. 33 (3) (1985) 291–296.
[29] C.A. Sobsey, J. Han, K. Lin, W. Swardfager, A. Levitt, C.H. Borchers, Development and evaluation of a liquid chromatography-mass spectrometry method for rapid,

accurate quantitation of malondialdehyde in human plasma, J. Chromatogr. B 1029–1030 (2016) 205–212.
[30] K. Fujioka, T. Shibamoto, Improved malonaldehyde assay using headspace solid-phase microextraction and its application to the measurement of the antioxidant activity of phytochemicals, J. Agric. Food Chem. 53 (12) (2005) 4708–4713.
[31] P. Maboudou, D. Mathieu, H. Bachelet, J.F. Wiart, M. Lhermitte, Detection of oxidative stress. Interest of GC-MS for malondialdehyde and formaldehyde monitoring, Biomed. Chromatogr. 16 (3) (2002) 199–202.
[32] D. Tsikas, S. Rothmann, J.Y. Schneider, M.T. Suchy, A. Trettin, D. Modun, N. Stuke, N. Maassen, J.C. Frölich, Development, validation and biomedical applications of stable-isotope dilution GC-MS and GC-MS/MS techniques for circulating malondialdehyde (MDA) after pentafluorobenzyl bromide derivatization: MDA as a biomarker of oxidative stress and its relation to 15(*S*)-8-*iso*-prostaglandin $F_{2\alpha}$ and nitric oxide ($^{\bullet}$NO), J. Chromatogr. B 1019 (2016) 95–111.
[33] U. Dreissigacker, M.T. Suchy, N. Maassen, D. Tsikas, Human plasma concentrations of malondialdehyde (MDA) and the F_2-isoprostane 15(S)-8-iso-$PGF_{2\alpha}$ may be markedly compromised by hemolysis: evidence by GC-MS/MS and potential analytical and biological ramifications, Clin. Biochem. 43 (2010) 159–167.
[34] J. Grace van der Gugten, Tandem mass spectrometry in the clinical laboratory: A tutorial Overview, Clinical Mass Spectrometry 15 (2020) 36–43.
[35] D. Tsikas, Pentafluorobenzyl bromide-A versatile derivatization agent in chromatography and mass spectrometry: I. Analysis of inorganic anions and organophosphates, J. Chromatogr. B Anal. Technol. Biomed. Life Sci. 104 (2017) 187–201, https://doi.org/10.1016/j.jchromb.2016.08.015.
[36] D. Tsikas, Simultaneous derivatization and quantification of the nitric oxide metabolites nitrite and nitrate in biological fluids by gas chromatography/mass spectrometry, Anal. Chem. 72 (2000) 4064–4072, https://doi.org/10.1021/ac9913255.
[37] D. Tsikas, A. Wolf, A. Mitschke, F.M. Gutzki, W. Will, M. Bader, GC-MS determination of creatinine in human biological fluids as pentafluorobenzyl derivative in clinical studies and biomonitoring: Inter-laboratory comparison in urine with Jaffé, HPLC and enzymatic assays, J. Chromatogr. B Anal. Technol. Biomed. Life Sci. 878 (27) (2010 Oct 1) 2582–2592, https://doi.org/10.1016/j.jchromb.2010.04.025.
[38] D. Tsikas, J Application of gas chromatography-mass spectrometry and gas chromatography-tandem mass spectrometry to assess in vivo synthesis of prostaglandins, thromboxane, leukotrienes, isoprostanes and related compounds in humans, J. Chromatogr. B Biomed. Sci. Appl. 717 (1998) 201–245, https://doi.org/10.1016/s0378-4347(98)00210-2.
[39] R.D. Velásquez, G. Brunner, M. Varrentrapp, D. Tsikas, J.C. Frölich, Helicobacter pylori produces histamine and spermidine, Z. Gastroenterol. 34 (1996) 116–122.
[40] E. Hanff, S. Ruben, M. Kreuzer, A. Bollenbach, A.A. Kayacelebi, A.M. Das, F. von Versen-Höynck, C. von Kaisenberg, D. Haffner, S. Ückert, D. Tsikas, Development and validation of GC-MS methods for the comprehensive analysis of amino acids in plasma and urine and applications to the HELLP syndrome and pediatric kidney transplantation: evidence of altered methylation, transamidination, and arginase activity, Amino Acids 51 (2019) 529–547, https://doi.org/10.1007/s00726-018-02688-w.
