Szkoła Główna Gospodarstwa Wiejskiego

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Instytut Nauk o Żywieniu Człowieka

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Analiza zależności między wielkością spożycia wybranych składników pokarmowych a profilem metabolicznym osób dorosłych środowiskowo narażonych na arsen

Analysis of the relationship between the intake of selected nutrients and the metabolic profile of adults environmentally exposed to arsenic

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Streszczenie

Analiza zależności między wielkością spożycia wybranych składników pokarmowych a profilem metabolicznym osób dorosłych środowiskowo narażonych na arsen

Duża liczba osób narażonych na arsen oraz negatywny wpływ na zdrowie tego pierwiastka, skłaniają do głębokiej analizy mechanizmów jego toksyczności oraz do poszukiwania sposobu zmniejszenia niekorzystnego wpływu arsenu na organizm człowieka. Badania w tym zakresie koncentrują się m.in. na składnikach pokarmowych zaangażowanych w metabolizm arsenu. Są to metionina i foliany (jako donory grup metylowych w metabolizmie arsenu) oraz witaminy B_2, B_6, B_{12} i cynk (jako kofaktory reakcji metabolizmu arsenu), które mogą mieć modulujący wpływ na efektywność metylacji arsenu i zmniejszanie jego toksycznego działania.

W związku z powyższym celem pracy była analiza dostępnych wyników badań w obszarze wpływu składników pokarmowych na zmniejszanie toksyczności i poprawę efektywności metylacji arsenu oraz analiza zależności między wielkością spożycia wybranych składników pokarmowych a nasileniem zmian w profilu metabolicznym u osób dorosłych środowiskowo narażonych na arsen. Zakres pracy obejmował przegląd wyników badań z zakresu wpływu składników pokarmowych zaangażowanych w metabolizm arsenu na efektywność jego metylacji oraz nasilenie niekorzystnych zmian związanych z narażeniem na ten pierwiastek w badaniach na modelach zwierzęcych i przeprowadzonych z udziałem ludzi. Następnie dokonano ilościowej analizy spożycia składników pokarmowych zaangażowanych w metabolizm arsenu oraz przeprowadzono badania z zakresu metabolomiki niecelowanej w grupie mężczyzn narażonych na arsen. Uzyskane dane wykorzystano do określenia zależności między wielkością spożycia składników pokarmowych zaangażowanych w metabolizm arsenu a profilem metabolicznym osób dorosłych środowiskowo narażonych na arsen. Sformułowano hipotezę główną, która zakładała, że istnieje zależność między nasileniem zmian w profilu metabolicznym osób środowiskowo narażonych na arsen a wielkością spożycia składników pokarmowych, które są zaangażowane w metabolizm arsenu. Przedstawiono hipotezy szczegółowe, według, których narażenie na arsen indukuje zmiany w profilu metabolicznym, a także, że istnieje zależność między wielkością spożycia metioniny, folianów, witamin B_2, B_6, B_{12} i cynku a nasileniem zmian w profilu metabolicznym mężczyzn narażonych na arsen.

W niniejszej rozprawie podsumowano wyniki badań modelowych na zwierzętach (publikacja 1) i przeprowadzonych z udziałem ludzi (publikacja 2). W większości badań na modelach zwierzęcych narażonych na arsen nieorganiczny suplementacja niektórych składników pokarmowych (w szczególności kwasu foliowego i cynku) zwiększała efektywność metylacji i zmniejszała szereg niekorzystnych zmian w wielu układach: pokarmowym, moczowym, limfatycznym, krwionośnym, nerwowym i rozrodczym. Mimo, że badania te wskazywały potencjalnie korzystny wpływ składników pokarmowych, wiele aspektów ograniczało porównanie wyników i sformułowanie jednoznacznych wniosków. Ponadto, badania przeprowadzone na modelach zwierzęcych nie są równoważne badaniom przeprowadzonym z udziałem ludzi, ale wskazały kierunek i były przyczynkiem do dalszych rozważań. Biorąc pod uwagę obiecujące wyniki badań na modelach zwierzęcych *in vivo* i *in vitro*, przeanalizowano również wpływ tych składników pokarmowych na efektywność procesu metylacji arsenu, a także na zmniejszenie nasilenia całego spektrum zaburzeń związanych z narażeniem na arsen w populacji narażonej na ten pierwiastek. Podobne korzystne wyniki zaobserwowano w badaniach przeprowadzonych z udziałem ludzi środowiskowo

narażonych na arsen. Badania wykazały, że wielkość spożycia i stężenie niektórych składników pokarmowych we krwi (metioniny, choliny, folianów, witamin B_2, B_6, B_{12}, cynku) może mieć korzystny wpływ na poprawę metylacji arsenu i na zmniejszenie nasilenia niekorzystnych skutków zdrowotnych. Jednak w tych badaniach analizowano jedynie zależności między spożyciem, suplementacją lub stężeniem we krwi składników pokarmowych a stężeniem metabolitów arsenu w moczu oraz ryzykiem rozwoju chorób. Ponadto, dotychczasowe badania przeprowadzone z udziałem ludzi są niejednoznaczne, wiele zmiennych determinowało uzyskane wyniki. Przegląd badań wskazał potrzebę dalszych analiz, aby określić rolę tych składników pokarmowych jako kofaktorów reakcji i donorów grup metylowych, a także aby przeanalizować szczegółowe zmiany w metabolizmie wynikające z narażenia na arsen oraz ich powiązania ze spożyciem składników pokarmowych zaangażowanych w metabolizm arsenu.

W odpowiedzi na te potrzeby zrealizowano badania, których celem była analiza zależności między intensywnością sygnału metabolitów a wielkością spożycia składników pokarmowych zaangażowanych w metabolizm arsenu (metioniny, folianów, witamin B_2, B_6, B_{12}, cynku) u osób dorosłych środowiskowo narażonych na arsen. W ramach publikacji 3 zrealizowano badania w grupie pracowników: grupę WN (n = 75) stanowili pracownicy ze stężeniem arsenu całkowitego (tAs) w granicach wartości dopuszczalnych, grupę WH (n = 41) stanowili pracownicy ze stężeniem tAs powyżej wartości dopuszczalnych. Analizy z zakresu metabolomiki niecelowanej wykonano przy użyciu systemu ultraefektywnej chromatografii cieczowej sprzężonej z wysokorozdzielczym spektrometrem mas (LC/HRMS). Wielkość spożycia wybranych składników pokarmowych zaangażowanych w metabolizm arsenu oceniono na podstawie danych z kwestionariusza 3-dniowego bieżącego notowania spożywanych produktów, potraw i napojów. W grupie WH mężczyzn odnotowano wyższą intensywność sygnału pochodzącą od dwudziestu pięciu metabolitów. Potencjalnie zidentyfikowane metabolity należały do głównych ścieżek przemian metabolizmu aminokwasów, węglowodanów, lipidów, glikanów, witamin i nukleotydów. U mężczyzn w grupie WN wielkość spożycia składników pokarmowych (metioniny, witamin B_2, B_6 i B_{12}, folianów i cynku) wykazała ujemne zależności z sześcioma metabolitami (cytozyną, kwasem D-glukuronowym, N-acetylo-D-glukozaminą, kwasem piroglutaminowym, urydyną i kwasem urokanowym), w grupie WH z pięcioma metabolitami (kwasem D-glukuronowym, kwasem L-glutaminowym, N-acetylo-D-glukozaminą, kwasem N-acetyloneuraminowym i urydyną). Ponadto, w grupie WH zaobserwowano dodatnie zależności między wielkością spożycia metioniny, folianów i cynku a intensywnością sygnału kwasu bursztynowego i kwasu 3-merkaptomlekowego.

Wyniki przedstawione w opublikowanych pracach przeglądowych oraz wyniki z badań eksperymentalnych wskazały na kluczową rolę składników pokarmowych (metioniny, folianów, witamin B_2, B_6, B_{12}, cynku) w zmniejszaniu niekorzystnych skutków związanych z narażeniem na arsen. Badania własne wykazały, że narażenie na arsen indukuje zmiany w profilu metabolicznym, manifestujące się wyższą intensywnością sygnału pochodzącą od metabolitów należących do wielu ścieżek przemian m.in. węglowodanów, aminokwasów, lipidów, nukleotydów. W grupach pracowników narażonych na arsen zaobserwowano występowanie zależności między wielkością spożycia składników pokarmowych zaangażowanych w metabolizm arsenu a nasileniem zmian w profilu metabolicznym. Biorąc pod uwagę ujemne zależności między spożyciem metioniny i folianów (donorów grup metylowych w metabolizmie arsenu) oraz witamin B_2, B_6, B_{12} i cynku (kofaktorów reakcji metabolizmu arsenu) a intensywnością sygnału metabolitów, wydaje się, że wyższe

spożycie składników pokarmowych może zmniejszyć nasilenie niekorzystnych zmian związanych z narażeniem na arsen. Podkreśla to potrzebę edukacji ukierunkowanej na odpowiednie, adekwatne do norm spożycie składników pokarmowych zaangażowanych w metabolizm arsenu, w szczególności w populacjach środowiskowo narażonych na arsen. Uzyskane wyniki mogą przyczynić się do dalszych rozważań podczas opracowywania zaleceń dietetycznych dla osób narażonych na arsen.

Słowa kluczowe: arsen, arsen nieorganiczny, metabolizm arsenu, narażenie, metabolomika niecelowana, składniki pokarmowe

Summary

Analysis of the relationship between the intake of selected nutrients and the metabolic profile of adults environmentally exposed to arsenic

The large number of people exposed to arsenic and the adverse effects of this element on health have prompted an in-depth analysis of the mechanisms of its toxicity and the search for ways to reduce the adverse effects of arsenic on the human body. Research in this area focuses, among others, on nutrients involved in arsenic metabolism. These include methionine and folate (as donors of methyl groups in arsenic metabolism) and vitamins B_2, B_6, B_{12}, and zinc (as cofactors of arsenic metabolism reactions), which may have a modulating effect on the efficiency of arsenic methylation and the reduction of its toxic effects.

Accordingly, the aim of this study was to review the available results of research in the field of the effect of nutrients on reducing toxicity and improving the efficiency of arsenic methylation, and to analyze the relationship between the amount of intake of selected nutrients and the severity of changes in the metabolic profile of adults environmentally exposed to arsenic. The aim of the study was to provide an overview of the research findings on the effects of nutrients involved in arsenic metabolism on the efficiency of arsenic methylation and the severity of adverse changes associated with exposure to this element in animal models and human studies. This was followed by a quantitative analysis of the intake of nutrients involved in arsenic metabolism and a non-targeted metabolomics study in a group of men exposed to arsenic. The data obtained were used to determine the relationship between nutrient intake involved in arsenic metabolism and the metabolic profile of adults environmentally exposed to arsenic. The main hypothesis was formulated, which was that there is a relationship between the severity of changes in the metabolic profile of people environmentally exposed to arsenic and the amount of intake of nutrients that are involved in arsenic metabolism. Specific hypotheses were presented, according to which arsenic exposure induces alterations in the metabolic profile and that there is a relationship between the intake of methionine, folate, vitamin B_2, B_6, B_{12}, and zinc and the severity of these changes in the metabolic profile of men exposed to arsenic.

This dissertation summarizes the results of animal model studies (publication 1) and those conducted with humans (publication 2). In most studies on animal models exposed to inorganic arsenic, supplementation with certain nutrients (particularly folic acid and zinc) increased methylation efficiency and reduced various adverse changes in a number of systems: gastrointestinal, urinary, lymphatic, circulatory, nervous, and reproductive. Although these studies indicated a potentially beneficial effect of nutrients, many aspects limited the comparison of results and the formulation of clear conclusions. In addition, studies conducted on animal models are not equivalent to those conducted with humans, but they guided further research and contributed to further considerations. Considering the promising results of studies on animal models in vivo and in vitro, the effects of these nutrients the efficiency of arsenic methylation and reduction of severity of the entire spectrum of disorders associated with arsenic exposure in arsenic-exposed population were also analyzed. Similarly, favorable results have been observed in studies conducted on humans environmentally exposed to arsenic. Studies have shown that the amount of intake and blood concentrations of certain nutrients (methionine, choline, folate, vitamins B_2, B_6, B_{12}, and zinc) can have a beneficial effect on improving arsenic methylation and reducing the severity of adverse health effects. However, these studies only analyzed the relationships between dietary intake, supplementation, or blood concentrations of nutrients, and urinary arsenic metabolite concentrations and the risk of disease development. In addition, previous studies conducted on humans are inconclusive, with numerous variables influencing the outcomes. A review of the studies indicated the need for further analysis to determine the role of these nutrients as reaction cofactors in biochemical reactions and as methyl group donors,

as well as to analyze the specific metabolic changes resulting from arsenic exposure and their relationship to the intake of nutrients involved in arsenic metabolism.

In response to these needs, a study was conducted to analyze the relationship between the intensity of the metabolite signal and the intake of nutrients involved in arsenic metabolism (methionine, folate, vitamins B_2, B_6, B_{12}, and zinc) in adults environmentally exposed to arsenic. In publication 3, the study was performed on a group of workers: the WN group (n = 75) consisted of workers with total arsenic (tAs) concentrations within the limits, the WH group (n = 41) consisted of workers with tAs concentrations above the limits. Untargeted metabolomics analyses were performed using an ultraperformance liquid chromatography system coupled to a high-resolution mass spectrometer (LC/HRMS). The amount of intake of selected nutrients involved in arsenic metabolism was assessed using data from 3-day dietary records. In both studies, groups with higher urinary tAs concentrations had higher signal intensities from twenty-five metabolites in WH men and from eighteen metabolites in WH women. The potentially identified metabolites belonged to the main metabolism pathways of amino acids, carbohydrates, lipids, glycans, vitamins, and nucleotides. In men in the WN group, the amount of nutrient intake (methionine, vitamins B_2, B_6, and B_{12}, folate, and zinc) showed negative relationships with six metabolites (cytosine, D-glucuronic acid, N-acetyl-D-glucosamine, pyroglutamic acid, uridine, and urocanic acid), in the WH group with five metabolites (D-glucuronic acid, L-glutamic acid, N-acetyl-D-glucosamine, N-acetylneuraminic acid, and uridine). Moreover, in the WH group, positive correlations were observed between the amount of methionine, folate, and zinc intake and the signal intensity of succinic acid and 3-mercaptolactic acid.

The results presented in review papers and results from our experimental studies indicated the crucial role of nutrients (methionine, folate, vitamin B_2, B_6, B_{12}, zinc) in reducing adverse effects associated with arsenic exposure. Our study demonstrated that arsenic exposure induces changes in the metabolic profile, manifested by increased signal intensity from metabolites involved in various metabolic pathways, including carbohydrates, amino acids, lipids, and nucleotides. In groups of man exposed to arsenic, correlations were observed between the amount of intake of nutrients involved in arsenic metabolism and the severity of changes in the metabolic profile. Notably, the negative correlations between the intake of methionine and folate (methyl group donors in arsenic metabolism) and vitamins B_2, B_6, B_{12}, and zinc (cofactors of the arsenic metabolism reaction) and the intensity of the metabolite signals suggest that a higher nutrient intake may alleviate the severity of adverse changes associated with arsenic exposure. This underscores the need for education aimed at adequate, standards-appropriate intake of nutrients involved in arsenic metabolism, particularly in populations environmentally exposed to arsenic. The results may contribute to further considerations when developing dietary recommendations for arsenic-exposed individuals.

Keywords: arsenic, inorganic arsenic, arsenic metabolism, exposure, non-target metabolomics, nutrients

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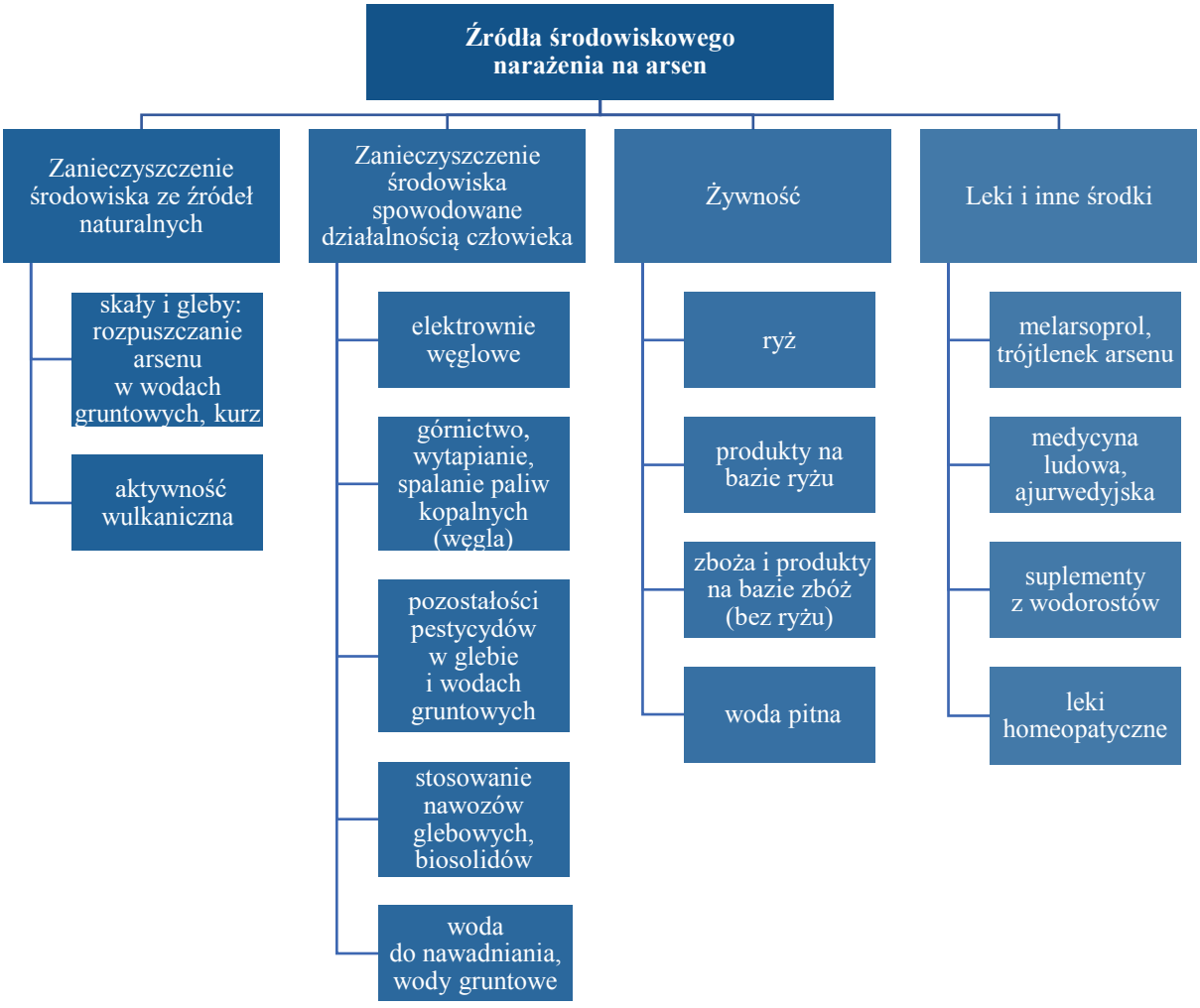
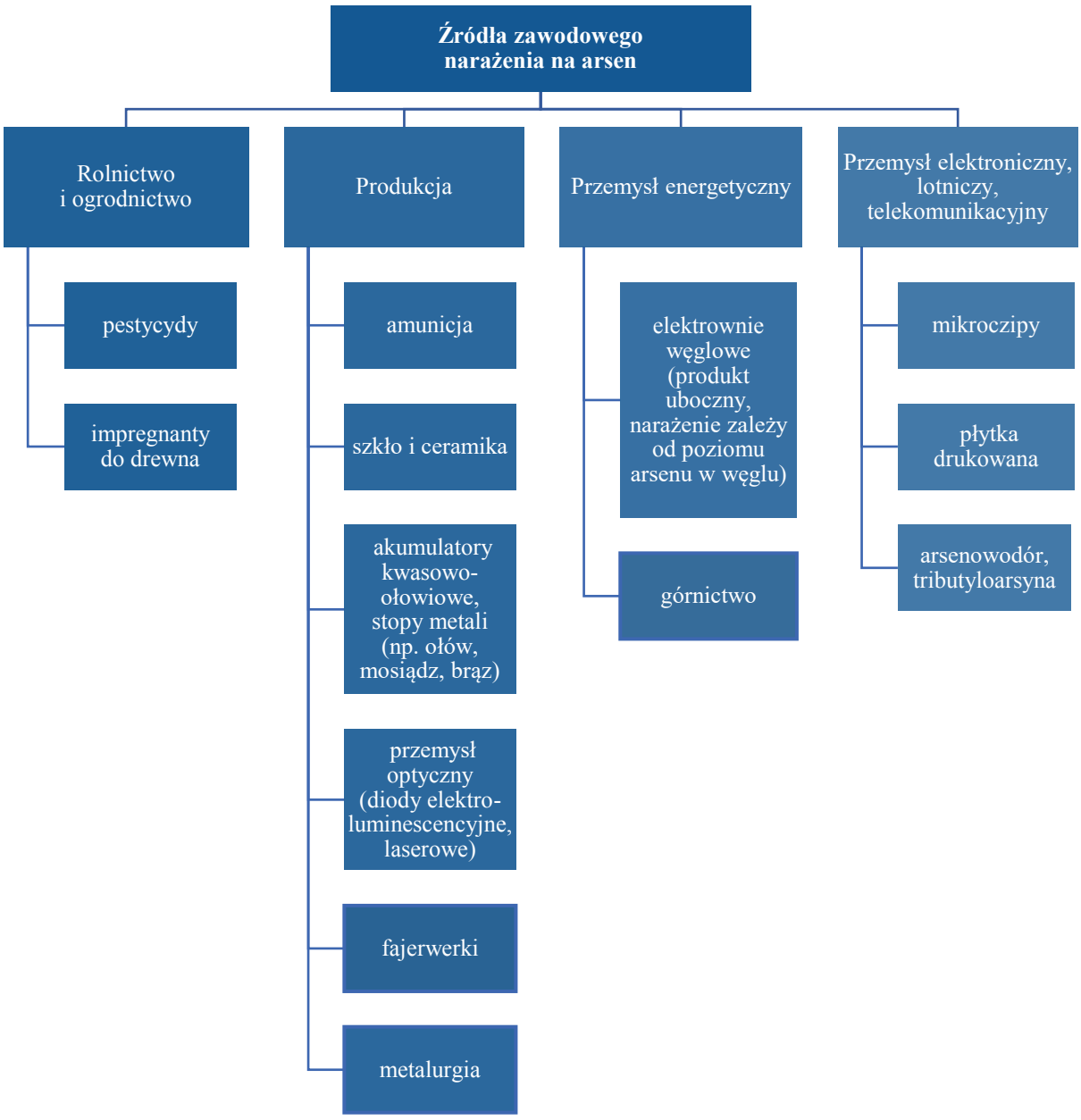
1. Uzasadnienie podjęcia tematu

Szacuje się, że od 94 do 220 milionów ludzi na całym świecie jest narażonych na arsen, który jest obecny w wodzie pitnej, a zanieczyszczenie arsenem wód gruntowych może występować aż w 107 krajach, w tym 31 w Europie (Podgorski i Berg, 2020, Shaji i wsp., 2021). Problem ten dotyczy ludności wielu regionów świata m.in. Chile, Argentyny, Bangladeszu, Meksyku, również w Europie istnieją obszary zagrożone tym problemem np. Węgry, Serbia, Rumunia, Chorwacja, Czechy, Polska, Grecja, Finlandia i Turcja (Medunic i wsp., 2020). Dokładna liczba osób narażonych na arsen może być wyższa, ponieważ woda pitna nie jest jego jedynym źródłem. Inne źródła narażenia środowiskowego oraz zawodowego na różne formy chemiczne arsenu (Baker i wsp., 2018, EFSA, 2021) przedstawiono na Rycinie 1 i 2.



Rycina 1. Potencjalne źródła środowiskowego narażenia na arsen.
Źródło: opracowano na podstawie Baker i wsp., 2018, EFSA 2021.



Rycina 2. Potencjalne źródła zawodowego narażenia na arsen.
Źródło: opracowano na podstawie Baker i wsp., 2018.

Arsen może występować na różnych stopniach utlenienia: As^{3-}, As^{0}, As^{3+}, As^{5+}. Najbardziej toksyczne formy arsenu występują na trzecim i piątym stopniu utlenienia. Arsen może tworzyć połącznia ze związkami nieorganicznymi (iAs) i organicznymi. Spośród tych związków, to połączenia arsenu ze związkami nieorganicznymi są bardziej toksyczne i są to m.in. trójtlenek arsenu, pięciotlenek arsenu, kwas arsenowy(III), kwas arsenowy(V), arsenian sodu. Z kolei w grupie związków organicznych wyróżnia się np. kwas monometyloarsenowy (MMA), kwas dimetyloarsenowy (DMA), arsenocholinę, asrenolipidy, arsenocukry (Styblo i wsp., 2000, Vahter, 2002).

Narażenie na arsen wiąże się z wieloma negatywnymi skutkami zdrowotnymi. Toksyczność arsenu jest zależna w dużej mierze od formy jego występowania. Międzynarodowa Agencja Badań nad Rakiem uznała arsen za kancerogen i sklasyfikowała jego związki do poszczególnych grup (IARC, 2012), (Tabela 1).

Tabela 1. Klasyfikacja arsenu i jego związków według Międzynarodowej Agencji Badań nad Rakiem.

Grupa	Związki arsenu
Grupa 1 – czynniki rakotwórcze dla ludzi	arsen (metaliczny), nieorganiczne związki arsenu
Grupa 2B – czynniki przypuszczalnie rakotwórcze dla ludzi	kwas dimetyloarsynowy, kwas monometyloarsonowy
Grupa 3 – czynniki nie podlegają klasyfikacji ze względu na ich rakotwórczość dla ludzi	arsenobetaina, inne organiczne związki arsenu, które nie są metabolizowane u ludzi

Źródło: opracowano na podstawie IARC, 2012.

Narażenie na iAs może prowadzić do rozwoju nowotworów płuc, pęcherza moczowego i skóry, a ponadto zaobserwowano zależność między narażeniem a występowaniem innych nowotworów narządów: nerek, wątroby, prostaty (IRAC 2012). Długotrwałe narażenie na arsen zwiększa ryzyko zachorowania między innymi na choroby układu krążenia, a w tym na chorobę niedokrwienną serca (Chowdhury i wsp., 2018), chorobę naczyń obwodowych (Tseng i wsp., 2005) oraz na cukrzycę typu 2 (Rahimi i wsp., 2023), przewlekłą chorobę nerek (Jalili i wsp., 2021), choroby neurodegeneracyjne (Escudero-Lourdes, 2016), zespół policystycznych jajników (Liang i wsp., 2022) oraz choroby tarczycy (Kim i wsp., 2022). Ponadto, narażenie na arsen u kobiet w ciąży wiąże się z niekorzystnymi wynikami porodowymi u dziecka m.in. niższą masą urodzeniową (Liu i wsp., 2018), a w późniejszym okresie negatywnie wpływa na zdolności poznawcze oraz może indukować zaburzenia neurologiczne u dzieci (Tyler i Allan, 2014).

Metabolizm iAs w organizmie człowieka jest złożony i może obejmować różne ścieżki. Najbardziej poznany mechanizm metabolizmu iAs stanowią etapy przekształceń chemicznych, obejmujące naprzemienne reakcje utleniania oraz redukcji do MMA i DMA. Utlenianie wraz z metylacją iAs do form MMA i DMA, katalizuje enzym – metylotransferaza arsenu (AS3MT) przy użyciu S-adenozylometiony (SAM) jako donora grup metylowych. SAM syntetyzowana jest w szlaku jednowęglowym, w którym zaangażowane są niektóre składniki pokarmowe. Są to donory grup metylowych (metionina, cholina, betaina, foliany) i kofaktory reakcji (witaminy B_2, B_6, B_{12} i cynk). Z kolei reakcja redukcji katalizowana

jest przez S-transferazę glutationową omega 1 ($GSTO_1$) z wykorzystaniem glutationu jako środka redukującego (Challenger, 1945, Thomas i wsp., 2007). W alternatywnych ścieżkach metabolizmu, iAs łączy się z białkami lub glutationem. W każdej z tych ścieżek jest zaangażowany enzym AS3MT (Hayakawa i wsp., 2005, Naranmandura i wsp., 2006, El-Ghiaty i El-Kadi, 2023). Mimo, iż metabolizm iAs jest przedmiotem dyskusji i nadal jest poznawany, to produkty metabolizmu są dobrze opisane; iAs jest wydalany z moczem, w postaci niezmienionej stanowi 10-30%, w formie MMA 10-20%, a jako DMA 60-80% (Vahter, 2002).

Duża liczba osób narażonych na arsen oraz negatywny wpływ tego pierwiastka na zdrowie, skłaniają do głębokiej analizy mechanizmów toksyczności oraz do poszukiwania sposobu zmniejszenia niekorzystnego wpływu arsenu na organizm człowieka. Badania w tym zakresie koncentrują się między innymi na składnikach pokarmowych zaangażowanych w metabolizm arsenu, które mogą mieć modulujący wpływ na efektywność metylacji arsenu i zmniejszanie jego toksycznego działania. W literaturze dostępnych jest wiele badań, w których analizowano korzystny wpływ tych składników pokarmowych. W niniejszej rozprawie dokonano podsumowania dostępnych wyników badań modelowych na zwierzętach i przeprowadzonych z udziałem ludzi. W badaniach na modelach zwierzęcych narażonych na iAs suplementacja niektórych składników pokarmowych zwiększyła efektywność metylacji i zmniejszyła szereg niekorzystnych skutków zdrowotnych. Podobne korzystne wyniki zaobserwowano w badaniach przeprowadzonych z udziałem ludzi środowiskowo narażonych na arsen. Jednak w tych badaniach analizowano jedynie zależności między spożyciem, suplementacją lub stężeniem we krwi składników pokarmowych a stężeniem metabolitów arsenu w moczu oraz ryzykiem rozwoju chorób. Należy podkreślić, że wyniki badań na modelach zwierzęcych nie są równoważne badaniom przeprowadzonym z udziałem ludzi, a w tych z kolei wyniki były niejednoznaczne. Dlatego potrzebne są dalsze badania w tym zakresie, aby określić rolę tych składników pokarmowych jako kofaktorów reakcji i donorów grup metylowych. Ponadto w przeprowadzonych badaniach nie wykonywano analizy szczegółowych zmian zachodzących w metabolizmie pod wpływem narażenia na arsen i powiązania tych zmian ze spożyciem składników pokarmowych zaangażowanych w metabolizm arsenu, co uzasadnia konieczność przeprowadzenia takich badań.

2. Cel, zakres badań, hipotezy badawcze

Cel badań:

Przegląd wyników badań w obszarze wpływu składników pokarmowych na zmniejszanie toksyczności i poprawę efektywności metylacji arsenu oraz analiza zależności między wielkością spożycia wybranych składników pokarmowych a nasileniem zmian w profilu metabolicznym u osób dorosłych środowiskowo narażonych na arsen.

Zakres badań:

1. Przegląd wyników badań z zakresu wpływu składników pokarmowych zaangażowanych w metabolizm arsenu na efektywność jego metylacji oraz na nasilenie niekorzystnych zmian związanych z narażeniem na ten pierwiastek w badaniach na modelach zwierzęcych i przeprowadzonych z udziałem ludzi.
2. Ilościowa analiza wielkości spożycia składników pokarmowych zaangażowanych w metabolizm arsenu w grupie mężczyzn narażonych na arsen.
3. Przeprowadzenie badań z zakresu metabolomiki niecelowanej w grupie mężczyzn narażonych na arsen.
4. Analiza zależności między wielkością spożycia składników pokarmowych zaangażowanych w metabolizm arsenu a profilem metabolicznym mężczyzn narażonych na arsen.

Hipoteza główna:

Istnieje zależność między nasileniem zmian w profilu metabolicznym osób środowiskowo narażonych na arsen a wielkością spożycia składników pokarmowych, które są zaangażowane w metabolizm arsenu.

Hipotezy szczegółowe:

H1. Narażenie na arsen indukuje zmiany w profilu metabolicznym mężczyzn narażonych na arsen.

H2. Istnieje zależność między wielkością spożycia metioniny i folianów (jako donorów grup metylowych w metabolizmie arsenu) a nasileniem zmian w profilu metabolicznym mężczyzn narażonych na arsen.

H3. Istnieje zależność między wielkością spożycia witamin B_2, B_6, B_{12} i cynku (jako kofaktorów reakcji metabolizmu arsenu) a nasileniem zmian w profilu metabolicznym mężczyzn narażonych na arsen.

3. Materiał i metody badawcze

3.1. Prace przeglądowe

W pracach tych wykonano analizę dostępnych wyników badań z zakresu wpływu donorów grup metylowych (metioniny, choliny, betainy, kwasu foliowego) i kofaktorów reakcji (witamin B_2, B_6, B_{12} i cynku) na efektywność procesu metylacji arsenu, a także na zmniejszenie nasilenia całego spektrum zaburzeń związanych z narażeniem na arsen: wykonanych na modelach zwierzęcych (publikacja 1), przeprowadzonych z udziałem ludzi (publikacja 2). W obu pracach wykorzystano elektroniczną bazę danych PubMed (https://pubmed.ncbi.nlm.nih.gov). Do wyszukiwania artykułów użyto następujących słów kluczowych: „arsen" oraz „metionina", „betaina", „cholina", „kwas foliowy", „foliany", „cynk", „witamina B", „witamina B_2", „witamina B_6", „witamina B_{12}", „ryboflawina", „pirydoksyna", „kobalamina". Uwzględniono oryginalne, recenzowane artykuły w języku angielskim opublikowane w latach 1980–2020. Mając na uwadze wyżej wymienione kryteria analizie poddano 58 badań wykonanych na modelach zwierzęcych *in vivo* oraz *in vitro* (publikacja 1) oraz 62 badania przeprowadzone z udziałem ludzi, w tym 4 badania *in vitro* (publikacja 2).

3.2. Badana grupa mężczyzn

Badania przeprowadzono w grupie pracowników huty miedzi, położonej w południowo-zachodnim regionie Polski. Do kryteriów włączenia należały: narażenie zawodowe na arsen, wiek powyżej 18 lat oraz obecność w pracy w trakcie badania. Do analizy włączono uczestników, od których uzyskano kompletne dane: kwestionariusz 3-dniowego bieżącego notowania spożywanych produktów, potraw i napojów, ankietę (zawierającą dane dotyczące wieku, wzrostu, masy ciała i ogólnej charakterystyki osoby badanej) oraz próbkę moczu. Ostatecznie z grupy 119 pracowników, do badań zakwalifikowano 116, wyłączono dwie osoby ze względu na brak kwestionariusza 3-dniowego bieżącego notowania spożywanych produktów, potraw i napojów, jedną osobę z powodu braku danych na temat specjacji arsenu. Uczestników podzielono na dwie grupy na podstawie stężenia arsenu całkowitego w moczu (tAs), zgodnie z zalecanymi biologicznymi wartościami dopuszczalnymi dla narażenia zawodowego wynoszącymi 35 µg/l moczu (American Conference of Governmental Industrial Hygienists, 2014). Grupę WN (n = 75) stanowili pracownicy ze stężeniem tAs w granicach wartości dopuszczalnych to znaczy

do 35 µg/l moczu, natomiast grupę WH (n = 41) stanowili pracownicy ze stężeniem tAs powyżej wartości dopuszczalnych.

3.3. Ocena wielkości spożycia składników pokarmowych zaangażowanych w metabolizm arsenu

Ocenę wielkości spożycia wybranych składników pokarmowych zaangażowanych w metabolizm arsenu przeprowadzono na podstawie danych z kwestionariusza 3-dniowego bieżącego notowania spożywanych produktów, potraw i napojów. Uczestnicy zostali poinstruowani w jaki sposób wypełniać kwestionariusz (otrzymali standardowy wzór, który zawierał poprawnie zapisane przykłady uwzględniające: czas i miejsce spożycia posiłku, nazwę posiłku, składniki, ilość lub miary domowe). Poproszono o dokładne zapisywanie wszystkich produktów spożywczych, potraw i napojów, sposobu przygotowania potraw i wielkości porcji (określonej za pomocą wagi kuchennej lub typowych miar domowych). Wielkość spożycia składników pokarmowych zaangażowanych w metabolizm arsenu (metioniny, folianów, witamin B_2, B_6, B_{12}, cynku) wyliczono przy pomocy programu komputerowego Dieta 6.0. Do precyzyjnego określenia wielkości porcji wykorzystano „Album fotografii produktów i potraw" (Szponar i wsp., 2000). Spożycie wybranych składników pokarmowych przedstawiono jako średnią lub medianę spożycia z trzech dni oraz w przeliczeniu na dzień i kilogram masy ciała. Wielkość spożycia składników pokarmowych zaangażowanych w metabolizm arsenu (metioniny, folianów, witamin B_2, B_6, B_{12}, cynku) porównano z normami (Jarosz i wsp., 2020) na poziomie szacowanego średniego zapotrzebowania (EAR).

3.4. Oznaczenia stężenia arsenu całkowitego oraz jego form specjacyjnych

Do analizy stężenia arsenu całkowitego w moczu wykorzystano spektrometr mas z plazmą indukcyjnie sprzężoną i wyposażony w dynamiczną komorę reakcyjną (Perkin Elmer, SCIEX, USA). Analizy stężeń iAs i arsenobetainy wykonano przy pomocy spektrometru mas z plazmą indukcyjnie sprzężoną w połączeniu z wysokosprawną chromatografią cieczową (Perkin Elmer, SCIEX, USA).

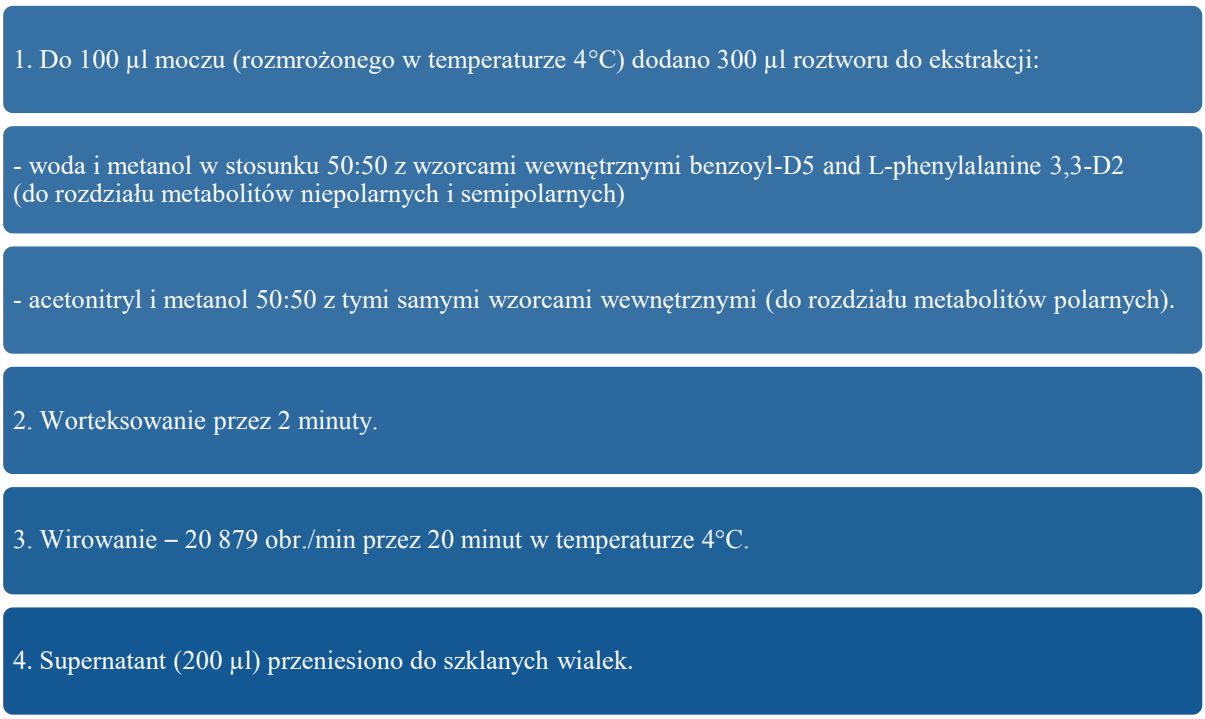
3.5. Metodyka badań z zakresu metabolomiki niecelowanej

Badania z zakresu metabolomiki niecelowanej obejmowały cztery etapy, które przedstawia Rycina 3.

Rycina 3. Etapy badań z zakresu metabolomiki niecelowanej.
LC/HRMS – chromatografia cieczowa z wysokorozdzielczą spektrometrią mas.

Przygotowanie próbek moczu

W pierwszym etapie analiz przygotowano próbki moczu zgodnie z protokołem Southam i wsp. (2020). Procedurę przygotowania próbek przedstawiono na Rycinie 4.



Rycina 4. Schemat ekstrakcji próbek moczu do analiz z zakresu metabolomiki niecelowanej.

Próbki randomizowano i podzielono na partie. Każda partia składała się z następujących próbek: 10 próbek do równoważenia systemu, 7 próbek kontrolnych (QC), 2 próbki blank (ultraczysta woda), 58-60 analizowanych próbek moczu. Próbki QC przygotowano mieszając równe objętości (100 µl) moczu od wszystkich osób z danej grupy. Próbkę QC stosowano do monitorowania stabilności systemu oraz sprawdzania poprawności wykonywanych analiz.

Analizy LC/HRMS

Analizy wykonywano przy użyciu systemu ultraefektywnej chromatografii cieczowej (Waters Acquity™ Ultra Performance LC system – Waters Corp., Milford, USA) sprzężonej z wysokorozdzielczym spektrometrem mas (HRMS), (Synapt G2Si Q-TOF – Waters

MS Technologies, Manchester, UK) ze źródłem jonizacji typu elektrosprej (ESI source – Waters, Manchester, UK). Rozdział chromatograficzny przeprowadzano na dwóch kolumnach:

- ACQUITY UPLC HSS T3 – do rozdziału metabolitów niepolarnych i semipolarnych
- ACQUITY UPLC BEH Amide – do rozdziału metabolitów polarnych.

W Tabeli 2 scharakteryzowano podstawowe parametry przeprowadzonych analiz.

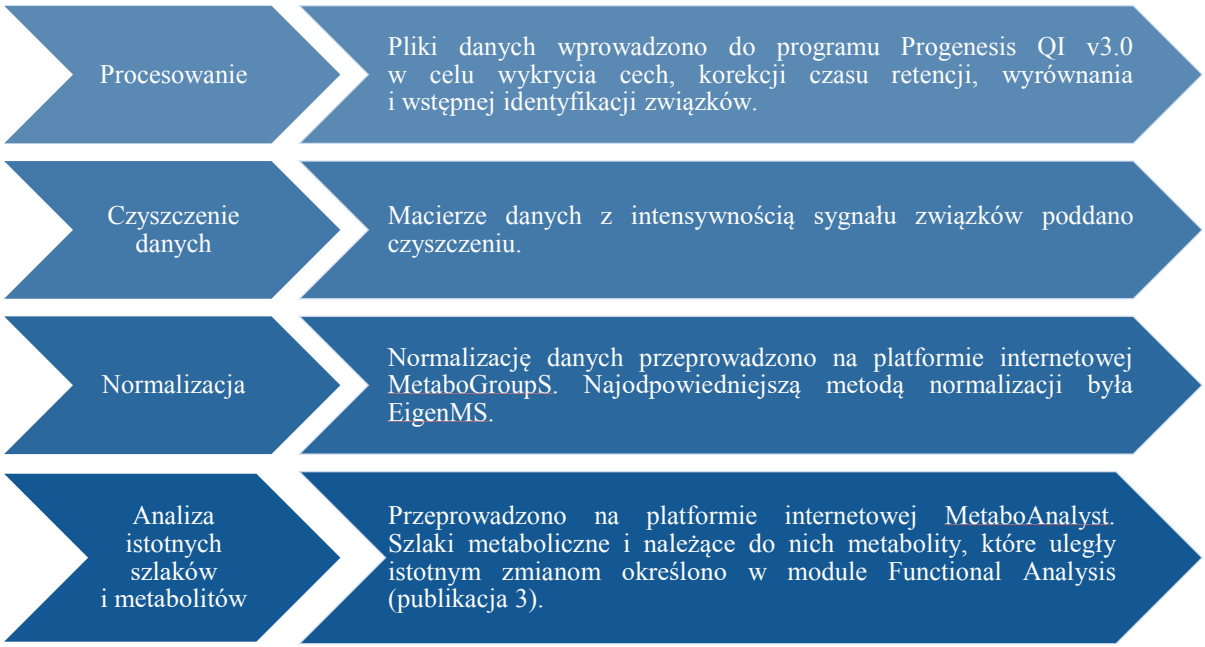
Tabela 2. Charakterystyka rozdziału chromatograficznego metabolitów.

Parametry	Metabolity niepolarne i semipolarne	Metabolity polarne
Kolumna	ACQUITY UPLC HSS T3 (1.8 µm, 2.1 × 100 mm)	ACQUITY UPLC BEH Amide (1.7 µm, 2.1 × 100 mm)
Prekolumna	ACQUITY UPLC HSS T3 (1.8 µm, 2.1 × 5 mm)	ACQUITY UPLC BEH Amide (1.7 µm, 2.1 × 5 mm)
Objętość nastrzyku	4.0 µl	2.5 µl
Fazy ruchome	A – ultraczysta woda, 0.1% kwas mrówkowy B – acetonitryl, 0.1% kwas mrówkowy	A – 95% acetonitryl, 5% ultraczysta woda, 10 mmol mrówczan amonu, 0.1% kwas mrówkowy B – 50% acetonitryl, 50% ultraczysta woda, 10 mmol mrówczan amonu, 0.1% kwas mrówkowy

Wszystkie analizy były wykonywane w trybie jonizacji dodatnim i ujemnym. Czas analizy jednej próbki w danym trybie jonizacji wynosił 15 minut. Zastosowane odczynniki chemiczne, dokładny przepływ faz chromatograficznych oraz parametry pracy spektrometru opisano w publikacji 3.

Analizy danych metabolomicznych

Rycina 5 przedstawia etapy przetwarzania danych metabolomicznych obejmujące procesowanie, czyszczenie, normalizację oraz analizę istotnych szlaków i metabolitów. Szczegółowy opis przetwarzania danych zawarto w publikacji 3 wchodzącej w skład rozprawy.



Rycina 5. Etapy przetwarzania danych metabolomicznych.

Potencjalna identyfikacja metabolitów

W celu potencjalnej identyfikacji, metabolity, których intensywność sygnału była istotnie statystycznie różna między porównywanymi grupami, poddano fragmentacji metodą Fast Data Dependent Acquisition z zastosowaniem średniej energii kolizyjnej 25 V. Do fragmentacji związków stosowano próbkę QC, a parametry chromatograficzne i pracy spektrometru były takie same jak przy analizie prób w poszczególnych grupach badanych osób. Otrzymane widma fragmentacyjne związków porównywano z widmami w bazie danych HMDB (The Human Metabolome Database), (Wishart i wsp., 2022).

3.6. Analizy statystyczne

Analizy statystyczne przeprowadzono przy użyciu oprogramowania Statistica w wersji 13.0 (StatSoft, Tulsa, OK, USA). Wartość przyjętego poziomu istotności statystycznej wynosiła $p \leq 0.05$. Normalność rozkładu danych oceniano za pomocą testu Shapiro–Wilka i wyrażano jako średnia ± odchylenie standardowe (SD) dla danych o rozkładzie normalnym lub mediana oraz minimum–maksimum dla danych odbiegających od rozkładu normalnego. Do porównania zmiennych między dwiema grupami wykorzystano test t-Studenta (dla danych o rozkładzie normalnym) i test U Manna–Whitneya (dla danych odbiegających od rozkładu normalnego). Analizy zależności między spożyciem składników pokarmowych zaangażowanych w metabolizm arsenu i potencjalnie zidentyfikowanymi metabolitami przeprowadzono przy użyciu współczynnika korelacji Pearsona lub współczynnika korelacji rang Spearmana.

4. Najważniejsze wyniki

4.1. Publikacja przeglądowa: Influence of Dietary Compounds on Arsenic Metabolism and Toxicity. Part I – Animal Model Studies

W wielu badaniach na modelach zwierzęcych *in vitro* oraz *in vivo* analizowano wpływ składników pokarmowych – metioniny, choliny, kwasu foliowego, witaminy B_6, witaminy B_{12}, i cynku, zaangażowanych w metabolizm arsenu na efektywność jego metylacji i zmniejszanie toksycznego działania. W Tabeli 3 przedstawiono główne wyniki tych badań.

Tabela 3a. Podsumowanie wyników badań modelowych na zwierzętach *in vivo i in vitro* dotyczących wpływu składników pokarmowych na metabolizm i toksyczność arsenu.

Parametry	Główne wyniki	Podsumowanie wyników
Metionina	• zmniejszenie stężenia tAs, iAs, MMA we krwi, wątrobie, mózgu	Suplementacja metioniny zwiększała efektywność metabolizmu iAs (3 badania *in vivo*).
	• zmiany w stężeniach różnych form arsenu w wątrobie, nerkach, a także zmniejszenie stężenia w moczu tAs, DMA, zwiększenie iAs	Dieta o niskiej zawartości metioniny zmniejszała efektywność metabolizmu iAs (2 badania *in vivo*).
	• zmniejszenie nasilenia peroksydacji lipidów w nerkach i wątrobie (zmniejszenie stężenia MDA) • zmniejszenie nasilenia stresu oksydacyjnego (zwiększenie aktywności CAT) w nerkach i wątrobie • obniżenie stężenia glukozy we krwi oraz kwasu pirogronowego w wątrobie, wolnego azotu aminokwasowego w wątrobie i nerkach oraz aktywności transaminazy glutaminianowo-pirogronianowej w nerkach • zwiększenie stężenia tlenku azotu w mózgu	Suplementacja metioniny wykazywała działanie hepatoprotekcyjne, nefroprotekcyjne oraz korzystnie wpływała na metabolizm glukozy, a także złagodzenie negatywnych skutków toksyczności iAs w mózgu (3 badania *in vivo*).
Cholina	• zmniejszenie stężenia tAs i DMA w moczu, zwiększenie stężenia tAs w narządach	Dieta o niskiej zawartości choliny zmniejszała efektywność metabolizmu iAs (2 badania *in vivo*).
	• zmniejszenie wydłużenia odstępu QT w sercu (*in vivo*), zmniejszenie wydłużenia czasu trwania potencjału czynnościowego i prądów wapniowych typu L w miocytach (*in vitro*) • zwiększenie wskaźnika przeżycia • zmniejszenie występowania wad zamknięcia cewy nerwowej • zmniejszenie metylacji DNA w rdzeniu kręgowym (zwiększenie ekspresji DNMT1 i DNMT3a) • zmniejszenie nasilenia apoptozy w rdzeniu kręgowym (zwiększenie ekspresji Bcl-2, zmniejszenie ekspresji Bax)	Suplementacja choliny wykazywała efekt kardioprotekcyjny i neuroprotekcyjny (2 badania *in vitro*, 1 *in vivo*).
	• zmniejszenie migracji DNA w komórkach skóry i uszkodzeń chromosomalnych w szpiku kostny	Dieta o niskiej zawartości choliny zwiększała uszkodzenia DNA (1 badanie *in vivo*).

Tabela 3b. Podsumowanie wyników badań modelowych na zwierzętach *in vivo i in vitro* dotyczących wpływu składników pokarmowych na metabolizm i toksyczność arsenu.

Parametry	Główne wyniki	Podsumowanie wyników
Witamina B_2 (z selenem)	• zmniejszenie stężenia tAs w wątrobie, skrzelach, mózgu, nerkach i mięśniach	Suplementacja witaminą B_2 zmniejszała bioakumulację tAs w narządach (2 badania *in vivo*).
	• zmniejszenie nasilenia stresu oksydacyjnego (zmniejszenie podwyższonej aktywności SOD, CAT, GST, GPx w wątrobie, skrzelach, mózgu, nerkach, zmniejszenie stężenia kortyzolu we krwi, HSP 70 w skrzelach i wątrobie, zwiększenie aktywności AChE w mózgu i wątrobie) • zmniejszenie nasilenia peroksydacji lipidów w wątrobie, skrzelach, mózgu, nerkach (zmniejszenie stężenia TBARS) • zwiększenie odporności (zwiększenie stężenia całkowitej immunoglobuliny, mieloperoksydazy, zmniejszenie stosunku albumina:globulina we krwi)	Suplementacja witaminą B_2 zmniejszała nasilenie stresu oksydacyjnego w układzie pokarmowym, nerwowym, oddechowym, moczowym i zwiększała odporność ryb (2 badania *in vivo*).
Witamina B_{12}	• zwiększenie stężenia tAs w moczu, zmniejszenie stężenia tAs we krwi i wątrobie	Suplementacja zwiększała efektywność metabolizmu iAs (1 badanie *in vivo*).
	• zmniejszenie nasilenia peroksydacji lipidów w wątrobie, jelitach, płucach (zmniejszenie stężenia TBARS, OH^-, CD) • obniżenie stężeń enzymów ALT, AST we krwi • zmniejszenie nasilenia apoptozy w wątrobie (m.in. zmniejszenie aktywności kaspazy 3, Ca^{2+}-ATPazy) • zmniejszenie uszkodzeń DNA w wątrobie • zmniejszenie nasilenia zmian zwyrodnieniowych w nerkach i wątrobie • zmniejszenie stężenia metalotioneiny w wątrobie	Suplementacja zmniejszała niekorzystne zmiany w układzie pokarmowym, moczowym i oddechowym (3 badania *in vivo*).
Kwas foliowy	• zwiększenie stężenia tAs w moczu, zmniejszenie stężenia tAs we krwi, kale, wątrobie • zmniejszenie stężenia w moczu iAs i zwiększenie DMA (efekt przy zastosowaniu kwasu foliowego z dietą o niskiej zawartości tłuszczu, z dietą wysokotłuszczową przeciwny efekt) • zmniejszenie stężenia iAs w wątrobie	Suplementacja kwasu foliowego zwiększała efektywność metabolizmu iAs (5 badań *in vivo*).
	• zmniejszenie stężenia tAs w moczu	Dieta o niskiej zawartości kwasu foliowego zmniejszała efektywność metabolizmu iAs (2 badania *in vivo*).
	• zmniejszenie nasilenia stresu oksydacyjnego (zwiększenie aktywności SOD, CAT, GSH we krwi, wątrobie, trzustce) • zmniejszenie nasilenia peroksydacji lipidów (zmniejszenie stężenia TBARS, OH^- we krwi, wątrobie, trzustce) • zmniejszenie nasilenia apoptozy (zmniejszenie aktywności kaspazy 3, Ca^{2+}-ATPazy w wątrobie) • obniżenie stężeń enzymów ALT, AST, ACP we krwi • zmniejszenie uszkodzeń wątroby (wyniki badań histopatologicznych) • zmniejszenie uszkodzeń DNA w wątrobie, trzustce, limfocytach (zmniejszenie rozmazania pasma DNA) • poprawa funkcji nerek (zmniejszenie stężenia mocznika we krwi)	Suplementacja zmniejszała nasilenie niekorzystnych zmian w układzie pokarmowym, moczowym i krwionośnym (8 badań *in vivo*), a także wykazywała działanie kardioprotekcyjne, neuroprotekcyjne i antycytotoksyczne (3 badania *in vitro*).

Tabela 3c. Podsumowanie wyników badań modelowych na zwierzętach *in vivo i in vitro* dotyczących wpływu składników pokarmowych na metabolizm i toksyczność arsenu.

Parametry	Główne wyniki	Podsumowanie wyników
	• zmniejszenie uszkodzeń chromosomów (zmniejszenie liczby mikrojąder w erytrocytach) • poprawa wzrostu i rozwoju embrionalnego, zmniejszenie embrionalnych wad serca • zwiększenie przeżywalności komórek	
	• zmniejszenie liczby bakterii w kale • nasilenie zaburzeń w metylacji DNA (zmiana metylacji wysp CpG, w tym genów związanych z nowotworami i rozwojem płodowym, zmiany metylacji genów w szlaku sygnalizacyjnym Wnt) • wzrost śmiertelności zarodków i matek	Suplementacja wykazywała niekorzystny wpływ na florę jelitową, metylację DNA, przeżywalność płodu i matki (3 badania *in vivo*).
	• zwiększenie stężenia insuliny na czczo we krwi i wskaźnika HOMA-IR • wzrost występowania określonych wad cewy nerwowej • zmniejszenie ekspresji genów związanych z rozwojem i różnicowaniem komórek skóry, zwiększenie ekspresji genów związanych z ruchem komórki • zmniejszony transport neurofilamentów	Dieta o niskiej zawartości kwasu foliowego nasilała zaburzenia homeostazy glukozy, rozwoju, proliferacji skóry (3 badania *in vivo*) oraz neurotoksyczność (1 badanie *in vitro*).
Witamina B_{12} i kwas foliowy	• zwiększenie stężenia tAs w moczu, a zmniejszenie we krwi i wątrobie • zwiększenie stężenia MMA i DMA w moczu (przy niskiej dawce iAs)	Suplementacja zwiększała efektywność metabolizmu iAs (3 badania *in vivo*).
	• zmniejszenie nasilenia stresu oksydacyjnego w wątrobie i trzustce (zwiększenie aktywności SOD, CAT, GSH) • zmniejszenie nasilenie peroksydacji lipidów w wątrobie i trzustce (zmniejszenie stężenia MDA) • zmniejszenie nasilenia apoptozy w wątrobie (zmniejszenie aktywności kaspazy 3, Ca^{2+}-ATPazy) • zmniejszenie uszkodzeń DNA w wątrobie, trzustce, nerkach (zmniejszenie pęknięć DNA i rozmazania pasma DNA) • obniżenie stężeń enzymów ALT, AST, ACP we krwi • zmniejszenie uszkodzeń wątroby i nerek (wyniki badań histologicznych) • zmniejszenie stężenia insuliny we krwi na czczo oraz wskaźnika HOMA-IR	Suplementacja zmniejszała uszkodzenia w układzie pokarmowym i moczowym (6 badań *in vivo*).
	• zmniejszenie stężenia tAs m.in. w wątrobie, nerkach, śledzionie, jelicie cienkim, mózgu, sercu	Suplementacja zmniejszała akumulację arsenu w wielu narządach (6 badań *in vivo*) i komórkach (1 badanie *in vitro*).
Cynk	• zmniejszenie uszkodzeń w jelitach, nerkach (wyniki badań histopatologicznych) • zmniejszenie ekspresji markerów stanu zapalnego w jelitach i nerkach (IL-1β, IL-6, IL-10, TNF-α) • zwiększenie poziomów mRNA białek połączeń ścisłych w jelitach i nerkach (okludyny, klaudyny i zonuliny) • poprawa funkcji wątroby (korzystne zmiany stężeń ALT, AST, ALP we krwi, w wątrobie) • zmniejszenie nasilenia stresu oksydacyjnego w nerkach, sercu, mózgu (zwiększenie aktywności CAT, SOD, GSH, zmniejszenie generacji ROS)	

Tabela 3d. Podsumowanie wyników badań modelowych na zwierzętach *in vivo i in vitro* dotyczących wpływu składników pokarmowych na metabolizm i toksyczność arsenu.

Parametry	Główne wyniki	Podsumowanie wyników
	• zmniejszenie nasilenia autofagii w nerkach, śledzionie, sercu (zwiększenie ekspresji białka p62, p-AKT, p-mTOR, Bekliny-1, zmniejszenie ekspresji LC3, LC3-II, ATG-5) • zmniejszenie nasilenia stanu zapalnego w nerkach (zmniejszenie ekspresji nNF-κB, zwiększenie ekspresji cNF-κB) • zmniejszenie nasilenia stresu retikulum endoplazmatycznego w nerkach i śledzionie (zmniejszenie ekspresji GRP78, GRP94, PERK, CHOP) • zmniejszenie nasilenia zaburzeń homeostazy białek w nerkach (zmniejszenie ekspresji białek HSP60, HSP70, HSP90, zmniejszenie stężenia PC) • zmniejszenie nasilenia peroksydacji lipidów w wątrobie, nerkach, sercu, mózgu (zmniejszenie stężenia MDA) • zwiększenie ekspresji metalotioneiny w wątrobie, nerkach • zmniejszenie nasilenia apoptozy w wątrobie, nerkach, śledzionie, sercu, mózgu (zmniejszenie ekspresji kaspazy-3, kaspazy-8, kaspazy-9, Bax, a zwiększenie Bcl-2) • zmniejszenie uszkodzeń DNA w nerkach (zmniejszenie stężenia 8-OGdG) • zmniejszanie nasilenia zaburzeń behawioralnych u szczurów • zmniejszenie nasilenia zaburzeń w układzie cholinergicznym (zwiększenie aktywności acetylocholiny i zmniejszenie stężenia acetylocholiny w mózgu) • zmniejszenie nasilenia zaburzeń w seminogramie (zwiększenie proporcji prawidłowych plemników, zmniejszenie występowania nieprawidłowości w plemnikach)	Suplementacja zmniejszała uszkodzenia w układach: pokarmowym, moczowym, limfatycznym, krwionośnym, nerwowym i rozrodczym (17 badań *in vivo*), a także wykazywała działanie antyapoptotyczne (1 badanie *in vitro*).
	• zwiększenie nasilenia stanu zapalnego w wątrobie (zwiększenie ekspresji HO-1, IL-6, Ccl2, ICAM1) • nasilenie apoptozy w linii komórkowej INS-1 (zwiększenie ekspresji przeciętego PARP) • zwiększenie uszkodzeń DNA w linii komórkowej INS-1 (zwiększenie ekspresji γ-H2AX) • obniżenie żywotności komórek (zwiększenie procentu żywych komórek, zmniejszenie liczby żywych komórek) • zwiększenie zaburzeń funkcji nerek (zwiększenie stężenia kwasu moczowego i mocznika we krwi, zwiększenie aktywności arginazy w nerkach) • nasilenie stresu oksydacyjnego (zmniejszenie ekspresji nrf2a, nrf2b, ogg1) i zaburzeń związanych z produkcją insuliny (zmniejszenie ekspresji insa) w zarodkach • zmniejszony wzrost zwierząt, podwyższony hematokryt i aktywność fosfatazy zasadowej we krwi	Dieta o niskiej zawartości cynku nasilała niekorzystne zmiany m.in. poprzez nasilenie stanu zapalnego w wątrobie, nasilenie zaburzeń w układzie moczowym, zmniejszenie wzrostu i wpływ na ekspresję genów odpowiedzialnych za nasilenie stresu oksydacyjnego i syntezę insuliny (4 badania *in vivo*), nasilała apoptozę i uszkodzenia DNA oraz zmniejszała żywotność komórek (1 badanie *in vitro*).

8-OGdG – 8-hydroksy-deoksyguanozyna; AChE – acetylocholinoesteraza; ACP – fosfataza kwaśna; ALP – fosfataza alkaliczna; ALT – aminotransferaza alaninowa; AST – aminotransferaza asparaginianowa; ATG-5 – gen związany z autofagią 5; Bax – białko X związane z Bcl-2; Bcl-2 – rodzina endogennych białkowych

regulatorów apoptozy; Ca2+-ATPaza – adenozynotrifosfataza wapniowa; CAT – katalaza; Ccl2 – ligand 2 chemokiny o motywie CC; CD – sprzężone dieny; CHOP – białko homologiczne do C/EBP; cNF-κB – jądrowy czynnik transkrypcyjny NF kappa B w cytoplazmie; DMA – kwas dimetyloarsenowy; DNA – kwas deoksyrybonukleinowy; DNMT1 – metylotransferaza DNA 1; DNMT3a – metylotransferaza DNA 3A; γ-H2AX – fosforylowana forma histonu H2AX w pozycji seryny139; GPx – peroksydaza glutationowa; GRP78 – białko regulowane glukozą o masie 78 kDa; GRP94 – białko regulowane glukozą o masie 94 kDa; GSH – glutation; GST – S-transferaza glutationowa; HO-1 – oksygenaza hemowa 1; HOMA-IR – wskaźnik oceny modelu homeostatycznego insulinooporności; HSP 60 – białka szoku termicznego o masie 60 kDa; HSP 70 – białka szoku termicznego o masie 70 kDa; HSP 90 – białka szoku termicznego o masie 60 kDa; iAs – arsen nieorganiczny; ICAM1 – cząsteczka adhezji międzykomórkowej 1; IL-1β – interleukina 1β; IL-10 – interleukina 10; IL-6 – interleukina 6; insa – insulina-a; LC3 – lekki łańcuch 3 białek związanych z mikrotubulami 1A/1B; MDA – dialdehyd malonowy; MMA – kwas monometyloarsenowy; nNF-kappaB – jądrowy czynnik transkrypcyjny NF kappa B; nrf2 – jądrowy czynnik transkrypcyjny pochodzenia erytroidalnego typu 2; ogg1– glikozylaza DNA 8-oksoguaniny; $OH^{\cdot}$ – rodnik hydroksylowy; p-AKT – fosforylowana kinaza białkowa B; p-mTOR – fosforylowany ssaczy cel rapamycyny; PARP – polimeraza poli(ADP)rybozy; PC – białka karbonylowane; PERK – kinaza białkowa retikulum endoplazmatycznego; ROS – reaktywne formy tlenu; SOD – dysmutaza ponadtlenkowa; tAs – arsen całkowity; TBARS – substancje reagujące z kwasem tiobarbiturowym; TNF-α – czynnik martwicy nowotworów alfa.

W większości badań przeprowadzonych na modelach zwierzęcych wykazano, że składniki pokarmowe zaangażowane w metabolizm arsenu mogą zwiększyć efektywność metylacji arsenu, jak również zmniejszać nasilenie całego spektrum zaburzeń związanych z narażeniem na ten pierwiastek. Kwas foliowy i cynk to składniki pokarmowe, które były analizowane w największej liczbie badań (19 kwas foliowy, 25 cynk), co pozwoliło na pełniejsze określnie ich wpływu na metabolizm i toksyczność arsenu. Wykazano, że suplementacja tych składników pokarmowych zwiększała efektywność metylacji arsenu oraz zmniejszała niekorzystne zmiany w wielu układach: pokarmowym, moczowym, limfatycznym, krwionośnym, nerwowym i rozrodczym. Liczba badań dotyczących wpływu metioniny, choliny, witamin B_2 i B_{12}, kwasu foliowego w połączeniu z witaminą B_{12}, była ograniczona, łącznie 21 badań. Te nieliczne badania wskazywały na ich potencjalnie korzystny wpływ. Jednak wiele aspektów tych badań uniemożliwia porównanie otrzymanych wyników i sformułowanie jednoznacznych wniosków. W protokołach badań wykorzystywano różne linie komórkowe i modele zwierzęce, które różniły takie czynniki jak płeć, wiek, różnice w metabolizmie arsenu między gatunkami. Ponadto, występowało duże zróżnicowanie dotyczące narażenia na arsen (forma, dawka, czas trwania narażenia, sposób podawania), jak również związane ze składnikami pokarmowymi (dawka, sposób i czas podania; przed, jednocześnie lub po narażeniu na iAs, początkowe stężenia składników pokarmowych w organizmie).

Badania przeprowadzone na liniach komórkowych i modelach zwierzęcych nie są równoważne badaniom przeprowadzonym z udziałem ludzi, ale wskazały kierunek i były przyczynkiem do dalszych rozważań. Biorąc pod uwagę obiecujące wyniki badań na modelach zwierzęcych *in vivo* i *in vitro*, przeanalizowano również wpływ tych składników pokarmowych na efektywność procesu metylacji arsenu, a także na zmniejszenie nasilenia całego spektrum zaburzeń związanych z narażeniem na arsen w populacjach narażonych na ten pierwiastek.

4.2. Publikacja przeglądowa: Influence of Dietary Compounds on Arsenic Metabolism and Toxicity. Part II – Human Studies

W badaniach *in vitro* na ludzkich liniach komórkowych (4 badania) oraz przeprowadzonych z udziałem ludzi narażonych na arsen analizowano zależności między spożyciem (22 badania), suplementacją (kwasu foliowego, cynku; 9 badań) lub stężeniem składników pokarmowych we krwi (29 badań) a metabolizmem arsenu oraz zaburzeniami związanymi z tym narażeniem.

4.2.1. Badania in vitro – kwas foliowy i cynk a metabolizm i toksyczność arsenu

W 4 badaniach na ludzkich liniach komórkowych analizowano ochronne działanie kwasu foliowego i cynku przy ekspozycji na iAs.
W jednym z badań kwas foliowy nie miał wpływu na metabolizm arsenu. Z kolei w 3 badaniach, kwas foliowy i cynk zmniejszały nasilenie procesu apoptozy, stresu oksydacyjnego oraz uszkodzeń DNA. W badaniach tych uzyskano korzystne wyniki pomimo dużego zróżnicowania metodycznego (różne linie komórkowe, różne dawki kwasu foliowego, cynku i iAs).
W warunkach niedoboru kwasu foliowego zaobserwowano zmniejszenie efektywności procesu metylacji arsenu (1 badanie). W 2 badaniach niedobory kwasu foliowego i cynku nasilały apoptozę i prowadziły do wzrostu stężenia markerów stresu oksydacyjnego i stanu zapalnego. W tych badaniach zarówno krótki okres niedoboru kwasu foliowego, jak i dłuższy okres niedoboru cynku prowadziły do niekorzystnych zmian.

4.2.2. Wielkość spożycia i suplementacji składników pokarmowych a metabolizm i toksyczność arsenu

Zależność między wielkością spożycia składników pokarmowych a metabolizmem arsenu analizowano w 4 badaniach przeprowadzonych w grupie dzieci oraz w 10 badaniach w grupie osób dorosłych.

W 3 badaniach przeprowadzonych z udziałem dzieci zaobserwowano ujemne zależności między wielkością spożycia witaminy B_6, folianów a %MMA w moczu oraz dodatnie zależności między wielkością spożycia folianów i %DMA. Zależności z pozostałymi składnikami pokarmowymi a stężeniem metabolitów arsenu w moczu były nieistotne statystycznie.

W części badań z udziałem osób dorosłych wykazano, że wielkość spożycia składników pokarmowych (metioniny, choliny, witamin B_2, B_6, B_{12}, folianów i cynku) była skorelowana ze stężeniem metabolitów arsenu w moczu (głównie ujemnie z MMA i iAs oraz dodatnio z DMA). Wyniki te są niejednoznaczne, ponieważ w większości analizowanych badań były to jedynie pojedyncze zależności, których nie stwierdzono w pozostałych badaniach. Ilościowe podsumowanie opisywanych zależności przedstawiono w Tabeli 4.

Tabela 4. Podsumowanie wyników badań dotyczących zależności między wielkością spożycia składników pokarmowych zaangażowanych w metabolizm arsenu a stężeniem metabolitów arsenu w moczu w badaniach przeprowadzonych w grupie osób dorosłych.

Parametry	Metionina	Betaina	Cholina	Witamina B_2	Witamina B_6	Witamina B_{12}	Foliany	Cynk
Liczba badań	3	2	2	7	7	6	8	2
%MMA	(+); (1)	NS	-	(+/-); (2)	(–); (1)	(+); (1)	(–); (1)	(–); (2)
%DMA	(+); (1)	NS	(+); (1)	(+); (1)	(+); (1)	(+); (1)	NS	(+); (2)
%iAs	(–); (2)	NS	(–); (1)	(–); (1)	(–); (2)	(–); (2)	(–); (1)	(–); (1)

W nawiasie podano liczbę badań, w których wynik był statystycznie istotny. (+) – zależność dodatnia; (–) – zależność ujemna; DMA – kwas dimetyloarsenowy; iAs – arsen nieorganiczny; MMA – kwas monometyloarsenowy; NS – nieistotne statystycznie.

W kilku badaniach analizowano zależności między wielkością spożycia składników pokarmowych a niekorzystnymi skutkami zdrowotnymi. Zaobserwowano zależności między wysokim spożyciem witamin z grupy B i folianów a zmniejszeniem niekorzystnych zmian związanych z narażeniem na arsen (między innymi zmniejszenie stresu oksydacyjnego, zmniejszenie ryzyka zmian skórnych u osób dorosłych oraz zwiększeniem ogólnych zdolności poznawczych u dzieci). Tylko w jednym badaniu wysokie spożycie witaminy B_6 wiązało się ze zwiększonym ryzykiem rozwoju cukrzycy i zespołu metabolicznego, dlatego aspekt ten wymaga dalszych badań. Z kolei niskie spożycie (choliny, witamin z grupy B, folianów i cynku) zwiększało ryzyko wysokiego ciśnienia tętna oraz ryzyko wystąpienia zmian skórnych. Jednak w kilku badaniach uzyskano sprzeczne wyniki. Ponadto, nie wykazano jednoznacznych zależności między wielkością spożycia witamin z grupy B i folianów a ryzykiem wystąpienia zmian skórnych, rozwoju cukrzycy i raka pęcherza moczowego, a także poziomem różnych umiejętności szkolnych u dzieci. Autorzy badań sugerowali, że brak lub niejasny efekt może wynikać z małej zmienności spożycia tych witamin lub tego,

że u większości uczestników wielkość spożycia była wyższa od zalecanych norm. Dodatkowo uzyskane wyniki mogły być determinowane zmiennością genetyczną lub różnicami w metabolizmie wynikającymi z wpływu takich czynników jak płeć, wiek, stan zdrowia.

Przeprowadzono niewiele badań, w których analizowano wpływ suplementacji składników pokarmowych na metabolizm arsenu i skutki zdrowotne związane z narażeniem na ten pierwiastek. W 4 badaniach suplementacja kwasem foliowym zwiększała efektywność metylacji arsenu u osób dorosłych (głównie poprzez zwiększenie stężenia DMA w moczu i zmniejszenie stężenia tAs w moczu i krwi). Z kolei suplementacja cynku u dzieci spowodowała odmienny efekt (zmniejszenia stężenia DMA w moczu, brak wpływu na stężenie pozostałych metabolitów arsenu). W odniesieniu do skutków zdrowotnych w 2 badaniach suplementacja kwasem foliowym zmniejszała niekorzystne objawy związane z zatruciem arsenem, podczas gdy w 1 badaniu suplementacja kwasem foliowym nie miała wpływu na regulację epigenetyczną. Krótki okres suplementacji, zastosowanie niskiej dawki kwasu foliowego lub zastosowanie filtrów do usuwania arsenu z wody to czynniki, które według autorów badań mogły wpływać na uzyskane wyniki.

4.2.3. Stężenie składników pokarmowych a metabolizm i toksyczność arsenu

Zależność między stężeniem składników pokarmowych we krwi a metabolizmem arsenu analizowano w 8 badaniach przeprowadzonych z udziałem dzieci oraz w 12 badaniach z udziałem osób dorosłych.

W badaniach z udziałem dzieci tylko stężenie witaminy B_{12} i folianów we krwi korelowało dodatnio z %DMA i ujemnie %MMA oraz z %iAs. Podobne wyniki zaobserwowano u osób dorosłych, u których w 5 badaniach wykazano występowanie istotnych zależności między stężeniem witaminy B_{12}, folianów we krwi a stężeniem metabolitów arsenu w moczu (ujemne z %MMA i %iAs oraz dodatnie z %DMA). W kilku badaniach przeprowadzonych z udziałem kobiet w ciąży nie wykazano zależności między stężeniem witaminy B_{12}, folianów a stężeniem metabolitów arsenu w moczu, a jedynie ze stężeniem różnych form arsenu we krwi i we krwi pępowinowej.

W części badań wykazano, że stężenie witaminy B_{12} i folianów może mieć wpływ na efektywność metylacji arsenu. Jednak w innych badaniach zależności między stężeniem składników pokarmowych we krwi (witamin B_6, B_{12}, folianów i cynku) a stężeniem różnych form arsenu w moczu nie były istotne statystycznie.

Analizowano również zależności między stężeniem składników pokarmowych we krwi a nasileniem różnych zaburzeń związanych z narażeniem na arsen. W 3 badaniach wykazano, że wyższe stężenia we krwi choliny, witaminy B_{12}, folianów były powiązane ze zmniejszeniem zaburzeń metylacji DNA, modyfikacji histonów, z wyższym poziomem umiejętności naukowych u dzieci. W kilku badaniach zaobserwowano także zależność między stężeniem składników pokarmowych we krwi (foliany – niskie i wyższe stężenia, witamina B_{12} – niskie stężenie) a zwiększonym ryzykiem rozwoju chorób i niekorzystnych zmian w organizmie związanych z narażeniem na arsen (zwiększona metylacja DNA, zwiększone ryzyko rozwoju cukrzycy typu 1, raka urotelialnego, zmian skórnych u osób dorosłych i opóźnionego rozwoju u dzieci). W 4 badaniach nie wykazano zależności między stężeniem metioniny, witamin B_6, B_{12}, folianów we krwi a metylacją DNA, ryzykiem rozwoju cukrzycy typu 2 i zmianami skórnymi. Ponadto, zaobserwowano obniżone stężenie cynku w moczu, krwi i włosach u osób narażonych na arsen (kobiety w ciąży, pacjenci z chorobą czarnej stopy, pracownicy huty i recyklingu metali). Wyniki tych badań wskazują, że odpowiedni stan odżywienia w odniesieniu do tych witamin, może łagodzić niekorzystne skutki zdrowotne związane z narażeniem na arsen. Z kolei stężenie składników pokarmowych we krwi w zakresach powyżej i poniżej norm może być związane z nasileniem tych niekorzystnych skutków zdrowotnych.

Podsumowując badania przeprowadzone z udziałem ludzi wykazały, że wielkość spożycia i stężenie niektórych składników pokarmowych we krwi (metioniny, choliny, folianów, witamin B_2, B_6, B_{12}, cynku) może mieć korzystny wpływ na poprawę metylacji arsenu i na zmniejszenie nasilenia niekorzystnych skutków zdrowotnych. Podobnie jak w badaniach na modelach zwierzęcych wielkość spożycia składników pokarmowych, jak i ich stężenie we krwi miało istotny wpływ na obserwowane efekty. Jednakże, dotychczasowe badania przeprowadzone z udziałem ludzi są niejednoznaczne (wiele zmiennych determinowało uzyskane wyniki). W większości badań wykazano jedynie pojedyncze zależności między wielkością spożycia lub stężeniem danego składnika pokarmowego a stężeniem metabolitów arsenu w moczu czy też skutkami zdrowotnymi. Przegląd badań przeprowadzonych z udziałem ludzi narażonych na arsen wskazuje na konieczność dokładniejszej analizy zależności między wielkością spożycia składników pokarmowych zaangażowanych w metabolizm arsenu a zmianami w organizmie jakie zachodzą pod wpływem narażenia na ten pierwiastek.

4.3. Publikacja: Metabolic Changes and Their Associations with Selected Nutrients Intake in the Group of Workers Exposed to Arsenic

4.3.1. Ogólna charakterystyka badanej grupy mężczyzn i wielkość spożycia wybranych składników pokarmowych zaangażowanych w metabolizm arsenu

Grupę badaną stanowili pracownicy zawodowo narażeni na arsen (pracownicy huty miedzi). Charakterystykę grupy badanej przedstawiono w Tabeli 5. Nie wykazano różnić w zakresie wieku, wzrostu oraz okresu narażenia między grupą WN i WH. Odnotowano istotnie wyższe wartości masy ciała i wskaźnika masy ciała w grupie WH w porównaniu do grupy WN.

Tabela 5. Ogólna charakterystyka grupy mężczyzn.

Parametry	Obie grupy (n = 116)	WN (n = 75)	WH (n = 41)	*p**
Wiek (lata)	43.5 (21.0-62.0)	42.1 ± 10.0	44.0 (23.0-56.0)	0.4772
Wzrost (cm)	177.0 (165.0-198.0)	176.5 (165.0-198.0)	178.3 ± 6.1	0.9673
Masa ciała (kg)	89.4 ± 14.8	86.7 ± 14.0	94.0 ± 15.1	0.0109
BMI (kg/m^2)	28.1 ± 4.3	27.2 ± 4.1	29.5 ± 4.2	0.0047
Okres narażenia na iAs (lata)	17.5 (1.0-44.0)	17.0 (1.0-44.0)	18.5 (2.0-38.0)	0.7867

Wartości przedstawiono jako średnią ± odchylenie standardowe dla rozkładu normalnego lub jako medianę z wartościami minimalnymi i maksymalnymi dla zmiennych odbiegających od rozkładu normalnego (zweryfikowano testem Shapiro–Wilka). * – do porównania zmiennych pomiędzy dwiema grupami wykorzystano test t-Studenta dla danych o rozkładzie parametrycznym lub test U Manna–Whitneya dla danych o rozkładzie nieparametrycznym; BMI – wskaźnik masy ciała; iAs – arsen nieorganiczny; *p* – poziom istotności; WN – grupa pracowników ze stężeniem arsenu całkowitego w moczu w granicach normy; WH – grupa pracowników ze stężeniem arsenu całkowitego w moczu powyżej normy.

Stężenie tAs w moczu różniło się istotnie między porównywanymi grupami, w grupie WN wynosiło 20.6 µg/l (zakres 1.5-33.9 µg/l), a w grupie WH 54.7 µg/l (zakres 35.9-498.1 µg/l). Wielkość spożycia wybranych składników pokarmowych zaangażowanych w metabolizm arsenu (metioniny, folianów, witamin B_2, B_6, B_{12}, cynku) w przeliczeniu na dzień jak i w przeliczeniu na dzień i kilogram masy ciała przedstawiono odpowiednio w Tabeli 6 i 7.

Tabela 6. Spożycie wybranych składników pokarmowych zaangażowanych w metabolizm arsenu w grupie mężczyzn w odniesieniu do norm na poziomie EAR.

Parametry	Obie grupy (n = 116)	WN (n = 75)	WH (n = 41)	EAR	*p**
Metionina (mg/dzień)	2205.3 (843.1-4955.0)	2184.8 (843.1-4502.8)	2411.2 (1267.1-4955.0)	–	0.5103
Witamina B_2 (mg/dzień)	1.6 (0.5-3.8)	1.6 (0.5-3.8)	1.6 ± 0.4	1.1	0.8989
Witamina B_6 (mg/dzień)	2.0 (0.7-4.3)	2.0 ± 0.6	1.9 (1.1-3.8)	1.1 (19-50 lat) 1.4 (>50 lat)	0.6235
Witamina B_{12} (µg/dzień)	3.1 (0.6-16.9)	3.0 (0.6-16.9)	3.3 (1.3-8.0)	2.0	0.5328
Foliany (µg/dzień)	250.3 (94.9-700.8)	255.6 (107.1-700.8)	257.8 ± 73.1	320.0	0.7816
Cynk (mg/dzień)	10.8 (3.5-20.2)	10.5 (3.5-19.2)	11.5 ± 3.2	9.4	0.4956

Wartości przedstawiono jako średnią ± odchylenie standardowe dla rozkładu normalnego lub jako medianę z wartościami minimalnymi i maksymalnymi dla zmiennych odbiegających od rozkładu normalnego (zweryfikowano testem Shapiro–Wilka). * – do porównania zmiennych między dwiema grupami wykorzystano test U Manna–Whitneya; EAR – poziom średniego zapotrzebowania; *p* – poziom istotności; WN – grupa pracowników ze stężeniem arsenu całkowitego w moczu w granicach normy; WH – grupa pracowników ze stężeniem arsenu całkowitego w moczu powyżej normy.

Tabela 7. Spożycie wybranych składników pokarmowych w przeliczeniu na kilogram masy ciała zaangażowanych w metabolizm arsenu w grupie mężczyzn.