[41] D. Tsikas, B. Beckmann, F.M. Gutzki, J. Jordan, Simultaneous gas chromatography-tandem mass spectrometry quantification of symmetric and asymmetric dimethylarginine in human urine, Anal. Biochem. 413 (2011) 60–62, https://doi.org/10.1016/j.ab.2011.02.002.
[42] D. Tsikas, T. Thum, T. Becker, V.V. Pham, K. Chobanyan, A. Mitschke, B. Beckmann, F.M. Gutzki, J. Bauersachs, D.O. Stichtenoth, Accurate quantification of dimethylamine (DMA) in human urine by gas chromatography-mass spectrometry as pentafluorobenzamide derivative: evaluation of the relationship between DMA and its precursor asymmetric dimethylarginine (ADMA) in health and disease, J. Chromatogr. B Anal. Technol. Biomed. Life Sci. 851 (2007) 229–239.
[43] K. Chobanyan, A. Mitschke, F.M. Gutzki, D.O. Stichtenoth, D. Tsikas, Accurate quantification of dimethylamine (DMA) in human plasma and serum by GC-MS and GC-tandem MS as pentafluorobenzamide derivative in the positive-ion chemical ionization mode, J. Chromatogr. B Anal. Technol. Biomed. Life Sci. 851 (2007) 240–249, https://doi.org/10.1016/j.jchromb.2007.03.006.
[44] J.Y. Schneider, S. Rothmann, F. Schröder, J. Langen, T. Lücke, F. Mariotti, J. F. Huneau, J.C. Frölich, D. Tsikas, Effects of chronic oral L-arginine administration on the L-arginine/NO pathway in patients with peripheral arterial occlusive disease or coronary artery disease: L-Arginine prevents renal loss of nitrite, the major NO reservoir, Amino Acids 47 (9) (2015) 1961–1974, https://doi.org/10.1007/s00726-015-2031-0.
[45] A.A. Kayacelebi, J. Willers, V.V. Pham, A. Hahn, J.Y. Schneider, S. Rothmann, J. C. Frölich, D. Tsikas, Plasma homoarginine, arginine, asymmetric dimethylarginine and total homocysteine interrelationships in rheumatoid arthritis, coronary artery disease and peripheral artery occlusion disease, Amino Acids 47 (9) (2015) 1885–1891, https://doi.org/10.1007/s00726-015-1915-3.
[46] A.A. Kayacelebi, J. Langen, K. Weigt-Usinger, K. Chobanyan-Jürgens, F. Mariotti, J.Y. Schneider, S. Rothmann, J.C. Frölich, D. Atzler, C.U. Choe, E. Schwedhelm, J. F. Huneau, T. Lücke, D. Tsikas, Biosynthesis of homoarginine (hArg) and asymmetric dimethylarginine (ADMA) from acutely and chronically administered free L-arginine in humans, Amino Acids 47 (9) (2015) 1893–1908, https://doi.org/10.1007/s00726-015-2012-3.
[47] D. Tsikas, S. Rothmann, J.Y. Schneider, F.M. Gutzki, B. Beckmann, J.C. Frölich, Simultaneous GC-MS/MS measurement of malondialdehyde and 4-hydroxy-2-nonenal in human plasma: Effects of long-term L-arginine administration, Anal. Biochem. 524 (2017) 31–44, https://doi.org/10.1016/j.ab.2016.08.009.
[48] Jessica Y. Lachmuth (Dr. rer. biol. hum.). Die APAV-Pilotstudie: „L-Arginin bei Patienten mit peripherer arterieller Verschlusskrankheit“. Hannover Medical School. 2008.
[49] Sabine Rothmann (Dr. rer. biol. hum.). Pilotstudie zur Wirkung von L-Arginin bei Patienten mit koronarer Herzkrankheit nach Angioplastie und Methoden-Entwicklung zur Bestimmung von MDA in Plasma als PFB-Derivat. Hannover Medical School. 2010.
[50] D. Tsikas, M. Mikuteit, N-Acetyl-L-cysteine in human rheumatoid arthritis and its effects on nitric oxide (NO) and malondialdehyde (MDA): analytical and clinical considerations, Amino Acids 54 (2022) 1251–1260, https://doi.org/10.1007/s00726-022-03185-x.