Parametry	Obie grupy (n = 116)	WN (n = 75)	WH (n = 41)	*p**
Metionina (mg/kg m.c.)	25.05 (8.43–70.48)	25.23 (8.43–70.48)	23.75 (14.69–54.47)	0.5220
Witamina B_2 (mg/kg m.c.)	0.02 (0.00–0.04)	0.02 (0.00–0.04)	0.02 ± 0.01	0.3554
Witamina B_6 (mg/kg m.c.)	0.02 (0.01–0.06)	0.02 (0.01–0.06)	0.02 ± 0.01	0.1506
Witamina B_{12} (µg/kg m.c.)	0.04 (0.01–0.20)	0.03 (0.01–0.20)	0.04 ± 0.01	0.8515
Foliany (µg/kg m.c.)	2.94 (1.03–8.98)	3.04 (1.13–8.98)	2.78 ± 0.80	0.1819
Cynk (mg/kg m.c.)	0.12 (0.03–0.30)	0.12 (0.03–0.30)	0.12 ± 0.04	0.5418

Wartości przedstawiono jako średnią ± odchylenie standardowe dla rozkładu normalnego lub jako medianę z wartościami minimalnymi i maksymalnymi dla zmiennych odbiegających od rozkładu normalnego (zweryfikowano testem Shapiro–Wilka). * – do porównania zmiennych między dwiema grupami wykorzystano test U Manna–Whitneya; m.c. – masa ciała; *p* – poziom istotności; WN – grupa pracowników ze stężeniem arsenu całkowitego w moczu w granicach normy; WH – grupa pracowników ze stężeniem arsenu całkowitego w moczu powyżej normy.

Wielkość spożycia składników pokarmowych: metioniny, folianów, witamin B_2, B_6, B_{12}, cynku w przeliczeniu na jeden dzień i jak i w przeliczeniu na dzień i kilogram masy ciała nie różniła się istotnie między porównywanymi grupami WN i WH. Średnie spożycie lub mediana spożycia wszystkich składników pokarmowych były powyżej poziomu EAR, z wyjątkiem folianów, których spożycie kształtowało się poniżej normy EAR w obu grupach. W grupie WN odsetek ten wynosił 78.7%, a w grupie WH – 80.5%. Szczególnie w grupie WN, a co za tym idzie również w obu grupach wielkość spożycia była bardzo zróżnicowana i u znaczącego odsetka osób spożycie było niższe od normy na poziomie EAR.W grupie WN spożycie witaminy B_2 poniżej normy odnotowano u 14.7% mężczyzn, witaminy B_6 u 6.7%, witaminy B_{12} u 16.0%, cynku u 28.0%. Natomiast w grupie WH odsetek mężczyzn z niższym niż norma spożyciem witaminy B_2 wynosił 9.8%, witaminy B_6 – 4.9%, witaminy B_{12} – 9.8%, cynku – 19.5%.

4.3.2. Wybrane wyniki

W grupie WH w porównaniu do grupy WN wykazano istotnie wyższą intensywność sygnału pochodząca od 25 metabolitów należących do pięciu głównych ścieżek przemian: aminokwasów, węglowodanów, glikanów, witamin i nukleotydów. Rycina 6 przedstawia ścieżki przemian oraz liczbę metabolitów należących do danej ścieżki.

Rycina 6. Główne ścieżki przemian oraz liczba metabolitów ze statystycznie istotną różnicą w intensywności sygnału WN vs WH.
WN – grupa pracowników ze stężeniem arsenu całkowitego w moczu w granicach normy; WH – grupa pracowników ze stężeniem arsenu całkowitego w moczu powyżej normy.

Analizowano zależności między wielkością spożycia wybranych składników pokarmowych zaangażowanych w metabolizm arsenu a intensywnością sygnału potencjalnie zidentyfikowanych metabolitów. Analizę zależności przeprowadzono w całej grupie badanych mężczyzn oraz oddzielnie w grupach WN i WH. Wykazano kilka zależności między wielkością spożycia składników pokarmowych a intensywnością sygnału pochodzącą od 11 metabolitów (Tabela 8).

Tabela 8a. Zależności między wielkością spożycia składników pokarmowych zaangażowanych w metabolizm arsenu a intensywnością sygnału potencjalnie zidentyfikowanych metabolitów.

Parametry	Obie grupy		WN		WH	
	R	*p*	R	*p*	R	*p*
witamina B_2 & cytozyna	-0.1886*	0.0464	-0.2554**	0.0316	NS	
witamina B_6 & cytozyna	-0.2175*	0.0213	-0.2346**	0.0489	NS	
witamina B_2 & kwas D-glukuronowy	NS		NS		-0.3956*	0.0100
witamina B_6 & kwas D-glukuronowy	NS		NS		-0.3646*	0.0190
witamina B_{12} & kwas D-glukuronowy	NS		NS		-0.3479*	0.0260
foliany & kwas D-glukuronowy	-0.1996*	0.0349	-0.2603*	0.0283	NS	
metionina & kwas hydroksypropionowy	-0.2163*	0.0220	NS		NS	
witamina B_{12} & kwas hydroksypropionowy	-0.2017*	0.0330	NS		NS	
witamina B_2 & kwas L-glutaminowy	NS		NS		-0.4211**	0.0061
witamina B_6 & kwas L-glutaminowy	NS		NS		-0.3653**	0.0188
foliany & kwas L-glutaminowy	NS		NS		-0.3794**	0.0144
metionina & N-acetylo-D-glukozamina	-0.1954*	0.0390	-0.2728*	0.0214	NS	
witamina B_2 & N-acetylo-D-glukozamina	-0.2504*	0.0077	-0.3150*	0.0075	-0.3253*	0.0380
witamina B_6 & N-acetylo-D-glukozamina	-0.2374*	0.0117	NS		NS	
witamina B_{12} & N-acetylo-D-glukozamina	-0.1871*	0.0482	-0.3261*	0.0055	NS	
cynk & N-acetylo-D-glukozamina	-0.1917*	0.0429	-0.2710*	0.0223	NS	
witamina B_2 & kwas N-acetyloneuraminowy	-0.1915*	0.0431	NS		NS	
witamina B_6 & kwas N-acetyloneuraminowy	-0.2246*	0.0173	NS		NS	
witamina B_{12} & kwas N-acetyloneuraminowy	NS		NS		-0.3171*	0.0430
witamina B_2 & kwas piroglutaminowy	NS		-0.2832*	0.0167	NS	
witamina B_6 & kwas piroglutaminowy	NS		-0.2430*	0.0412	NS	
witamina B_{12} & kwas piroglutaminowy	NS		-0.2667*	0.0246	NS	
foliany & kwas piroglutaminowy	-0.1894*	0.0454	NS		NS	

Tabela 8b. Zależności między wielkością spożycia składników pokarmowych zaangażowanych w metabolizm arsenu a intensywnością sygnału potencjalnie zidentyfikowanych metabolitów.

Parametry	Obie grupy		WN		WH	
	R	*p*	R	*p*	R	*p*
cynk & kwas piroglutaminowy	NS		-0.2548*	0.0320	NS	
witamina B_2 & urydyna	-0.1944*	0.0400	NS		NS	
witamina B_6 & urydyna	-0.1955*	0.0388	NS		NS	
witamina B_{12} & urydyna	NS		-0.2559*	0.0313	-0.3308*	0.0350
metionina & kwas urokanowy	NS		-0.2569*	0.0305	NS	
witamina B_6 & kwas urokanowy	NS		-0.2457*	0.0389	NS	
foliany & kwas urokanowy	-0.2067*	0.0288	-0.2665*	0.0247	NS	
cynk & kwas urokanowego	NS		-0.2722*	0.0217	NS	
foliany & kwas 3-merkaptomlekowy	NS		NS		0.3337*	0.0330
cynk & kwas 3-merkaptomlekowy	NS		NS		0.3658*	0.0190
metionina & kwas bursztynowy	NS		NS		0.3284**	0.0361
foliany & kwas bursztynowy	NS		NS		0.3359*	0.0320
cynk & kwas bursztynowy	NS		NS		0.3498*	0.0250

Wielkość spożycia składników pokarmowych uwzględniono w przeliczeniu na kilogram masy ciała. * – współczynnik korelacji Pearsona; ** – współczynnik korelacji rang Spearmana; R – współczynnik korelacji; *p* – poziom istotności; WN – grupa pracowników ze stężeniem arsenu całkowitego w moczu w zakresie normy; WH – grupa pracowników ze stężeniem arsenu całkowitego w moczu powyżej normy.

W całej grupie wykazano tylko ujemne zależności między intensywnością sygnału pochodzącą od ośmiu metabolitów (cytozyny, kwasu D-glukuronowego, kwasu hydroksypropionowego, N-acetylo-D-glukozaminy, kwasu N-acetyloneuraminowego, kwasu piroglutaminowego, urydyny i kwasu urokanowego) a wielkością spożycia składników pokarmowych takich jak metionina, foliany, witaminy B_2, B_6, B_{12} oraz cynk.

Ujemne zależności między intensywnością sygnału pochodzącą od sześciu metabolitów (cytozyny, kwasu D-glukuronowego, N-acetylo-D-glukozaminy, kwasu piroglutaminowego, urydyny i kwasu urokanowego) a wielkością spożycia wszystkich analizowanych składników pokarmowych zaobserwowano w grupie WN.

Z kolei w grupie WH intensywność sygnału pochodząca od pięciu metabolitów (kwasu D-glukuronowego, kwasu L-glutaminowego, N-acetylo-D-glukozaminy, kwasu N-acetyloneuraminowego, urydyny) była ujemnie skorelowana z wielkością spożycia prawie wszystkich analizowanych składników pokarmowych (z wyjątkiem cynku). Ponadto w tej grupie mężczyzn zaobserwowano dodatnie zależności między intensywnością sygnału pochodzącą od dwóch metabolitów: kwasu bursztynowego i kwasu 3-merkaptomlekowego a wielkością spożycia metioniny, folianów i cynku.

Intensywność sygnału trzech metabolitów (kwasu D-glukuronowego, N-acetylo-D-glukozaminy i urydyny) była ujemnie skorelowana z wielkością spożycia składników pokarmowych w obu grupach WN i WH. W grupie WN zaobserwowano istotne korelacje z intensywnością sygnału trzech metabolitów (cytozyny, kwasu piroglutaminowego i kwasu urokanowego), które nie były istotne w grupie WH. Natomiast w grupie WH istotne korelacje występowały z intensywnością sygnału dwóch metabolitów (kwasu L-glutaminowego i kwasu N-acetyloneuraminowego), które w grupie WN nie były istotne statystycznie.

5. Podsumowanie wyników badań eksperymentalnych i dyskusja

5.1. Wyniki analiz z zakresu metabolomiki niecelowanej

Potencjalnie zidentyfikowane metabolity w grupie mężczyzn należały do głównych ścieżek przemian: aminokwasów, węglowodanów, lipidów i nukleotydów. Wyniki tych badań są zgodne z wcześniejszymi analizami metabolomicznymi wykonanymi w próbkach moczu, które wykazały, że ekspozycja na arsen wiąże się z licznymi zmianami w metabolizmie u osób dorosłych (Zhang i wsp., 2014, Wu i wsp., 2018, Kozłowska i wsp., 2019). W badaniu Zhang i wsp. (2014), przeprowadzonym w grupie mężczyzn, odnotowano zmiany w intensywności sygnałów metabolitów należących do metabolizmu aminokwasów (seryny, hipuranu, acetylo-N-formylo-5-metoksy kynurenaminy), lipidów (testosteronu), nukleotydów (guaniny). W grupie kobiet i mężczyzn z Bangladeszu również zaobserwowano zmiany w metabolizmie aminokwasów (m.in. glicyny, L-treoniny, seryny, kwasu piroglutaminowego), węglowodanów (m.in. kwasu bursztynowego, kwasu fosforowego, kwasu cytrynowego), nukleotydów (uracylu, kwasu moczowego), (Wu i wsp., 2018). Podobnie w badaniu przeprowadzonym w grupie kobiet i mężczyzn środowiskowo narażonych na arsen zidentyfikowano wiele metabolitów związanych z metabolizmem aminokwasów (m.in. kwasu wanilinowego, kwasu argininobursztynowego, ketoleucyny), węglowodanów (siarczanu, L-threo-2-pentulozy, kwasu D-mlekowego), lipidów (m.in. pregnenolonu, dihydrotestosteronu, dehydroepiandrosteronu, 4-hydroksynonenalu) i nukleotydów (adeniny, adenozyno-5′-monofosforanu).

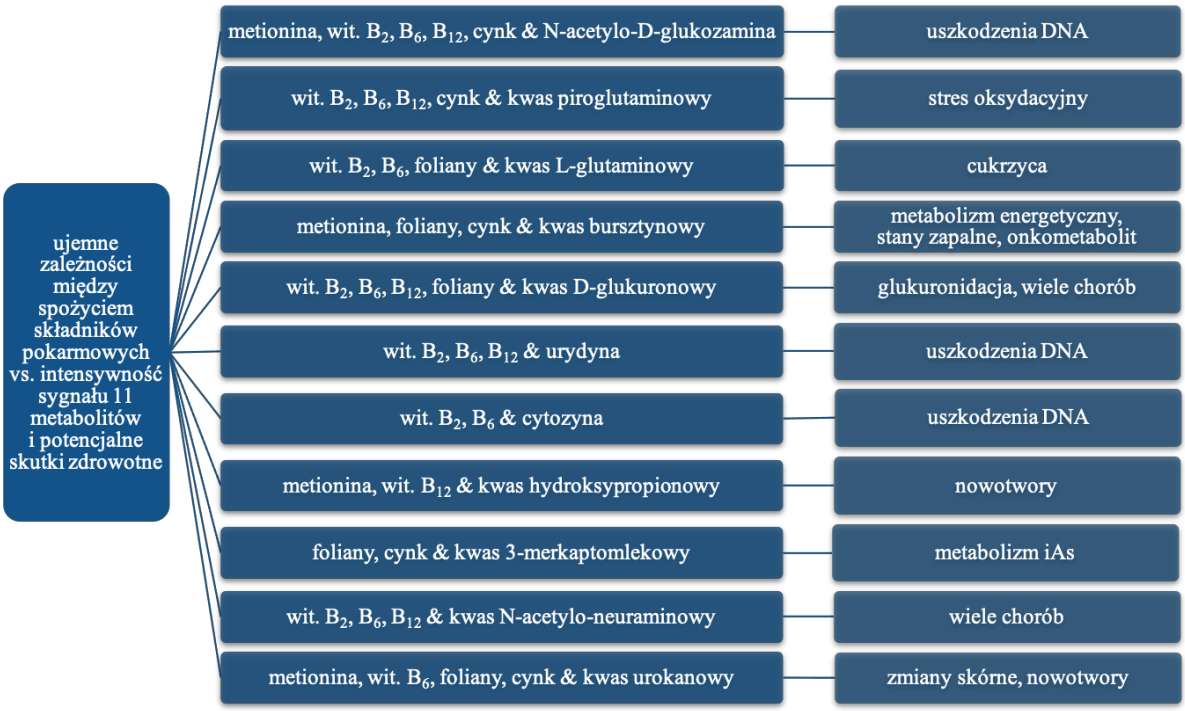
W ramach każdej z głównych ścieżek przemian wyróżnia się liczne podścieżki. Porównując zmiany w podścieżkach z danymi innych badań metabolomicznych również zauważono zbliżone wyniki. W badanej grupie mężczyzn potencjalnie zidentyfikowano kwas piroglutaminowy, związany z metabolizmem glutationu, którego obecność stwierdzono również w moczu kobiet i mężczyzn w badaniu Wu i wsp. (2018). Ponadto, w naszym badaniu, podobnie jak w badaniu Wu i wsp. (2018), potencjalnie zidentyfikowano kwas bursztynowy, zaangażowany w liczne podścieżki, w tym m.in. metabolizm tyrozyny oraz cykl kwasów trikarboksylowych. W podścieżce metabolizmu pirymidyn, w badanej grupie mężczyzn odnotowano urydynę, tyminę podczas gdy w badaniach innych autorów wykryto guaninę (Zhang i wsp., 2014) i adeninę (Kozłowska i wsp., 2019) należące do metabolizmu puryn. Mimo zaobserwowanych podobieństw, w naszych oraz wcześniejszych badaniach

zaobserwowano zmiany w wielu innych różnych podścieżkach m.in. w metabolizmie metioniny, cysteiny, tryptofanu, tyrozyny, histydyny, witamin.

Uzyskane wyniki mogą być efektem różnic w źródłach i poziomie narażenia na arsen, a także różnic metabolicznych związanych z płcią oraz innymi czynnikami wpływającymi na metabolizm arsenu (Tseng, 2009).

5.2. Zależności między wielkością spożycia wybranych składników pokarmowych a intensywnością sygnału metabolitów w grupie mężczyzn

Według naszej najlepszej wiedzy, są to pierwsze tego typu badania, w których połączono wyniki badań metabolomicznych z wielkością spożycia wybranych składników pokarmowych w grupach osób narażonych na arsen. Wyniki przeprowadzonych badań wykazały istotne ujemne zależności między wielkością spożycia składników pokarmowych zaangażowanych w metabolizm iAs (metioniny, folianów, witamin B_2, B_6 i B_{12} i cynku) a 11 potencjalnie zidentyfikowanymi metabolitami w grupie mężczyzn. Metionina, witaminy B_2, B_6 i B_{12}, foliany i cynk są kofaktorami i donorami grup metylowych w procesach przemiany iAs do MMA i DMA. Wiele badań wykazało korelacje między wydajnością metylacji iAs a spektrum niekorzystnych zmian wynikających z ekspozycji na ten pierwiastek, co zostało szczegółowo omówione w publikacjach przeglądowych. Z tego względu zaobserwowane zależności między spożyciem wybranych składników pokarmowych a intensywnością sygnału metabolitów mogą również pośrednio odzwierciedlać efektywność procesu metylacji iAs, a tym samym nasilenie negatywnych skutków zdrowotnych związanych z ekspozycją na arsen. Ze względu na brak podobnych badań dotyczących korelacji między wielkością spożycia składników pokarmowych a zmianami w profilu metabolicznym osób narażonych na arsen, dyskusja zawarta w artykułach koncentrowała się na analizie skutków podwyższonych stężeń potencjalnie zidentyfikowanych metabolitów. Ponadto, omawiane były potencjalne korzyści wynikających ze zwiększonego spożycia donorów grup metylowych i kofaktorów uczestniczących w metabolizmie iAs. Zależności między wielkością spożycia wybranych składników pokarmowych a intensywnością sygnału metabolitów oraz potencjalne niekorzystne skutki zdrowotne związane z tymi zmianami w profilu metabolicznym mężczyzn przedstawiono odpowiednio na Rycinie 8.



Rycina 8. Statystycznie istotne ujemne zależności między wielkością spożycia składników pokarmowych zaangażowanych w metabolizm arsenu a intensywnością sygnału metabolitów oraz potencjalne niekorzystne skutki zdrowotne związane z tymi zmianami w grupie pracowników.
DNA – kwas deoksyrybonukleinowy, iAs – arsen nieorganiczny; wit. – witamina.

W badaniach innych autorów wyższa intensywność sygnału lub stężenie we krwi lub moczu potencjalnie zidentyfikowanych metabolitów, które wykazały zależności z analizowanymi składnikami pokarmowymi, była związana z różnymi zmianami i skutkami zdrowotnymi. Najczęściej obserwowane efekty obejmowały uszkodzenia DNA, nasilenie stresu oksydacyjnego, nasilenie stanów zapalnych oraz zaburzenia w szlakach (m.in. glukuronidacji, metabolizmu energetycznego). Ponadto, zaobserwowano zmiany skórne, cukrzycę, przewlekłą chorobę nerek, a także nowotwory różnych narządów (płuc, żołądka, wątroby, jelita grubego, pęcherza moczowego). Nasilenie powyższych, niekorzystnych zmian i skutków zdrowotnych związanych z potencjalnie zidentyfikowanymi metabolitami może być zmniejszone poprzez wyższe spożycie składników pokarmowych, z którymi wykazano zależności (metioniny, witamin B_2, B_6 i B_{12}, folianów i cynku).

Wykazane zależności między spożyciem składników pokarmowych a zmianami w metabolizmie mogą stanowić podstawę do opracowania skutecznych strategii profilaktycznych i interwencyjnych, ukierunkowanych na zmniejszenie negatywnych skutków zdrowotnych wynikających z narażenia na arsen. Nasze badania wskazały na kluczową rolę metioniny, folianów, witamin z grupy B (B_2, B_6, B_{12}) oraz cynku jako kofaktorów i donorów

grup metylowych w procesie przemian arsenu. Spożycie tych składników pokarmowych może potencjalnie zwiększyć wydajność metylacji iAs, co jest kluczowe dla jego eliminacji z organizmu w formie mniej toksycznych metabolitów. Poprawa wydajności metylacji iAs poprzez odpowiednie interwencje dietetyczne może potencjalnie przyczynić się do zmniejszenia nasilenia niekorzystnych zmian oraz ryzyka rozwoju chorób przewlekłych związanych z narażeniem na arsen, takich jak choroby sercowo-naczyniowe, cukrzyca typu 2, oraz różnego rodzaju nowotwory. Na podstawie wyników badań można rozważyć wdrożenie programów edukacyjnych, które promowałyby spożycie produktów bogatych w metioninę, foliany, witaminy B_2, B_6 i B_{12} oraz cynk. Takie interwencje są szczególnie potrzebne w populacjach o zwiększonym ryzyku narażenia na arsen, takich jak pracownicy czy mieszkańcy obszarów przemysłowych. Włączenie edukacji żywieniowej do programów zdrowia publicznego w regionach wysokiego ryzyka, mogłoby tym samym stanowić istotny krok w kierunku poprawy ogólnego stanu zdrowia populacji.

Podsumowując, wyniki badań mogą stanowić podstawę do opracowania skutecznych strategii prewencyjnych i interwencyjnych, które mogą złagodzić negatywne skutki zdrowotne związane z narażeniem na arsen, poprzez optymalizację diety i monitorowanie biomarkerów narażenia.

6. Mocne strony oraz ograniczenia przeprowadzonych badań

Przeprowadzone badania eksperymentalne są pierwszymi, w których połączono dane o wielkości spożycia składników pokarmowych z wynikami z zakresu metabolomiki niecelowanej. W badaniach wykorzystano wyniki stężeń tAs w moczu, oceniono spożycie wybranych składników pokarmowych zaangażowanych w metabolizm arsenu, zastosowano jedną z najnowocześniejszych technik analitycznych w celu kompleksowej analizy profilu metabolicznego. Pomimo starań, badania mają kilka ograniczeń.

Badania przeprowadzono jedynie u osób dorosłych narażonych na arsen. Doskonałym uzupełnieniem byłyby badania przeprowadzone w grupach dzieci. Takie analizy zostały wykonane w grupach dzieci środowiskowo narażonych na arsen, jednak mała liczebność grup spowodowała, że nie można było sformułować z tych badań rzetelnych wniosków. W opublikowanych badaniach skupiono uwagę wyłącznie na narażenie na arsen, bez uwzględnienia innych czynników, które mogły mieć wpływ na uzyskane wyniki. Kolejnym ograniczeniem było wykorzystanie kwestionariusza 3-dniowego bieżącego notowania spożywanych produktów, potraw i napojów, który podobnie jak inne metody oceny sposobu żywienia oprócz zalet (m.in. łatwość wykonania, niskie koszty, nie wymaga odtwarzania sposobu żywienia z pamięci) ma również wady. Uczestnicy zostali poinstruowani i poproszeni o staranny zapis, mimo to mogły wystąpić błędy w zapisie wynikające np. ze zmian sposobu żywienia związanych z koniecznością notowania lub nieprecyzyjnego szacowania wielkości porcji. Od uczestników badania zebrano tylko jedną próbkę moczu, co może stanowić kolejne ograniczenie. Zebranie kilku próbek w różnych odstępach czasu uwzględniłoby narażenie długotrwałe. Nie uwzględniono również analizy stężeń form specjacyjnych arsenu w moczu we wszystkich analizowanych próbkach.

Wyżej wymienione ograniczenia, mogły mieć wpływ na uzyskane wyniki. Przeprowadzone badania pozwoliły jednak na głębsze zrozumienie zmian w metabolizmie pod wpływem narażenia na arsen oraz uwypukliły rolę wielkości spożycia składników pokarmowych w prewencji tych zmian. Badania te stanowią punkt wyjścia do przyszłych badań analizujących zależność między sposobem żywienia a profilem metabolicznym osób środowiskowo narażonych, a także do celowanych analiz metabolomicznych.

7. Wnioski

Na podstawie przeprowadzonego przeglądu piśmiennictwa oraz uzyskanych wyników z przeprowadzonych badań eksperymentalnych pozytywnie zweryfikowano hipotezę główną oraz hipotezy szczegółowe, co pozwoliło na sformułowanie poniższych wniosków.

1. Wyniki przedstawione w opublikowanych pracach przeglądowych oraz wyniki własnych badań eksperymentalnych wskazują na kluczową rolę składników pokarmowych zaangażowanych w metabolizm arsenu (metioniny, folianów, witamin B_2, B_6, B_{12}, cynku) w zmniejszaniu niekorzystnych zmian w metabolizmie związanych z narażeniem na arsen. Podkreśla to znaczenie odpowiedniego spożycia tych składników pokarmowych, szczególnie w populacjach środowiskowo narażonych na arsen.
2. Badania własne wykazały, że narażenie na arsen indukuje zmiany w profilu metabolicznym. W grupie osób środowiskowo narażonych na arsen vs. grupa kontrolna wykazano wyższą intensywność sygnału pochodzącą od metabolitów należących do wielu ścieżek przemian m.in. węglowodanów, aminokwasów, lipidów, nukleotydów.
3. W grupach mężczyzn narażonych na arsen zaobserwowano występowanie zależności między wielkością spożycia składników pokarmowych zaangażowanych w metabolizm arsenu a nasileniem zmian w profilu metabolicznym. Biorąc pod uwagę ujemne zależności między spożyciem metioniny i folianów (donorów grup metylowych w metabolizmie arsenu) oraz witamin B_2, B_6, B_{12} i cynku (kofaktorów reakcji metabolizmu arsenu) a intensywnością sygnału metabolitów, wydaje się, że wyższe spożycie składników pokarmowych może zmniejszyć nasilenie niekorzystnych zmian związanych z narażeniem na arsen. Z kolei dodatnie zależności między spożyciem tych składników pokarmowych a intensywnością sygnału metabolitów wymagają dalszych wyjaśnień.
4. Istnieje potrzeba edukacji ukierunkowanej na odpowiednie, adekwatne do norm spożycie składników pokarmowych zaangażowanych w metabolizm arsenu, w szczególności w populacjach narażonych na arsen. Uzyskane wyniki mogą przyczynić się do dalszych rozważań podczas opracowywania zaleceń dietetycznych dla osób narażonych na arsen.

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9. Załączniki

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Review

Influence of Dietary Compounds on Arsenic Metabolism and Toxicity. Part I—Animal Model Studies

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Abstract: Population and laboratory studies indicate that exposure to various forms of arsenic (As) is associated with many adverse health effects; therefore, methods are being sought out to reduce them. Numerous studies focus on the effects of nutrients on inorganic As (iAs) metabolism and toxicity, mainly in animal models. Therefore, the aim of this review was to analyze the influence of methionine, betaine, choline, folic acid, vitamin B_2, B_6, B_{12} and zinc on the efficiency of iAs metabolism and the reduction of the severity of the whole spectrum of disorders related to iAs exposure. In this review, which includes 58 (in vivo and in vitro studies) original papers, we present the current knowledge in the area. In vitro and in vivo animal studies showed that methionine, choline, folic acid, vitamin B_2, B_{12} and zinc reduced the adverse effects of exposure to iAs in the gastrointestinal, urinary, lymphatic, circulatory, nervous, and reproductive systems. On the other hand, it was observed that these compounds (methionine, choline, folic acid, vitamin B_2, B_{12} and zinc) may increase iAs metabolism and reduce toxicity, whereas their deficiency or excess may impair iAs metabolism and increase iAs toxicity. Promising results of in vivo and in vitro on animal model studies show the possibility of using these nutrients in populations particularly exposed to As.

Keywords: vitamins; minerals; inorganic arsenic; exposure; detoxification; metal toxicity; methylation

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1. Introduction

The metabolism of inorganic arsenic (iAs) was mainly analyzed in animal model studies, in vivo and in vitro. The iAs metabolism may vary considerably depending on the animal species [1]. Most mammals have the metabolism ability of iAs, and a limited metabolism ability has been observed in chimpanzees and marmoset monkeys [1,2]. The iAs metabolism in mice is much higher than in rats; this may be due to the fact that a large amount of DMA (dimethylarsinic acid) is accumulated in the red blood cells of rats [3]. The iAs metabolism involves alternate reactions of methylation and reduction to MMA (monomethylarsonic acid), DMA, and then these forms it are excreted by the kidneys. In most animal species, the major form of iAs excretion is DMA, and in humans, it can be excreted unchanged and as MMA and DMA [4–6]. The methylation reactions of iAs are catalyzed by an enzyme—arsenic (+3 oxidation state) methyltransferase [7]. S-adenosyl-methionine, which is the donor of methyl groups, is important in the methylation process. S-adenosyl-methionine is synthesized in the one-carbon-metabolism (OCM) pathway. Various dietary compounds are involved in the OCM, mainly as methyl group donors: methionine, choline, betaine, folic acid, and moreover, cofactors of the reaction—e.g., vitamin B_2, B_6, B_{12} and zinc (Figure 1) [8].

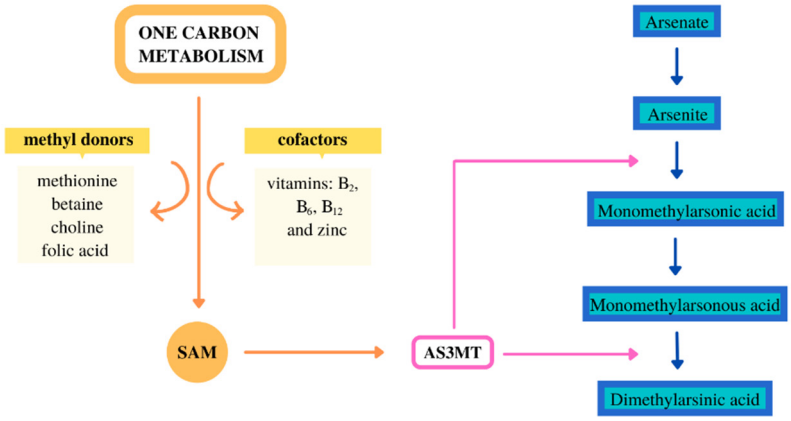

Figure 1. Arsenic metabolism and one carbon metabolism. AS3MT—arsenic (+3 oxidation state) methyltransferase; SAM—S-adenosylmethionine.

The many negative health effects caused by exposure to iAs lean, among others, to a deep analysis of the spectrum of health effects associated with As exposure and a search for ways that could reduce them on the effect of key dietary compounds on iAs metabolism and on reduction the adverse effects caused by iAs exposure. Therefore, the aim of this paper was to review and analyze the results of in vivo and in vitro studies on animal model on the influence of donors of methyl groups (methionine, choline, betaine, folic acid) and cofactors of reaction (vitamins B_2, B_6, B_{12} and zinc) on the efficiency of the metabolism process, as well as the reduction of the severity of the whole spectrum of disorders related to iAs exposure.

2. Methods

In this review, the electronic database PubMed was used. The following keywords were used to search for articles: arsenic and: methionine, betaine, choline, folic acid, folate, zinc, vitamin B, vitamin B_2, vitamin B_6, vitamin B_{12}, riboflavin, pyridoxine, cobalamin. The review was based on: The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate health care interventions: explanation and elaboration [9]. The search results in 2434 articles, excluding those unrelated to the topic of the study and those that examined the effects of complex plant extracts. Overall, 58 (in vitro and in vivo studies on animal models) original peer-reviewed articles in English were included in the analysis, which studied the effects of: methionine, betaine, choline, folic acid, vitamin B_2, B_6, B_{12}, zinc on iAs metabolism and As-induced toxicity. Articles published between 1980 and 2020 were used, of which 87.9% were published after 2000.

3. Results

3.1. Methionine

Six studies were conducted to examine the influence of methionine on iAs metabolism and decrease of iAs-induced toxicity using animal models. The experimental protocol included supplementation with different doses of methionine and administering a low-methionine diet with exposure to iAs for different time periods. Table 1 presents the results of these studies.

Table 1. Results of in vivo studies with iAs exposure and methionine treatment.

Reference	Research Model	Study Description	Main Results
Jin et al., 2010 [10]	Mice, Albino, adult, female	CG (n = 8)—sodium arsenite through drinking water 50 mg/L (orally, for 5 weeks) G1 (n = 8)—sodium arsenite through drinking water 50 mg/L (orally, for 4 weeks) and after that methionine 200 mg/kg bw/day (IP injection, for 7 days) and sodium arsenite through drinking water 50 mg/L (orally, for 7 days)	G1 vs. CG blood: DMA↑, %iAs↓, PMI↑ liver: SMI↑
Zhao et al., 2011 [11]	Mice, Albino, adult, female	CG (n = 8)—sodium arsenite through drinking water 50 mg/L (orally, for 4 weeks) G1 (n = 8)—methionine 100 mg/kg bw/day (IP injection, for 4 weeks) and sodium arsenite through drinking water 50 mg/L (orally, for 4 weeks) G2 (n = 8)—methionine 200 mg/kg bw/day (IP injection, for 4 weeks) and sodium arsenite through drinking water 50 mg/L (orally, for 4 weeks) G3 (n = 8)—methionine 400 mg/kg bw/day (IP injection, for 4 weeks) and sodium arsenite through drinking water 50 mg/L (orally, for 4 weeks)	G1, G2, G3 vs. CG blood: iAs↓, MMA↓, tAs↓ brain: DMA↓, tAs↓ G2, G3 vs. CG liver: DMA↑, %iAs↓, %DMA↑, %PMR↑ G3 vs. CG brain NO↑
Nandi et al., 2005 [12]	Rats, Wistar albino, adult, male	CG (n = 6)—sodium m-arsenite (III) 10 ppm in drinking water (orally, for 12 weeks) G1 (n = 6)—sodium m-arsenite (III) 10 ppm in drinking water (orally, for 12 weeks) and methionine solved in distilled water 25 mg/kg bw/day (orally, for 12 weeks)	G1 vs. GC blood: tAs↓ erythrocytes: LPO↔, SOD↔, CAT↔ liver: tAs↓, LPO↓, SOD↔, CAT↑ kidney: tAs↓, LPO↓, SOD↔, CAT↑
Vahter and Marafante 1987 [13]	Rabbits, Swedish loop, adult, male	CG (n = 4)—standard diet (orally, for 6 weeks) and after that [^{76}As] arsenite 0.4 mg/kg bw (IV injection, single dose) and sacrificed after 72 h G1 (n = 4)—low methionine diet 1.3 mg/kg (orally, for 6 weeks) and after that [^{76}As] arsenite 0.4 mg/kg bw (IV injection, single dose) and sacrificed after 72 h	G1 vs. CG liver and microsomes: ^{76}As↑ urine: tAs↓, iAs↑, DMA↓
Canet et al., 2012 [14]	Mice, C57BL/6, adult, male	CG (n = 3)—control diet (orally, for 8 weeks) and after that arsenic trioxide 0.2 mg/kg (orally, single dose) and sacrificed after 24 h G1 (n = 5)—methionine-and choline deficient diet (orally, for 8 weeks) and after that arsenic trioxide 0.2 mg/kg (orally, single dose) and sacrificed after 24 h	G1 vs. CG liver: %MMA↑, %DMA↓, %pentavalent iAs↑, expression of Mrp1 protein↑ kidney: % iAs↑, %DMA↓ urine: %tAs↑, %iAs↓, %trivalent iAs↑
		CG (n = 3)—control diet (orally, for 8 weeks) and after that sodium arsenate 0.75 mg/kg (orally, single dose) and sacrificed after 24 h G1 (n = 5)—methionine-choline deficient diet (orally, for 8 weeks) and after that sodium arsenate 0.75 mg/kg (orally, single dose) and sacrificed after 24 h	G2 vs. CG liver: %MMA↑, %DMA↓, %pentavalent iAs↑, expression of Mrp1 protein↑ kidney: %iAs↑, %DMA↓ urine: %tAs↔, %iAs↔, %trivalent iAs↔
Pal and Chatterjee 2004 [15]	Rats, Wistar, adult, male	CG (n = 6)—sodium arsenite 5.55 mg/kg bw/day (IP injection, for 21 days) G1 (n = 6)—sodium arsenite 5.55 mg/kg bw/day (IP injection, for 21 days) and after that 18% protein diet supplemented with 0.8% methionine (orally, for 5 days prior to sacrifice)	G1 vs. CG blood: glucose↑ liver: free amino acid nitrogen↓, pyruvic acid↑ kidney: free amino acid nitrogen↑, GPT↑

↑—significant increase; ↓—significant decrease; ↔—no significant changes; ALT—alanine aminotransferase; CAT—catalase; CG—control group; DMA—dimethylarsinic acid; G1—Group 1; G2—group 2; G3—group 3; GPT—glutamate-pyruvate transaminase; iAs—inorganic arsenic; IP—intraperitoneally; IV–intravenous; LPO—lipid peroxidation; MMA—monomethylarsonic acid; Mrp1—multidrug resistance-associated protein 1; NO—nitric oxide; PMI—primary methylation index; PMR—primary methylation ratio; SMI—secondary methylation index; SOD—superoxide dismutase; tAs—total arsenic species.

3.1.1. Methionine—iAs Metabolism

The effects of supplementation and deficiency of methionine on iAs metabolism were evaluated by conducting five studies. In three studies, the metabolism iAs was altered by supplementation with methionine. The results showed a decrease in the levels of total arsenic (tAs), iAs, MMA in the blood, levels of tAs and %iAs in the liver, as well as levels of DMA and tAs in the brain. This study also revealed increased levels of DMA, primary methylation index in the blood, as well as enhanced levels of %DMA, primary methylation ratio, and secondary methylation index in the liver [10–12]. Two studies analyzed the influence of a low-methionine diet on iAs metabolism. This diet showed a negative effect which can be attributed to the disposition of iAs species in the tissues, resulting in increased concentration of iAs, %monomethyl-As, and pentavalent iAs, but decreased levels of dimethyl-As in the liver, and increased %iAs and decreased dimethyl-As in the kidney [13,14]. These studies also showed differences in the concentrations of As species in the urine sample. In the study by Vahter and Marafante [13], decreased levels of tAs, DMA and increased levels of iAs were observed. Furthermore, the urine sample of mice exposed to arsenic trioxide (accounting for clinical exposure) exhibited increased excretion of tAs and trivalent iAs species, but no statistically significant differences in arsenic excretion were observed in mice exposed to sodium arsenate (accounting for environmental exposure). These two groups of mice also revealed the increased expression of multidrug resistance-associated protein 1 (arsenic species transporter) [14].

3.1.2. Methionine—Toxicity of iAs

The protective effect of methionine on the toxicity of iAs was analyzed in three studies. In a study using an animal model, exposure to iAs and supplementation with methionine showed hepatoprotective and renoprotective effects (decreased lipid peroxidation, and increased activity of antioxidant enzymes in kidney and liver) [12]. Methionine supplementation also had a beneficial effect on glucose homeostasis (restored normal blood glucose level, liver pyruvic acid level, free amino acid nitrogen concentration in the liver and kidney, glutamate-pyruvate transaminase activity in the kidney) in the group of rats fed with a methionine-rich diet and exposed to iAs [15].

In a study by Zhao et al. [11], it was shown that administration of methionine alleviated the negative effects of iAs exposure associated with elevated levels of nitric oxide in the brain.

3.1.3. Methionine—Summary

In summary, the in vivo studies in an animal model exposed to iAs showed that methionine supplementation had hepatoprotective, renoprotective, neuroprotective, and antidiabetic effects, and potentially may also increase iAs metabolism. The beneficial effect was observed regardless of the method of administration, time of administration and dose of both methionine (intraperitoneally or orally for 5 days or 1, 4, 12 weeks; in different doses in range 25–400 mg/kg bw day and 0.8% of diet), as well as iAs (intraperitoneally or orally; for 21 days or 4, 12 weeks; in different doses: 50 mg/L, 10 ppm in drinking water, 5 mg/kg bw/day).

In turn, in two studies, the use of low-methionine diet for a long period of time (orally for 6 or 8 weeks) showed a negative effect on iAs metabolism (after just one dose of iAs—orally or intravenously in range 0.2–0.75 mg/kg bw).

3.2. Choline

The effect of choline on metabolism and on the reduction of iAs-induced toxicity was analyzed in four studies (two in vivo using animal models and two in vitro). The results are summarized in Table 2.

Table 2. Results of in vivo and in vitro studies with iAs exposure and choline treatment.

Reference	Research Model	Study Description	Main Results
Vahter and Marafante 1987 [13]	Rabbits, Swedish loop, adult, male	CG (n = 4)—standard diet (orally, for 6 weeks) and after that [^{76}As] arsenite 0.4 mg/kg bw (IV injection, single dose) and sacrificed after 72 h G1 (n = 4)—choline deprived diet 1.3 mg/kg (orally, for 6 weeks) and after that [^{76}As] arsenite 0.4 mg/kg bw (IV injection, single dose) and sacrificed after 72 h	G1 vs. CG liver, lung, microsomes: ^{76}As↑ urine: tAs↓, iAs↔, MMA↑, DMA↓
Tice et al., 1997 [16]	Mice, B6C3Fl, adult, male	G1 (n = 4)—choline-sufficient diet (orally, for 1 week) after that choline-sufficient diet (orally, for 2 weeks) and sodium arsenite 2.5 or 5 or 10 mg/kg (orally, single dose) G2 (n = 4)—choline-sufficient diet (orally, for 1 week) and after that choline-deficient diet (orally, for 2 weeks) and sodium arsenite 2.5 or 5 or 10 mg/kg (orally, single dose)	in G1 bone marrow: MN-PCE↔, %PCE↔ liver parenchymal cells: DNA migration↓ bladder cells: DNA migration↓ lung cells: DNA migration↔ skin cells: DNA migration↔ bone marrow: MN-PCE↔, %PCE↓
			in G2 urine: tAs↓, DMA↓ liver parenchymal cells: DNA migration↔ bladder cells: DNA migration↔ lung cells: DNA migration↔ skin cells: DNA migration↓ bone marrow: MN-PCE↔, %PCE↔
		G1 (n = 4)—choline-sufficient diet (orally, for 1 week) after that choline-sufficient diet (orally, for 2 weeks) and sodium arsenite 2.5 or 5 or 10 mg/kg/day (orally, for 4 days) G2 (n = 4)—choline-sufficient diet (orally, for 1 week) and after that choline-deficient diet (orally, for 2 weeks) and sodium arsenite 2.5 or 5 or 10 mg/kg/day (orally, for 4 days)	in G1 liver parenchymal cells: DNA migration↓ bladder cells: DNA migration↓ lung cells: DNA migration↔ skin cells: DNA migration↔ bone marrow: MN-PCE↑, %PCE↓
			in G2 liver parenchymal cells: DNA migration↔ bladder cells: DNA migration↔ lung cells: DNA migration↔ skin cells: DNA migration↓ bone marrow: MN-PCE↑, PCE↔

Table 2. *Cont.*

Reference	Research Model	Study Description	Main Results
Sun et al., 2006 [17]	cardiomiocyte (Guinea Pig, adult, female and male)	CG (n = 8)—arsenic trioxide 1.6 mg/kg (IV injection, single dose) G1 (n = 7)—choline 8 mg/kg (single dose) and after that arsenic trioxide 1.6 mg/kg (IV injection, single dose)	G1 vs. CG in vivo: after 120 min—QTc prolongation↓
		CG (n = 8)—arsenic trioxide 50 μM (single dose) G1 (n = 8)—choline 1 mM (single dose) and after that arsenic trioxide 50 μM (single dose)	G1 vs. CG in vitro: APD prolongation↓, $I_{Ca\text{-}L}$↓
		CG (n = 8)—arsenic trioxide 50 μM (single dose) G1 (n = 8)—choline 1 mM (single dose) and arsenic trioxide 50 μM (single dose) and KCl 60 mM (single dose)	G1 vs. CG in vitro: changes of $[Ca^{2+}]_i$↓
Song et al., 2012 [18]	Chick embryos, White Leghorn	CG (n = 8)—sodium arsenite 100 nM (injected into the center of the egg yolk, incubation for 3 days) G1 (n = 6)—choline 25 μg/μL (injected into the center of the egg yolk, incubation for 3 days) and sodium arsenite 100 nM (injected into the center of the egg yolk, incubation for 3 days) G2 (n = 6)—choline 50 μg/μL (injected into the center of the egg yolk, incubation for 3 days) and sodium arsenite 100 nM (injected into the center of the egg yolk, incubation for 3 days)	G1 vs. CG survival rate↑, body weight↑, relative extraembryonic vascular area↑, neural tube closure defects↓ whole embryo, brain, spine: fluorescence signals of Nanog↓, fluorescence intensity of SCP1↓, fluorescence signals of Tuj-1↑, fluorescence intensity of 5-mec↑ spinal cord: positive signal of Nanog↓, positive signal of SCP1↓, positive signal of 5-mec↑, % of survival cells↑ expression of: DNMT3a↑, DNMT1↑, Bcl-2↑, Bax↓, caspase-3↑ protein level of: DNMT3a↑, DNMT1↑
			G2 vs. CG survival rate↔, body weight↔, relative extraembryonic vascular area↔, neural tube closure defects↔ whole embryo, brain, spine: fluorescence signals of Nanog↔, fluorescence intensity of SCP1↔, fluorescence signals of Tuj-1↔, fluorescence intensity of 5-mec↔, % of MOD↔ spinal cord: positive signal of Nanog↔, positive signal of SCP1↔, positive signal of 5-mec↔, % of survival cells↔ expression of: DNMT3a↔, DNMT1↔, Bcl-2↔, Bax↔, caspase-3↔ protein level of: DNMT3a↔, DNMT1↔

↑—significant increase; ↓—significant decrease; ↔—no significant changes; $[Ca^{2+}]$i—intracellular calcium; 5-mec—anti-5-methylcytidine; APD—action potential duration; Bax—BCL2-associated X protein; Bcl-2—B-cell lymphoma protein 2 alpha; CG—control group; DMA—dimethylarsinic acid; DNA—deoxyribonucleic acid; DNMT1—DNA methyltransferase 1; DNMT3a—DNA methyltransferases 3A; G1—group 1; G2—group 2; iAs—inorganic arsenic; ICa-L—L type calcium currents; IV—intravenous; MMA—monomethylarsonic acid; MN-PCE—micronuclei in polychromatic erythrocytes; MOD—mean optical densities; PCE—polychromatic erythrocyte; QTc—corrected QT interval; SCP1—Small C-terminal domain phosphatase 1; tAs—total arsenic species; Tuj-1—β3 Tubulin.

3.2.1. Choline—iAs Metabolism

The association between choline-deficient diet and iAs metabolism was analyzed in two studies. Overall, treatment with a choline-deficient diet resulted in adverse effects, like decreased urinary excretion of tAs and DMA in the urine [13,16]. In addition, Vahter and Marafante [13] observed higher levels of MMA in urine, but increased tAs concentration in the liver, microsomes, and lungs.

3.2.2. Choline—Toxicity of iAs

An in vitro study with guinea pig cardiomyocytes revealed the cardioprotective effect of choline (decreased QT prolongation, L-type calcium currents, and intracellular calcium concentration, which were enhanced by iAs) [17].

In chick embryos, neuroprotective effects of different doses of choline (25 μg/μL and 50 μg/μL) on halting the development of a neural tube defect were analyzed [18]. Only lower doses of choline were found to be effective in reducing the negative effects of iAs (increasing the survival rate and relative extra-embryonic vascular area, and reducing neural tube closure defects). In addition, it has been shown to alleviate the adverse effects of iAs exposure, such as the inhibition of differentiation of neural stem cells into neurons (which decreased the fluorescence signals of nanog and small C-terminal domain phosphatase 1, but increased the fluorescence signals of β3-Tubulin), decreased hypomethylation in the nervous system and spinal cord (which resulted, among other effects, in increased fluorescence intensity of anti-5-methylcytidine, and enhanced expression of DNA (deoxyribonucleic acid) methyltransferase 3A and DNA methyltransferase 1), and decreased apoptosis in chick embryos (which resulted in decreased expression of BCL2-associated X protein and increased expression of B-cell lymphoma protein 2 alpha).

Tice et al. [16] analyzed the influence of choline-deficient diet on iAs-induced toxicity. In the group of rats fed a choline-deficient diet and exposed to iAs (once or four times), DNA damage, particularly in skin cells (decreased DNA migration) and chromosomal damage in bone marrow (increased frequency of micronucleated polychromatic erythrocytes), was observed.

3.2.3. Choline—Summary

Both in vitro and in vivo studies have shown that choline supplementation can reduce the adverse changes induced by iAs in the cardiovascular and nervous systems. In vivo and in vitro studies showed the cardioprotective effect, which was noticeable after a single dose of choline (8 mg/kg or 1 mM before, simultaneously with the exposure to iAs—1.6 mg/kg or 50 μM). In turn, neuroprotective effect was depended on the dose of choline (25 or 50 μg/μL), and was only observed at the lower dose.

Moreover, choline deficient diet (for a long period of time—2 or 6 weeks) and exposure to one dose of iAs (orally or intravenously in range 0.4–10 mg/kg) was found to increase DNA damage and decrease iAs excretion.

In these studies, not only deficiency, but also the dose of choline, appears to have a significant effect on the obtained results. High doses increased adverse effects.

3.3. Vitamin B_2 with Selenium

Two studies evaluated the protective effect of riboflavin (and selenium nanoparticles) on reducing the adverse effects of iAs in *Pangasianodon hypophthalmus* reared in the presence of iAs and high temperature [19,20] (Table 3).

Table 3. Results of in vivo studies with iAs exposure and vitamin B_2 treatment.

Reference	Research Model	Study Description	Main Results
Kumar et al., 2019 [19]	*Pangasianodon hypophthalmus*	CG (n = 6)—control diet (orally, for 95 days) and sodium arsenite in experimental water at 1/10th of LC_{50} (2.8 mg/L), (orally, added at 96 h) and temperature 34 °C (for 95 days) G1 (n = 6)—Se-NPs 0.5 mg/kg diet and vitamin B_2 5 mg/kg diet (orally, for 95 days) and sodium arsenite in experimental water at 1/10th of LC_{50} (2.8 mg/L), (orally, added at 96 h) and temperature 34 °C (for 95 days) G2 (n = 6)—Se-NPs 0.5 mg/kg diet and vitamin B_2 10 mg/kg diet (orally, for 95 days) and sodium arsenite in experimental water at 1/10th of LC_{50} (2.8 mg/L), (orally, added at 96 h) and temperature 34 °C (for 95 days) G3 (n = 6)—Se-NPs 0.5 mg/kg diet and vitamin B_2 15 mg/kg diet (orally, for 95 days) and sodium arsenite in experimental water at 1/10th of LC_{50} (2.8 mg/L), (orally, added at 96 h) and temperature 34 °C (for 95 days)	G1, 2, 3 vs. CG tAs: muscle↓ CTMin↓, LTMin↓, CTMax↑, LTMax↑ liver, gill, brain, kidney: CAT↓(during LTMin and LTMax) liver, gill, kidney: SOD↓ (during LTMin) brain: SOD↔ (during LTMin) liver: SOD↓ (during LTMax) gill, brain, kidney: SOD↔ (during LTMax) liver, gill, brain, kidney: GST↓, GPx↓ (during LTMin and LTMax) brain: AChE↑
Kumar et al., 2020 [20]	*Pangasianodon hypophthalmus*	CG (n = 6)—control diet (orally, for 90 days) and sodium arsenite in experimental water at 1/10th of LC_{50} (2.68 mg/L), (orally, added at 96 h) and temperature 34 °C (for 90 days) G1 (n = 6)—Se-NPs 0.5 mg/kg diet and vitamin B_2 5 mg/kg diet (orally, for 90 days) and sodium arsenite in experimental water at 1/10th of LC_{50} (2.68 mg/L), (orally, added at 96 h) and temperature 34 °C (for 90 days) G2 (n = 6)—Se-NPs 0.5 mg/kg diet and vitamin B_2 10 mg/kg diet (orally, for 90 days) and sodium arsenite in experimental water at 1/10th of LC_{50} (2.68 mg/L), (orally, added at 96 h) and temperature 34 °C (for 90 days) G3 (n = 6)—Se-NPs 0.5 mg/kg diet and vitamin B_2 15 mg/kg diet (orally, for 90 days) and sodium arsenite in experimental water at 1/10th of LC_{50} (2.68 mg/L), (orally, added at 96 h) and temperature 34 °C (for 90 days)	G1, 2, 3 vs. CG tAs: muscle↔, gill↓, kidney↓, brain↓ serum: cortisol↓, HSP 70↓, TP↑, albumin↓, globulin↑, A:G ratio↓, NBT↑, total immunoglobulin↑, myeloperoxidase↑ blood: glucose↓ liver, gill, brain, kidney: CAT↓, GST↓, GPx↓, LPO↓ liver, gill: SOD↓ brain, kidney: SOD↔ brain, muscle: AChE↑, vitamin C↑ FWG%↑, FER↑, PER↑, SGR↑, relative survival↑, cumulative mortality↓

↑—significant increase; ↓—significant decrease; ↔—no significant changes; A:G ratio—albumin globulin ratio; AChE—acetylcholine esterase; CAT—catalase; CG—control group; CTMax—critical thermal maximum; CTMin—critical thermal minimum; FER—feed efficiency ratio; FWG—final weight gain; G1—group 1; G2—group 2; G3—group 3; GPx—glutathione peroxidase; GST—glutathione-s-transferase; HSP 70—heat shock proteins 70; LC_{50}—lethal concentration; LPO—lipid peroxidation; LTMax—lethal thermal maximum; LTMin—lethal thermal minimum; NBT—nitroblue tetrazolium; PER—protein efficiency ratio; Se-NPs—selenium nanoparticles; SGR—specific growth rate; SOD—superoxide dismutase; tAs—total arsenic species; TP—total protein.

3.3.1. Vitamin B_2 with Selenium—iAs Metabolism

In the studies involving *Pangasianodon,* only tAs bioaccumulation in various organs was analyzed. Vitamin B_2 supplementation caused a reduction in tAs concentration in the liver, gills, brain, kidneys, and muscles [19,20].

3.3.2. Vitamin B_2 with Selenium—Toxicity of iAs

Supplementation with these ingredients reduced oxidative stress, increased the levels of acetylcholine in the brain, and enhanced thermal tolerance, growth performance, and immunity [19,20].

3.3.3. Vitamin B_2 with Selenium—Summary

To sum up, the two in vivo studies show that vitamin B_2 (with selenium) has a beneficial effect associated with the reduction of tAs bioaccumulation and with reduction of adverse iAs effect in digestive, nervous, respiratory, immune, and urinary systems. These two studies were carried out in the same animal model and used the same vitamin B_2 and iAs doses, as well as exposure time. Three doses of vitamin B_2 were used (5, 10, 15 mg/kg diet) and the beneficial effect was already demonstrated at the lowest dose. The favorable results could also be influenced by the long duration of the vitamin administration, the study lasted up to 96 days. It is not known whether supplementation with this vitamin would be equally effective in studies in other animal models and under different experimental protocols.

3.4. Vitamin B_{12}

In the three studies performed using animal models exposed to iAs, protective effects of vitamin B_{12}, such as enhanced metabolism and reduced toxicity, are analyzed (Table 4).

3.4.1. Vitamin B_{12}—iAs Metabolism

In one study, the association between vitamin B_{12} and iAs metabolism was examined. In this study conducted in a rat model, supplementation with vitamin B_{12} increased the excretion of tAs in the urine, which subsequently resulted in reduced tAs levels in the blood and liver [21].

3.4.2. Vitamin B_{12}—Toxicity of iAs

The protective action of vitamin B_{12} on the changes induced by iAs in the digestive system has been studied. In a study carried out in an animal model exposed to iAs, vitamin B_{12} was shown to have a hepatoprotective effect. In the liver of rats, increased activity of antioxidant markers, decreased activity of pro-oxidative markers and liver enzymes, beneficial effect on apoptotic changes, and decreased histopathological damage were observed [21]. In a rat model, Chen and Whanger [22] analyzed, inter alia, the relationship between vitamin B_{12} intake and levels of metallothionein. In the group that received vitamin B_{12} and was exposed to iAs, reduced levels of metallothionein in the liver were observed compared to the group that did not receive vitamin B_{12}.

Acharyya et al. [23] showed that vitamin B_{12} supplementation caused a slight decrease in adverse effect of iAs not only in the digestive, but also urinary, and respiratory systems. The following changes were observed in the liver: decreased lipid peroxidation, decrease in the levels of liver function markers, DNA breakage, extensive damage to the histoarchitecture, and increased activity of antioxidants enzymes. In the kidney, improved levels of function marker and decreased tissue degeneration were noticed. In addition, decreased concentration of free radical products and increased activity of antioxidants were observed in the intestine and lungs.

Table 4. Results of in vivo studies with iAs exposure and vitamin B_{12} treatment.

Reference	Research Model	Study Description	Main Results
Majumdar et al., 2010 [21]	Rats, Albino, adult, male	CG (n = 6)—arsenic trioxide 3 mg/kg bw/day (orally, for 30 days) G1 (n = 6)—vitamin B_{12} 0.63 µg/kg bw/day (orally, for 30 days) and arsenic trioxide 3 mg/kg bw/day (orally, for 30 days)	G1 vs. CG urine: tAs↑ blood, liver: tAs↓ hepatic mitochondria: NO↓, TBARS↓, OH^-↓, SOD↔, CAT↔, GSH↑ damaging changes in liver histology↓ liver: ALT↓, AST↓, ACP↓, iNOS↔ Mitochondrial Swelling↓, Mitochondrial Cytochrome c oxidase↑, Mitochondrial Calcium↑, Mitochondrial Ca^{2+}-ATPase activity↑, Mitochondrial Caspase 3 activity↓ hepatic cell DNA smearing↓
Chen and Whanger 1994 [22]	Rats, Weanling and Sprague-Dawley, adult, male	CG (n = 5)—arsenite 0–150 µg/g (orally, for 8 weeks) G1 (n = 5)—vitamin B_{12} sufficient diet 100 µg/kg (orally, for 8 weeks) and arsenite 0–150 µg/g (orally, for 8 weeks)	G1 vs. CG liver: levels of MT↓
Acharyya et al., 2015 [23]	Rats, Albino, adult, female	CG (n = 6)—drinking water (orally, for 28 days) G1 (n = 6)—vitamin B_{12} 0.07 µg/100 g bw/day dissolved in water 200 µL/day (orally, for 28 days) and sodium arsenite 0.6 ppm/100 g bw/day (orally, for 28 days)	G1 vs. CG serum: ALP↔, AST↔, LDH↔, uric acid↔ hepato-somatic index↔, reno-somatic index↑ liver: MDA↔, XO↔, CAT↔ lung: CD↔, NPSH↔ intestine: MDA↔, CD↔, CAT↔

↑—significant increase; ↓—significant decrease; ↔—no significant changes; ACP—acid phosphatase; ALP—alkaline phosphatase; ALT—alanine aminotransferase; AST—aspartate amino transferase; Ca^{2+}-ATPase—calcium adenosine triphosphatase; CAT—catalase; CD—conjugated diene; CG—control group; DNA—deoxyribonucleic acid; G1—group 1; GSH—glutathione; iNOS—inducible nitric oxide synthase; LDH—lactate dehydrogenase; MDA—malondialdehyde; MT—metallothionein; NO—nitric oxide; NPSH—nonprotein-soluble thiol; OH—-hydroxide; tAs—total arsenic species; TBARS—thiobarbituric acid reactive substances; XO—xanthine oxidase.

3.4.3. Vitamin B_{12}—Summary

The aforementioned in vivo studies provide evidence for the beneficial effects of vitamin B_{12} supplementation on iAs metabolism and reduction of unfavorable changes in the digestive as well as, to a lesser extent, in the urinary and respiratory systems. The beneficial effect in the study on the rat model was observed regardless of the experimental protocol—the same method of vitamin B_{12} and iAs administration (orally), duration (for 28, 30 days or 8 weeks), but different doses of vitamin B_{12} administration (0.07 µg/100 g bw/day, 63 µg bw/day, 100 µg/kg diet) and iAs (3 mg/kg bw/day, 150 µg/g, 0.6 ppm/100 g bw/day).

3.5. Folic Acid

The influence of folic acid on metabolism and reduction of iAs-induced toxicity was investigated in 15 animal model studies and in 4 in vitro studies (Table 5).

3.5.1. Folic Acid—iAs Metabolism

The studies performed on rats exposed to iAs proved the beneficial effect of folic acid supplementation, which was associated with increased tAs content in the urine samples and decreased levels in the blood, liver, and stool samples [21,24,25]. A similar effect was observed in a study conducted by Huang et al. [26] in mice (wild type and As3mt knockout) fed a low or high fat diet with supplementation with folic acid and exposed to iAs. This outcome was evident only in wild-type female mice exposed to iAs and higher dose of folic acid. A low-fat diet supplemented with folic acid increased iAs metabolism (decreased %iAs and increased %dimethyl As in urine), whereas a high-fat diet decreased its metabolism (decreased tAs, %iAs and increased %monomethyl and %dimethyl As in the liver). The group of mice (As3mt knockout) which have a limited metabolism capacity of iAs exhibited no significant differences in the levels of As species in the urine and liver. However, in another study, supplementation with folic acid increased iAs metabolism in the maternal livers, resulting in a reduced concentration of iAs and increased methylation ratios, but no influence on plasma concentrations of S-adenosylmethionine and S-adenosylhomocysteine was noticed. In the fetal livers, the beneficial effect of folic acid on iAs metabolism was not observed, and increased concentration of only S-adenosylhomocysteine was detected [27].

The adverse effects of folic acid deficiency were observed in two studies carried out in mice exposed to iAs. A folate-deficient diet decreased the urinary excretion of As in the wild-type mice (decreased %tAs in the urine) [28,29]. Moreover, folate-binding protein-1 (Folbp1) mice showed less efficient iAs metabolism—which was evidenced by the decreased percentage of arsenate and increased %DMA in the urine, but did not affect the plasma concentrations of S-adenosylmethionine and S-adenosylhomocysteine compared to wild-type mice [28]. However, no significant changes were observed in the group of Folbp2 mice, which showed no differences in the excretion of As, but revealed altered plasma concentrations of S-adenosylmethionine and S-adenosylhomocysteine [29].

3.5.2. Folic Acid—Toxicity of iAs

The protective effect of folic acid following exposure to iAs has been analyzed with respect to a reduction in the damage caused to the digestive system. The two animal model studies demonstrated that folic acid exhibits hepatoprotective effect in rats exposed to iAs by decreasing the oxidative stress levels, lipid peroxidation, apoptosis, elevated serum levels of liver function markers, and tissue damage [21,23].

Table 5. Results of in vivo and in vitro studies with iAs exposure and folic acid treatment.

Reference	Research Model	Study Description	Main Results
Majumdar et al., 2010 [21]	Rats, Albino, adult, male	CG (n = 6)—arsenic trioxide 3 mg/kg bw/day (orally, for 30 days) G1 (n = 6)—folic acid 36 µg/kg bw/day (orally, for 30 days) and arsenic trioxide 3 mg/kg bw/day (orally, for 30 days)	G1 vs. CG urine: tAs↑ blood, liver: tAs↓ hepatic mitochondria: NO↓, TBARS↓, OH^-↓, SOD↑, CAT↑, GSH↑ damaging changes in liver histology↓ liver: iNOS↓, ALT↓, AST↓, ACP↓ Mitochondrial Swelling↓, Mitochondrial Cytochrome c oxidase↑, Mitochondrial Calcium↑, Mitochondrial Ca^{2+}-ATPase activity↑, Mitochondrial Caspase 3 activity↓ hepatic cell DNA smearing↓
Majumdar et al., 2009 [24]	Rats, Albino, adult, male	CG (n = 6)—arsenic trioxide 3 mg/kg bw/day (orally, for 30 days) G1 (n = 6)—folic acid 36 µg/kg bw/day (orally, for 30 days) and arsenic trioxide 3 mg/kg bw/day (orally, for 30 days)	G1 vs. CG urine: tAs↑ plasma and pancreatic islet cell mitochondria: NO↓, MDA↓, OH^-↓, SOD↑, CAT↑, GSH↑ pancreatic islet cell mitochondria and lymphocyte: DNA smearing↓
Choudhry et al., 2009 [25]	Rats, Long Evans Norwegian Strains, adult, male	CG (n = 6)—arsenic 1 mg/L in drinking water (orally, for 2 weeks) G1 (n = 6)—folic acid 200 µg/day (orally, for 2 weeks) and arsenic 1 mg/L in drinking water (orally, for 2 weeks)	G1 vs. CG stool: bacterial count↓ tAs: in stool↓, in liver↓
Huang et al., 2018 [26]	Mice, C57BL/6J adult, male and female	CG (n = 16)—low-fat diet with folate 0.2 mg/kg/diet (orally, 6 weeks) for and arsenite 100 ppb in drinking water (orally, for 6 weeks) G1 (n = 16)—low-fat diet with folate 10 mg/kg/diet (orally, for 6 weeks) and arsenite 100 ppb (orally, for 6 weeks)	G1 vs. CG urine: tAs↔, %iAs↔, %DMAs↔ (in the male group) urine: tAs↔, %iAs↓, %DMAs↑ (in the female group)
	Mice, As3mt-KO, adult, male and female	CG (n = 16–20)—low-fat diet with folate 0.2 mg/kg/diet (orally, 6 weeks) for and arsenite 100 ppb in drinking water (orally, for 6 weeks) G1 (n = 16–20)—low-fat diet with folate 10 mg/kg/diet (orally, for 6 weeks) and arsenite 100 ppb in drinking water (orally, for 6 weeks)	G1 vs. CG urine: tAs↔, %DMAs (not detected), %MMAs (not detected)

Table 5. *Cont.*

Reference	Research Model	Study Description	Main Results
Huang et al., 2018 [26]	Mice, C57BL/6J adult, male and female	CG (n = 16)—low-fat diet with folate 0.2 mg/kg/diet (orally, for 24 weeks) and arsenite 100 ppb (orally, for 24 weeks) after that high-fat diet with folate 0.2 mg/kg/diet (orally, for 8 weeks) and arsenite 100 ppb in drinking water (orally, for 13 weeks) G1 (n = 16)—low-fat diet with folate 10 mg/kg/diet (orally, for 24 weeks) and arsenite 100 ppb in drinking water (orally, for 24 weeks) after that high-fat diet with folate 10 mg/kg/diet (orally, for 8 weeks) and arsenite 100 ppb in drinking water (orally, for 13 weeks)	G1 vs. CG FPI↔, HOMA-IR↔ (after 24 weeks on a low fat diet) FPI↓, HOMA-IR↓ (marginally significant, after 8 week on a high fat diet) liver: tAs↔, %iAs↔, %MMAs↔, %DMAs↔ (in the male group) liver: tAs↓, %iAs↓, %MMAs↑, %DMAs↑ (in the female group)
	Mice, As3mt-KO, adult, male and female	CG (n = 16–20)—low-fat diet with folate 0.2 mg/kg/diet (orally, for 24 weeks) and arsenite 100 ppb in drinking water (orally, for 24 weeks) after that high-fat diet with folate 0.2 mg/kg/diet (orally, for 8 weeks) and arsenite 100 ppb in drinking water (orally, for 13 weeks) G1 (n = 16–20)—low-fat diet with folate 10 mg/kg/diet (orally, for 24 weeks) and arsenite 100 ppb in drinking water (orally, for 24 weeks) after that high-fat diet with folate 10 mg/kg/diet (orally, for 8 weeks) and arsenite 100 ppb in drinking water (orally, for 13 weeks)	G1 vs. CG FPI↔, HOMA-IR↔ (after 24 weeks on a low fat diet and after 8 week on a high fat diet) liver: tAs↔
Tsang et al., 2012 [27]	Fetal mice (mice, CD1, adult, female)	CG (n = 12)—control diet with folate 2.2 mg/kg/diet (orally, from gestation day 1 to 18) and sodium meta-arsenite 85 ppm in drinking water (orally, from gestation day 8 to 18) G1 (n = 12)—control diet with folate 11.0 mg/kg/diet (orally, from gestation day 5 to 18) and sodium meta-arsenite 85 ppm in drinking water (orally, from gestation day 8 to 18)	G1 vs. CG maternal livers: iAs↓, methylation ratios of DMAs/MAs↑, methylation ratios of (MAs+DMAs)/iAs↑ fetal livers: tAs↔, speciated As↔, SAH↑, SAM/SAH↔, mRNA level of Dnmt3↔ body weights of fetuses↓ in G1: changed the CpG island methylation including genes associated with cancer and fetal development, altered methylation status of genes involved in the Wnt-signaling pathway

Table 5. *Cont.*

Reference	Research Model	Study Description	Main Results
Spiegelstein et al., 2003 [28]	Mice, Folbp1^{+}/$^{+}$, adult, male	CG (n = 5)—control diet with folate 2.7 mg/kg/diet (orally, for 20 days) and sodium arsenate 10 µL/g bw (IP injection, once dose) G1 (n = 5)—control diet with folate 2.7 mg/kg/diet (orally, for 20 days) and sodium arsenate 10 µL/g bw (IP injection, once dose) after that folate deficient diet 0.3 mg/kg/diet (orally, for 27 days)	G1 vs. CG urine: %As(V)↔, %As(III)↔, MMA(V), (not detected), %DMA(V)↔, %tAs↓ plasma: folate↓, SAM↔, SAH↔, SAM/SAH↔
	Mice, Folbp1^{-}/$^{-}$, adult, male	CG (n = 6)—control diet with folate 2.7 mg/kg/diet (orally, for 20 days) and sodium arsenate 10 µL/g bw (IP injection, once dose) G2 (n = 6)—control diet with folate 2.7 mg/kg/diet (orally, for 20 days) and sodium arsenate 10 µL/g bw (IP injection, once dose) after that folate deficient diet 0.3 mg/kg/diet (orally, for 27 days)	G2 vs. CG urine: %As(V)↔, %As(III)↔, MMA(V), (not detected), %DMA(V)↔, %tAs↔ plasma: folate↓, SAM↔, SAH↔, SAM/SAH↔
			G2 vs. G1 urine: %As(V)↓, %As(III)↔, MMA(V), (not detected), %DMA(V)↑, %tAs↔ plasma: folate↓, SAM↔, SAH↔, SAM/SAH↔
Spiegelstein et al., 2005 [29]	Mice, wild type, adult, male	CG (n = 5)—control diet with folate 2.7 mg/kg/diet (orally, for 20 days) and sodium arsenate 10 µL/g bw (IP injection, once dose) G1 (n = 5)—control diet with folate 2.7 mg/kg/diet (orally, for 20 days) and sodium arsenate 10 µL/g bw (IP injection, once dose) after that folate deficient diet 0.3 mg/kg/diet (orally, for 27 days)	G1 vs. CG urine: %As(V)↔, %As(III)↔, %MMA(V), (not detected), %DMA(V)↔, %tAs↓ plasma: folate↓, SAH↔, SAM↔, SAM/SAH↔
	Mice, Folbp2^{-}/$^{-}$, adult, male	CG (n = 5)—control diet with folate 2.7 mg/kg/diet (orally, for 20 days) and sodium arsenate 10 µL/g bw (IP injection, once dose) G2 (n = 5)—control diet with folate 2.7 mg/kg/diet (orally, for 20 days) and sodium arsenate 10 µL/g bw (IP injection, once dose) after that folate deficient diet 0.3 mg/kg/diet (orally, for 27 days)	G2 vs. CG urine: %As(V)↔, %As(III)↔, %MMA(V), (not detected), %DMA(V)↔, %tAs↔ plasma: folate↓, SAH↑, SAM↔, SAM/SAH↓
			G2 vs. G1 urine: %As(V)↔, %As(III)↔, %MMA(V), (not detected), %DMA(V)↔, %tAs↔ plasma: folate↓, SAH↑, SAM↓, SAM/SAH↓

Table 5. *Cont.*

Reference	Research Model	Study Description	Main Results
Acharyya et al., 2015 [23]	Rats, Albino, adult, female	CG (n = 6)—drinking water (orally, for 28 days) G1 (n = 6)—folic acid 4 µg/100 g bw/day dissolved in water 200 µL/day (orally, for 28 days) and sodium arsenite 0.6 ppm/100 g bw/day (orally, for 28 days)	G1 vs. CG serum: ALP↔, ALT↔, urea↔ hepato-somatic index↔, reno-somatic index↑ liver: MDA↔, XO↔, CAT↔
Mukherjee et al., 2006 [30]	Rats, Albino, adults, male	CG (n = 5)—arsenic trioxide 3 mg/kg bw/day (orally, for 30 days) G1 (n = 5)—folic acid 36 µg/kg bw/day (orally, for 30 days) and arsenic trioxide 3 mg/kg bw/day (orally, for 30 days)	G1 vs. CG pancreatic tissue: NO↓, MDA↓, OH^-↓, SOD↑, GSH↑, CAT↑ serum: TNF-α↔, IL-6↔ islet cell counts↑
McDorman et al., 2002 [31]	Mice, C57Bl/6J, adult, male	CG (n = 5)—folate deficient diet (orally, for 7 weeks) and sodium arsenite 0, 2.5, 5, 10 mg/kg bw/day (orally, during week 7 for 4 days at 24 h intervals) G1 (n = 5)—folic acid 5 mg/kg diet (orally, for 7 weeks) and sodium arsenite 0, 2.5, 5, 10 mg/kg bw/day (orally, during week 7 for 4 days at 24 h intervals)	G1 vs. CG after 10 mg/kg bw/day sodium arsenite: RBC folate↑, MN-PCEs/1000↓, MN-NCEs↓
Lin et al., 2018 [32]	Fetal rats (Rats, Sprague Dawley, adult, female)	CG (n = 10)—sodium arsenic 75 mg/L in drinking water (orally, for 6 weeks) G1 (n = 10)—folate 0.53 mg/kg bw/day (orally, for 6 weeks) and sodium arsenic 75 mg/L in drinking water (orally, for 6 weeks) G2 (n = 10)—folate 5.3 mg/kg bw/day (orally, for 6 weeks) and sodium arsenic 75 mg/L in drinking water (orally, for 6 weeks) G3 (n = 10)—folate 10.6 mg/kg bw/day (orally, for 6 weeks) and sodium arsenic 75 mg/L in drinking water (orally, for 6 weeks)	G1 vs. CG weight of fetus↔, weight of placenta↔, heart malformation↔ embryonic heart: relative mRNA level and relative protein level of: NkX2.5↔, GATA-4↔, TBX5↑ G2 vs. CG weight of fetus↑, weight of placenta↑, heart malformation↔ embryonic heart: relative mRNA level of: NkX2.5↑, GATA-4↑, TBX5↑ embryonic heart: relative protein level of: NkX2.5↑, GATA-4↑, TBX5↔ G3 vs. CG weight of fetus↑, weight of placenta↑, heart malformation↔ embryonic heart: relative mRNA level and relative protein level of: NkX2.5↑, GATA-4↑, TBX5↑

Table 5. *Cont.*

Reference	Research Model	Study Description	Main Results
Na et al., 2020 [33]	Fetal rats (Rats, Sprague Dawley, adult, female)	CG (n = 12)—sodium arsenic 75 mg/L in drinking water (orally, for 6 weeks) G1 (n = 12)—folic acid 0.53 mg/kg bw/day (orally, for 6 weeks) and sodium arsenic 75 mg/L in drinking water (orally, for 6 weeks) G2 (n = 12)—folic acid 5.3 mg/kg bw/day (orally, for 6 weeks) and sodium arsenic 75 mg/L in drinking water (orally, for 6 weeks) G3 (n = 12)—folic acid 10.6 mg/kg bw/day (orally, for 6 weeks) and sodium arsenic 75 mg/L in drinking water (orally, for 6 weeks)	G1 vs. CG weight of fetus↓, weight of placenta↔, heart malformation↓ fetal heart: mRNA expression levels of Mef2C↓, levels of H3AcK9↓ G2 vs. CG weight of fetus↑, weight of placenta↑, heart malformation↓ fetal heart: mRNA expression levels of Mef2C↓, levels of H3AcK9↓ G3 vs. CG weight of fetus↑, weight of placenta↑, heart malformation↓ fetal heart: mRNA expression levels of Mef2C↓, levels of H3AcK9↓
Gefrides et al., 2002 [34]	Fetal mice (Mice, heterozygotus Splotch, adult, female)	CG—arsenic acid 40 mg/kg bw/day (IP injection, once on each of gestational day 7.5 and 8.5) and sacrificed on gestational day 18.5 G1—folic acid 25 mg/kg bw/day (IP injection, gestational day 6.5 and 10.5) and arsenic acid 40 mg/kg bw/day (IP injection, once on each of gestational day 7.5 and 8.5) and sacrificed on gestational day 18.5 G2—folinic acid 2 mg/kg bw/day (IP injection, from gestational day 6.5 and 10.5) and arsenic acid 40 mg/kg bw/day (IP injection, once on each of gestational day 7.5 and 8.5) and sacrificed on gestational day 18.5	G1 vs. CG maternally lethal↑ G2 vs. CG NTDs↔
	Fetal mice (Mice wild-type Splotch, adult, female)	CG—arsenic acid 40 mg/kg bw/day (IP injection, once on each of gestational day 7.5 and 8.5) and sacrificed on gestational day 18.5 G1—folic acid 25 mg/kg bw/day (IP injection, gestational day 6.5 and 10.5) and arsenic acid 40 mg/kg bw/day (IP injection, once on each of gestational day 7.5 and 8.5) and sacrificed on gestational day 18.5 G2—folinic acid 2 mg/kg bw/day (IP injection, from gestational day 6.5 and 10.5) and arsenic acid 40 mg/kg bw/day (IP injection, once on each of gestational day 7.5 and 8.5) and sacrificed on gestational day 18.5	G1 vs. CG maternally lethal↑ G2 vs. CG NTDs↓ embryo/fetal lethality↑

Table 5. *Cont.*

Reference	Research Model	Study Description	Main Results
Gefrides et al., 2002 [34]	Fetal mice (Mice, LM/Bc and SWV litters, adult, female)	CG—arsenic acid 40 mg/kg bw/day (IP injection, once on each of gestational day 7.5 and 8.5) and sacrificed on gestational day 18.5 G1—folic acid 25 mg/kg bw/day (IP injection, gestational day 6.5 and 10.5) and arsenic acid 40 mg/kg bw/day (IP injection, once on each of gestational day 7.5 and 8.5) and sacrificed on gestational day 18.5 G2—folinic acid 2 mg/kg bw/day (IP injection, from gestational day 6.5 and 10.5) and arsenic acid 40 mg/kg bw/day (IP injection, once on each of gestational day 7.5 and 8.5) and sacrificed on gestational day 18.5	G1 vs. CG maternally lethal↑ G2 vs. CG NTDs↔ embryo/fetal lethality↔
Wlodarczyk et al., 2001 [35]	Fetal mice (Mice, Folbp2^{-}/$^{-}$, adult, female) Fetal mice (Mice, Folbp2^{+}/$^{+}$, adult, female)	CG (n = 6)—control diet with folate 2.7 mg/kg (orally, for 2–3 weeks prior to the first attempts at mating and for all pregnancy) and sodium arsenate 40 mg/kg (IP injection on gestational days 7.5 and 8.5) G1 (n = 6)—folate deficient diet 0.3 mg/kg (orally, for 2–3 weeks prior to the first attempts at mating and for all pregnancy) and sodium arsenate 40 mg/kg (IP injection on gestational days 7.5 and 8.5) G2 (n = 6)—folate deficient diet 0.3 mg/kg/diet (orally, for 2–3 weeks prior to the first attempts at mating and for all pregnancy) and sodium arsenate 40 mg/kg (IP injection on gestational days 7.5 and 8.5)	G1 vs. CG rate of exencephaly↑ G2 vs. CG rate of exen cephaly↔ G1 vs. G2 rate of exencephaly↑
Ma et al., 2015 [36]	Wild-type AB strain and Tg (cmlc2:GFP) zebrafish, embryos	CG—sodium arsenite 2 mM G1—folic acid 50 µM and sodium arsenite 2 mM G2—folic acid 100 µM and sodium arsenite 2 mM	G1, G2 vs. CG after 96 h: hatched embryos↑, survival↑, affected embryos↓ G2 vs. CG abnormal development↓, ventricle development↑, cardiac looping↑, normal erythropoiesis↑, axons in all areas of the brain↑, mRNA level of Dvr1↑
Dubey and Shea 2007 [37]	NB2a/d1 cells	CG—sodium arsenite 0.07 µm (for 24 h) G1—absence folate and sodium arsenite 0.07 µm (for 24 h)	G1 vs. CG neurofilament transport↓ perikaryal RT97↑ perikaryal phospho-NF immunoreactivity↑

Table 5. *Cont.*

Reference	Research Model	Study Description	Main Results
Nelson et al., 2007 [38]	Mice, C57BL/6, adult	CG (n = 4)—folate sufficient diet 5 mg/kg (orally, for 30 days) and sodium arsenite 1 ppm in drinking water (orally, for 30 days) G1 (n = 4)—folate deficient diet (orally, for 30 days) and sodium arsenite 1 ppm in drinking water (orally, for 30 days)	G1 vs. CG serum: folate↓ blood: homocysteine↑ zinc-finger transcription factors↓ expression of epidermal development and differentiation (hair and skin) genes↓ expression of cellular movement genes↑
Ruan et al., 2000 [39]	Fibroblasts embryo, (SWV/Fnn, adult, female)	CG—folic acid to final concentration 9 μM (for 24 h) and sodium arsenite 10 μM (for 24 h) G1—folic acid to final concentration 270 μM (for 24 h) and sodium arsenite 10 μM (for 24 h)	G1 vs. CG viability↑
		G1—folic acid to final concentration 100 μM (for 24 h) and sodium arsenite 6 or 10 μM (for 24 h)	in the G1: viability↔
		CG—folic acid to final concentration 9 μM and sodium arsenite 1 μM G1—folic acid to final concentration 90 μM and sodium arsenite 1 μM	G1 vs. CG at day 6: number of cells↔ at day 7: number of cells↑
		CG—folic acid to final concentration 9 μM (for 24 h) and dimethylarsinic acid 10 mM (for 24 h) G1—folic acid to final concentration 270 μM (for 24 h) and dimethylarsinic acid 10 mM (for 24 h)	G1 vs. CG viability↑
		G1—folic acid to final concentration 100 μM (for 24 h) and dimethylarsinic acid 3 or 10 mM (for 24 h)	in the G1: viability↔

Table 5. *Cont.*

Reference	Research Model	Study Description	Main Results
Crandall and Vorce 2002 [40]	Fibroblast Folbp2^{-}/$^{-}$	CG—sodium arsenite 100 μM (for 24 h) G1—folic acid 30 μM (for 1 week) and after that sodium arsenite 100 μM (for 24 h)	G1 vs. CG survival↔
		CG—sodium arsenite 0.3 or 3 μM G1—folic acid 7 nM (for 2 h) and sodium arsenite 0.3 or 3 μM	G1 vs. CG folic acid uptake↓
	Fibroblast Folbp2^{+}/$^{+}$	CG—sodium arsenite 100 μM (for 24 h) G1—folic acid 30 μM (for 1 week) and after that sodium arsenite 100 μM (for 24 h)	G1 vs. CG survival↑
		CG—sodium arsenite 0.3 or 3 μM G1—folic acid 7 nM (for 2 h) and sodium arsenite 0.3 or 3 μM	G1 vs. CG folic acid uptake↔

↑—significant increase; ↓—significant decrease; ↔—no significant changes; ACP—acid phosphatase; ALP—alkaline phosphatase; ALT—alanine aminotransferase; As(III)—arsenite; As(V)—arsenate; AST—aspartate amino transferase; Ca^{2+}-ATPase—calcium adenosine triphosphatase; CAT—catalase; CG—control group; DMA(V)—dimethylarsonic acid; DMAs—dimethylarsenic; DMG—dimethylglycine; DNA—deoxyribonucleic acid; Dnmt3—DNA(cytosine-5)-methyltransferase 3-alpha; Dvr1—decapentaplegic and Vg-related-1; Folbp—folate binding proteins; FPI—fasting plasma insulin; G1—group 1; G2—Group 2; G3—group 3; GSH—glutathione; H3AcK9—acetytalion of histone H3 lysine 9; IL-6—Interleukin 6; iNOS—inducible nitric oxide synthase; IP—intraperitoneally; MAs—monomethylarsenic; MDA—malondialdehyde; Mef2C—myocyte enhancer factor-2C; MMA(V)—monomethylarsonic acid; MN—micronuclei; NCEs—normochromatic erythrocytes; NF—neurofilament; NO—nitric oxide; NTDs—neural tube defects; OH—-hydroxide; PCEs—polychromatic erythrocytes; RBC—red blood cell; SAH—S-adenosylhomocysteine; SAM—S-adenosylmethionine; SOD—superoxide dismutase; tAs—total arsenic species; TBARS—thiobarbituric acid reactive substances; TNF-α—tumor necrosis factor-α; XO—xanthine oxidase.