[51] R.A. Bowen, G.L. Hortin, G. Csako, O.H. Otañez, A.T. Remaley, Impact of blood collection devices on clinical chemistry assays, Clin. Biochem. 43 (2010) 4–25, https://doi.org/10.1016/j.clinbiochem.2009.10.001.
[52] M. Davids, J.H. Peters, S. de Jong, T. Teerlink, Measurement of nitric oxide-related amino acids in serum and plasma: effects of blood clotting and type of anticoagulant, Clin. Chim. Acta 421 (2013) 164–167, https://doi.org/10.1016/j.cca.2013.03.009.
[53] G. Lima-Oliveira, D. Monneret, F. Guerber, G.C. Guidi, Sample management for clinical biochemistry assays: Are serum and plasma interchangeable specimens? Crit. Rev. Clin. Lab. Sci. 55 (2018) 480–500, https://doi.org/10.1080/10408363.2018.1499708.
[54] V.L. Stevens, E. Hoover, Y. Wang, K.A. Zanetti, Pre-Analytical Factors that Affect Metabolite Stability in Human Urine, Plasma, and Serum: A Review, Metabolites 9 (2019) 156, https://doi.org/10.3390/metabo9080156.
[55] M. Plebani, G. Banfi, S. Bernardini, F. Bondanini, L. Conti, R. Dorizzi, F.E. Ferrara, R. Mancini, T. Trenti, Serum or plasma? An old question looking for new answers, Clin. Chem. Lab. Med. 58 (2020) 178–187, https://doi.org/10.1515/cclm-2019-0719.
[56] D. Kratz, A. Sens, S.M.G. Schäfer, L. Hahnefeld, G. Geisslinger, D. Thomas, R. Gurke, Pre-analytical challenges for the quantification of endocannabinoids in human serum, J. Chromatogr. B Anal. Technol. Biomed. Life Sci. 1190 (2022), 123102, https://doi.org/10.1016/j.jchromb.2022.123102.
[57] D. Tsikas, N. Maassen, A. Thorns, A. Finkel, M. Lützow, M.A. Röhrig, L.S. Blau, L. Dimina, F. Mariotti, B. Beckmann, V. Shushakov, M. Jantz, Short-Term Supplementation of Sodium Nitrate vs. Sodium Chloride Increases Homoarginine Synthesis in Young Men Independent of Exercise, Int. J. Mol. Sci. 23 (2022) 10649, https://doi.org/10.3390/ijms231810649.
[58] G. de Gaetano, G. Rajtar, M. Livio, J. Merino, Arachidonic acid-induced malondialdehyde formation in rat platelets. Kinetic aspects and inhibition by acetylsalicylic acid and indomethacin, Naunyn Schmiedebergs Arch. Pharmacol. 312 (1980) 85–89.
[59] A.M. Jentzsch, H. Bachmann, P. Fürst, H.K. Biesalski, Improved analysis of malondialdehyde in human body fluids, Free Radic. Biol. Med. 20 (1996) 251–256.
[60] W. Wasowicz, J. Nève, A. Peretz, Optimized steps in fluorometric determination of thiobarbituric acid-reactive substances in serum: importance of extraction pH and influence of sample preservation and storage, Clin. Chem. 39 (1993) 2522–2526.
[61] D.M. Lee, Malondialdehyde formation in stored plasma, Biochem. Biophys. Res. Commun. 95 (1980) 1663–1670.
[62] D. Tsikas, A. Mitschke, F.M. Gutzki, S. Engeli, J. Jordan, Evidence by gas chromatography-mass spectrometry of ex vivo nitrite and nitrate formation from air nitrogen oxides in human plasma, serum, and urine samples, Anal. Biochem. 397 (1) (2010) 126–128, https://doi.org/10.1016/j.ab.2009.09.047.
[63] V. Turcu, P. Wild, M. Hemmendinger, J.J. Sauvain, E. Bergamaschi, N.B. Hopf, I. G. Canu, Towards Reference Values for Malondialdehyde on Exhaled Breath Condensate: A Systematic Literature Review and Meta-Analysis, Toxics. 10 (5) (2022) 258, https://doi.org/10.3390/toxics10050258.
[64] B. Antus, Oxidative Stress Markers in Sputum, Oxid. Med. Cell. Longev. 2016 (2016) 2930434, https://doi.org/10.1155/2016/2930434.