In the two studies conducted in rats exposed to iAs, the protective effect of folic acid was associated with a reduction in oxidative stress and lipid peroxidation in the pancreatic tissue [24,30]. One of the studies demonstrated a decrease in DNA damage, which was evident by reduced DNA smearing in pancreatic islet cell mitochondria and lymphocytes [24].

In one study, the supplementation of folic acid in rats exposed to iAs showed an adverse effect on gut flora, as it resulted in a decreased bacterial count in the stool [25]. In wild-type mice exposed to iAs and fed a high-fat diet with low folate dose, adverse effects on glucose homeostasis (marginal increase in fasting plasma insulin levels and homeostasis model assessment of insulin resistance) were observed [26].

A study by Tsang et al. [27] showed that supplementation with a high dose of folic acid may exert an adverse effect on the mouse fetal liver exposed to iAs, which is manifested through changes in DNA methylation and genes associated with cancer and development.

Supplementation with folic acid alleviated the adverse effect of iAs in the urinary system by improving the reno-somatic index values and decreasing the biochemical marker of kidney function (serum urea level) in the rat kidney [23].

Three studies performed on animal models exposed to iAs demonstrated a cardioprotective effect of folic acid. Folate-sufficient diet decreased genotoxicity in mice exposed to iAs (decreased chromosomal damage by reducing the incidence of micronuclei formation in polychromatic erythrocytes and normochromatic erythrocytes) in comparison to mice fed with a folate-deficient diet and exposed to iAs [31]. Two studies conducted in female rats exposed to iAs showed that folic acid supplementation exerted a protective effect on embryonic cardiac defects. This outcome was more prominent when supplemented with higher doses of folic acid (5.3 and 10.6 mg/kg bw/day) [32,33]. Supplementation with this vitamin has been shown to increase embryonic growth and development [32,33], via upregulating the gene expression of cardiac transcription factors [32], downregulating the expression of genes involved in cardiac development, and decreasing the incidence of protein acetylation and cardiac malformation [33].

The influence of folic acid on the changes induced by iAs in the nervous system has also been studied. Supplementation with folic acid in three mice strains exposed to iAs exhibited no protective effect on reducing the frequency of neural tube defects in embryos but rather was found to increase embryo/fetal lethality; on the other hand, folate supplementation caused increased maternal lethality [34]. The study on female mice with genotypes Folbp2^{+}/$^{+}$ and Folbp2^{-}/$^{-}$ showed an increase in the incidence of exencephaly, and elevated growth was noticeable in Folbp2^{-}/$^{-}$ mice and in the group of mice fed with a folate-deficient diet [35]. In an in vitro study performed on zebrafish embryos, folic acid showed a protective effect against adverse effects of iAs. In the embryo, a beneficial effect was associated with increased survival and maintenance of normal development, via decreasing the defects in cardiac and nervous systems and upregulating the expression of decapentaplegic and Vg-related-1 protein [36]. In addition, folic acid deficiency caused an increase in the iAs-induced neurotoxicity in NB2a/dl cells by inducing changes in the neurofilament dynamics, thus resulting in decreased neurofilament transport and increased immunoreactivity of perikaryal RT97 and perikaryal phospho-NF [37].

The protective action of folic acid on the adverse effects of iAs was also analyzed in skin cells. In the one study on mice exposed to iAs, a folic acid-deficient diet negatively affected the skin cell proliferation and differentiation by decreasing the expression of key genes involved in this process (including those involved in epidermal development and differentiation) and increasing the expression of cancer-related genes (cellular movement genes) [38].

A reduction in iAs-induced toxicity was observed in SWV/Fnn embryo fibroblasts following folic acid treatment, but the effect was found to be dose-dependent. A significant reduction in cytotoxicity, resulting in the increased viability of cells, was noted at a folic acid concentration of 270 μM in the media, containing a constant concentration of As or

DMA. Furthermore, when cells were treated with higher doses of iAs or DMA, folic acid supplementation did not affect the viability of cells. Moreover, the treatment of cells with iAs and 90 μM concentration of folic acid caused an increase in the number of cells in the media at 7 days, but not at 6 days of treatment [39]. Exposure of Folbp2 null fibroblasts to iAs and supplementation with folic acid did not show any protective effect on survival, but exposure to this element certainly decreased the uptake of folic acid. In contrast, exposure of Folbp2 wild-type fibroblasts to folic acid increased the survival rate, but did not affect the folic acid uptake [40].

3.5.3. Folic Acid—Summary

The studies performed in vivo on animal models exposed to iAs have shown not only alleviating effects of folic acid on digestive, urinary, and circulatory systems, but also potentially can increase iAs metabolism. These beneficial effects were observed during oral administration with different time and doses of folic acid (for 28, 30 days; 6, 7 weeks; 5.3, 10.6 or 36 μg/kg bw/day; 5 or 10 mg/kg diet; 4 μg/100g bw/day), as well as iAs (for 4, 28, 30 days; 6 weeks; 2.5, 3, 5, 10 mg/kg bw/day; 100 ppb in drinking water; 0.6 ppb/100 g bw/day; 75 mg/L).

However, the negative effects were also observed. In the four in vivo studies, the supplementation of folic acid had an adverse effect on metabolism iAs, gut flora, DNA methylation, neural development and viability. Various modes of administration, exposure time, and dose of folic acid (orally or intraperitoneally; for 2, 13 days or 2, 8 weeks; 200 μg/day; 10, 11 mg/kg/diet; 2 or 25 mg/kg bw/day) and iAs (orally or intraperitoneally; for 2, 10 days; 2, 13 weeks; 1 mg/L; 85 ppm in drinking water; 40 mg/kg bw/day; 100 ppb in drinking water) were used in these studies. In the case of an unfavorable effect of supplementation with folic acid on iAs metabolism, the effect may result from the different diets (the combination of a high-fat diet and folic acid supplementation decreased the iAs metabolism, as opposed to a low-fat diet). The results were also influenced by species differences and different models of animals (wild-type mice and mice with limited capacity to methylation), as well as the stage of development, were also important (beneficial effect in maternal, but not in the fetal). In the remaining studies, an adverse effect may result from the use of high doses of folic acid.

The five studies carried out in mice exposure to iAs and fed with a folate-deficient diet showed adverse effects related to iAs metabolism, glucose homeostasis, development, and skin proliferation. In three studies, iAs was administered intraperitoneally (a single dose—10 μL/g bw; twice at dose—40 mg/kg), and in the two other studies, it was administered orally for 30 days or 13 weeks at a dose of 1 ppm; 100 ppb in drinking water. Despite differences in study protocols, folic acid deficiency exacerbated the adverse effects of iAs. The results could also be influenced by animal species differences (the studies were carried out in wild-type and mice Folbp1$^{-}/^{-}$ and 2$^{-}/^{-}$ mice), as well as by diet (a high-fat diet had adverse effects).

In the in vitro studies (iAs exposure ranged from 0.3–100 μM and 2–10 mM of iAs; duration of exposure 24 h), folic acid showed cardioprotective, neuroprotective and anti-cytotoxic effects. One study showed a beneficial effect with two (50 and 100 μM) doses of folic acid, with the higher dose showing a more pronounced beneficial effect. In the second of these studies, the constant concentration of folic acid brought the expected positive results, and in the third study, the beneficial effect may be due to the length of treatment with folic acid (up to 1 week). In one in vitro study, folic acid, did not increase survival, but the result was influenced by the type of cells used (cells lacking Folbp 2). In one in vitro study, it was found that folate deficiency enhanced the neurotoxicity of iAs at dose 0.07 μm for 24 h.

3.6. Vitamin B_{12} and Folic Acid

Five studies have been conducted to study the modulating effect of simultaneous supplementation with vitamin B_{12} and folic acid on iAs metabolism and toxicity in animal models (Table 6).

Table 6. Results of in vivo studies with iAs exposure and simultaneous vitamin B_{12} and folic acid treatment.

Reference	Research Model	Study Description	Main Results
Majumdar et al., 2010 [21]	Rats, Albino, adult, male	CG (n = 6)—arsenic trioxide 3 mg/kg bw/day (orally, for 30 days) G1 (n = 6)—vitamin B_{12} 0.63 µg/kg bw/day, folic acid 3 mg/kg bw/day (orally, for 30 days) and arsenic trioxide 3 mg/kg bw/day (orally, for 30 days)	G1 vs. CG urine: tAs↑ blood, liver: tAs↓ hepatic mitochondria: NO↓, TBARS↓, OH^-↓,SOD↑, CAT↑, GSH↑ damaging changes in liver histology↓ liver: iNOS↓, ALT↓, AST↓, ACP↓ Mitochondrial Swelling↓, Mitochondrial Cytochrome c oxidase↑, Mitochondrial Calcium↑, Mitochondrial Ca^{2+}-ATPase activity ↑, Mitochondrial Caspase 3 activity↓ hepatic cell DNA smearing↓
Majumdar et al., 2009 [24]	Rats, Albino, adult, male	CG (n = 6)—arsenic trioxide 3 mg/kg bw/day (orally, for 30 days) G1 (n = 6)—vitamin B_{12} 0.63 µg/kg bw/day, folic acid 36 µg/kg bw/day (orally, for 30 days) and arsenic trioxide 3 mg/kg bw/day (orally, for 30 days)	G1 vs. CG urine: tAs↑ plasma and pancreatic islet cell mitochondria: NO↓, MDA↓, OH^-↓, SOD↑, CAT↑, GSH↑ pancreatic islet cell mitochondria and lymphocyte: DNA smearing↓
Huang et al., 2018 [41]	Mice, C57BL/6J, adult, female	CG (n = 6)—vitamin B_{12} 10 µg/kg/diet, folate 2 mg/kg/diet (adequate diet) and deionized water (orally, for 1 week) after that adequate diet and sodium arsenite 100 or 1000 ppb in drinking water (orally, for 1 week) after that mating for 1 week and diet and exposure (the same that before mating) until parturition, after giving birth vitamin adequate diet and deionized water G1 (n = 6)—vitamin B_{12} 10 µg/kg/diet, folate 2 mg/kg/diet and deionized water (orally, for 1 week) and after that vitamin B_{12} 50 µg/kg/diet, folate 6 mg/kg/diet (supplemented diet) and sodium arsenite 100 or 1000 ppb in drinking water (orally, for 1 week) after that mating for 1 week and diet and exposure (the same that before mating) until parturition after giving birth adequate diet and deionized water	G1 vs. CG in the group with 100 ppb sodium arsenite: urine: MAs↑, DMAs↑, iAs↔, tAs↔ in the group with 1000 ppb sodium arsenite: urine: MAs↔, DMAs↔, iAs↔, tAs↔

Table 6. *Cont.*

Reference	Research Model	Study Description	Main Results
Huang et al., 2018 [41]	Mice, C57BL/6J, offspring, male	CG (n = 5–16)—prenatally exposed to adequate diet and sodium arsenite 100 or 1000 ppb in drinking water G1 (n = 5–16)—prenatally exposed to supplemented diet and sodium arsenite 100 or 1000 ppb in drinking water	G1 vs. CG in the group with 100 ppb sodium arsenite: 13-week-old: AUC↓ (glucose tolerance test) 14-week- old: FPI↓, HOMA-IR↓ in the group with 1000 ppb sodium arsenite: 14-week- old: FPI↓, HOMA-IR↓ liver: fraction of methylated DNA↑
	Mice, C57BL/6J, offspring, female	CG (n = 5–16)—prenatally exposed to adequate diet and sodium arsenite 100 or 1000 ppb in drinking water G1 (n = 5–16)—prenatally exposed to supplemented diet and sodium arsenite 100 or 1000 ppb in drinking water	G1 vs. CG 14-week- old: FPI↔, HOMA-IR↔
Chattopadhyay et al., 2012 [42]	Rats, Wistar, adult, female	CG (n = 6)—sodium arsenite 0.4 ppm/100 g bw/day (orally, for 24 days) G1 (n = 6)—vitamin B_{12} 0.07 µg with folic acid 4.0 µg dissolved in 0.1 mL of distilled water/100 g bw (by gavage, for 24 days) and sodium arsenite 0.4 ppm/100 g bw/day (orally, for 24 days)	G1 vs. CG hepatosomatic index↓ hepatic histoarchitecture↑ liver: ALT↓, AST↓, TP↑, MDA,↓ CD↓, SOD↑, CAT↑, NPSH↑, DNA fragmentation↓ plasma: TCH↓, TG↓, LDL↓, HDL↑
Mukherjee et al., 2006 [30]	Rats, Albino, adults, male	CG (n = 5)—arsenic trioxide 3 mg/kg bw/day (orally, for 30 days) G1 (n = 5)—vitamin B_{12} 0.63 µg/kg bw/day with folic acid 36 µg/kg bw/day (orally, for 30 days) and arsenic trioxide 3 mg/kg bw/day (orally, for 30 days)	G1 vs. CG pancreatic tissue: NO↓, MDA↓, OH^-↓, SOD↑, GSH↑, CAT↑ serum: TNF-α↓, IL-6↓ islet cell counts↑
Acharyya et al., 2015 [23]	Rats, Albino, adult, female	CG (n = 6)—drinking water (orally, for 28 days) G1 (n = 6)—vitamin B_{12} 0.07 µg/100 g bw/day with folic acid 4.0 µg/100 g bw/day dissolved in water 200 µL/day (orally, for 28 days) and sodium arsenite 0.6 ppm/100 g bw/day (orally, for 28 days)	G1 vs. CG serum: ALP↔, AST↔, ALT↔, LDH↔, uric acid↔, urea↔, creatinine↔ liver: MDA↔, NPSH↔, CAT↔, XO↔ lung: CD↔ intestine: MDA↔, CD↔, CAT↔ liver and kidney: DNA breakage↓ hepatic and renal histoarchitecture↑, reno-somatic index↑

↑—significant increase; ↓—significant decrease; ↔—no significant changes; ACP—acid phosphatase; ALP—alkaline phosphatase; ALT—alanine aminotransferase; AST—aspartate amino transferase; AUC—area under the curve; CAT—catalase; CD—conjugated diene; CG—control group; DMAs—dimethylarsenic; DNA—deoxyribonucleic acid; FPI—fasting plasma insulin; G1—group 1; GSH—glutathione; HDL—high-density lipoprotein; HOMA-IR—homeostasis model assessment-insulin resistance; iAs—inorganic arsenic; IL-6—Interleukin 6; iNOS—inducible nitric oxide synthase; LDH—lactate dehydrogenase; LDL—low-density lipoprotein; MAs—monomethylarsenic; MDA—malondialdehyde; NO—nitric oxide; NPSH—nonprotein-soluble thiol; OH^-—hydroxide; SOD—superoxide dismutase; tAs—total arsenic species; TBARS—thiobarbituric acid reactive substances; TCH—total cholesterol; TG—triglyceride; TNF-α—tumor necrosis factor-α; TP—total protein; XO—xanthine oxidase.

3.6.1. Vitamin B_{12} and Folic Acid—iAs Metabolism

The study performed on rats exposed to iAs demonstrated that the beneficial effect of supplementation with vitamin B_{12} and folic acid was associated with the more efficient excretion of tAs, which was evident by increased concentration of tAs in urine and decreased levels in blood and liver [21,24]. However, in another study, no significant changes in tAs excretion were observed in the female adult mice that were supplemented with these vitamins. In the group which received supplementation and was exposed to low doses of iAs, only an increase in the concentrations of monomethylarsenic and dimethylarsenic in the urine was shown [41].

3.6.2. Vitamin B_{12} and Folic Acid—Toxicity of iAs

Five studies using animal models aimed to determine the influence of the simultaneous application of vitamin B_{12} and folic acid on the adverse effects of iAs in the digestive system. These vitamins demonstrated hepatoprotective effects in mice, which were associated with a reduction in oxidative stress levels, lipid peroxidation, DNA fragmentation, damage to the histoarchitecture of the liver, as well as restored the serum level of liver function markers [21,42]. Moreover, they improved the lipid profile [42] and decreased hepatic mitochondrial apoptotic changes [21].

Two studies analyzed the protective effect of vitamin B_{12} and folic acid in reducing the damage to pancreatic islet cells of rats exposed to iAs. Supplementation with these vitamins decreased the production of reactive oxidants, reduced lipid peroxidation, increased the activity of antioxidative enzymes [24,30], decreased DNA damage [24], decreased the levels of inflammatory markers, and increased islet cell counts [30].

In another study, the protective effect of simultaneous application of vitamin B_{12} and folic acid on disorders associated with glucose metabolism was investigated in the offspring of mice prenatally exposed to iAs. Glucose metabolism disorders were mainly reported in male offspring, but prenatal vitamin B_{12} and folic acid supplementation reduced hyperglycemia and insulin resistance in the mice exposed to lower and higher doses of iAs, resulting in a marginal decrease in the fasting plasma insulin levels and Homeostasis Model Assessment of Insulin Resistance. Moreover, in the group including male offspring mice exposed to 1000 ppm of iAs, supplementation with these vitamins increased global DNA methylation in the liver [41].

The study by Acharyya et al. [23] investigated the influence of cotreatment of vitamin B_{12} with folic acid on the adverse effect of iAs, and found to be highly effective in restoring the damage to the digestive system. The vitamins restored normal levels of liver and renal function markers, decreased oxidative stress and lipid peroxidation in the liver, intestine, kidney, and lungs, as well as repaired DNA damage in the liver and kidney. Additionally, supplementation with these vitamins prevented hepatic and renal tissue degeneration.

3.6.3. Vitamin B_{12} and Folic Acid—Summary

The in vivo studies in animal models revealed that simultaneous supplementation with vitamin B_{12} and folic acid exerted a positive effect on iAs metabolism and alleviated adverse effects, not only in the digestive system, but also in the urinary and respiratory systems. In one of these studies, the supplementation of these vitamins had a positive effect on iAs metabolism only at a lower exposure dose, which may indicate that the beneficial properties of these vitamins did not exceed the negative effects of a higher dose of iAs, or showed a synergistic effect with this element. In the remaining studies on rat models, different doses of vitamins (vitamin B_{12}: 0.63 µg/kg bw/day; 0.07 µg/0.1 mL water/100 g bw/day; 0.07 µg/100 g bw/day and folic acid: 3 mg/kg bw/day; 36 µg/kg bw/day; 4 µg/100 g bw/day; 4 µg/0.1 mL water/100 g bw) and iAs (3 mg/kg bw/day; 0.4 or 0.6 ppm/100 g bw/day; 100, 1000 ppb in drinking water) and similar exposure times (24, 28,

30 days) were used, however, using each of these protocols, beneficial effects in reducing the adverse effects of iAs were obtained.

3.7. Zinc

The protective effect of zinc has been analyzed by performing studies in 21 animal models and 6 cell lines that were exposed to iAs. The results of these experiments are summarized in Table 7.

3.7.1. Zinc—iAs Metabolism

Zinc supplementation in the animal models exposed to iAs decreased tAs levels in liver [43], kidney [44], spleen [45], and brain [46,47], but it had no effect on tAs concentration in the blood, liver, and kidney in two studies [48,49]. Pretreatment with zinc enhanced the elimination of As, which was evidenced by a reduced concentration of As-73 in the blood, skin, muscle, and organs such as heart, lung, kidney, and small intestine, but similar effects were not observed in the liver, brain, and large intestine [50]. In one in vitro study conducted in SA7 cells exposed to iAs, zinc pretreatment resulted in decreased tAs accumulation and increased excretion [51].

3.7.2. Zinc—Toxicity of iAs

Many studies analyzed the protective effect of zinc on the digestive system. In a study conducted in common carp exposed to iAs, the anterior and mid-intestines showed increased activity of superoxide dismutase and tight junction proteins (inter alia, mRNA levels of occludin, claudin, and zonula occludens), as well as decreased levels of inflammatory markers (mRNA levels of interleukins [1β, 6], phosphorylation of inhibitor of nuclear factor kappa B, and nuclear factor kappa B nuclear translocation) and histological changes in intestines [52].

In three studies, zinc has been shown to exhibit a hepatoprotective effect, which is accompanied by a reduction in oxidative stress [48,53,54], lipid peroxidation [48,54], apoptosis [53], and damage of the liver structure [53,54], as well as with the elevation of blood levels of alanine and aspartate transaminases [48], metallothionein expression [54], and activity of δ-aminolevulinic acid dehydratase [48].

In one study, zinc supplementation after exposure of male mice to iAs did not reduce the adverse effects (oxidative stress, lipid peroxidation in the liver), while simultaneous administration showed a partial mitigating effect, mediated by increased δ-aminolevulinic acid dehydratase activity in blood, and decreased lipid peroxidation and oxidized glutathione in liver [49].

Zinc supplementation in the group of rats exposed to iAs showed an attenuating effect in regulating the biokinetics of ^{65}Zn, which was impaired in the group of rats exposed to iAs, but not treated with zinc. It was observed that zinc decreased the fast component in the liver and decreased the uptake of ^{65}Zn in the brain and liver [43].

Furthermore, the in vivo (rat liver) study carried out in the presence of iAs demonstrated that zinc deficiency increased inflammatory response (inter alia through increased production of inflammatory markers) [55].

An in vitro study showed that zinc deficiency and iAs exposure adversely affected pancreatic beta cells. In cells, deficiency resulted in, inter alia, increased apoptosis (increased poly(ADP) polymerase), DNA damage (increased the levels of a marker of DNA breaks), and decreased proliferation (decreased viable cells) were observed [56].

Table 7. Results of in vivo and in vitro studies with iAs exposure and zinc treatment.

Reference	Research Model	Study Description	Main Results
Kumar et al., 2011 [43]	Rats, Wistar, adult, male	CG (n = 8)—sodium arsenite 100 ppm in drinking water (orally, for 3 months) G1 (n = 8)—zinc sulfate 227 mg/L in drinking water (orally, for 3 months) and sodium arsenite 100 ppm in drinking (orally, for 3 months)	G1 vs. CG liver: tAs↓, Zn↑ % uptake values of ^{65}Zn: brain↓, liver↓, kidney↔, intestine↔, spleen↔, lungs↔ biological half-lives slow and fast component of ^{65}Zn in whole body↔ biological half-lives fast component of ^{65}Zn in liver↓
Wang et al., 2020 [44]	Cyprinus carpio	CG (n = 30)—arsenic trioxide 2.83 mg/L (orally, for 1 month) G1 (n = 30)—zinc chloride 1 mg/L and (orally, for 1 month) and arsenic trioxide 2.83 mg/L (orally, for 1 month)	G1 vs. CG kidney: tAs↓, Zn↔, CAT↑ protein levels of: HSP60↓, HSP70↓, HSP90↓, Beclin-1↑, LC3↓, p62↑, GRP78↔, p-PERK↓, p-eIF2a↔, pI3K↔, p-AKT↑, p-mTOR↑ mRNA levels of: Occludin↑, ZO-1↑, ZO-2↑, Claudin 3↑, Claudin 4↑, Claudin 7↑, Claudin 11↔, Claudin 15↔, GRP78↓, ATF-6↓, IRE1↓, CHOP↓, MT↑, ZnT1↑, ZnT5↑, ZIP7↑, ZIP8↑, ZIP10↑
Wang et al., 2021 [45]	Cyprinus carpio	CG—arsenic 2.83 mg/L G1—zinc 1 mg/L and arsenic 2.83 mg/L	G1 vs. CG spleen tissues: tAs↓, Ca↓, Bax/Bcl-2 ratio↓, LC3-II/LC3-I ratio↓, mRNA levels of: ATPα↑, Na^+/K^+-ATPase↑, Ca^{2+}-Mg^{2+}-ATPase↑, GRP78↓, GRP94↓, PERK↓, eIF2α↔, IRE1↔, ATF6↔, CHOP↓, Fas↓, caspase 8↓, caspase 9↓, caspase 3↓, Bax↓, Bcl-2↑, Beclin1↓, ATG-5↓, p62↑, LC3-I↔, LC3-II↓, protein levels of: caspase-3↓, p-eIF2α↓, p-PERK↓, p62↑, Beclin1↓ changes in spleen tissues (apoptosis, endoplasmic reticulum damage)↓
Kumar and Reddy 2017 [46]	Rats, Wistar, young, adult, old, male	CG (n = 6)—sodium arsenite 10 mg/kg bw/day (orally, for 1 week) G1 (n = 6)—zinc chloride 0.02% through drinking deoinized water (orally, for 1 week) and sodium arsenite 10 mg/kg bw/day (orally, for 1 week)	G1 vs. CG cerebral cortex, cerebellum, hippocampus: tAs↓, level of AChE↓, activity of AChE↑ open field behavioral tasks↑, total locomotor activity↑, exploratory behavior↑, grip strength↑, behavioral assessments on water maze↑

Table 7. *Cont.*

Reference	Research Model	Study Description	Main Results
Kadeyala et al., 2013 [47]	Rats, Wistar, 3 months old, 21 and 28 postnatal days	CG (n = 6)—sodium arsenite 100 ppm in sterile distilled water (orally, from gestation day 6 to 21 postnatal day) G1 (n = 6)—zinc 10 ppm in sterile distilled water (orally, from gestation day 6 to 21 postnatal day) and sodium arsenite 100 ppm in sterile distilled water (orally, from gestation day 6 to 21 postnatal day)	G1 vs. CG cerebral cortex, cerebellum, hippocampus: tAs↓, Mn-SOD↑, Cu/Zn-SOD↑, CAT↑, GPx↑, GR↑, GST↓, MDA↓, mRNA expression of: caspase 3↓, caspase 9↓
Modi et al., 2006 [48]	Rats, Wistar, adult, male	CG (n = 6)—sodium arsenite 2 mg/kg bw/day (orally, for 3 weeks) G1 (n = 6)—zinc sulfate 5 mg/kg bw/day (orally, for 3 weeks) and sodium arsenite 2 mg/kg bw/day (orally, for 3 weeks)	G1 vs. CG blood: tAs↔, Zn↔, ALAD↑ serum: ALT↓, AST↓ liver: tAs ↔, Zn↔, GSH↔, TBARS↓, CAT↓, ALP↑, ACP↔ kidney: tAs↔, Zn↔, GSH↔, TBARS↔, CAT↔
Modi et al., 2005 [49]	Mice, Swiss, adult, male	CG (n = 5)—sodium arsenite 2 mg/kg bw/day (IP, for 5 days) G1 (n = 5)—zinc acetate 10 mg/kg bw/day (orally, 2 h after arsenic for 5 days) and sodium arsenite 2 mg/kg bw/day (IP, for 5 days)	G1 vs. CG blood: tAs↔, Zn↔, ALAD↑, GSH↔, ZPP↔ liver: tAs↔, Zn↔, GSH↔, GSSG↓, TBARS↓ kidney: tAs↔, Zn↔, GSH↔, GSSG↔, TBARS↓
		CG (n = 5)—sodium arsenite 2 mg/kg bw/day (IP, for 5 days) after that saline (orally, for 3 days) G1 (n = 5)—sodium arsenite 2 mg/kg bw/day (IP, for 5 days) after that zinc acetate 10 mg/kg bw/day (orally, for 3 days)	G1 vs. CG blood: tAs↔, Zn↔, ALAD↔, GSH↔, ZPP↔ liver: tAs↔, Zn↔, GSH↔, GSSG↔, TBARS↔ kidney: tAs↔, Zn↔, GSH↔, GSSG↔, TBARS↔
Kreppel et al.1994 [50]	Mice, CF1, adult, male	CG (n = 6)—saline (sc injected, one dose) and after that arsenite-73 115 or 85 µmol/kg bw (sc injected, 24 h after, one dose) G1 (n = 6)—zinc acetate 1000 µmol/kg bw (sc injected, one dose) and after that arsenite-73 115 or 85 µmol/kg bw (sc injected, 24 h after zinc, one dose)	in the group with dose 115 µmol/kg bw—arsenic-73: liver↔, blood↓, kidney↓, skin↓, heart↓, brain↔, lung↓, small intestine↓, large intestine↔, muscle↓ in the group with 85 µmol/kg bw—arsenic-73: liver (nuc↔, Mit↔, Mic↑, Cyt↓), kidney (nuc↔, Mit↔, Mic↔, Cyt↔), small intestine (nuc↔, Mit↑, Mic↑, Cyt↔) arsenic-73 bound to MT↔
		CG (n = 15)—sodium arsenite 130 µmol/kg bw (sc injected, one dose) G1 (n = 15)—zinc acetate 100 µmol/kg bw (sc injected, one dose) and sodium arsenite 130 µmol/kg bw (sc, 96 h after zinc, one dose)	G1 vs. CG at 96 h: survival↑
		CG (n = 20–40)—saline (sc injected, one dose) and after that sodium arsenite 130 µmol/kg bw (sc injected, 24 h after, one dose) G1 (n = 20–40)—zinc acetate 1000 µmol/kg bw (sc injected, one dose) and sodium arsenite 130 µmol/kg bw (sc injected, 96 h after zinc, one dose)	G1 vs. CG correlation between MT induction and protection against the lethal effects of arsenic↔

Table 7. *Cont.*

Reference	Research Model	Study Description	Main Results
Wang and Lee 1993 [51]	SA7N cells	CG—sodium arsenite 200 μM (for 120 min) G1—zinc sulfate 200 μM (for 24 h) and after that sodium arsenite 200 μM (for 120 min)	G1 vs. CG tAs: accumulation↓
		CG—sodium arsenite 200 μM (for 30 min) G1—zinc sulfate 200 μM (for 24 h) and after that sodium arsenite 200 μM (for 30 min) and after that incubated in normal medium (for 120 min)	G1 vs. CG tAs: excrection↑
Zhao et al., 2019 [52]	Cyprinus carpio	CG (n = 30)—arsenic trioxide 2.83 mg/L (orally, for 1 month) G1 (n = 30)—zinc 1 mg/L and (orally, for 1 month) and arsenic trioxide 2.83 mg/L (orally, for 1 month)	G1 vs. CG anterior, mid intestines: SOD↑, mRNA levels of: IL-1β↓, IL-6↓, IL-8↔, phosphorylation of IkB-α↓, NF-kB nuclear translocation↓ histological changes in intestines↓ anterior intestines: mRNA levels of: Occludin↑, Claudin 3↑, Claudin 4↑, Claudin 7↑, Claudin 11↑, Claudin 15↑, ZO-1↑, ZO-2↑ mid intestines: mRNA levels of: Occludin↔, Claudin 3↔, Claudin 4↔, Claudin 7↑, Claudin 11↑, Claudin 15↑, ZO-1↑, ZO-2↑
Zhao et al., 2019 [53]	Cyprinus carpio	CG (n = 30)—arsenic trioxide 2.83 mg/L (orally, for 1 month) G1 (n = 30)—zinc 1 mg/L and (orally, for 1 month) and arsenic trioxide 2.83 mg/L (orally, for 1 month)	G1 vs. CG liver: ROS↓, Na^+/K^+-ATPase↑, Ca^{2+}-Mg^{2+}-ATPase↑, AST↑, ALT↑, ALP↓ activity of CYP1A↑, protein and mRNA levels of CYP1A↑, capase 3↓, caspase 8↓, Bax↓, Bcl-2↑, TNF-α↓, Fas↓ damage in nucleus and mitochondria↓
Ganger et al., 2016 [54]	Rats, Sprague Dawley, adult, male	CG (n = 6)—sodium arsenite 75 μmol/kg (sc injected, for 1 day) G1 (n = 6)—zinc sulfate 153 μmol/kg (sc injected, for 1 day) and sodium arsenite 75 μmol/kg (sc injected, for 1 day) after that zinc sulfate 153 μmol/kg (sc injected, for 1 day)	G1 vs. CG liver: MDA↓, GSH↑, NADPH↑, CAT↔, SOD↓, ALP↑, protein expression of MT↑, mRNA expression of MT-1↑, iron↑, Zn↑, potassium↓, chlorine↑, sulfur↑ histopathological changes (cytoplasmic vacuolization, sinusoidal expansions)↓
Wong et al., 2019 [55]	Mice, C57Bl/6, adult, female)	CG (n = 7)—zinc carbonate 30 mg/kg/diet (orally, for 6 weeks) and sodium arsenite 50 or 500 ppb in drinking water (orally, for 6 weeks) G1 (n = 7)—zinc carbonate 6 mg/kg/diet (orally, for 6 weeks) and sodium arsenite 50 or 500 ppb in drinking water (orally, for 6 weeks)	G1 vs. CG plasma: Zn↓ liver: transcript abundance of: HO-1↑, IL6↑, Ccl2↑, ICAM1↑

Table 7. *Cont.*

Reference	Research Model	Study Description	Main Results
Cao et al., 2019 [56]	INS-1 (rat insulinoma pancreatic beta cells)	CG—zinc sulfate 4 μM (for 5 days) and after that sodium arsenite 50 ppm (for 24 h) G1—zinc sulfate 0 μM (for 5 days) and after that sodium arsenite 50 ppm (for 24 h)	G1 vs. CG Zn↓, calcium↔, copper↔, selenium↔, iron↔, magnesium↔, viable cells↓, % dead cells↔, insulin↔ mRNA level of: cleaved PARP↔, BAX/Bcl-2↔, Mt1↓, Mt2↔, HO-1↔, Ogg1↔, p53↔, 8-OHdG↔, γ-H2AX↔, Ins1↑, Pdx1↓, Neurod1↔, Znt8↔
		CG—zinc sulfate 4 μM (for 5 days) and after that sodium arsenite 500 ppm (for 24 h) G1—zinc sulfate 0 μM (for 5 days) and after that sodium arsenite 500 ppm (for 24 h)	G1 vs. CG Zn↓, calcium↔, copper↔, selenium↔, iron↔, magnesium↔, viable cells↓, % dead cells↑, insulin↔ mRNA level of: cleaved PARP↑, BAX/Bcl-2↔, Mt1↔, Mt2↔, HO-1↓, Ogg1↔, p53↔, 8-OHdG↔, γ-H2AX↑, Ins1↔, Pdx1↔, Neurod1↔, Znt8↔
Wang et al., 2020 [57]	Cyprinus carpio	CG (n = 30)—arsenic trioxide 2.83 mg/L (orally, for 1 month) G1 (n = 30)—zinc chloride 1 mg/L and (orally, for 1 month) and arsenic trioxide 2.83 mg/L (orally, for 1 month)	G1 vs. CG kidney: BUN↓, creatinine↓, histopathological changes↓, DNA breaks↓, ROS↓, MDA↓, PC↓, 8-OHdG↓, SOD↑, GSH↑ mRNA and protein levels of: Bcl-2↑, Bax↓, Caspase 3↓, Caspase 8↓, Caspase 9↓, p53↓, PUMA↓, NRf2↓, GCL↓, NQO1↓, HO-1↓, iNOS↓, TNF-α↓, Il-10↓, Il-6↓, IkB-α↑, nNF-kB↓, cNF-kB↑, p-ERK↔, p-p38↓, p-JNK1↔, p-JNK-2↔
Nasiry Zarrin Ghabaee et al., 2017 [58]	Rats, Wistar, adult, female	CG (n = 6)—sodium meta-arsenite 5 mg/kg bw/day (orally, for 42 days) G1 (n = 6)—zinc sulfate 20 mg/kg bw/day and (orally, for 42 days) and sodium meta-arsenite 5 mg/kg bw/day (orally, for 42 days)	G1 vs. CG infant total weight↑, infant tissue weight↑, total birth numbers↑, infant dead↓ kidney from pups: MDA↓, GSH↑, tubular damage↓, histopathological changes↓
Uthus and Nielsen 1985 [59]	Chicks, Golden Giant, cockerel	CG (n = 15)—zinc acetate 25 μg/g/diet (orally, for 28 days) and disodium arsenate 2 μg/g (orally, for 28 days) G1 (n = 15)—zinc acetate 2.5 μg/g/diet (orally, for 28 days) and disodium arsenate 2 μg/g (orally, for 28 days)	G1 vs. CG plasma: uric acid↑, urea↑ kidney: arginase↑
		CG (n = 15)—zinc acetate 25 μg/g/diet (orally, for 28 days) and disodium arsenate 2 μg/g (orally, for 28 days) G1 (n = 15)—zinc acetate 2.5 μg/g/diet for 9 day after that 10 μg/g for 19 days (orally) and disodium arsenate 2 μg/g (orally, for 28 days)	G1 vs. CG plasma: uric acid↑, urea↑ kidney: arginase↑

Table 7. *Cont.*

Reference	Research Model	Study Description	Main Results
Zhao et al., 2019 [60]	Cyprinus carpio	CG (n = 30)—arsenic trioxide 2.83 mg/L (orally, for 1 month) G1 (n = 30)—zinc 1 mg/L and (orally, for 1 month) and arsenic trioxide 2.83 mg/L (orally, for 1 month)	G1 vs. CG heart: ROS↓, CAT↑, SOD↑, MDA↓, protein level of: Bax↓, BCl-2↑, Caspase 9↓, Caspase 3↓, LC3II/LC3I↓, p62↑, pI3K↑, p-AKT/AKT↑, p-mTOR/mTOR↑, p-38/p38↓, p-ERK/ERK↔, p-JNK/JNK↓ injury symptoms (agglutinated chromatin, damaged mitochondria and autophagosome)↓
Bhardwaj and Dhawan 2019 [61]	Rats, Wistar, adult, male	CG (n = 6)—sodium arsenite 100 mg/L in drinking water (orally, for 12 weeks) G1 (n = 6)—zinc sulfate in drinking water 227 mg/L (orally, for 12 weeks) and sodium arsenite 100 mg/L in drinking water (orally, for 12 weeks)	G1 vs. CG serum: Zn↑, Hb↔, reduced glutathione↑, CAT↑, MDA↓, GST↑, lymphocyte count↔, neutrophils↑, monocyte↔, eosinophilis↔, TLC↔ morphology of erythrocytes↑ morphological index of erythrocytes↓
Ahmad et al., 2013 [62]	Mice, Swiss-Webster, adult, female	CG (n = 10)—sodium arsenate 40 mg/kg bw/day in drinking water (orally, during pregnancy and until postnatal day 15) G1 (n = 10)—zinc sulfate 40 mg/kg bw/day in drinking water (orally, during pregnancy and until postnatal day 15) and sodium arsenate 40 mg/kg bw/day in drinking water (orally, during pregnancy and until postnatal day 15)	G1 vs. CG pups on postnatal days 21: bw↑, body hair appearance↓, eye opening↓, mean rotating reflex↓, mean righting reflex↓, mean cliff avoidance↓ male adolescent offspring (postnatal day 22): motor activity (number of squares crossed, wall rears, rears, movement duration)↑ serum: GGT↓, TBARS↓, GSH↑
Milton et al., 2004 [63]	2.3D cells (neuronal cell line)	CG—arsenic trioxide 20 μM G1—zinc sulfate 75 μM and arsenic trioxide 20 μM	G1 vs. CG DEVD-caspase activity↓
		CG—arsenic trioxide 20 μM G1—zinc sulfate 50 μM and arsenic trioxide 20 μM	G1 vs. CG DEVD-caspase activity↓
		CG—arsenic trioxide 20 μM G1—zinc sulfate 25 μM and arsenic trioxide 20 μM	G1 vs. CG DEVD-caspase activity↔

Table 7. *Cont.*

Reference	Research Model	Study Description	Main Results
Fascineli et al., 2002 [64]	Mice, Swiss, adult, female	CG (n = 10)—sodium arsenate 45 mg/kg bw (IP, single dose on 8th gestation day) G1 (n = 10)—zinc sulfate 20 mg/kg bw (orally, on 7th and 8th gestation day) and after that sodium arsenate 45 mg/kg bw (IP, single dose on 8th gestation day) G2 (n = 10)—zinc sulfate 40 mg/kg bw (orally, on 7th and 8th gestation day) and after that sodium arsenate 45 mg/kg bw (IP, single dose on 8th gestation day)	G1 vs. CG maternal weight gain↓, fetal weight↔, signs of delayed ossification↔, placental weight↔, external, visceral and skeletal malformation↔, vertebrae skeletal anomalies↑ G2 vs. CG maternal weight gain↔, fetal weight↔, signs of delayed ossification↔, placental weight↔, external, visceral and skeletal malformation↔, vertebrae skeletal anomalies↑
	Mice, Swiss, adult, female	CG (n = 10)—sodium arsenate 45 mg/kg bw (IP, single dose on 8th gestation day) G1 (n = 10)—zinc sulfate 5 mg/kg bw (orally, on 8th gestation day) and sodium arsenate 45 mg/kg bw (IP, single dose on 8th gestation day) G2 (n = 10)—zinc sulfate 10 mg/kg bw (orally, on 8th gestation day) and sodium arsenate 45 mg/kg bw (IP, single dose on 8th gestation day)	G1 vs. CG maternal weight gain↔, fetal weight↔, signs of delayed ossification↔, placental weight↔, external, visceral and skeletal malformation↔, vertebrae skeletal anomalies↑ G2 vs. CG maternal weight gain↓, fetal weight↔, signs of delayed ossification↔, placental weight↓, external, visceral and skeletal malformation↔, vertebrae skeletal anomalies↑
	Mice CD-1, embryo culture	CG—sodium arsenite 5 μM G1—zinc chloride 500 μM and sodium arsenite 5 μM (6 h after zinc or simultaneously)	G1 vs. CG dysmorphology↔, lethality↔, neutral tube closure defects↔, pharyngeal arch dysmorphology↔, heart conotruncal dysmorphology↔
Beaver et al., 2017 [65]	Zebrafish, *Danio rerio*, embryos	CG—parental adults fish fed zinc 33.81 μg/g of diet (orally, for 8 weeks) and after that embryos was exposed to sodium arsenite 50 ppb (at 4 h to 120 h post fertilization) G1—parental adults fish fed zinc 14.45 μg/g of diet (orally, for 8 weeks) and after that embryos was exposed to sodium arsenite 50 ppb (at 4 h to 120 h post fertilization)	G1 vs. CG Zn↓, mRNA levels of: zip1↔, zip8↔, znt7↔ mortality↔, developmental malformation↔, activity of the embryos↓, at 24 h post fertilization: pax4↓ at 48 h post fertilization: mRNA levels of: nrf2a↔, nrf2b↓, Mt2↔, Ogg1↔, insa↔ at 120 h post fertilization: mRNA levels of: nrf2a↓, nrf2b↔, Mt2↔, Ogg1↔, insa↓, pdx1↔