[65] D. Tsikas, M.T. Suchy, Protocols for the measurement of the F2-isoprostane, 15 (S)-8-iso-prostaglandin F2α, in biological samples by GC-MS or GC-MS/MS coupled with immunoaffinity column chromatography, J. Chromatogr. B Anal. Technol. Biomed. Life Sci. 1019 (2016) 191–201, https://doi.org/10.1016/j.jchromb.2014.12.019.
[66] F. Mariotti, M. Valette, C. Lopez, H. Fouillet, M.H. Famelart, V. Mathé, G. Airinei, R. Benamouzig, C. Gaudichon, D. Tomé, D. Tsikas, J.F. Huneau, Casein Compared with Whey Proteins Affects the Organization of Dietary Fat during Digestion and Attenuates the Postprandial Triglyceride Response to a Mixed High-Fat Meal in Healthy, Overweight Men, J. Nutr. 145 (2015) 2657–2664, https://doi.org/10.3945/jn.115.216812.
[67] I. Dalle-Donne, R. Rossi, R. Colombo, D. Giustarini, A. Milzani, Biomarkers of oxidative stress in human disease, Clin. Chem. 52 (2006) 601–623.
[68] D. Giustarini, I. Dalle-Donne, D. Tsikas, R. Rossi, Oxidative stress and human diseases: Origin, link, measurement, mechanisms, and biomarkers, Crit. Rev. Clin. Lab. Sci. 46 (5-6) (2009) 241–281.
[69] F. Nielsen, B.B. Mikkelsen, J.B. Nielsen, H.R. Andersen, P. Grandjean, Plasma malondialdehyde as biomarker for oxidative stress: reference interval and effects of life-style factors, Clin. Chem. 43 (1997) 1209–1214.
[70] M. Wilking, M. Ndiaye, H. Mukhtar, N. Ahmad, Circadian rhythm connections to oxidative stress: implications for human health, Antioxid. Redox Signal. 19 (2) (2013) 192–208.

[71] F. Fimognari, G. Piccirillo, J. Lama, P. Marzio, S. Morelli, G. Monteleone, V. Mollace, F. Violi, Nocturnal rise of urinary thromboxane B_2 in patients with coronary artery disease, Thromb. Haemost. 93 (03) (2005) 618–619.
[72] P.D. Rubin, V.S. Murthy, R.G. Hansen, C. Pietrowiak, Dissociation and dirnal variation of prostaglandin E2, 6-keto-prostaaglandin-F1-alpha, and thrpmboxane B2 excretion in healthy females, Prostaglandins Leukotrienes Med. 27 (1987) 105–117.
[73] A.B. Bidges, M. McLaren, A. Saniabadi, T.C. Fisher, J.J.F. Belch, Circadian variation of endothelial cell function, red blood cell deformability and dehydro-thromboxane B2 in healthy humans, Blood Coagulation Fibrinolysis 2 (1991) 447–452.
[74] M. Sani, N. Ghanem-Boughanmi, W. Gadacha, H. Sebai, N.A. Boughattas, A. Reinberg, M. Ben-Attia, Malondialdehyde content and circadian variations in brain, kidney, liver, and plasma of mice, Chronobiol. Int. 24 (4) (2007) 671–685.
[75] E.L. Kanabrocki, D. Murray, R.C. Hermida, G.S. Scott, W.F. Bremner, M.D. Ryan, D.E. Ayala, J.L. Third, P. Shirazi, B.A. Nemchausky, D.C. Hooper, Circadian variation in oxidative stress markers in healthy and type II diabetic men, Chronobiol. Int. 19 (2002) 423–439.
[76] J. Kalra, A.K. Chaudhary, K. Prasad, Increased production of oxygen free radicals in cigarette smokers, Int. J. Exp. Pathol. 72 (1991) 1–7.
[77] A.B. Bridges, N.A. Scott, G.J. Parry, J.J.F. Belch, Age, sex, cigarette smoking and indices of free radical activity in healthy humans, Eur. J. Med. 205–208 (1993).
[78] K.M. Brown, P.C. Morrice, G.G. Duthie, Vitamin E suppresses indexes of lipid peroxidation and platelet count in blood of smokers and nonsmokers but plasma lipoprotein concentrations remain unchanged, Am. J. Clin. Nutr. 60 (1994) 383–387.
[79] J. Pré, A.L. Floch, Lipid-peroxidation products and antioxidants in plasma of cigarette smokers, Clin. Chem. 36 (1990) 1849–1850.