Table 7. *Cont.*

Reference	Research Model	Study Description	Main Results
Beaver et al., 2017 [65]		CG—parental adults fish fed zinc 33.81 μg/g of diet (orally, for 8 weeks) and after that embryos was exposed to sodium arsenite 500 ppb (at 4 h to 120 h post fertilization) G1—parental adults fish fed zinc 14.45 μg/g of diet (orally, for 8 weeks) and after that embryos was exposed to sodium arsenite 500 ppb (at 4 h to 120 h post fertilization)	G1 vs. CG Zn↓, mRNA levels of: zip1↔, zip8↔, znt7↔ mortality↔, developmental malformation↔, activity of the embryos↓ at 24 h post fertilization: pax4↔ at 48 h post fertilization: mRNA levels of: nrf2a↔, nrf2b↓, Mt2↔, Ogg1↓, insa↔ at 120 h post fertilization: mRNA levels of: nrf2a↔, nrf2b↔, Mt2↔, Ogg1↔, insa↔, pdx1↔
Nielsen et al., 1980 [66]	Chicks, day-old	CG—zinc acetate 40 μg/g/diet (orally, for 32 days) and sodium arsenate 2 μg/g (orally, for 32 days) G1—zinc acetate 5 μg/g/diet (orally, for 32 days) and sodium arsenate 2 μg/g (orally, for 32 days)	G1 vs. CG bw↓, liver wt/body wt ratio↔, hematocrit↑, growth↓ plasma: ALP↑
Altoe et al., 2016 [67]	Rats, Wistar, adult, male	CG (n = 6)—sodium arsenite 5 mg/kg bw/day (orally, for 60 days) G1 (n = 6)—zinc chloride 20 mg/kg bw/day (orally, for 60 days) and sodium arsenite 5 mg/kg bw (orally, for 60 days)	G1 vs. CG normal sperm morphology↑ abnormalities in spermatoza (wrong-angled hooks, folded sperm, amorphous head and normal tail, two heads)↓

↑—significant increase; ↓—significant decrease; ↔—no significant changes; γ-H2AX—gamma-H2A histone family member X; 8-OHdG—8-hydroxy-deoxyguanosine; Ache—acetylocholine; ACP—acid phosphatase; AKT—protein kinase B; ALAD—δ-aminolevulinic acid dehydratase; ALP—alkaline phosphatase; ALT—alanine transaminases; AST—aspartate transaminases; ATF6—activating transcription factor 6; ATG-5—autophagy related 5; Bax—Bcl-2-associated X protein; Bcl-2—B-cell lymphoma 2; BUN—blood urea nitrogen; bw—body weight; Ca—calcium; $Ca^{2+/}Mg^{2+}$-ATPase—calcium–magnesium adenosine triphosphatase; CAT—catalase; Ccl2—C-C motif chemokine ligand 2; CG—control group; CHOP—C/EBP homologous protein; cNF-kB—cytoplasmic nuclear factor kappa-light-chain-enhancer of activated B cells; Cu/Zn-SOD—copper zinc superoxide dismutase; CYP1A—cytochrome P-1A; Cyt—cytosols; DNA—deoxyribonucleic acid; eIF2α—eukaryotic initiation factor 2α; Fas—apoptosis antigen 1; G1—group 1; G2—group 2; GCL—glutamate cysteine ligase; GGT—γ-glutamyl transferase; GPx—glutathione peroxidase; GR—glutatione reductase; GRP78—glucose-regulating protein 78; GRP78—glucose-related protein 78; GRP94—glucose-related protein 94; GSH—glutathione; GSSG—oxidized glutathione; GST—glutathione s transferase; Hb—hemoglobin; HO-1—*heme oxygenase-1*; HSP60—heat shock response 60; HSP70—heat shock response 70; HSP90—heat shock response 90; ICAM1—intercellular adhesion molecule 1; IkB-α—inhibitor of nuclear factor kappa B; Il-10—Interleukin 10; IL-1β—Interleukin 1β; IL-6—Interleukin 6; IL-8—Interleukin 8; iNOS—inducible nitric oxide synthase; IP—intraperitoneally; insa—insulin-a; Ins1—insulin-1; IRE1—inositol-requiring enzyme 1; LC3—microtubule-associated protein 1 light chain 3; MDA—malondialdehyde; Mic—microsomes; Mit—mitochondria; Mn-SOD—manganase superoxide dismutase; MT—metallothionein; Mt1—metallothionein 1; Mt2—metallothionein 2; mTOR—mammalian target of rapamycin; Na^+/K^+-ATPase—sodium-potassium adenosine triphosphatase; NADPH—nicotinamide adenine dinucleotide phosphate; Neurod1—neurogenic differentiation-1; NF-kB—nuclear factor kappa-B; nNF-kB—nuclear factor kappa-light-chain-enhancer of activated B cells; NQO1—NAD(P)H quinone dehydrogenase 1; nrf2—nuclearfactor(erythroid-derived2)-like 2; Nuc—nuclei; Ogg1—8-oxoguanine DNA-glycosylase 1; P62—sequestosome 1; PARP—poly(ADP) polymerase; pax4—paired box4; PC—protein carbonylation; pdx1—pancreatic and duodenal homeobox 1; p-eIF2a—Phospho-eukaryotic initiation factor 2 alpha; p-ERK—Phosphorylated Extracellular Signal-Regulated Kinase; PERK—PKR-like reticulum kinase; pI3K—phosphatidylinositol-3-kinase; p-JNK1—phosphorylated c-Jun N-terminal kinase 1; p-JNK-2 –phosphorylated c-Jun N-terminal kinase 2; p-PERK–Phospho- PKR-like ER kinase; PUMA—p53 upregulated modulator of apoptosis; ROS—reactive oxygen species; sc—subcutaneous; SOD—superoxide dismutase; tAs—total arsenic species; TBARS—thiobarbituric acid reactive substances; TLC—total leukocyte count; TNF-α—tumor necrosis factor-α; zip1 –zinc importer 1; zip8—zinc importer 7; Zn—zinc; ZnT—zinc transporter; znt7—zinc exporter 7; Znt8—zinc transporter 8; ZO-1—Zonula occludens-1; ZO-2—Zonula occludens-2; ZPP—zinc protoporphyrin.

Six studies analyzed the potential role of zinc in the reduction of iAs-induced toxicity in the urinary system. The renoprotective effect of zinc was observed in three studies carried out in animal models exposed to iAs [44,57,58]. The protective effect of zinc on the kidneys was not only by reducing oxidative stress, but also by reducing tight junction damage, mitigating disturbances in protein homeostasis, and reducing autophagy [44]. In the second study, zinc also reduced oxidative stress, lipid peroxidation, protein and DNA damage, apoptosis, inflammation, and kidney histopathological changes [57]. Supplementation with zinc during gestation and lactation in female rats also reduced the adverse effect of iAs in the offspring (by reducing lipid peroxidation and changes in the structure of the kidney) [58]. In two studies, zinc did not exhibit renoprotective effects [48,49].

In turn, zinc deficiency in chickens exposed to iAs increased plasma levels of uric acid and urea and enhanced arginase activity in the kidney [59].

Zinc showed a protective effect on the lymphatic system of carps exposed to iAs by decreasing the toxicity-related changes in the spleen, inter alia, by decreasing harmful changes in spleen tissue, the expression of genes related to endoplasmic reticulum stress (glucose-related protein 78 and 94, PKR-like reticulum kinase, C/EBP homologous protein), apoptosis (apoptosis antigen 1, caspases 3, 8, 9, Bcl-2-associated X protein), and autophagy (Beclin-1, autophagy-related 5, microtubule-associated protein 1 light chain 3) [45].

The protective effect of zinc on the circulatory system has also been demonstrated. The heart tissue of common carp exposed to iAs revealed reduced oxidative stress (through decreased production of reactive oxygen species and increased activity of antioxidant enzymes), lipid peroxidation (through decreased content of malondialdehyde), apoptosis (through increased expression of Bcl-2 and decreased expression of Bax and caspases), autophagy (by decreasing the level of proteins involved in the previously mentioned pathways), and injury symptoms [60]. In rats exposed to iAs, zinc supplementation reduced the damage to erythrocytes, mediated through the increased activity of antioxidant enzymes, decreased lipid peroxidation in the serum, and decreased morphological changes in red blood cells [61].

Three studies conducted in animal models exposed to iAs have also shown that zinc has a protective effect on the nervous system. Zinc decreased neurotoxicity in the rats belonging to three age groups (young, adult, and old) by lowering behavioral perturbations and alleviating perturbations in the cholinergic system (through increased activity of acetylcholine and decreased amount of acetylcholine in the brain) [46]. The protective effect of zinc was also observed in a study performed in groups of rats belonging to different age groups (21 and 28 postnatal days and 3 months old). In all age groups, zinc reduced oxidative stress (by increasing the activity of antioxidant enzymes), decreased lipid peroxidation (by reducing malondialdehyde concentration), and also decreased apoptosis (by decreasing mRNA expression of caspase) [47]. In another study, zinc also reduced adverse effect of iAs in the offspring mice (through increased morphological development, decreased early development of sensory–motor reflexes, increased motor behavior, and decreased oxidative stress in the serum) [62]. In an in vitro study, higher doses of zinc (50 and 75 μM) reduced apoptosis in a neuronal cell line (by reducing DEVD-caspase activity), but such an effect was not observed at lower doses of Zn (25 μM) [63].

A study carried out by Kreppel et al. [50] in an animal model revealed that zinc pretreatment reduced As-induced lethality, but no significant correlation between metalothionein induction in the liver and protection against the lethal effect of As by zinc was noted. In another study, both pretreatment with zinc and simultaneous administration did not reduce teratogenicity in mice and embryos exposed to iAs, which was evident by no significant changes in maternal, placental, and fetal weight, and no reduction of malformation in the fetuses and morphological development in the embryo [64].

Zinc deficiency in the embryos of zebrafish exposed to iAs did not affect mortality and development, but an adverse effect was observed with regard to the reduction of activity of the embryos and genes associated with oxidative stress and insulin production (decreased

mRNA levels of 8-oxoguanine DNA glycosylase, nuclear factor (erythroid-derived 2)-like 2, and paired box 4) [65]. In the chickens that were fed a zinc-deficient diet and exposed to iAs, slower growth and increased hematocrit and activity of plasma alkaline phosphatase in the plasma were observed [66].

One of the studies showed that zinc reduced damage to the reproductive system of rats exposed to iAs, mainly through increasing the proportion of normal sperm and decreasing the abnormalities in spermatozoa [67].

3.7.3. Zinc—Summary

In vivo studies have been shown that zinc reduced adverse changes induced by iAs in many systems, including digestive, urinary, lymphatic, cardiovascular, nervous, and reproductive, and can reduce the bioaccumulation of tAs in the many organs. The same methods of administration and exposure time (orally or subcutaneously; one dose or for 5, 15, 42, 60 days; 1, 3, 12 weeks; 1, 3 months), but different doses of zinc (1, 227 mg/L; 0.02% or 10 ppm in drinking water; 5, 10, 20 mg/kg bw/day; 153, 1000 μmol/kg bw) and iAs (10, 100 ppm in drinking water; 2.83, 100 mg/L; 2, 5, 10, 40 mg/kg bw/day; 75, 85, 115 μmol/kg bw), were used in the experimental protocols, although beneficial effects were obtained.

However, in four in vivo studies, zinc did not decrease: oxidative stress, teratogencity and tAs accumulation in the blood, liver, kidney, brain, large intestine. In these studies, the ingredients were administered for different periods of time and doses—zinc (for 1, 2, 5 days or 3 weeks; 5, 10, 20, 40 mg/kg bw/day) and iAs (for 1, 5 days; 3 weeks; 2, 45 mg/kg bw/day). The reason for the lack of beneficial effects in these 4 studies in comparison to the studies where the zinc effect was satisfactory may be the method of iAs administration. When iAs was administered orally for 3 weeks (1 study), a beneficial effect was seen only in the liver, but not in the kidneys (which may indicate differences in iAs metabolism in these organs). In the other studies, where iAs was administered intraperitoneally and subcutaneously (only one dose or one dose per day for 5 days), no beneficial effect of zinc was observed. Perhaps when iAs was taken orally, its absorption was limited, and therefore, in the case of direct administration (intraperitoneally and subcutaneously), the oral administration of zinc cannot reduce its negative effects.

In the four in vivo studies, zinc deficiency intensified the adverse effect of exposure to iAs (inflammation in the liver, disturbance in the urinary system, decreased growth and influence on expression of genes responsible to oxidative stress and insulin production). These adverse effects were observed in different experimental protocols. In three studies, zinc and iAs were administered orally, the time of exposure was the same (for 28, 32 days; 6, 8 weeks), but doses of zinc (6 mg/kg/diet; 2.5, 5, 14.45 μg/g/diet,) and iAs (50, 500 ppb in drinking water; 2μg/g) were different. The results of these studies may have been influenced by long exposure to iAs and a long period of zinc deficiency. Moreover, in one of these in vivo studies, the protocol of the experiment could have had a major impact on the results, zinc deficiency was applied to parental fish, and then embryos were exposed to iAs.

Furthermore, in two in vitro studies, zinc increased the excretion of tAs, as well as showed antiapoptotic effects. In one of these studies, the time of administration could be crucial—zinc was given before exposure to iAs. In the second study, the zinc dose appeared to be the outcome determinant. Antiapoptotic effects were shown with higher doses (50 μM or 70 μM), but a lower dose (25 μM) did not have much of an effect.

In one in vitro study after 5 days of zinc deficiency and after 24 h of exposure to iAs, intensified apoptosis and DNA damage, as well as decreased proliferation, were observed.

4. Conclusions

The results of the in vitro and in vivo animal model studies indicate that dietary compounds involved in iAs metabolism may have beneficial effects in reducing the severity of the entire spectrum of disorders associated with exposure to iAs. Numerous studies

where the effects of folic acid and zinc have been analyzed allow one to draw some conclusions in terms of the role of these nutrients in iAs metabolism and the adverse effect reduction. Folic acid and zinc supplementation improved iAs metabolism and reduced adverse changes induced by iAs in many systems: digestive, urinary, cardiovascular, lymphatic, nervous and reproductive. Adverse effects of folic acid supplementation were also observed, and were mainly connected with reduction iAs metabolism, intensification of oxidative stress, and disturbances in: DNA methylation, gut flora composition, neural development, and viability. These adverse effects were determined by such factors as: type of diet (high-fat diet), type of animal model (mice with limited capacity to methylation), animal species (wild-type mice) and folic acid dose (high dose). Moreover, folate and zinc deficiency intensified the adverse effect of iAs exposure. The folate-deficient diet induced adverse effects related to iAs methylation, glucose homeostasis, development, and skin proliferation. Meanwhile, zinc deficiency intensified such adverse effects as: inflammation in the liver, disturbance in the urinary system, decreased growth, expression of genes responsible to oxidative stress and insulin production. The amount of research on the role of methionine, choline, vitamin B_2, B_{12}, and a combination of vitamin B_{12} and folic acid, zinc is very limited, and therefore, no meaningful conclusions can be drawn. Nevertheless, these few studies provide evidence for beneficial effects of methionine, choline, vitamin B_2, B_{12}, combination of vitamin B_{12} and folic acid on iAs metabolism and reduction unfavorable changes in digestive, urinary, nervous, cardiovascular, respiratory, immune systems. Among these ingredients, it has been shown that higher doses of choline are not effective in the reduction of adverse effects of iAs, and the deficiency of methionine and choline may impair iAs metabolism and contribute to DNA damages. In the case of these compounds, further studies are needed to fully determine their role in terms of iAs metabolism and reduction of the adverse health effects. Nevertheless, taking into consideration the promising results of in vivo and in vitro animal model studies, it seems reasonable to analyze the effect of these dietary components in populations exposed to As.

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Oświadczenie o współautorstwie

Niniejszym oświadczam, że w pracy Sijko, M., Kozłowska, L. (2021). Influence of Dietary Compounds on Arsenic Metabolism and Toxicity. Part I—Animal Model Studies. *Toxics*, *9*(10), 258 mój indywidualny udział w jej powstaniu polegał na utworzeniu koncepcji treści i układu pracy, opracowaniu metodyki przeglądu piśmiennictwa, wyszukiwaniu artykułów w bazie danych, analizie i kategoryzacji artykułów, napisaniu oryginalnego draftu artykułu, prowadzeniu korespondencji z wydawcą.

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Lucyna Kozłowska

(Podpis)

toxics

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Review

Influence of Dietary Compounds on Arsenic Metabolism and Toxicity. Part II—Human Studies

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Abstract: Exposure to various forms of arsenic (As), the source of which may be environmental as well as occupational exposure, is associated with many adverse health effects. Therefore, methods to reduce the adverse effects of As on the human body are being sought. Research in this area focuses, among other topics, on the dietary compounds that are involved in the metabolism of this element. Therefore, the aim of this review was to analyze the influence of methionine, betaine, choline, folic acid, vitamin B_2, B_6, B_{12} and zinc on the efficiency of inorganic As (iAs) metabolism and the reduction in the severity of the whole spectrum of disorders related to As exposure. In this review, which included 62 original papers (human studies) we present the current knowledge in the area. In human studies, these compounds (methionine, choline, folic acid, vitamin B_2, B_6, B_{12} and zinc) may increase iAs metabolism and reduce toxicity, whereas their deficiency may impair iAs metabolism and increase As toxicity. Taking into account the results of studies conducted in populations exposed to As, it is reasonable to carry out prophylactic activities. In particular nutritional education seems to be important and should be focused on informing people that an adequate intake of those dietary compounds potentially has a modulating effect on iAs metabolism, thus, reducing its adverse effects on the body.

Keywords: vitamins; minerals; inorganic arsenic; exposure; detoxification; metal toxicity; methylation

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1. Introduction

Exposure to arsenic (As) affects people living in many regions of the world. This problem occurs not only in less affluent areas (such as Chile, Argentina, Bangladesh, and Mexico), but also in several hotspot regions of Europe (Poland, Hungary, Serbia, Romania, Czech Republic, Croatia, Finland, Greece, and Italy) [1]. The source of exposure to different chemical forms of As can be both environmental and occupational. Environmental pollution can be of natural origin (rocks, soils, and volcanoes, among others) and can be caused by human activities (e.g., mining, burning fossil fuels, residues of agricultural chemicals in the soil and groundwater) [2,3]. Environmental sources also include water and food contamination. The As content in food products varies and depends on the type of product and its origin. Foods that contribute most to general population exposure to inorganic arsenic (iAs) include rice and rice-based products, grains and grain-based processed products, and drinking water [4]. The second source of As exposure is occupational exposure. As is used in many industries (agriculture; horticulture; mining; wood preservation; and production of, e.g., ammunition, glass, semiconductors, dyes) [2,3].

Both environmental and occupational exposures include exposure to inorganic as well as organic forms. Inorganic forms (such as As trioxide, As pentoxide, arsenous acid, and arsenic acid) are more toxic than organic forms (monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA), arsenobetaine). Among iAs, As on the third and fifth oxidation state is the most toxic, with forms of the third oxidation state being more toxic [5–7].

Exposure to As can cause many adverse health effects. iAs has been classified as a carcinogenic compound [8]. Epidemiological studies in individuals exposed to As have shown an increased incidence of cancers including lung, kidney, liver, bladder, and skin cancers [9,10]. Long-term exposure to As in adults increased the risk of type 2 diabetes [11,12] and the risk of developing peripheral vascular disease [13], increased the incidence of skin lesions [14], and impaired lung function [15]. Furthermore, As exposure in children had a negative impact on cognitive abilities and induced neurological disorders [16,17].

The large number of people exposed to As and the many negative health effects caused by this exposure warrant, among other investigations, a deep analysis of the spectrum of health effects associated with As exposure and a search for ways that could reduce these adverse effects on the human body. Research in this area has focused, among other topics, on dietary compounds. There is no systematic summary of the results of human studies that have analyzed the effects of key dietary compounds on iAs metabolism and reduction in adverse effects caused by As exposure. Therefore, in this paper, we analyzed the results of studies in which the influence of methionine, choline, betaine, folic acid as donors of methyl groups and vitamins B_2, B_6, B_{12} and zinc as reaction cofactors in the aspect of efficiency of the metabolism process as well as reduction in the severity of the whole spectrum of disorders related to As exposure.

2. Methods

In this review, the electronic database PubMed was used. The following keywords were used to search for articles: arsenic and: methionine, betaine, choline, folic acid, folate, zinc, vitamin B, vitamin B_2, vitamin B_6, vitamin B_{12}, riboflavin, pyridoxine, cobalamin. The review was based on the PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate health care interventions: explanation and elaboration [18]. The search resulted in 2434 articles, excluding those unrelated to the topic of the study and those that examined the effects of complex plant extracts. Sixty-two (4 in vitro studies and 58 human studies) original peer-reviewed articles in English were included in the analysis, which studied the effects of methionine, betaine, choline, folic acid, vitamin B_2, B_6, B_{12}, zinc on iAs metabolism and As-induced toxicity. Articles published between 1980 and 2020 were used, 95.2% of which were published after 2000.

3. Results

3.1. Folic Acid and Zinc as Modulators of iAs Metabolism and Toxicity—In Vitro Studies

The protective effect of folic acid (two studies) and zinc (two studies) has been analyzed in four studies on cell lines that were exposed to iAs. The results are summarized in Table 1.

3.1.1. Folic Acid—iAs Metabolism

The association between folic acid and iAs metabolism was analyzed only in one in vitro study. In a study performed in Chang human hepatocytes, folate deficiency decreased the levels of methylated arsenicals, and supplementation with folic acid showing no effects on iAs metabolism [19].

3.1.2. Folic Acid and Zinc—Toxicity of iAs

In one of the in vitro studies in Chang human hepatocyte folate supplementation decreased apoptosis, lipid peroxidation, and oxidative stress. Furthermore, folate deficiency enhanced the adverse effect of iAs, resulting in, inter alia, increased apoptosis, lipid peroxidation, and oxidative stress [19].

Table 1. Results of in vitro studies with iAs exposure and folic acid or zinc treatment.

Reference	Research Model	Study Description	Main Results
Xu et al., 2010 [19]	Chang human hepatocytes	CG—normal folate medium 2.3 μM (1 h) and sodium arsenite 20 μM (for 24 h) G1—folate-deficient medium (1 h) and sodium arsenite 20 μM (for 24 h) G2—folate-supplemented medium 10 μM (1 h) and sodium arsenite 20 μM (for 24 h)	G1 vs. CG intracellular tAs↔, methylated arsenicals↓ viability↓, early apoptosis↑, late apoptosis↑, caspase-3 cleavage↑, PARP cleavage↑, percentage of cells with collapsed mitochondrial membrane potential↑, ROS↑, TBARS↔, GSH↓, CAT↓, SOD↔ cytochrome c: in mitochondria↓, in cytosol↑
			G2 vs. CG intracellular tAs↔, methylated arsenicals↔ viability↑, early apoptosis↔, late apoptosis↔, caspase-3 cleavage↓, PARP cleavage↓, percentage of cells with collapsed mitochondrial membrane potential↓, ROS↓, TBARS↓, GSH↑, CAT↔, SOD↔ cytochrome c: in mitochondria↑, in cytosol↓
Ma et al., 2015 [20]	HEK293ET cells (human embryonic kidney 293 cells)	CG—sodium arsenite 5 mM G1—folic acid 100 μM and sodium arsenite 5 mM	G1 vs. CG cell viability↑, mRNA level of GDF1↑, ROS↓, expression of p66Shc↓
Wong et al., 2019 [21]	THP-1 (human monocyte cell line)	CG—zinc sulfate 4 μM (for 4 weeks) and after that sodium arsenite 10 μM (for 24 h) G1—zinc sulfate 0 μM (for 4 weeks) and after that sodium arsenite 10 μM (for 24 h)	G1 vs. CG Zn total and intracellular↓, ROS↑
		CG—zinc sulfate 4 μM (for 4 weeks) and after that sodium arsenite 10 μM (for 4 h) G1—zinc sulfate 0 μM (for 4 weeks) and after that sodium arsenite 10 μM (for 4 h)	G1 vs. CG HO-1↑, SOD↔, CAT↔
		CG—zinc sulfate 4 μM (for 4 weeks) and after that sodium arsenite 1 μM (for 4 h) G1—zinc sulfate 0 μM (for 4 weeks) and after that sodium arsenite 1 μM (for 4 h)	G1 vs. CG intracellular zinc↓, transcript levels of: ICAM1↑, IL6↑, CXCL8↑
Sun et al., 2014 [22]	HaCaT cells (human keratinocyte cell line)	CG—sodium arsenite 2 μM (for 24 h) G1—zinc chloride 2 μM (for 24 h) and sodium arsenite 2 μM (for 24 h)	G1 vs. CG zinc content in PARP-1↑, PARP-1 activity↑

↑—significant increase; ↓—significant decrease; ↔—no significant changes; CAT—catalase; CG—control group; CXCL8—C-X-C motif chemokine ligand 8; G1—group 1; GDF 1—growth differentiation factor 1; GSH—glutathione; HO-1—*heme oxygenase-1*; ICAM1—intercellular adhesion molecule 1; IL-6—Interleukin 6; PARP—poly(ADP) polymerase; PARP-1—poly(ADP) polymerase-1; ROS—reactive oxygen species; SOD—superoxide dismutase; tAs—total arsenic species; TBARS—thiobarbituric acid reactive substances.

In the in vitro study performed on human embryonic kidney 293 cells, folic acid showed a protective effect against adverse effect of iAs by increasing the viability of cells and decrease the oxidative stress levels through decreasing the levels of reactive oxygen species and p66Shc expression [20].

Furthermore, a study with a human monocyte cell line, carried out in the presence of iAs, demonstrated that zinc deficiency increased oxidative stress (increased reactive oxygen species production) and inflammatory response (inter alia, through increased production of inflammatory markers) [21].

In the human keratinocyte cell lines exposed to iAs, zinc reduced DNA damage by affecting the production of poly(ADP-ribose) polymerase-1 (increased content zinc in this protein and its activity; decreased As binding with this protein) [22].

3.1.3. Folic Acid and Zinc—Summary

In vitro studies have shown that folic acid deficiency decreased iAs metabolism and supplementation had no influence. Folic acid deficiency (1 h) could exacerbate the adverse effect of exposure to iAs in a short period of time, and the addition of folic acid (also only for 1 h) did not improve iAs metabolism; in both cases, a long exposure time to iAs (24 h) was used.

However, these compounds reduced the adverse changes induced by iAs in three cell lines, mainly through decreased apoptosis, oxidative stress and DNA damage. Although the studies were carried out on different cell lines and different doses of folic acid (10, 100 μM) zinc (2 μM) and iAs (2, 20 μM; 5 mM) with the same exposure times iAs (24 h), favorable results were obtained.

In two in vitro studies with iAs exposure (for 4 or 24 h), folic acid and zinc deficiency intensified oxidative stress, apoptosis and inflammation. In these cases, both short folic acid deficiency (1 h) and long zinc deficiency (4 weeks) had an adverse effect.

3.2. Relationship between Dietary Intake of Selected Compounds and iAs Metabolism and Toxicity

Twenty-two studies in subjects exposed to iAs (mainly in drinking water) analyzed the relationships between the intake of a whole range of nutrients and iAs metabolism as well as toxicity (Table 2).

3.2.1. Nutrient Intake—iAs Metabolism

The main results of studies that analyzed the relationships between intakes of methionine, betaine, choline, vitamins B_2, B_6, B_{12}, folate, zinc and biomarkers of iAs metabolism in children and then in adults are presented below.

The relationship between nutrient intake and iAs metabolism was reported in four studies conducted with children.

In the case of vitamin B_2, no significant relationship was observed between its intake and the urinary excretion of As metabolites (%MMA, %DMA, and %iAs) [23]. A negative association between vitamin B_6 intake and %MMA in urine was observed only in one study involving children, and there were no significant relationships between vitamin B_6 intake and the percentage of DMA and iAs in the urine [23]. In contrast, in the study by Kurzius-Spencer et al. [24], no significant association was observed between vitamin B_6 intake and the urinary excretion of iAs metabolites. Three studies showed no relationship between vitamin B_{12} intake and iAs metabolism in children [23–25]. Folate intake was negatively associated with urinary %MMA [25,26] and, additionally, in one of these studies, a positive association with urinary %DMA was observed [26]. In these studies, no association was observed between intake of folic acid and %iAs, tAs in the urine [25,26] and urinary excretion of iAs metabolites [24].

The relationship between nutrient intake and iAs metabolism was also analyzed in the adult population (10 studies).

In the study by Heck et al. [27], methionine intake (in groups of men and women) was positively associated with urinary %MMA, whereas no such relationship was observed in

another study involving women [28]. In the study by Heck et al. [28], methionine intake also was positively associated with urinary %DMA [28], but in a subsequent study by these authors, this relationship was not significant [27]. Additionally, these two studies showed a negative association between methionine intake and %iAs in the urine [27,28]. Moreover, in the study by Heck et al. [29], a higher methionine intake was associated with an increased excretion of tAs in the urine. Another analyzed ingredient was betaine; there was no relationship between betaine intake and iAs metabolism [27,28].

Regarding choline, two studies showed intake of this compound was not associated with urinary %MMA, but a positive association was observed with the DMA/MMA ratio [27,28]. In contrast, one study demonstrated associations with the other iAs metabolites in urine (positive association of choline intake with %DMA and negative association with %iAs) [28].

The relationships between vitamin B_2 intake and iAs metabolism have been analyzed in several studies. One study showed a positive association between vitamin B_2 intake and urinary %MMA [27], while another study showed a negative association with this form [30]. Additionally, in several studies, there were no relationships [28,31–33]. In the study by Spratlen et al. [30], vitamin B_2 intake was positively related with urinary %DMA, while no such relationship was shown in other studies [27,28,31–33]. In contrast, a negative association with %iAs was shown in the study by Spratlen et al. [30], while no such relationship was observed in other studies [27,28,31–33]. There was no significant relationship between vitamin B_2 intake and urinary excretion of tAs [34]. One study revealed a positive association between vitamin B_2 intake and first methylation step (MMA/iAs), and negative with the second methylation step (DMA/MMA) [27].

The relationship between vitamin B_6 intake and iAs metabolism was analyzed in seven studies. In the study by Spratlen et al. [30], vitamin B_6 intake was negatively associated with urinary %MMA and positively with %DMA. In the study by Kurzius-Spencer et al. [24], these relationships were not significant. Both studies showed a negative association with urinary %iAs [24,30], while the study by Argos et al. [34] showed a positive association with tAs in the urine. On the other hand, in several studies, there was no relationship between vitamin B_6 intake and urinary %iAs metabolite excretion [27,28,31,33].

Vitamin B_{12} intake correlated positively with urinary %MMA in the study by Heck et al. [27]; other studies did not show such a relationship [24,28,30,32]. The study by Lopez-Carillo et al. [28] also showed a positive association with urinary %DMA, which was not observed in the studies of other authors [27,30,32]. Only in two studies, the intake of this vitamin was negatively associated with %iAs in the urine [27,28]. This relationship was not confirmed in other studies [24,30,32]. Positive associations with ratios of various forms of As in the urine were also observed in studies by Heck et al. [27] and Lopez-Carillo et al. [28]. However, the intake of this vitamin did not affect the urinary excretion of tAs [34].

None of the studies observed a relationship between folic acid intake and urinary %MMA and %DMA [27,28,30–33]. In contrast, the study by Howe et al. [35] showed that intake of sum of vitamins B_2, B_6, B_{12} and folate had a negative association with the proportion of monomethyl arsenic species. In turn, folate intake was negatively associated with urinary %iAs in one study [28], which was not shown in other studies [27,30–33]. Folate intake was positively associated with ratio DMA/iAs [28]. No relationship between folate intake and tAs in the urine was reported in the study by Argos et al. [34].

Associations between zinc intake and iAs metabolism were demonstrated in two studies. They included one negative association with urinary %MMA and a positive association with urinary %DMA [28,33]. Moreover, the study by Lopez Carillo et al. [28] demonstrated a negative association with urinary %iAs and a positive association with ratios DMA/MMA and DMA/iAs.

3.2.2. Nutrient Intake—Toxicity of As

Several studies have analyzed the relationship between nutrient intake and the severity of adverse changes in the body and the risk of developing diseases associated with exposure to As.

In one study involving adults and children, an adverse effect of high vitamin B_6 intake was observed; it was associated with an increased risk of diabetes and metabolic syndrome [31]. On the other hand, in another study, a low intake of vitamin B_2, B_6, B_{12} and folic acid in subjects exposed to iAs with drinking water was associated with worse cardiovascular outcomes (through increased pulse pressure and marginally systolic hypertension) [36]. A protective effect of nutrients was demonstrated in one study, in which a reduction in the severity of oxidative stress (reduced urinary 15-F_{2t}-isoprostane) was observed in adults who had a higher intake of B vitamins [35].

However, no consistent effect was observed between folic acid intake and bladder cancer risk in adults [37]. Three studies demonstrated an increased risk of skin lesions in individuals who had low intakes of choline, vitamin B_2, folate and zinc [38–40]. In turn, a reduced risk of As-related skin lesions was observed in adults who consumed higher amounts of vitamin B_2, B_6 and folic acid [41].

Low folic acid intake was not associated with cognitive performance in children, but higher intake was positively associated with cognitive abilities. In this study, moreover, several relationships were observed between tAs in the urine and cognitive performance depending on the level of folic acid intake, but it was an inconsistent effect [42]. In another study by the same author, there was no relationship between urinary tAs and achievement (broad math and reading scores) among children and B vitamin intake [43].

In turn, negative associations between vitamin B_{12} intake and tAs concentrations in toenails were observed in the study by Gruber et al. [44].

3.2.3. Nutrient Intake—Summary

The results of human studies on the relationship between nutrient intake and iAs metabolism are inconclusive. They indicate that some nutrient intake may contribute to iAs elimination from the body. In several studies, the intake of such nutrients as methionine, choline, vitamin B_2, vitamin B_6, B_{12}, folate, zinc was observed to be correlated with iAs metabolism. However, some of the correlations between nutrient intakes (betaine, choline, vitamin B_2, vitamin B_6, vitamin B_{12}, and folate) and the urinary content of various forms of iAs suggest that these nutrients may or may not impair iAs metabolism.

The authors also observed a relationship between a high intake of B vitamins and folic acid and a reduction in the adverse changes associated with exposure to iAs (decreased oxidative stress, reduced risk of As-related skin lesions, and increased cognitive abilities). In turn, low intake (choline, B vitamins, folic acid, and zinc) was associated with the deterioration of cardiovascular outcomes and an increased risk of skin lesions. The differences in these results may be due to the levels of nutrient intake (higher intake was connected with a reduction in the negative effects associated with iAs exposure, while a low intake may exacerbate these effects).

However, in several studies, there are conflicting data that require clarification. No consistent effect was observed between B vitamins and folic acid intake, and risk of diabetes and bladder cancer, as well as cognitive performance and achievements in children. In these studies, the author suggested that the lack of, or unclear, effect may be due to the low variability in vitamin intake, or that intake was above a sufficient level in the majority of participants. Additionally, the results could also be influenced by additional metabolic differences (e.g., genetic variation or differences in iAs metabolism—the study was conducted on children, adults and patients with bladder cancer). In one of these studies, a high intake of vitamin B_6 was associated with diabetes-related outcomes, but this effect was unclear because, in most studies, low consumption contributed to the severity of the negative effects and, therefore, this aspect requires further analysis.

Table 2. Relationship between dietary compound intake and iAs metabolism and health effects related to As exposure.

Reference	Population	Dietary Assessment Methods	Component	Main Results
Desai et al., 2020 [23]	*n* = 290 Montevideo (Uruguay), children ~7 years	2 nonconsecutive 24 h recalls	vitamin B_6—dietary intake	urine: %DMA (NS), %MMA (−), %iAs (NS)
			vitamin B_2 and B_{12}—dietary intake	urine: %DMA (NS), %MMA (NS), %iAs (NS)
Desai et al., 2020 [25]	*n* = 307 Montevideo (Uruguay), children ~7 years	2 nonconsecutive 24 h recalls	vitamin B_{12}—dietary intake	urine: %DMA (NS), %MMA (NS), %iAs (NS)
			folate—dietary intake	urine: %DMA (NS), %MMA (−), %iAs (NS)
Kordas et al., 2016 [26]	*n* = 357 Montevideo (Uruguay), children ~5–8 years	2 nonconsecutive 24 h recalls	folate—dietary intake	urine: %DMA (+), %MMA (−), %iAs (NS), tAs (NS)
Kurzius-Spencer et al., 2017 [24]	*n* = 2420 U.S., adults and children > 6 years	24 h recall	vitamin B_6—dietary intake	urine: %DMA (NS), %MMA (NS), %iAs (NS), DMA/MMA (NS) (in the group of children) urine: %DMA (NS), %MMA (NS), %iAs (−), DMA/MMA (NS) (in the group of adults)
			vitamin B_{12}—dietary intake	urine: %DMA (NS), %MMA (NS), %iAs (NS), DMA/MMA (NS) (in the groups of adults and children)
			folate—dietary intake	urine: %DMA (NS), %MMA (NS), %iAs (NS), DMA/MMA (NS) (in the group of children) urine: %DMA (+), %MMA (NS), %iAs (−), DMA/MMA (NS) (in the group of adults)
Spratlen et al., 2018 [31]	*n* = 935 Arizona, Oklahoma, North Dakota, South Dakota, men and women >14 aged	FFQ	vitamin B_2, vitamin B_6, folic acid—dietary intake	urine: %DMAs (NS), %MMAs (NS), %iAs (NS)
			vitamin B_6—dietary intake	risk for metabolic syndrome (+), risk for diabetes (+), HOMA2-IR (+)
			vitamin B_2, folic acid—dietary intake	risk for metabolic syndrome (NS), risk for diabetes (NS), HOMA2-IR (NS)
Lopez-Carillo et al., 2016 [28]	*n* = 1027 Mexico, women	FFQ	methionine—dietary intake	urine: %DMA (+), %MMA (NS), %iAs (−), DMA/MMA (+), DMA/iAs (+)
			betaine—dietary intake	urine: %DMA (NS), %MMA (NS), %iAs (NS), MMA/iAs (NS), DMA/MMA (NS)
			choline—dietary intake	urine: %DMA (+), %MMA (NS), %iAs (−), DMA/MMA (+), DMA/iAs (+)
			vitamin B_2, vitamin B_6—dietary intake	urine: %DMA (NS), %MMA (NS), %iAs (NS), DMA/MMA (NS), DMA/iAs (NS)
			vitamin B_{12}—dietary intake	urine: %DMA (+), %MMA (NS), %iAs (−), DMA/MMA (+), DMA/iAs (+)
			folate—dietary intake	urine: %DMA (NS), %MMA (NS), %iAs (−), DMA/MMA (NS), DMA/iAs (+)
			zinc—dietary intake	urine: %DMA (+), %MMA (−), %iAs (−), DMA/MMA (+), DMA/iAs (+)
Heck et al., 2009 [29]	*n* = 10,402 Bangladesh, men and women	FFQ	methionine—dietary intake	urine: tAs↑ and (+)

Table 2. *Cont.*

Reference	Population	Dietary Assessment Methods	Component	Main Results
Heck et al., 2007 [27]	n = 1016 Bangladesh, men and women	FFQ	methionine—dietary intake	urine: %DMA (NS), %MMA (+), %iAs (−), MMA/iAs (+), DMA/MMA (NS)
			betaine—dietary intake	urine: %DMA (NS), %MMA (NS), %iAs (NS), MMA/iAs (NS), DMA/MMA (NS)
			choline—dietary intake	urine: %DMA (NS), %MMA (NS), %iAs (NS), MMA/iAs (NS), DMA/MMA (+)
			vitamin B_2—dietary intake	urine: %DMA (NS), %MMA (+), %iAs (NS), MMA/iAs (+), DMA/MMA (−)
			vitamin B_6—dietary intake	urine: %DMA (NS), %MMA (NS), %iAs (NS), MMA/iAs (NS), DMA/MMA (NS)
			vitamin B_{12}—dietary intake	urine: %DMA (NS), %MMA (+), %iAs (−), MMA/iAs (+), DMA/MMA (NS)
			folate—dietary intake	urine: %DMA (NS), %MMA (NS), %iAs (NS), MMA/iAs (NS), DMA/MMA (NS)
Bommarito et al., 2019 [32]	n = 1166 Chihuahua (Mexico), men and women	FFQ	vitamin B_2, vitamin B_{12}, folate—sufficient and insufficient	urine: %DMAs↔, %MMAs↔, %iAs↔
Spratlen et al., 2017 [30]	n = 405 Arizona, Oklahoma, North Dakota, South Dakota, men and women	FFQ	vitamin B_2, B_6—dietary intake	urine: %DMA (+), %MMA (−), %iAs (−)
			vitamin B_{12}, folate– dietary intake	urine: %DMA (NS), %MMA (NS), %iAs (NS)
Steinmaus et al., 2005 [33]	n = 87 U.S., men and women	HHHQ	zinc—dietary intake	urine: %DMA (+), %MMA (−), %iAs (NS)
			vitamin B_2, vitamin B_6, folate—dietary intake	urine: %DMA (NS), %MMA (NS), %iAs (NS)
Argos et al., 2010 [34]	n = 9833 Araihazar (Bangladesh), men and women	FFQ	vitamin B_6—dietary intake	urine: tAs (+)
			vitamin B_2, vitamin B_{12}, folate—dietary intake	urine: tAs (NS)
Chen et al., 2007 [36]	n = 10,910 Bangladesh, men and women	FFQ	vitamin B_2, B_6, B_{12}, folate—low intake level	ORs of high pulse pressure↑, ORs of systolic hypertension↑ (weak association)
Howe et al., 2017 [35]	n = 418 New Hampshire, men and women	FFQ	vitamin B_2, vitamin B_6, vitamin B_{12}, folate—sum of B vitamin—dietary intake	urine: proportion of MMAs (−), 15-F_{2t}-IsoP (−)
Koutros et al., 2018 [37]	n = 2366 Maine, New Hampshire, Vermont, case with bladder cancer and control group	DHQ	folate—high and low intake level	ORs of risk of bladder cancer↔ (weak association)
Melkonian et al., 2012 [38]	n = 16,391 Araihazar (Bangladesh), cases with skin lesions and control group	FFQ	vitamin B_2, folate—low intake level	keratotic skin lesion risk (+)

Table 2. *Cont.*

Reference	Population	Dietary Assessment Methods	Component	Main Results
Deb et al., 2012 [39]	*n* = 208 West Bengal, cases with skin lesions and control group	24 h recall	choline, vitamin B_2, zinc—low intake level	ORs of skin lesions↑ (in the group of women)
			choline, zinc—low intake level	ORs of skin lesions↑ (in the group of men)
			vitamin B_6, vitamin B_{12}, folate	ORs of skin lesions↔ (in the group of women and men)
Mitra et al., 2004 [40]	*n* = 384 West Bengal (India), cases with skin lesions and control group	24 h recall	folate—low intake level	ORs of skin lesions↑
			vitamin B_2, vitamin B_6, zinc	ORs of skin lesions↔
Zablotska et al., 2008 [41]	*n* = 10,628 Araihazar (Bangladesh), men and women	FFQ	vitamin B_2, B_6, folic acid—high intake level	PORs risk for skin lesions↓
			vitamin B_{12}	PORs risk for skin lesions↔
Desai et al., 2018 [42]	*n* = 328 Montevideo (Uruguay), children ~5–8 years	2 nonconsecutive 24 h recalls	folate—low intake level	cognitive performance (NS) tAs—concept formation (−), tAs—scores of numbers reversed subtest (+), tAs—cognitive efficiency (+)
			folate—mean intake level	scores on: verbal comprehension (+), visual auditory learning (+), verbal ability (+), general intellectual abilities (+) tAs—sound integration scores (+)
			folate—high intake level	scores on: visual auditory learning (−), concept formation (+), numbers reversed (+), cognitive efficiency (+) tAs—concept formation (+)
Desai et al., 2020 [43]	*n* = 239 Montevideo (Uruguay), children ~5–8 years	2 nonconsecutive 24 h recalls	vitamin B_2, vitamin B_6, vitamin B_{12}, folate—dietary intake	broad math and reading scores (calculation, math facts fluency, applied problems, sentence reading fluency, letter word identification, passage comprehension) and urinary tAs (NS)
Gruber et al., 2012 [44]	*n* = 920 New Hampshire, men and women	FFQ	vitamin B_{12}—dietary intake	toenail: tAs (−)

↑—significant increase; ↓—significant decrease; ↔—no significant changes; (+)—positively association; (−)—negatively association; (NS)—no significant association; DHQ—diet history questionnaire; DMA—dimethylarsinic acid; DMAs—monomethyl arsenic species; FFQ—food frequency questionnaire; HHHQ—health habits and history questionnaire; HOMA2-IR—Homeostatic Model Assessment Index 2; iAs—inorganic arsenic; MMA—monomethylarsonic acid; MMAs—monomethyl arsenic species; ORs—odds ratios; PORs—prevalence odds ratios; tAs—total arsenic species; 15-F_{2t}-IsoP—15-F_{2t}-isoprostane.