[80] B. Frei, T.M. Forte, B.N. Ames, C.E. Cross, Gas phase oxidants of cigarette smoke induce lipid peroxidation and changes in lipoprotein properties in human blood plasma, Biochem. J. 277 (1991) 133–138.
[81] A.-A. Kamal, M.E. Khafif, S. Koraah, A. Massoud, J.-F. Caillard, Blood superoxide dismutase and plasma malondialdehyde among workers exposed to asbestos, Am. J. Ind. Med. 21 (3) (1992) 353–361.
[82] S. Ramírez-Zamora, M.L. Méndez-Rodríguez, M. Olguín-Martínez, L. Sánchez-Sevilla, M. Quintana-Quintana, N. García-García, R. Hernández-Muñoz, M. Pietropaolo, Increased erythrocytes by-products of arginine catabolism are associated with hyperglycemia and could be involved in the pathogenesis of type 2 diabetes mellitus, PLoS One 8 (6) (2013) e66823.
[83] R. Pamplona, E. Dalfó, V. Ayala, M.J. Bellmunt, J. Prat, I. Ferrer, M. Portero-Otín, Proteins in human brain cortex are modified by oxidation, glycoxidation, and lipoxidation, Effects of Alzheimer disease and identification of lipoxidation targets, J. Biol. Chem. 280 (2005) 21522–21530.
[84] L.H. Sanders, J.T. Greenamyre, Oxidative damage to macromolecules in human Parkinson disease and the rotenone model, Free Radic. Biol. Med. 62 (2013) 111–120.
[85] G. Aldini, I. Dalle-Donne, R.M. Facino, A. Milzani, M. Carini, Intervention strategies to inhibit protein carbonylation by lipoxidation-derived reactive carbonyls, Med. Res. Rev. 27 (6) (2007) 817–868.
[86] S. Lenzen, Oxidative stress: the vulnerable beta-cell, Biochem. Soc. Trans. 2008 (36) (2008) 343–347.
[87] L.K. Niskanen, J.T. Salonen, K. Nyyssönen, Plasma lipid peroxidation and hyperglycaemia: a connection through hyperinsulinaemia? Diabet. Med. 12 (1995) 802–808.
[88] L. Arribas, I. Almansa, M. Miranda, M. Muriach, F.J. Romero, V.M. Villar, R. Khodarahmi, Serum Malondialdehyde concentration and glutathione peroxidase activity in a longitudinal study of gestational diabetes, PLoS One 11 (5) (2016) e0155353.
[89] S.K. Jain, K.S. Krueger, R. McVie, J.J. Jaramillo, M. Palmer, T. Smith, Relationship of blood thromboxane-B2 (TxB2) with lipid peroxides and effect of vitamin E and placebo supplementation on TxB2 and lipid peroxide levels in type 1 diabetic patients, Diabetes Care 21 (1998) 1511–1516.
[90] D.K. de Vries, J.H. Lindeman, D. Tsikas, E. de Heer, A. Roos, J.W. de Fijter, A. G. Baranski, J. van Pelt, A.F. Schaapherder, Early renal ischemia-reperfusion injury in humans is dominated by IL-6 release from the allograft, Am. J. Transplant. 9 (2009) 1574–1584, https://doi.org/10.1111/j.1600-6143.2009.02675.x.
[91] D.K. de Vries, K.A. Kortekaas, D. Tsikas, L.G. Wijermars, C.J. van Noorden, M. T. Suchy, C.M. Cobbaert, R.J. Klautz, A.F. Schaapherder, J.H. Lindeman, Oxidative damage in clinical ischemia/reperfusion injury: a reappraisal, Antioxid. Redox Signal. 19 (2013) 535–545, https://doi.org/10.1089/ars.2012.4580.
[92] L.G. Wijermars, J.A. Bakker, D.K. de Vries, C.J. van Noorden, J. Bierau, S. Kostidis, O.A. Mayboroda, D. Tsikas, A.F. Schaapherder, J.H. Lindeman, The hypoxanthine-xanthine oxidase axis is not involved in the initial phase of clinical transplantation-related ischemia-reperfusion injury, Am. J. Physiol. Renal Physiol. 312 (2017) F457–F464, https://doi.org/10.1152/ajprenal.00214.2016.