3.3. Folic Acid and Zinc Supplementation

Table 3 includes results from studies in which folic acid (eight studies) and zinc (one study) were supplemented in a population exposed to As.

3.3.1. Folic Acid and Zinc Supplementation—iAs Metabolism

Five studies analyzed the protective effect of folic acid and zinc supplementation on iAs metabolism. Changes in iAs metabolite concentrations (inter alia, decreased concentrations of MMA in the blood and increased urinary excretion of DMA) were observed in people taking folic acid supplementation [45]. In a study by Bozack et al. [46], folic acid supplementation in subjects with low and normal blood concentrations of this compound also altered iAs metabolism profile (by increased percentage dimethyl-arsenical species and decreased %iAs and monmethylarsenical species in the urine). In the study by Peters et al. [47] conducted on a Bangladeshi population, folic acid supplementation at a higher dose (800 µg/db) reduced the concentration of tAs in the blood. This effect persisted even after 12 weeks from the end of supplementation, but no such effect was observed with a lower dose of this vitamin (400 µg/db). Folic acid supplementation in participants with betaine concentrations below the median affected iAs metabolism (inter alia, through an increased percentage of dimethyl-arsenical species and decreased percentage of monomethyl arsenical species in the urine) [48]. In contrast, in a group of children, zinc supplementation decreased urinary %DMA, but did not affect the concentration of other As biomarkers in the urine [49]. In a group of Bangladeshi residents exposed to iAs in drinking water supplementation with folic acid, there was an influence on parameters related to iAs metabolism through increased plasma choline and betaine concentration and a percentage decrease in dimethylglycine in the plasma. There were no significant differences between groups receiving lower (400 µg/day) and higher (800 µg/day) doses of folic acid [50].

3.3.2. Folic Acid Supplementation—Toxicity of As

In one study, symptoms of chronic arsenic intoxication were analyzed in an adolescent girl who was taking globules (containing iAs). Treatment with thiamine and folic acid, among others, reduced the adverse symptoms and the concentration of iAs in urine and hair [51]. In contrast, folic acid supplementation did not alter post-translational histone modifications in peripheral blood mononuclear cells from Bangladeshi adults [52]. Ghose et al. [53] showed that folic acid supplementation reduced adverse symptoms in patients with chronic As toxicity.

3.3.3. Folic Acid and Zinc Supplementation—Summary

In four studies, folic acid supplementation (in doses 400 and 800 µg/day for 12 or 24 weeks) improved iAs metabolism in adults (mainly through increased urinary DMA concentration and decreased tAs concentration in the urine and the blood). In one of these studies, only a higher dose of folic acid improved iAs metabolism. In turn, zinc supplementation (30 mg/day for 6 months) in children resulted in a different effect (decreased urinary DMA concentration).

In two studies, folic acid supplementation (5 mg/day) reduced the adverse symptoms associated with As poisoning, while in one study, folic acid supplementation with a small dose (400 µg/day) had no effect on epigenetic regulation. A short period of supplementation (12 weeks) with folic acid or using a low dose of folic acid (400 mg/day) could make this effect invisible. The authors also indicate that the As-removing water filter was used during the study, which could counteract the effects caused by folic acid supplementation.

Table 3. Results of studies conducted in people with As exposure and supplementation with folic acid or zinc.

Reference	Research Model	Study Description	Main Results
Gamble et al., 2007 [45]	Bangladesh, adults	CG (*n* = 62)—placebo (orally, for 12 weeks) G1 (N = 68)—folic acid 400 µg/day (orally, for 12 weeks)	G1 vs. CG blood: MMA↓, tAs↓, DMA↔ urine: DMA↑ (after 1 week) DMA↔(after 12 week)
Bozack et al., 2019 [46]	Bangladesh, adults	CG (*n* = 90)—placebo (orally, for 12 weeks) G1 (*n* = 133)—folic acid 400 µg/day (orally, for 12 weeks) G2 (*n* = 129)—folic acid 800 µg/day (orally, for 12 weeks)	G1, G2 vs. CG plasma: folate↑, homocysteine↓ RBC folate↑ urine: %iAs↓, %MMAs↓, %DMAs↑
			G2 vs. G1 urine: %MMAs↓
		G1 (*n* = 68)—folic acid 400 µg/day (orally, for 12 weeks) and after that placebo (orally, for 12 weeks) G2 (*n* = 60)—folic acid 800 µg/day (orally, for 12 weeks) and after that placebo (orally, for 12 weeks) G1a (*n* = 65)—folic acid 400 µg/day (orally, for 24 weeks) G2a (*n* = 69)—folic acid 800 µg/day (orally, for 24 weeks)	G1a, G2a vs. G1, G2 urine: %iAs↓, %MMAs↓, %DMAs↑
Peters et al., 2015 [47]	Bangladesh, adults	CG (*n* = 102)—placebo (orally, for 12 weeks) G1 (*n* = 153)—folic acid 400 µg/day (orally, for 12 weeks) G2 (*n* = 151)—folic acid 800 µg/day (orally, for 12 weeks)	G1 vs. CG plasma folate↑, RBC folate↑, geometric mean of blood tAs↑, percentage decline in geometric mean blood tAs from baseline↓
			G2 vs. CG plasma folate↑, RBC folate↑, geometric mean of blood tAs↓, percentage decline in geometric mean blood tAs from baseline↑
		CG (*n* = 102)—placebo (orally, for 24 weeks) G1 (*n* = 76)—folic acid 400 µg/day (orally, for 12 weeks) and after that placebo (orally, for 12 weeks) G2 (*n* = 74)—folic acid 800 µg/day (orally, for 12 weeks) and after that placebo (orally, for 12 weeks) G1a (*n* = 77)—folic acid 400 µg/day (orally, for 24 weeks) G2a (*n* = 77)—folic acid 800 µg/day (orally, for 24 weeks)	G2, G2a vs. CG geometric mean of blood tAs↓
			G1a vs. G1 geometric mean of blood tAs↔, percentage decline in geometric mean of urinary and blood tAs↔
			G2a vs. G2 geometric mean of blood tAs↔, percentage decline in geometric mean of urinary and blood tAs↔

Table 3. *Cont.*

Reference	Research Model	Study Description	Main Results
Bozack et al., 2020 [48]	Bangladesh, adults	CG (n = 104)—placebo (orally, for 12 weeks) G1 (n = 156)—folic acid 400 µg/day (orally, for 12 weeks) G2 (n = 154)—folic acid 800 µg/day (orally, for 12 weeks)	G1 vs. CG participants with betaine concentrations below the median: urine: decreases in ln(%iAs)↑, decrease in %MMAs↑, increases in %DMAs↑ G2 vs. CG participants with betaine concentrations below the median: urine: decreases in ln(%iAs)↑, increases in %DMAs↑
Kordas et al., 2017 [49]	Mexico, children (6–7 years)	CG (n = 151)—placebo (orally, for 6 months) G1 (n = 144)—zinc oxide 30 mg/day (orally, for 6 months)	G1 vs. CG urine: %DMA↓, %MMA↔, %iAs↔, tAs↔
Hall et al., 2016 [50]	Bagladesh, adults	CG (n = 101)—placebo (participants received arsenic-removal water filters and had been drinking water from wells with water As concentration >50 µg/L at least 3 years) G1 (n = 152)—folic acid 400 µg/day (orally, for 12 weeks) and after that placebo (participants received arsenic-removal water filters and had been drinking water from wells with water As concentration >50 µg/L at least 3 years) G2 (n = 149)—folic acid 800 µg/day (orally, for 12 weeks) and after that placebo (participants received arsenic-removal water filters and had been drinking water from wells with water As concentration >50 µg/L at least 3 years)	G1, G2 vs. CG plasma: choline↑, betaine↑, percentage decrease in DMG↑ G1 vs. G2 plasma: choline↔, betaine↔, percentage decrease in DMG↔
Dani, 2019 [51]	Women (16 year-old)—chronic arsenic intoxication	*ex juvantibus* therapy: torasemide 10–20 mg/day, thiamine 300 mg/day, magnesium 5 mg/day, folic acid 5 mg/day and metamizole and simeticon (on-demand)	nuchal scalp hair shafts: As—undetectable morning urine: As—undetectable afternoon urine: As 50 nmol/L symptoms (leg cramps, abdominal pains)↓
Howe et al., 2017 [52]	Bangladesh, adults	CG (n = 104)—placebo (orally, for 12 weeks) G1 (n = 156)—folic acid 400 µg/day (orally, for 12 weeks)	G1 vs. CG blood: PTHMs↔
Ghose et al., 2014 [53]	India, patients with symptoms of arsenic toxicity	CG (n = 45)—drinking arsenic free water (orally, for 6 months) G1 (n = 32)—folic acid 5 mg/day (orally, for 6 months)	G1 vs. CG skin score↓, systemic disease score↑ (overall: clinical symptoms of arsenicosis↓)

↑—significant increase; ↓—significant decrease; ↔—no significant changes; CG—control group; DMA—dimethylarsinic acid; DMAs—dimethyl arsenical species; DMG—dimethylglycine; G1—group 1; G2—group 2; iAs—inorganic arsenic; MMA—monomethylarsonic acid; MMAs—monomethyl arsenical species; PTHMs—post-translational histone modifications; RBC—red blood cell; tAs—total arsenic species.

3.4. Blood and Tissues Nutrients Concentration

Table 4 presents a summary of the results of 29 human studies in which the relationship between the concentrations of dietary compounds (in blood and other tissue) and As metabolism was analyzed, as well as severity of adverse health effects associated with As exposure.

3.4.1. Blood Nutrient Concentration—iAs Metabolism

Eight studies analyzed the relationship between blood micronutrient concentrations and urinary As metabolites in children. No relationship was observed between plasma vitamin B_6 levels and urinary excretion of As metabolites (%MMA, %DMA, and %iAs) [24]. The results of three studies [24,25,54] did not demonstrate the relationship between vitamin B_{12} concentration in the plasma/serum and As metabolites in the urine. In turn, the results of two studies demonstrated an increase in the %DMA and a decrease in the %MMA, %iAs in the urine of the group of children with high concentrations of vitamin B_{12} and folate in their plasma [55] in the second study showed a positive association between serum vitamin B_{12} concentration and urinary concentrations of DMA [56]. Additionally, results of three studies showed no relationship between the concentration of plasma/serum folate and urinary %MMA [24,54,57]. Plasma/serum folate concentration was positively associated with the percentage and concentration of DMA in the urine [56–58] and negatively with urinary %iAs [54,57]. In contrast, in the study by Kurzius-Spencer et al. [24], folate concentration in the serum had no relationship with urinary %DMA and %iAs, and in the study by Desai et al. [25], no relationship was observed with any urinary iAs metabolites.

Family iAs metabolism was only analyzed in one study. A strong pattern of metabolism was shown between siblings. Additionally, in siblings, positive correlations between urinary iAs/methylated As, monomethylarsonate/dimethylarsinate a methionine, vitamin B_6, B_{12} folate in the blood were observed. These correlations between parents and children were much lower [59].

The relationship between nutritional status and iAs metabolism has also been analyzed in the adult population (12 studies).

One study found that plasma vitamin B_6 concentration was not associated with the urinary excretion of As metabolites (%MMA, %DMA, and %iAs) [24].

A lower %MMA and higher %DMA in the urine were observed in adults with a low concentration of vitamin B_{12} in the plasma compared to individuals with a higher concentration of this vitamin. The study also noted that the concentration of vitamin B_{12} in the plasma was positively associated with urinary %MMA, but inversely associated with urinary %iAs. Such a relationship was stronger in the group of subjects with an adequate folate concentration in the plasma [60]. The study by Zhu et al. [56] demonstrated that serum vitamin B_{12} concentration was positively associated with urinary concentrations of DMA. However, the results of other studies showed no relationship between plasma and serum vitamin B_{12} concentration and urinary As metabolites [24,61].

In contrast, three studies found no relationship between plasma/serum vitamin B_{12} concentrations and urinary excretion of As metabolites (%MMA, %DMA, and %iAs) in pregnant women [62–64]. Only the study by Laine et al. [62] demonstrated a negative correlation between maternal serum vitamin B_{12} with tAs in the urine. Moreover, in women, the concentration of vitamin B_{12} in the plasma was inversely associated with arsenate [65] and positively associated with %iAs [62] in cord blood.

In one study, serum/plasma folate concentration was positively associated with urinary %MMA [58], while in the study by Gamble et al. [61], it was negatively associated with urinary %MMA. Two studies demonstrated a positive association between folate concentration in the serum/plasma and urinary %DMA [56,61]. In turn, the results of another study did not report such a relationship [58]. Moreover, in the study by Gamble et al. [61] plasma folate concentration was negatively associated with urinary %iAs. In turn, in the study by Kurzius-Spencer et al. [24] no relationship was shown between serum folate levels and urinary iAs metabolites.

In a Bangladeshi population, individuals with low plasma folate concentrations showed a correlation between decreased ratio of glutathione-to-glutathione disulfide and higher blood levels of tAs, as well as having increased %MMA and decreased %DMA in the urine [66].

The study by Chung et al. [67] analyzed the relationship between gene polymorphisms, iAs metabolism and plasma folate. Abnormal iAs metabolism and decreased plasma folate levels were observed in patients with urothelial carcinoma. Subjects with the 5,10-methylenetetrahydrofolate reductase CT or TT genotype had lower percentage dimethylarsenic acid in the urine and a lower folate concentration in the plasma than those with the CC genotype. A positive correlation was also observed between plasma folate concentration and percentage urinary dimethylarsenic acid in the control group.

In Bangladeshi women, folate concentration in the plasma/serum was inversely associated with arsenate in the maternal blood [65], and in pregnant women negatively correlated with percentage monomethyl arsenicals in the cord serum [62]. In contrast, three studies did not demonstrate significant correlations between plasma folate levels and urinary As metabolites in pregnant women [62–64]. Only in women with both higher As exposure level and plasma folate concentration was a reduced iAs level in the urine noted [63].

Two studies reported no significant relationship between plasma zinc concentrations and urinary As metabolites in pregnant women [62,64]. Only in subjects with the highest exposure level were higher plasma zinc concentrations associated with increased %MMA and %iAs, and decreased %DMA [63].

3.4.2. Blood and Tissue Nutrient Concentration—Toxicity of As

Many studies have focused on analyzing the relationship between nutritional status and As concentrations in various tissues and adverse health effects in individuals exposed to As.

The relationship between As exposure, nutritional status, and DNA methylation was analyzed in three studies. In elderly men, no association was observed between the plasma concentrations of vitamin B_6 and B_{12} and DNA methylation. However, in men with a low concentration of folate in the plasma, a positive association with one repetitive element (increased Alu DNA methylation) was observed, whereas in men with a higher concentration of plasma folate, the effect was the opposite [68]. Similarly, the study in Bangladeshi adults with high plasma folate concentration (above 9 nmol/L) showed negative correlation between urinary or plasma tAs and methylation of peripheral blood leukocyte DNA (lower [3H]-methyl incorporation) [69]. In turn, in women with folate deficiency in the plasma, an inversely association was observed between the tAs concentration in toenail and total histone 3 levels in the plasma [70].

Howe et al. [52] demonstrated positive associations between the plasma concentration of vitamin B_{12} (among women) and choline (among men) and global levels of post-translational histone modifications.

Several studies have reported the relationship between blood concentrations of nutrients and the risk of disease development or the severity of adverse body changes associated with an exposure to As.

A higher odds ratio of type 1 diabetes by the percentage of monomethylates As was observed in individuals with a higher concentration of plasma folate (stronger relationship between percentage monomethylated As and type 1 diabetes). No significant relationship was shown for type 2 diabetes between percentage monomethylates As and plasma folate or vitamin B_{12} concentration [71]. It was observed in the study by Chung et al. [72] that low a concentration of folate in the plasma, global 5-methyl-2′-deoxycytidine levels, and a high concentration of total urinary As levels was related to an increased risk of urothelial carcinoma. The study conducted in a West Bengal population, did not demonstrate any relationship between the concentration of folate, methionine, vitamin B_6, vitamin B_{12} in the blood and the susceptibility to develop skin lesions [73]. Subjects with low plasma

folate concentrations had an increased risk of skin lesions, whereas no such relationship was observed in subjects with lower vitamin B_{12} concentrations [74].

Low concentrations of folate and vitamin B_{12} in the plasma were indirectly (through association with decreased iAs metabolism) related to an increased childhood developmental delay (increase in odds ratio of developmental delay) [55]. In the study by Desai et al. [43], higher broad math scores were observed in subjects with higher vitamin B_{12} in the serum and tAs concentration in the urine.

In subjects exposed to As, zinc and tAs concentrations in different tissues were analyzed. In pregnant women living in Wuhan (the largest industrial city), a positive correlation between tAs and zinc concentration in blood was observed [75]. On the other hand, in patients with blackfoot disease, decreased zinc concentration in the hair, urine, blood as well as increased tAs concentration in hair and urine were observed [76–78]. On the other hand, in groups of workers, a higher concentration of tAs in the lung tissue and in the blood was observed, but no decreased concentration of zinc in these tissues was shown [79,80]. Moreover, in the study by Tutkun et al. [80], the correlation between Zn and tAs was negative and in the workers group higher levels of inflammatory markers (interleukins 6, 10 and tumor necrosis factor-α) was also observed, but no correlation was observed between these cytokines and Zn.

3.4.3. Concentration of Nutrients in Blood and Other Tissues—Summary

The results of human studies on the relationship between the blood concentrations of dietary compounds and iAs metabolism are also inconclusive. They indicate that the concentration of dietary compounds in the blood may be related to iAs metabolism and contribute to its elimination from the body. This is indicated by several studies in which it was observed that the blood concentration of vitamin B_{12} and folate correlated with iAs metabolism. However, some of the correlations between the blood concentration of these nutrients (folate, B_6, B_{12}, and zinc) and the urinary content of various forms of iAs suggest that these nutrients may or may not impair iAs methylation.

Three studies showed that nutritional status (choline, vitamin B_{12}, folate—higher plasma/serum concentrations) may be related to a decrease in adverse health effects (affected methylation DNA, histone modification, and children achievement) in individuals exposed to As. The results of this study are in line with expectations, since the appropriate status of these nutrients can alleviate the adverse health effects associated with exposure to As. Numerous studies also observed a relationship between concentration of these nutrients (folate—low and higher plasma concentration; vitamin B_{12}—low plasma concentration) and increased risk of disease and adverse body changes associated with an exposure to As (increased DNA methylation, risk of type 1 diabetes and urothelial carcinoma, skin lesions, and developmental delay). Folate deficiency (five studies) and vitamin B12 deficiency (one study) enhanced adverse effects of iAs exposure, and only in one study higher concentration of folate exacerbated them. These results indicate that both a deficiency and an excess of these compounds may contribute to enhancing the toxicity of As. The level of exposure to iAs does not appear to be a differentiating factor, as the adverse effects associated with folic acid deficiency occurred in populations with low (including elderly men, preschool children, and women) and high exposure (cases with skin lesions and urothelial carcinoma). In several studies, no relationships (or the relationship was unclear) between blood/plasma concentration of methionine, vitamins B_6, B_{12}, folate a DNA methylation, risk of type 2 diabetes and skin lesions were found. Moreover, in pregnant women, patients with blackfoot disease and workers exposed to As, elevated tAs concentrations in many tissues were connected with disturbances in zinc concentrations.

Table 4. Relationship between concentration of dietary compound in blood and other tissues and iAs metabolism and health effects related to As exposure.

Reference	Population	Measure of Component Status	Main Results
Hall et al., 2009 [54]	*n* = 165 Bangladesh, children (6 years old)	plasma: vitamin B_{12}	urine: %DMA (NS), %MMA (NS), %iAs (NS)
		plasma: folate	urine: %DMA (NS), %MMA (NS), %iAs (−)
Skroder Loveborn et al., 2016 [57]	*n* = 488 Bangladesh, children (9 years old)	plasma: folate	urine: %DMA (+), %MMA (NS), %iAs (−)
Lin et al., 2019 [55]	*n* = 266 Taiwan, children (preschool aged)	plasma: vitamin B_{12} and folate	urine: %DMA↑, %MMA↓, %iAs↓ (in the group with high concentrations vitamin B_{12} and folate)
			ORs of development delay↑ (in the group with low concentrations vitamin B_{12} and folate)
Desai et al., 2020 [25]	*n* = 307 Montevideo (Uruguay), children ~7 years	serum: vitamin B_{12}, folate	urine: %DMA (NS), %MMA (NS), %iAs (NS)
Zhang et al., 2019 [58]	*n* = 11,016 US, adults and children (≤18 years)	serum: folate	urine: %DMA (+), MMA (NS) (in the group of children) urine: %DMA (NS), %MMA (+) (in the group of adults)
Zhu et al., 2018 [56]	*n* = 3099 U.S., adults and children (6–19 years)	serum: vitamin B_{12}, folate	urine: DMA (+) (in the group of children and in the group of adults)
Kurzius-Spencer et al., 2017 [24]	*n* = 2420 U.S., adults and children >6 years	plasma: vitamin B_6 serum: vitamin B_{12}, folate	urine: %DMA (NS), %MMA (NS), %iAs (NS), DMA/MMA (NS) (in the groups of adults and children)
Chung et al., 2002 [59]	*n* = 44 Chile, adults and children (6–14 years)	blood: methionine, vitamin B_6, vitamin B_{12}, folate	urine: iAs/methylated As (+), MMA/DMA (+)
Hall et al., 2009 [60]	*n* = 778 Bangladesh, adults	plasma: vitamin B_{12}	urine: %DMA↑, %MMA↓, %iAs↔ (in the vitamin B_{12} deficient group compared to vitamin B_{12} sufficient group) urine: %DMA (NS), %MMA (+), %iAs (−)
Gamble et al., 2005 [61]	*n* = 300 Bangladesh, adults	plasma: vitamin B_{12}	urine: %DMA (NS), %MMA (NS), %iAs (NS)
		plasma: folate	urine: %DMA (+), %MMA (−), %iAs (−)
Niedzwiecki et al., 2014 [66]	*n* = 376 Bangladesh, adults	plasma: folate	plasma: GSH/GSSG ratio association with urine: %DMA (−), %MMA (+), SMI (+), blood: tAs (−) (in the folate deficient group)
Chung et al., 2010 [67]	*n* = 450 cases with urothelial carcinoma and control group	plasma: folate	urine: %DMA↓, %MMA↑, %iAs↑, tAs↑ (cases with urothelial carcinoma) urine: %DMA↓ (controls with 5,10-methylenetetrahydrofolate reductase CT or TT genotype) urine: %DMA (+) (in the control group)
Hall et al., 2007 [65]	*n* = 30 pairs Bangladesh, women and children (newborn)	plasma: vitamin B_{12}	cord blood: percentage arsenate (−) (in the group of women)
		plasma: folate	blood: percentage arsenate (−) (in the group of women)

Table 4. *Cont.*

Reference	Population	Measure of Component Status	Main Results
Laine et al., 2018 [62]	*n* = 197 Mexico, women (pregnant)	serum: vitamin B_{12}	urine: %DMA (NS), %MMA (NS), %iAs (NS), tAs (−) cord serum: %iAs (+)
		serum: folate	urine: %DMA (NS), %MMA (NS), %iAs (NS), tAs (NS) cord serum: %MMAs (−)
Li et al., 2008 [63]	*n* = 753 Bangladesh, women (pregnant)	plasma: vitamin B_{12}, folate, Zn—high and low values	urine: %DMA↔, %MMA↔, %iAs↔ (in the group at the low As exposure level)
		plasma: folate—high values	urine: %iAs↓ (in the group at the highest As exposure level)
		plasma: Zn—high values	urine: %DMA↓, %MMA↑, %iAs↑, SMI↓ (in the group at the highest As exposure level)
Gardner et al., 2011 [64]	*n* = 324 Bangladesh, women (pregnant)	plasma: vitamin B_{12}, folate, Zn	urine: %DMA (NS), %MMA (NS), %iAs (NS)
Lambrou et al., 2012 [68]	*n* = 581 Boston, men (elderly)	plasma: vitamin B_6, vitamin B_{12}	blood: Alu (NS), Long Interspersed Nucleotide Element-1 (NS)
		plasma: folate	blood: Alu (+), Long Interspersed Nucleotide Element-1 (NS) (in the low folate group)
			blood: Alu (−), Long Interspersed Nucleotide Element-1 (NS) (in the high folate group)
Pilsner et al., 2007 [69]	*n* = 294 Bangladesh, adults	plasma: folate	[^{3}H]-methyl incorporation association with tAs in the urine, plasma (−) (in the high folate group)
Tauheed et al., 2017 [70]	*n* = 85 Bangladesh, women	plasma: folate	tAs concentration in toenail association with plasma total H3 (−) (in the folate deficient group)
Howe et al., 2017 [52]	*n* = 324 Bangladesh, adults	plasma: choline	peripheral blood mononuclear cells: H3K36me2 (+) (in the men group)
		plasma: vitamin B_{12}	peripheral blood mononuclear cells: H3K79me2 (+) (in the women group)
		plasma: folate	peripheral blood mononuclear cells: H3K36me2 (NS), H3K36me3 (NS), H3K79me2 (NS) (in the men and women group)
Grau-Perez et al., 2017 [71]	*n* = 688 U.S., adults and children	plasma: vitamin B_{12}	ORs of type 1 and type 2 diabetes by %monomethylated As↔
		plasma: folate—high values	ORs of type 1 diabetes by %monomethylated As↑ ORs of type 2 diabetes by %monomethylated As↔
Chung et al., 2019 [72]	*n* = 534 Taiwan, cases with urothelial carcinoma and control group	plasma: folate	ORs of urothelial carcinoma↑ (low folate level and global 5-MedC, high tAs in the urine)

Table 4. *Cont.*

Reference	Population	Measure of Component Status	Main Results
Chung et al., 2006 [73]	*n* = 372 West Bengal, cases with skin lesions and control group	blood: methionine, vitamin B_6, vitamin B_{12}, folate	ORs of skin lesions↔
Pilsner et al., 2009 [74]	*n* = 548 Bangladesh, cases with skin lesions and control group	plasma: vitamin B_{12}—low values	ORs for development skin lesions↔
		plasma: folate—low values	ORs for development skin lesions↑
Desai et al., 2020 [43]	*n* = 239 Montevideo (Uruguay), children ~5–8 years	serum: vitamin B_{12}	urine: tAs and broad math score (+)
Gong et al., 2020 [75]	*n* = 406 Wuhan, women (pregnant) and control group (non-pregnant)	blood: Zn	blood: tAs (+) (in the group of pregnant women)
Wang et al., 1994 [76]	*n* = 218 Taiwan, cases with blackfoot disease and control group	hair: Zn	hair: Zn↓, tAs↑ (in the group of patients with blackfoot disease)
Tsai et al., 2004 [77]	*n* = 136 Taiwan, cases with blackfoot disease and control group	urine: Zn	urine: Zn↓, tAs↑ (in the group of patients with blackfoot disease)
Lin and Yang, 1988 [78]	*n* = 56 cases with blackfoot disease and control group	blood, serum, urine: Zn	blood, serum, urine: Zn↓ (in the group of patients with blackfoot disease) urine: tAs↔(in the group of patients with blackfoot disease) hair: tAs↑ (in the group of patients with blackfoot disease)
Gerhardsson and Nordberg, 1993 [79]	*n* = 110 smelter workers and control group (from urban and rural area)	lung tissue: Zn	lung tissue: Zn↔, tAs↑
Tutkun et al., 2019 [80]	*n* = 135 Ankara, men—workers (exposed to As) and control group	serum: Zn	blood: tAs↑, Zn↔, IL-6↑, IL-10↑, TNF-α↑ (in the workers group) correlation tAs—Zn (−) (in the workers group)

↑—significant increase; ↓—significant decrease; ↔—no significant changes; (+)—positively association; (−)—negatively association; (NS)—no significant association; As—arsenic; DMA—dimethylarsinic acid; GSH/GSSG—glutathione/glutathione disulfide; H3—histone 3; H3K36me2—histone 3 lysine 36 dimethylation; H3K36me3—histone 3 lysine 36 trimethylation; H3K79me2—histone 3 lysine 79 dimethylation; iAs—inorganic arsenic; IL-6—Interleukin 6; IL-10—interleukin 10; MMA—monomethylarsonic acid; MMA/DMA—monomethylarsonate/dimethylarsinate; MMAs—monomethyl arsenical species; ORs—odds ratios; SMI—secondary methylation index; tAs—total arsenic species; TNF-α—tumor necrosis factor-α; Zn—zinc; 5-MedC—5-methyl-2′deoxycytidine.

4. Conclusions

Many studies have also focused on analyzing the relationship between dietary compounds (intake, supplementation mainly folic acid, and blood concentrations), and iAs metabolism, as well as exposure-related disorders. The intake and blood concentrations of certain dietary compounds (methionine, choline, vitamin B_2, B_6, B_{12}, folic acid, and zinc) showed a relationship with an improvement in iAs metabolism and were associated with reduction in adverse health effects. It was also shown that not only the deficiency (folate), but also the excess of some dietary compounds (vitamin B_{12}, folic acid, zinc) may impair iAs metabolism and may increase adverse health effects. Human studies, to date, are inconclusive, because many factors influenced the results. This signals the need for a more detailed analysis of the relationship between the nutrient intake of involved in iAs metabolism, nutritional status as well as the severity and source of iAs exposure. Considering the promising results of the studies conducted so far, it seems reasonable that individuals exposed to iAs should consume natural products rich in methionine, choline, folic acid, B vitamins (B_2, B_6, B_{12}) and zinc. Products rich in methionine (meat: turkey, beef, pork and milk, tofu, and Brazil nuts), choline (eggs, beans, and broccoli; meat: chicken, pork, and beef), folic acid (green leafy vegetables), B-group vitamins (meat, eggs, dairy product, leafy greens, and legumes), and zinc (meat, nuts, and cereal products). Nutrition education focusing on an adequate dietary intake of methionine, choline, zinc, folic acid, and B vitamins (B_2, B_6, and B_{12})—nutrients which potentially have modulating effects in iAs metabolism and toxicity—should be used in prevention efforts for populations exposed to iAs.

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Szkoły Głównej Gospodarstwa Wiejskiego w Warszawie

Oświadczenie o współautorstwie

Niniejszym oświadczam, że w pracy Sijko, M., Kozłowska, L. (2021). Influence of Dietary Compounds on Arsenic Metabolism and Toxicity. Part II—Human Studies. *Toxics*, *9*(10), 259 mój indywidualny udział w jej powstaniu polegał na utworzeniu koncepcji treści i układu pracy, opracowaniu metodyki przeglądu piśmiennictwa, wyszukiwaniu artykułów w bazie danych, analizie i kategoryzacji artykułów, napisaniu oryginalnego draftu artykułu, prowadzeniu korespondencji z wydawcą.

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Szkoły Głównej Gospodarstwa Wiejskiego w Warszawie

Oświadczenie o współautorstwie

Niniejszym oświadczam, że w pracy Sijko, M., Kozłowska, L. (2021). Influence of Dietary Compounds on Arsenic Metabolism and Toxicity. Part II—Human Studies. *Toxics*, *9*(10), 259 mój indywidualny udział w jej powstaniu obejmował: pomysł podjęcia badań obejmujących aspekt zmian w profilu metabolicznym w odniesieniu do wielkości spożycia składników pokarmowych zaangażowanych w metabolizm arsenu, konsultacje merytoryczne dotyczące tworzenia treści, układu pracy, metodyki, przeglądu piśmiennictwa, analizy i kategoryzacji artykułów oraz edycji i korekcie merytorycznej artykułu..

Lucyna Kozłowska
(Podpis)

metabolites

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Article

Metabolic Changes and Their Associations with Selected Nutrients Intake in the Group of Workers Exposed to Arsenic

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Abstract: Arsenic (As) exposure causes numerous adverse health effects, which can be reduced by the nutrients involved in the metabolism of iAs (inorganic As). This study was carried out on two groups of copper-smelting workers: WN, workers with a urinary total arsenic (tAs) concentration within the norm (n = 75), and WH, workers with a urinary tAs concentration above the norm (n = 41). This study aimed to analyze the association between the intake level of the nutrients involved in iAs metabolism and the signal intensity of the metabolites that were affected by iAs exposure. An untargeted metabolomics analysis was carried out on urine samples using liquid chromatography–mass spectrometry, and the intake of the nutrients was analyzed based on 3-day dietary records. Compared with the WN group, five pathways (the metabolism of amino acids, carbohydrates, glycans, vitamins, and nucleotides) with twenty-five putatively annotated metabolites were found to be increased in the WH group. In the WN group, the intake of nutrients (methionine; vitamins B2, B6, and B12; folate; and zinc) was negatively associated with six metabolites (cytosine, D-glucuronic acid, N-acetyl-D-glucosamine, pyroglutamic acid, uridine, and urocanic acid), whereas in the WH group, it was associated with five metabolites (D-glucuronic acid, L-glutamic acid, N-acetyl-D-glucosamine, N-acetylneuraminic acid, and uridine). Furthermore, in the WH group, positive associations between methionine, folate, and zinc intake and the signal intensity of succinic acid and 3-mercaptolactic acid were observed. These results highlight the need to educate the participants about the intake level of the nutrients involved in iAs metabolism and may contribute to further considerations with respect to the formulation of dietary recommendations for people exposed to iAs.

Keywords: inorganic arsenic; metabolic pathways; diet; untargeted metabolomic

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1. Introduction

There are two major sources of As (arsenic) exposure: environmental and occupational. Occupationally exposed people are also subject to environmental exposure as they are also part of the general population (whose exposure occurs through environmental pollution, anthropogenic sources, and diet). As is used in numerous industries; hence, many professional groups are exposed to As, including steelworkers, miners, farmers, gardeners, workers involved in the production of glass or ammunition, and e-waste-recycling workers. Inhalation and dermal contact are the primary routes of occupational exposure [1–3]. During the mining and smelting of ore, dusts containing As are emitted. Increased concentrations of total As (tAs) in the urine were observed among copper-smelting workers [4,5].

As occurs in organic and iAs (inorganic As) forms, of which the latter is the more toxic form; hence, it is the primary focus of research [6,7]. The harmful effects of iAs on the human body are well documented and are associated with various diseases, including diabetes and heart, kidney, and neurodegenerative diseases, and may cause neurodevelopmental problems in children [8–12]. The latest research also pointed to the possibility of the

accumulation of tAs in semen and blood serum in a group of men working in highly polluted areas, which is related to a decline in semen quality and, consequently, to male reproductive disorders [13]. Furthermore, iAs exposure is associated with lung, skin, bladder, and other cancers, and the carcinogenicity of iAs has been confirmed by the International Agency for Research Cancer (IARC) [14].

Many studies have investigated the influence of iAs on the human body with the aim of identifying methods for reducing its negative effects. One such approach is dietary modification, an area that is constantly being researched. Particular attention has been paid to the dietary compounds of methyl donors (methionine, betaine, choline, and folic acid) and cofactors of reactions (vitamins B2, B6, B12, and zinc), which are involved in carbon metabolism (OCM). S-adenosyl-methionine is synthesized via OCM - is used as a methyl group donor in iAs metabolism. In this process, iAs is converted to MMA (monomethylarsonic acid) and DMA (dimethylarsinic acid). iAs, MMA, and DMA may be the end products of these process and excreted in urine. The proportions of these metabolites reflect the efficiency of iAs metabolism. DMA is rapidly excreted in the urine compared to iAs and MMA; as such, its higher concentration in urine indicates more efficient metabolism [15,16]. Low iAs metabolism efficiency (higher urinary MMA concentrations) is associated with an increased risk of As-associated carotid atherosclerosis, urothelial carcinoma, and skin lesions, among other ailments [17–19]. Many studies, both on animal models and human populations exposed to iAs primarily via drinking water, have focused on the relationship between the intake of nutrients and the concentration of As metabolites in the urine. Some studies have shown that these nutrients can improve the efficiency of iAs metabolism (observed as decreased concentrations of iAs and MMA and an increased urinary DMA concentration) and reduce adverse health effects, but not all studies have provided conclusive results [20,21].

In addition, to the best of our knowledge, studies analyzing the association between nutrient intake and the severity of metabolic changes in copper-smelting workers are not available in the literature. Therefore, this study aimed to analyze the association between the intake level of the nutrients involved in iAs metabolism and the signal intensity of the metabolites in this group of workers.

2. Materials and Methods

2.1. Study Participants and Design

This study was conducted on a group of copper-smelting workers from the south-western region of Poland. The following inclusion criteria were enforced: occupationally exposed to iAs, male, age above 18, and presence at work during the study. Eligible workers from whom the following data were obtained were included in the analysis: 3-day dietary records of food and beverages consumed, questionnaire (with data on the subject's age, height, body mass, and general characteristics), and urine samples. A total of 116 workers were included (3 participants were excluded from the analysis—2 of which were excluded due to missing information on dietary intake and 1 due to the lack of data on As speciation). Inhalation and dermal contact were determined to be the primary routes of occupational exposure. Then, the participants were divided into two groups (WN and WH) based on the concentration of tAs, according to the recommended Biological Limit Values for occupational exposure of 35 μg/L of urine [22]. The WN group consisted of workers with tAs concentrations <35 μg/L (n = 75), whereas the WH group consisted of workers with tAs concentrations >35 μg/L (n = 41). The study design is presented in Figure 1. All participants gave their informed consent for the study. Ethical approval was obtained from the Ethics Committee of the Nofer Institute of Occupational Medicine in Lodz, Poland (NR 08/2020).