[93] M. Yepes-Calderón, C.G. Sotomayor, R.O.B. Gans, S.P. Berger, H.G.D. Leuvenink, D. Tsikas, R. Rodrigo, G.J. Navis, S.J.L. Bakker, Post-transplantation plasma malondialdehyde is associated with cardiovascular mortality in renal transplant recipients: a prospective cohort study, Nephrol. Dial. Transplant. 35 (2020) 512–519, https://doi.org/10.1093/ndt/gfz288.
[94] H. Maassen, M.Y. Said, A.S. Frenay, A. Koning, A. Post, I.J. Riphagen, M. R. Heiner-Fokkema, K. Drabert, B.O. Fernandez, R.O.B. Gans, E. van den Berg, G. Navis, D. Tsikas, M. Feelisch, S.J.L. Bakker, H. van Goor, Nitric oxide and long-term outcomes after kidney transplantation: Results of the TransplantLines cohort study, Nitric Oxide 125–126 (2022) 1–11, https://doi.org/10.1016/j.niox.2022.05.005.
[95] R. Pazdro, J.R. Burgess, The role of vitamin E and oxidative stress in diabetes complications, Mech. Ageing Dev. 131 (2010) 276–286.
[96] D. Tsikas, V.V. Pham, M.T. Suchy, M.A. van de Ree, M.V. Huisman, J.C. Frölich, H.M. Princen, DALI-Study Group. No effects of atorvastatin (10 mg/d or 80 mg/d) on nitric oxide, prostacyclin, thromboxane and oxidative stress in type 2 diabetes mellitus patients of the DALI study, Pharmacol. Res. 94 (2015) 1–8.
[97] A. Mezzetti, F. Cipollone, F. Cuccurullo, Oxidative stress and cardiovascular complications in diabetes: isoprostanes as new markers on an old paradigm, Cardiovasc. Res. 47 (2000) 475–488.
[98] M. Kunisak, F. Umeda, T. Inoguchi, J. Watanabe, H. Nawata, Effects of vitamin E administration on platelet function in diabetes mellitus, Diabetes Res. 14 (1990) 37–42.
[99] S.K. Jain, R. McVie, T. Smith, Vitamin E supplementation restores glutathione and malondialdehyde to normal concentrations in erythrocytes of type 1 diabetic children, Diabetes Care 23 (2000) 1389–1394.
[100] J.P. Allard, R. Kurian, E. Aghdassi, R. Muggli, D. Royall, Lipid peroxidation during n-3 fatty acid and vitamin E supplementation in humans, Lipids 32 (1997) 535–541.
[101] A.A. Kayacelebi, V.V. Pham, J. Willers, A. Hahn, D.O. Stichtenoth, J. Jordan, D. Tsikas, Plasma homoarginine (hArg) and asymmetric dimethylarginine (ADMA) in patients with rheumatoid arthritis: is homoarginine a cardiovascular corrective in rheumatoid arthritis, an anti-ADMA? Int. J. Cardiol. 176 (2014) 1129–1131.
[102] J. Willers, S. Fasse, N. Putschly, H. Zeidler, H.G. Pott, M. Bernateck, W. Demary, R. Hein, V.V. Pham, R. Stange, B. Uehleke, H.F. Weidemann, G. Hoese, R. Lichtinghagen, A. Hahn, Food Nutr. Sci. 2 (2011) 714–723.
[103] B. Halliwell, C.Y.J. Lee, Using isoprostanes as biomarkers of oxidative stress: some rarely considered issues, Antioxid. Redox Signal. 13 (2010) 145–156, https://doi.org/10.1089/ars.2009.2934.
[104] D. Tsikas, M.W. Duncan, Mass spectrometry and 3-nitrotyrosine: strategies, controversies, and our current perspective, Mass Spectrom. Rev. 33 (4) (2014) 237–276.
[105] K. Esalatmanesh, A. Jamali, R. Esalatmanesh, Z. Soleimani, A. Khabbazi, A. Malek Mahdavi, Effects of *N*-acetylcysteine supplementation on disease activity, oxidative stress, and inflammatory and metabolic parameters in rheumatoid arthritis patients: a randomized double-blind placebo-controlled trial, Amino Acids 54 (3) (2022) 433–440.
[106] L. Włodek, The reaction of sulfhydryl groups with carbonyl compounds, Acta Biochim. Pol. 35 (1988) 307–317.
[107] X. Hou, J. Song, X.N. Li, L. Zhang, X. Wang, L. Chen, Y.H. Shen, Metformin reduces intracellular reactive oxygen species levels by upregulating expression of the antioxidant thioredoxin via the AMPK-FOXO3 pathway, Biochem. Biophys. Res. Commun. 396 (2010) 199–205, https://doi.org/10.1016/j.bbrc.2010.04.017.
[108] N. Roustaei Rad, A. Movahedian, A. Feizi, A. Aminorroaya, M.H. Aarabi, Antioxidant effects of astaxanthin and metformin combined therapy in type 2 diabetes mellitus patients: a randomized double-blind controlled clinical trial, Res Pharma Sci 17 (2) (2022) 219.
[109] P. Hafner, U. Bonati, B. Erne, M. Schmid, D. Rubino, U. Pohlman, T. Peters, E. Rutz, S. Frank, C. Neuhaus, S. Deuster, M. Gloor, O. Bieri, A. Fischmann, M. Sinnreich, N. Gueven, D. Fischer, M.D. Cordero, Improved muscle function in Duchenne Muscular Dystrophy through L-arginine and metformin: An investigator-initiated, open-label, single-center, proof-of-concept-study, PLoS One 11 (1) (2016) e0147634.
[110] P. Hafner, U. Bonati, D. Rubino, V. Gocheva, T. Zumbrunn, N. Gueven, D. Fischer, Treatment with L-citrulline and metformin in Duchenne muscular dystrophy: study protocol for a single-centre, randomised, placebo-controlled trial, Trials 17 (2016) 389, https://doi.org/10.1186/s13063-016-1503-1.
[111] E. Hanff, P. Hafner, A. Bollenbach, U. Bonati, A.A. Kayacelebi, D. Fischer, D. Tsikas, Effects of single and combined metformin and L-citrulline supplementation on L-arginine-related pathways in Becker muscular dystrophy patients: possible biochemical and clinical implications, Amino Acids 50 (2018) 1391–1406, https://doi.org/10.1007/s00726-018-2614-7.
[112] E. Hanff, M.F. Eisenga, B. Beckmann, S.J. Bakker, D. Tsikas, Simultaneous pentafluorobenzyl derivatization and GC-ECNICI-MS measurement of nitrite and malondialdehyde in human urine: Close positive correlation between these disparate oxidative stress biomarkers, J. Chromatogr. B 1043 (2017) 167–175.
[113] K. Chobanyan-Jürgens, A. Schwarz, A. Böhmer, B. Beckmann, F.M. Gutzki, J. T. Michaelsen, D.O. Stichtenoth, D. Tsikas, Renal carbonic anhydrases are involved in the reabsorption of endogenous nitrite, Nitric Oxide 26 (2012) 126–131, https://doi.org/10.1016/j.niox.2012.01.005.
[114] M. Vogeser, C. Seger, LC-MS/MS in clinical chemistry, J. Chromatogr. B Anal. Technol. Biomed. Life Sci. 883–884 (2012) 1–2, https://doi.org/10.1016/j.jchromb.2012.01.001.
[115] M.W. Duncan, D. Nedelkov, R. Walsh, S.J. Hattan, Applications of MALDI Mass Spectrometry in Clinical Chemistry, Clin. Chem. 62 (2016) 134–143, https://doi.org/10.1373/clinchem.2015.239491.
[116] M.W. Duncan, Good mass spectrometry and its place in good science, J. Mass Spectrom. 47 (2012) 795–809, https://doi.org/10.1002/jms.3038.
[117] M. Vogeser, G. Schelling, Pitfalls in measuring the endocannabinoid 2-arachidonoyl glycerol in biological samples, Clin. Chem. Lab. Med. 45 (2007) 1023–1025, https://doi.org/10.1515/CCLM.2007.197.

[118] M. Vogeser, C. Seger, Pitfalls associated with the use of liquid chromatography-tandem mass spectrometry in the clinical laboratory, Clin. Chem. 56 (2010) 1234–1244, https://doi.org/10.1373/clinchem.2009.138602.
[119] D. Tsikas, A.A. Zoerner, Analysis of eicosanoids by LC-MS/MS and GC-MS/MS: a historical retrospect and a discussion, J. Chromatogr. B 964 (2014) 79–88.
[120] D. Tsikas, Quantitative analysis of eicosanoids in biological samples by LC-MS/MS: Mission accomplished? J. Chromatogr. B Anal. Technol. Biomed. Life Sci. 1012–1013 (2016) 211–214, https://doi.org/10.1016/j.jchromb.2015.04.018.