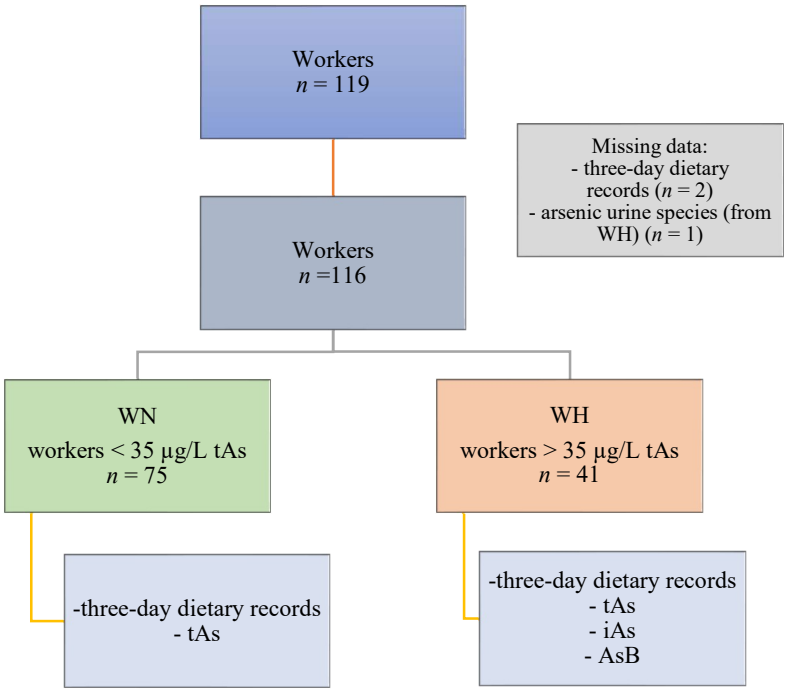

Figure 1. Study design. Abbreviations: AsB—arsenobetaine; iAs—inorganic arsenic; tAs—total arsenic; WN—the group of workers with urinary tAs concentration within the norm; WH—the group of workers with urinary tAs concentration above the norm.

2.2. Urine Collection and As Analysis

Urine samples were collected from workers after a shift at work. The samples were collected from May 2021 to June 2021 and were stored at −80 °C until As determination and untargeted analysis. In all urine samples, the concentrations of tAs and creatinine were determined. The concentrations of urinary tAs and As species were normalized to creatinine. In the WH group, speciation studies of two As forms—iAs and arsenobetaine (AsB)—were additionally performed. The concentration of urinary tAs was determined using an ELAN DRC-e ICP-MS with a Dynamic Reaction Cell (Perkin Elmer, SCIEX, Waltham, MA, USA), and the concentration of As species—iAs and AsB—were determined using the instrument Series 200 HPLC (Perkin Elmer, SCIEX, Waltham, MA, USA). Further description of As determination in the urine was provided in the study by Janasik et al. [23].

2.3. Diet Assessment

Dietary intake of the selected nutrients was analyzed based on 3-day dietary records of food and beverages consumed. The participants were instructed on how to fill in the dietary record (they received a standard template, which included time and place of consumption, name of the meal, ingredients, amount, or home measures) and were asked to accurately record all food products and beverages and report food's preparation and portion sizes (using kitchen scales or typical household measures). For quantitative analysis, data from 3-day dietary records of consumed food and beverages were entered into the Dieta 6.0 (Warsaw, Poland) computer program, as recommended by the National Centre of Nutritional Education. In accordance with the international methodology of dietary research, the portion size was determined based on photographs of products and dishes included in the "Album of photos of products and dishes" [24]. Mean daily values were calculated from the data obtained from 3-day dietary records. The intake level of the nutrients involved in As metabolism, calculated using the Dieta 6.0 program, was compared with the nutrition standards for the Polish population using EAR [25]. The intake of nutrients was calculated per kg of body mass.

2.4. Sample Preparations for Untargeted Metabolomics

Urine samples from the WH and WN groups were randomized and divided into two batches. Samples for metabolomics analysis were prepared using the latest protocol [26]. Two assays were used: one for the extraction of nonpolar and semipolar metabolites (assay 1) and the other for the extraction of polar metabolites (assay 2). The procedure for assay 1 was as follows: 300 µL solvent (ice-cold water and methanol at a 50:50 ratio with internal standards benzoyl-D5 and L-phenylalanine 3,3-D2) was added to 100 µL of urine (thawed at 4°C), vortexed for 2 min, and centrifuged at 20,879 rpm for 20 min at 4 °C. The same steps were followed for assay 2, except that a different solvent was used, acetonitrile and methanol (50:50), with the same internal standards. After centrifugation, the supernatants (200 µL) were aliquoted into vials. Quality control (QC) samples were prepared from all samples, and equal amounts of aliquots (100 µL) of each urine sample were mixed; this mixture was used to monitor system stability (injected every 10 experimental samples). Each batch comprised the following: samples for the equilibration system (10), analyzed samples (59, 58), QC samples (8), and blanks (2). More detailed information on sample preparation and analysis is available in our previous study [27].

2.5. Metabolomics Analysis

The equipment used in the study comprised a Waters ACQUITY™ Ultra Performance LC system (Waters Corp., Milford, MA, USA) connected to a Synapt G2Si Q-TOF mass spectrometer (Waters MS Technologies, Manchester, UK) with an electrospray (ESI) source (Waters, Manchester, UK). Analyses were performed in positive and negative ionization modes on two columns: an ACQUITY UPLC HSS T3 and an ACQUITY UPLC BEH Amide. The chemical reagent, metabolomics analysis parameters, and the fast data-dependent acquisition method were used as described in a study by Kozłowska et al. [27]. Compared with this study, only the gradient in the ACQUITY UPLC BEH Amide column for ESI was changed in the present study with respect to ESI+: t = 0.0–2.0 min, 1% B; t = 2.0–3.0 min, from 1% to 12% B; t = 3.0–6.0 min, from 12% to 50% B; t = 6.0–8.0 min, from 50% to 95% B; t = 8.0–8.5 min, from 95% to 99% B; t = 8.5–10.5 min, 99% B; t = 10.5–11 min, from 99% to 1% B; t = 11.0–14.0 min, 1% B. Gradient for ESI-: t = 0.0–2.0 min, 1% B; t = 2.0–3.2 min, from 1% to 6% B; t = 3.2–5.0 min, from 6% to 60% B; t = 5.0–5.5 min, from 60% to 95% B; t = 5.5–6.0 min, from 95% to 99% B; t = 6.0–9.5 min, 99% B; t = 9.5–10.5 min, from 99% to 1% B; t = 10.5–13.0 min, 1% B.

2.6. Bioinformatics and Statistical Analysis

For feature detection, retention time correction, alignment, and putative annotation of compound classes, files were loaded into the Progenesis software. The default parameters set for UPLC-High Res (Waters, Milford, MA, USA) were used. Then, the dataset was filtered; accordingly, metabolite features with a blank contribution >5%, QC relative standard deviation >25%, and QC count and sample count <60% were removed. Unwanted variations (signal drift and batch effects) were nullified by normalization performed on the web platform MetaboGroupS (https://www.omicsolution.com/wukong/MetaboGroupS/ accessed on 11 May 2022) [28]. Among the seven methods of normalization using the k-nearest neighbor algorithm (which imputes missing values) and log2 transformation (which removes data skewness), the most effective was the Eigen MS method.

Significant pathways were analyzed using the Functional Analysis module in MetaboAnalyst 5.0 platform (https://www.metaboanalyst.ca/home.xhtml accessed on 20 June 2022) [29]. A peak list with the retention time and *p* values was used, and the following parameters were used in each mode: mass tolerance 5 ppm, mummichog algorithms, adducts for positive and negative modes, and pathways/metabolite sets containing at least two entries. Compounds belonging to significantly changed pathways were fragmented. For putative annotation of compounds (level 2), the obtained fragmentation spectra of the compounds were compared with the spectra available in the Human Metabolome Database [30].

Statistica software, version 13.0 (StatSoft Inc., Tulsa, OK, USA), was used for statistical analyses. Normality of distribution was assessed using the Shapiro–Wilk test and expressed as means ± standard deviations for parametric distribution or medians and min–max for nonparametric distribution. Student's *t*-test for parametric distributions and Mann–Whitney U test for nonparametric distributions were used to compare variables between two groups. The Pearson correlation coefficient and the Spearman rank correlation coefficient were used to analyze the correlation between the intake of the nutrients involved in As metabolism and putatively annotated metabolites. Differences at $p < 0.05$ were considered significant.

3. Results

3.1. General Characteristic of the Workers

The overall characteristics of the participants are presented in Table 1. In brief, all the study participants (116) were male and worked in a copper-smelting plant. The participants were divided into two groups: WN, comprising workers with a urinary tAs concentration below 35 µg/L (n = 75), and WH, comprising workers with a urinary tAs concentration above 35 µg/L (n = 41). Between these groups, there were no significant differences in age, height, and period of iAs exposure; however, significant differences were observed concerning body mass and body mass index, with higher values in the WH group.

Table 1. General characteristics of the workers exposed to iAs.

Parameter *	Both Group	WN	WH	*p* Value **
	n = 116	*n* = 75	*n* = 41	
Age (years)	43.5 (21.0–62.0)	42.1 ± 10.0	44.0 (23.0–56.0)	0.4772
Height (cm)	177.0 (165.0–198.0)	176.5 (165.0–198.0)	178.3 ± 6.1	0.9673
Body mass (kg)	89.4 ± 14.8	86.7 ± 14.0	94.0 ± 15.1	0.0109
BMI (kg/m^2)	28.1 ± 4.3	27.2 ± 4.1	29.5 ± 4.2	0.0047
Period of iAs exposure (years)	17.5 (1.0–44.0)	17.0 (1.0–44.0)	18.5 (2.0–38.0)	0.7867

Abbreviations: *—Results are presented as means ± standard deviations for parametric distribution, or medians and min–max for nonparametric distribution (verified using Shapiro–Wilk test $p \leq 0.05$); **—Differences in parameters of groups WN and WH were assessed using Student's *t*-test for parametric distribution and Mann–Whitney U test for nonparametric distribution; BMI—Body Mass Index; iAs—inorganic arsenic; WN—the group of workers with urinary tAs concentration within the norm; WH—the group of workers with urinary tAs concentration above the norm.

The concentrations of tAs and its species in the urine are presented in Table 2. Significant differences were observed in the urinary tAs between the WN and WH groups. The same results were obtained while using creatinine-adjusted urinary tAs concentrations. The WH group showed significantly higher concentrations of urinary tAs, both when adjusted and not for urinary creatinine, in which $p = 0.0000$ in both cases. The concentration of iAs in the urine was determined only in the WH group, which was almost twofold higher than the determined urinary concentration of AsB. When adjusted for urinary creatinine, the urinary concentration of iAs was nearly 2.5 times higher than that of AsB.

The daily intake of selected nutrients (mg/kg bm or µg/kg bm) involved in iAs metabolism, such as methionine; vitamins B2, B6, and B12; folate; and zinc, did not differ between the two groups (Table 3). Only the mean dietary folate intake was lower than the estimated average requirement (EAR). The intake of the remaining analyzed nutrients was above the EAR norms. The average daily intake of nutrients as per the EAR norms is presented in the Supplementary Materials (Table S1).

Table 2. Between-group comparison of the urinary tAs and As metabolite concentrations.

Parameter *	Both Group	WN	WH	*p* Value **
	***n* = 116**	***n* = 75**	***n* = 41**	
tAs (μg/L)	27.3 (1.5–498.1)	20.6 (1.5–33.9)	54.7 (35.9–498.1)	0.0000
iAs (μg/L)	-	-	39.2 (10.0–87.8)	-
AsB (μg/L)	-	-	20.2 (2.5–433.0)	-
tAs (μg/g creat.)	19.0 (3.3–203.7)	14.6 (3.3–60.1)	30.4 (8.0–203.7)	0.0000
iAs (μg/g creat.)	-	-	20.1 (6.5–55.8)	-
AsB (μg/g creat.)	-	-	8.5 (0.8–170.8)	-

Abbreviations: *—Results are presented as medians and min–max for nonparametric distribution (verified using Shapiro–Wilk test $p \leq 0.05$); **—Differences in parameters of groups WN and WH were assessed using Student's *t*-test for parametric distribution and Mann–Whitney U test for nonparametric distribution; AsB—arsenobetaine; creat.—creatinine; iAs—inorganic arsenic; tAs—total arsenic; WN—the group of workers with urinary tAs concentration within the norm; WH—the group of workers with urinary tAs concentration above the norm.

Table 3. Dietary intake of selected nutrients involved in iAs metabolism.

Dietary Intake *	Both Groups	WN	WH	*p* Value **
	***n* = 116**	***n* = 75**	***n* = 41**	
Methionine (mg/kg bm)	25.05 (8.43–70.48)	25.23 (8.43–70.48)	23.75 (14.69–54.47)	0.5220
Vitamin B_2 (mg/kg bm)	0.02 (0.00–0.04)	0.02 (0.00–0.04)	0.02 ± 0.01	0.3554
Vitamin B_6 (mg/kg bm)	0.02 (0.01–0.06)	0.02 (0.01–0.06)	0.02 ± 0.01	0.1506
Vitamin B_{12} (μg/kg bm)	0.04 (0.01–0.20)	0.03 (0.01–0.20)	0.04 ± 0.01	0.8515
Folate (μg/kg bm)	2.94 (1.03–8.98)	3.04 (1.13–8.98)	2.78 ± 0.80	0.1819
Zinc (mg/kg bm)	0.12 (0.03–0.30)	0.12 (0.03–0.30)	0.12 ± 0.04	0.5418

Abbreviations: *—Results are presented as means ± standard deviations for parametric distribution or medians and min–max for nonparametric distribution (verified using Shapiro–Wilk test $p \leq 0.05$); **—Differences in parameters of groups WN and WH were assessed using Student's *t*-test for parametric distribution and Mann–Whitney U test for nonparametric distribution; bm—body mass; WN—the group of workers with urinary tAs concentration within the norm; WH—the group of workers with urinary tAs concentration above the norm.

3.2. Differences in Metabolic Profile beetwen WN and WH

Compared with the WN group, a higher signal intensity was observed for the metabolites belonging to the five pathways in the WH group: amino acid metabolism, carbohydrate metabolism, glycan biosynthesis and metabolism, vitamin metabolism, and nucleotide metabolism (Table 4).

In the amino acid metabolism pathway, significant changes were noted in the following sub-pathways: aspartate and asparagine metabolism ($p = 0.0168$), histidine metabolism ($p = 0.0168$), and methionine and cysteine metabolism ($p = 0.0176$). In the carbohydrate metabolism pathway, the following sub-pathways showed significant changes: butanoate metabolism ($p = 0.0135$), glycolysis and gluconeogenesis ($p = 0.0039$), pentose and glucuronate interconversions ($p = 0.0427$), and propanoate metabolism ($p = 0.0463/0.0003$). The following significant changes were noted in the sub-pathways of the following pathways, in which the sub-pathways are presented after each pathway: in the glycan biosynthesis and metabolism pathway—heparan sulfate degradation ($p = 0.0082$), keratan sulfate degradation ($p = 0.0313$), and hyaluronan metabolism ($p = 0.0063$); in the metabolism of vitamin pathways—vitamin B2 metabolism ($p = 0.0399$), vitamin B6 metabolism ($p = 0.0496$), and vitamin B9 metabolism ($p = 0.0047$); and in the nucleotide metabolism pathway—pyrimidine metabolism ($p = 0.0195$ in negative mode; 0.0312 in positive mode).

Table 4. Putatively annotated metabolites belonging to significantly changed pathways in analyses of differences between WN and WH.

Annotated Compounds' Names (ID in HMDB)	*p* Value (WN vs. WH)	Pathway Name	Sub-Pathway Name
gamma-glutamylcysteine (HMDB0001049)	0.0000 *	Amino acid metabolism	Aspartate and asparagine metabolism
pyroglutamic acid (HMDB0000267)	0.0000 **		
D-2-hydroxyglutaric acid (HMDB0000606)	0.0000 *		
4-acetamidobutanoic acid (HMDB0003681)	0.0000 *		
urocanic acid (HMDB0000301)	0.0000 *		Histidine metabolism
3-mercaptolactic acid (HMDB0002127)	0.0034 *		Methionine and cysteine metabolism
L-cystine (HMDB0000192)	0.0000 *		
succinic acid (HMDB0000254)	0.0020 *	Carbohydrate metabolism	Butanoate metabolism
D-glucose (HMDB0000122)	0.0286 **		Glycolysis and Gluconeogenesis
D-xylulose (HMDB0001644)	0.0000 *		Pentose and Glucuronate Interconversions
2-ketobutyric acid (HMDB0000005)	0.0000 *		Propanoate metabolism
hydroxypropionic acid (HMDB0000700)	0.0000 **		
iduronic acid (HMDB0002704)	0.0000 **	Glycan biosynthesis and metabolism	Heparan sulfate degradation
N-acetylneuraminic acid (HMDB0000230)	0.0000 *		Keratan sulfate degradation
riboflavin (HMDB0000244)	0.0000 **	Metabolism of vitamins	Vitamin B2 metabolism
pyridoxine (HMDB0000239)	0.0000 *		Vitamin B6 metabolism
thymine (HMDB0000262)	0.0000 *	Nucleotide metabolism	Pyrimidine metabolism
uridine (HMDB0000296)	0.0000 **		
cytosine (HMDB0000630)	0.0000 *		
cytidine (HMDB0000089)	0.0000 *		
adenosine monophosphate (HMDB0000045)	0.0000 *	Many pathways	Aspartate and asparagine metabolism, Histidine metabolism, Methionine and cysteine metabolism, Butanoate metabolism, Glycolysis and Gluconeogenesis, Propanoate metabolism, Vitamin B2 metabolism, Pyrimidine metabolism
L-glutamic acid (HMDB0000148)	0.0000 *		Aspartate and asparagine metabolism, Histidine metabolism, Butanoate metabolism, Vitamin B9 metabolism
2-hydroxybutyric acid (HMDB0000008)	0.0000 *		Butanoate metabolism, Propanoate metabolism
D-glucuronic acid (HMDB0000127)	0.0000 *		Pentose and Glucuronate Interconversions, Heparan sulfate degradation, Hyaluronan metabolism
N-acetyl-D-glucosamine (HMDB0000215)	0.0000 **		Heparan sulfate degradation, Keratan sulfate degradation, Hyaluronan metabolism

Abbreviations: *—Student's *t*-test; **—Mann–Whitney U test; HMDB—Human Metabolome Database; WN—the group of workers with urinary tAs concentration within the norm; WH—the group of workers with urinary tAs concentration above the norm.

3.3. Relationship between Dietary Nutrients Intake Involved in iAs Metabolism and Signal Intensity of Putatively Anotated Metabolites

The associations between the intake of dietary nutrients involved in iAs metabolism and the putatively annotated metabolites belonging to significantly changed pathways in the analyses of the differences between WN and WH groups were analyzed. A correlation analysis was conducted on the whole group as well as separately in the WH and WN groups. Several dependencies between the analyzed nutrients and 11 metabolites were observed (Table 5).

Table 5. Relationship between the intake of dietary nutrients involved in iAs metabolism and putatively annotated metabolites belonging to significantly changed pathways in analyses of differences between WN and WH.

Correlation between Nutrient Intake *** and Metabolite	Both Group		WN		WH	
	R	*p*	R	*p*	R	*p*
vitamin B2 with cytosine	−0.1886 *	0.0464	−0.2554	0.0316	NS	
vitamin B6 with cytosine	−0.2175 *	0.0213	−0.2346	0.0489	NS	
vitamin B2 with D-glucuronic acid	NS		NS		−0.3956 *	0.0100
vitamin B6 with D-glucuronic acid	NS		NS		−0.3646 *	0.0190
vitamin B12 with D-glucuronic acid	NS		NS		−0.3479 *	0.0260
folate with D-glucuronic acid	−0.1996 *	0.0349	−0.2603*	0.0283	NS	
methionine with hydroxypropionic acid	−0.2163 *	0.0220	NS		NS	
vitamin B12 with hydroxypropionic acid	−0.2017 *	0.0330	NS		NS	
vitamin B2 with L-glutamic acid	NS		NS		−0.4211 **	0.0061
vitamin B6 with L-glutamic acid	NS		NS		−0.3653 **	0.0188
folate with L-glutamic acid	NS		NS		−0.3794 **	0.0144
methionine with N-acetyl-D-glucosamine	−0.1954 *	0.0390	−0.2728 *	0.0214	NS	
vitamin B2 with N-acetyl-D-glucosamine	−0.2504 *	0.0077	−0.3150 *	0.0075	−0.3253 *	0.0380
vitamin B6 with N-acetyl-D-glucosamine	−0.2374 *	0.0117	NS		NS	
vitamin B12 with N-acetyl-D-glucosamine	−0.1871 *	0.0482	−0.3261 *	0.0055	NS	
zinc with N-acetyl-D-glucosamine	−0.1917 *	0.0429	−0.2710 *	0.0223	NS	
vitamin B2 with N-acetylneuraminic acid	−0.1915 *	0.0431	NS		NS	
vitamin B6 with N-acetylneuraminic acid	−0.2246 *	0.0173	NS		NS	
vitamin B12 with N-acetylneuraminic acid	NS		NS		−0.3171 *	0.0430
vitamin B2 with pyroglutamic acid	NS		−0.2832 *	0.0167	NS	
vitamin B6 with pyroglutamic acid	NS		−0.2430 *	0.0412	NS	
vitamin B12 with pyroglutamic acid	NS		−0.2667 *	0.0246	NS	
folate with pyroglutamic acid	−0.1894 *	0.0454	NS		NS	
zinc with pyroglutamic acid	NS		−0.2548 *	0.0320	NS	
vitamin B2 with uridine	−0.1944 *	0.0400	NS		NS	
vitamin B6 with uridine	−0.1955 *	0.0388	NS		NS	
vitamin B12 with uridine	NS		−0.2559 *	0.0313	−0.3308 *	0.0350
methionine with urocanic acid	NS		−0.2569 *	0.0305	NS	
vitamin B6 with urocanic acid	NS		−0.2457 *	0.0389	NS	
folate with urocanic acid	−0.2067 *	0.0288	−0.2665 *	0.0247	NS	
zinc with urocanic acid	NS		−0.2722 *	0.0217	NS	
folate with 3-mercaptolactic acid	NS		NS		0.3337 *	0.0330
zinc with 3-mercaptolactic acid	NS		NS		0.3658 *	0.0190
methionine with succinic acid	NS		NS		0.3284 **	0.0361
folate with succinic acid	NS		NS		0.3359 *	0.0320
zinc with succinic acid	NS		NS		0.3498 *	0.0250

Abbreviations: *—Pearson correlation coefficient; **—Spearman rank correlation coefficient; ***—intake of nutrients calculated per kg of body weight; NS–not statistically significant; R—correlation coefficient; *p*—*p* value; WN—the group of workers with urinary tAs concentration within the norm; WH—the group of workers with urinary tAs concentration above the norm.

Overall, in both groups, the intake of all the analyzed nutrients (methionine, vitamin B2, B6 and B12, folate, and zinc) was negatively associated with the following eight metabolites: cytosine, D-glucuronic acid, hydroxypropionic acid, N-acetyl-D-glucosamine, N-acetylneuraminic acid, pyroglutamic acid, uridine, and urocanic acid.

In the WN group, negative correlations were observed between the intake of all the analyzed nutrients and the following six metabolites: cytosine, D-glucuronic acid, N-acetyl-D-glucosamine, pyroglutamic acid, uridine, and urocanic acid.

In the WH group, the intake of nearly all the analyzed nutrients (except zinc) was negatively correlated with the following five metabolites: D-glucuronic acid, L-glutamic acid, N-acetyl-D-glucosamine, N-acetylneuraminic acid, and uridine. Moreover, in this group, positive correlations were observed between methionine, folate, and zinc intake, and the signal intensity of succinic acid and 3-mercaptolactic acid.

In both the WN and WH groups, the intake of the analyzed nutrients was negatively associated with the signal intensity of the following three metabolites: D-glucuronic acid, N-acetyl-D-glucosamine, and uridine. In contrast, the intake of the nutrients was negatively associated with the signal intensity of the following three metabolites: cytosine, pyroglutamic acid, and urocanic acid in the WN group, with no significant relationships in the WH group. However, negative correlations were observed in the WH group between the intake of the analyzed nutrients and the signal intensity of two metabolites, namely, L-glutamic acid and N-acetylneuraminic acid, which were not statistically significant in the WN group.

4. Discussion

To the best of our knowledge, this is the first metabolomics study on copper-smelting workers. Moreover, this is also the first study in which the association between the intake of the nutrients involved in iAs metabolism and changes in metabolic profile has been analyzed. Significant changes in metabolism were observed in the group of workers exposed to iAs. A total of 25 putatively annotated metabolites belonging to significantly changed pathways were detected in the analysis of differences between the WN and WH groups. In addition, associations between the intake of the nutrients involved in iAs metabolism and 11 putatively annotated metabolites were observed. To clearly understand the findings of this study, this article's discussion was divided into two parts. First, alterations in the metabolism under exposure to iAs were discussed, and secondly, associations between the intake of the nutrients and putatively annotated metabolites were discussed.

4.1. Urinary Metabolomics

The findings of this study are consistent with those of previous urinary metabolomics studies [31–33], which showed that As exposure is associated with numerous changes in metabolism in adults. In the present study, a higher signal intensity was observed in the WH group with respect to several metabolites belonging to the amino acid, carbohydrate, glycan, vitamin, and nucleotide pathways compared with the WN group. These results seem to be consistent with those obtained by Zhang et al. [32], who reported changes in nucleotide (guanine) and amino acid (serine, hippurate, and acetyl-N-formyl-5-methoxy kynurenamine) metabolism. Wu et al. [33] also found alterations in amino acid metabolism (glycine, L-threonine, and serine) and identified changes in the signal intensity of succinic acid and pyroglutamic acid; these findings are consistent with the results of the present study. Kozłowska et al. [31] reported that the signal intensity of several metabolites is higher in men and women with higher urinary tAs concentrations and that these metabolites are also associated with amino acid, vitamin, and nucleotide metabolism. In these three metabolomics studies, changes were also observed in other pathways, which were dependent on the differences in the source and level of As exposure. In a study by Zhang et al. [32], the median tAs concentration was 40.03 μg/g of creatinine in men with environmental exposure. However, in a study by Wu et al. [33] on men and women exposed to As via drinking water (<50 μg/L), higher baseline tAs concentrations were observed (194.30 μg/g of creatinine in the male group and 206.70 μg/g of creatinine in the

female group). Kozlowska et al. [31] conducted a study on a group of adults and children environmentally exposed to As and reported urinary tAs concentrations in a wide range, namely, 16.40–170.13 μg/g. Other factors such as the concentration of iAs in the urine, the duration of exposure to iAs, and the age and gender of the participants, as well as the use of different analytical techniques (HPLC–MS, UPLC–MS, and GC–MS), might have influenced the differences in the obtained results.

4.2. Association between Intake of Nutrients Involved in iAs Metabolism and Putatively Annotated Metabolites

The major finding of this study was the relationship between the intake level of the nutrients involved in iAs metabolism (methionine; vitamins B2, B6, and B12; folate; and zinc) and 11 putatively annotated metabolites belonging to significantly changed pathways in the analyses of differences between the WN and WH groups. Methionine; vitamins B2, B6, and B12; folate; and zinc are cofactors and donors of methyl groups in the metabolic changes of iAs to MMA and DMA. Many studies have reported correlations between the efficiency of iAs methylation and the spectrum of adverse changes associated with iAs exposure [21]. Thus, the relationships observed between dietary intake and the signal intensity of the metabolites may also indirectly reflect the efficiency of methylation and thus the severity of the adverse changes associated with iAs exposure. Due to the lack of findings regarding correlations between the intake of the aforementioned nutrients and changes in the metabolic profiles of the individuals exposed to iAs, this discussion was focused on analyzing the effects of increased concentrations of these metabolites and the benefits of increasing the intake of methyl group donors and cofactors of iAs metabolism.

The signal intensity of N-acetyl-D-glucosamine correlated negatively with the intake of methionine; vitamins B2, B6, and B12; and zinc. In vitro and in vivo studies have reported that As exposure increases the concentration of this metabolite. In a group of rats exposed to outdoor air pollution (containing, inter alia, As), a higher concentration of N-acetyl-D-glucosamine in the serum was also observed than in the control group. Moreover, this metabolite was positively related to the phosphorylation of H2AX at Ser 139 (γ-H2AX) in the lungs, which is one of the biomarkers of deoxyribonucleic acid (DNA) damage [34]. Interestingly, the oral administration of N-acetyl-D-glucosamine in mice increased DNA damage in the pancreas, brain, kidney, liver, lungs, and colon. In addition, in various nontumorigenic cell lines, treatment with N-acetyl-D-glucosamine for 3 days resulted in genome instability [35]. In a study by Ni et al. [36] on a group of workers exposed to iAs, DNA damage to the P21 gene fragments was observed, which was associated with the reduced methylation of iAs (positively associated with the percentage of MMA and negatively with the percentage of DMA in the urine). The results of the aforementioned study indicated that iAs exposure is associated with an increase in N-acetyl-D-glucosamine signal intensity. In accordance with this finding, based on the correlations observed in the present study between nutrient intake, N-acetyl-D-glucosamine, and DNA damage, it seems that a higher intake of these nutrients may be related to a reduction in the severity of these processes.

In this study, the intake of vitamins B2, B6, and B12 as well as zinc was negatively correlated with the signal intensity of pyroglutamic acid. This signal intensity was higher in the WH group than in the WN group. In metabolomics studies on populations exposed to various chemical compounds, differences in the signal intensity of this metabolite were observed between exposed groups and reference groups. A lower signal intensity of pyroglutamic acid in the urine was observed in Bangladeshi adults chronically exposed to As. Moreover, the signal intensity of this metabolite was inversely associated with the As concentration in the urine and drinking water [33]. In addition, in a urinary metabolomics study by Zeng et al. [37], pyroglutamic acid was downregulated in a group of women exposed to cadmium, which was negatively correlated with urinary cadmium concentrations. A serum metabolomics analysis also showed the downregulation of pyroglutamic acid in children and adolescents exposed to multiple carcinogens (including As). In ad-

dition, this metabolite was associated with biomarkers of early health effects (inversely with oxidative stress biomarkers, namely, 8-hydroxy-2′-deoxyguanosine and 4-hydroxy-2-nonenal-mercapturic acid, and inversely and positively with three acylcarnitines) [38]. The findings of the present study are consistent with those of a metabolomics study on women with higher cadmium concentrations, wherein a higher signal intensity of pyroglutamic acid was observed [39]. Similarly, in a study on rats exposed to iAs, an increased signal intensity of this metabolite was observed in the serum [40]. An in vitro study on rat brains showed that pyroglutamic acid may cause oxidative stress by reducing nonenzymatic antioxidant capacity, thus causing oxidative damage to proteins and increased reactive species in rat brain [41]. Another study reported oxidative stress in a group of workers exposed to As, which was related to decreased total and native thiol concentrations and an increased disulfide concentration in the serum [42]. The present findings suggest that As exposure is associated with a change in pyroglutamic acid signal intensity, which may also be related to the severity of oxidative stress. Based on these results and the negative correlations observed between the nutrient intake and the signal intensity of this metabolite, it seems that the higher intake may be related to a reduction in the severity of oxidative stress.

Negative relationships were observed between the intake of vitamin B2, vitamin B6, and folate and the signal intensity of L-glutamic acid. L-glutamic acid and pyroglutamic acid are related metabolites. Pyroglutamic acid can be produced by L-glutamic acid in the presence of enzymes, and the reaction can also proceed in the reverse direction, i.e., enzyme 5-oxoprolinase hydrolyzes pyroglutamic acid to yield L-glutamic acid [43]. In the present study, a higher L-glutamic acid signal intensity was observed in the WH group compared with the WN group, which is consistent with other studies. In murine models orally administered iAs, an increased signal intensity of L-glutamic acid was observed in the liver [44] as well as in the plasma [45]. In addition, in men and women exposed to cadmium, L-glutamic acid in the urine was upregulated and correlated with an increase in cadmium concentrations [37]. In a targeted metabolomics study in which dependencies between As metabolism and diabetes were analyzed, positive associations between the homeostasis model assessment index (HOMA2-IR), waist circumference, and the plasma level of L-glutamic acid were observed [46]. The relationship between diabetes and L-glutamic acid was confirmed in several studies [47–49]. Furthermore, two meta-analyses showed the association between iAs exposure and increased diabetes mellitus risk [8,50]. However, in a study by Spratlen et al. [46], L-glutamic acid was associated with more efficient As metabolism (through increased %DMA, decreased %MMA, and %iAs in the urine). Based on these findings, exposure to iAs is related to an increased signal intensity of L-glutamic acid. Considering the correlations observed in our study and the findings of other studies regarding the association between L-glutamic acid and diabetes, it seems that a higher intake of vitamins B2 and B6 and folate may be an important modulator of the development of the aforementioned disorders.

This study showed positive associations between the intake of methionine, folate, and zinc and the signal intensity of succinic acid, in which the signal intensity of this metabolite was higher in the WH group than in the WN group. Another urinary metabolomics study performed on a group of adults exposed to As reported that the signal intensity of succinic acid was inversely associated with As concentrations in urine and water, with a higher urinary tAs concentration (194.3 μg/g of creatinine in men and 206.7 μg/g of creatinine in women), which may affect the differences in the obtained results [33]. However, in another untargeted metabolomics study on an As-exposed population, an increased signal intensity of succinic acid and argininosuccinic acid was observed in boys and men with higher tAs concentrations, respectively [31]. In studies on animal models, increased concentrations of this metabolite in the urine were observed in diabetic mice [51], as well as in the plasma in an animal model of hypertension and metabolic disease, but such increases were not observed in hypertensive nor in diabetic human participants [52]. Under normal conditions, succinic acid undergoes various reactions. Apart from being involved in butanoate metabolism, it is involved in the tricarboxylic acid cycle (TCA cycle). In the TCA cycle, acetyl coenzyme

A is oxidized, which causes the release of energy in the form of adenosine triphosphate. On the other hand, succinic acid is converted into fumaric acid by the enzyme succinate dehydrogenase, and in case of a dysfunction of this step, succinic acid is accumulated and can act as an oncometabolite [53–55]. This metabolite may also contribute to inflammation (acting as immune signaling), which has been observed in mouse macrophages [56].

In the present study, a negative relationship was observed between the intake of vitamins B2, B6, and B12 and folate and the signal intensity of D-glucuronic acid. Disorders in the glucuronate degradation sub-pathway were also reported in another metabolomics study on a population exposed to As, in which a higher signal intensity of L-threo-2-pentulose belonging to this sub-pathway was observed in men, women, and boys with high As concentrations [31]. The signal intensity of D-glucuronic acid in the serum was associated with mortality in patients with cirrhosis [57]. Furthermore, in patients with diabetes mellitus, hepatocellular carcinoma, and liver cirrhosis, an increased level of glucuronic acid was observed in the serum [58,59]. In a study by Ho et al. [60], the level of glucuronic acid increased with age, and circulating glucuronic acid was considered to be a biomarker of biological aging and a predictor of all-cause mortality and health-related outcomes. Studies have shown that the microbiome can also affect the rapid cleavage of glucuronide conjugates by increasing the concentration of D-glucuronic acid and thus disrupting glucuronidation [60,61]. Considering the results of these studies, the higher signal intensity of D-glucuronic acid observed in the WH group in the present study may be associated with impaired glucuronidation and the risk of developing several diseases. The negative correlation of the consumption of cofactors of iAs metabolism and donors of methyl groups with this metabolite emphasizes the need for studies aimed at an in-depth understanding of these processes.

This study showed negative relationship between the intake of vitamins B2, B6, and B12 and the signal intensity of cytosine and uridine. The signal intensity of these metabolites was higher in the WH group than in the WN group. These findings are consistent with those of other studies, as shown by a study in which cytosine was upregulated in the urine of adults with high exposure to heavy metals (As, among others) and polycyclic aromatic hydrocarbons [62]. In men environmentally exposed to As, a positive correlation was reported between the concentration of As, male infertility, and uridine in the urine [63]. Cytosine, thymine, adenine, guanine, and uracil are the nitrogenous bases that build DNA and ribonucleic acid (RNA). Uracil, one of the nitrogenous bases of RNA, forms uridine when combined with ribose. A study by Zhang et al. [32] reported an increased signal intensity of one of the purine bases, guanine. These authors suggested that this change may indirectly indicate an increase in oxidative stress, which can lead to DNA damage. One marker of this damage is 8-oxoguanine, which is formed by the oxidation of guanine. Guanine combines by hydrogen bonding with cytosine, both of which are complementary nitrogenous bases, whereas 8-oxoguanine can combine with adenine, which leads to a point mutation [64]. Guanine-related disorders are an example of changes that can arise due to other nitrogenous bases that build DNA or RNA. Depending on the correlations between vitamin intake and the intensity of cytosine and uridine signaling observed in the present study, and the findings regarding guanine and DNA damage, it seems that a higher intake of these vitamins may have an indirect effect on reducing oxidative stress.

In the present study, negative relationships were observed between the intake of methionine and vitamin B12 and the signal intensity of hydroxypropionic acid. In metabolomics studies related to exposure to various compounds, no changes in the signal intensity of this metabolite were observed, but such changes have been reported in people with various types of cancer. A high urinary concentration of hydroxypropionic acid is a diagnostic biomarker of bladder and colorectal cancer [65,66]. In a study by Ikeda et al. [67], the signal intensity of hydroxypropionic acid in the serum was higher in gastric cancer patients compared with the control group. The IARC has classified iAs as carcinogens and indicated that iAs exposure can lead to lung, urinary bladder, and skin cancers [14]. In addition, studies have reported that long-term iAs exposure is associated with an increased risk of illness

or increased mortality from gastric and colorectal cancers, especially in high-exposure regions [68,69]. Therefore, hydroxypropionic acid may be one of the potential biomarkers of cancers, whose risk of development is increased in people exposed to iAs. These findings, as well as the negative correlations between iAs intake and this metabolite observed in the present study, suggest the need for further research.

In the present study, a positive relationship between folate and zinc intake and the signal intensity of 3-mercaptolactic acid was observed. This metabolite is involved in the cysteine metabolism pathway, and its high signal intensity may suggest the intensification of changes in this pathway. Only a few studies have been conducted on this metabolite, but it seems that cysteine is involved in the metabolism of As, as suggested by the results of a study by García-Sevillano et al. [44]. In this study, using an animal model exposed to iAs, an increased signal intensity of cysteine was observed in the liver [44]. Spratlen et al. [46] reported a positive association of cysteine with the percentage of DMA and a negative association with the percentages of MMA and iAs, as well as with diabetes-related adverse outcomes. However, a regression analysis did not confirm these associations. These authors suggested that as cysteine is part of glutathione, its increased intensity may reflect the need for increased glutathione synthesis. Other studies showed that a higher cysteine intake is associated with increased urinary tAs excretion [70] and with lower percentages of urinary iAs and a higher quantity of first methylation steps (MMA:iAs) [71]. The positive relationship between folate and zinc intake and the signal intensity of 3-mercaptolactic acid, with an indirect relationship with cysteine, may suggest that their higher intake may be a modulator of iAs metabolism.

Negative relationships were observed between the intake of vitamins B2, B6, and B12 and the signal intensity of N-acetylneuraminic acid. In another study, both the signal intensity and concentration of N-acetylneuraminic acid were higher in the plasma of coronary artery disease patients, and the authors indicated this metabolite as a possible marker of this disease's progression [72]. Lee et al. [73] conducted a meta-analysis revealing that urinary N-acetylneuraminic acid was associated with a higher risk of lung cancer; hence, these authors also indicated this metabolite as a potential biomarker of this disease. In addition, an increased concentration of N-acetylneuraminic acid in the urine has been observed in patients with diabetic nephropathy [74] and renal diseases [75]. These findings indicate that the upregulation of N-acetylneuraminic acid is related to coronary artery diseases, diabetic nephropathy, renal diseases, and lung cancer. These diseases can also develop as a result of exposure to iAs [8,9,14,76]. Given the results of these studies, in which a relationship between N-acetylneuraminic acid, the occurrence of the aforementioned disease, and iAs exposure was observed, it seems that vitamins B2, B6, and B12, being cofactors of many processes, can modulate the rate of many changes in their development.

In the present study, negative relationships were observed between the intake of methionine, vitamin B6, folate, and zinc and the signal intensity of urocanic acid. A higher signal intensity of this metabolite was observed in the WH group than in the WN group. In the metabolomics studies available in the literature, different results were reported: a lower signal intensity of urocanic acid in the urine was observed in psoriasis patients and atopic asthmatic children [77,78], whereas a higher signal intensity was observed in patients with endometrial carcinoma [79]. Urocanic acid is found in the skin, and it can be converted from the trans form to the cis form under the influence of ultraviolet radiation. It can have both beneficial and adverse effects on the body, for example, cis-urocanic acid can reduce cell-mediated immunity, leading to the development of skin cancer. However, due to urocanic acid's acidifying properties towards the cytosol of cancer cells, it can be used to treat some cancers [80]. In studies on human participants, keratinocytes led to cis-induced reactive oxygen species generation, lipid oxidation, increased cytokine protein production, and the upregulation of genes associated with apoptosis, cell growth arrest, cytokine synthesis, and oxidative stress [81,82]. Exposure to iAs (through drinking water) increases the risk of skin lesions [83,84], and many studies have focused on a deeper understanding of the mechanisms of As-induced skin lesions and cancers [85,86]. The

findings of studies on human keratinocytes, the relationship between iAs exposure and skin lesions, and the relationship between the intake of methionine, vitamin B6, folate, and zinc and the signal intensity of urocanic acid observed in the present study indicate the need for further research in this area.

Considering the observed negative correlations, it can be presumed that higher intakes of methionine; vitamins B2, B6, and B12; folate; and zinc, through their influence on the metabolism of iAs, may also have an indirectly beneficial effect by reducing the severity of the adverse effects of iAs exposure. Figure 2 summarizes the observed relationships between nutrient intake and potential adverse health effects associated with the putatively annotated metabolites.

Figure 2. Association between intake of nutrients involved in iAs metabolism vs. putatively annotated metabolites and related potential negative health effects. Abbreviations: DNA—deoxyribonucleic acid; iAs—inorganic arsenic; vit.—vitamin.

4.3. Strengths and Limitations

The strengths and limitations of this study should be considered while interpreting its results, which are listed below.

Strengths:

- The determination of urinary tAs concentration using well-developed methods;
- The analysis of several nutrients involved in As metabolism;
- Comprehensive analysis of the metabolic profile of workers exposed to iAs (not only urinary As metabolites), which allows for a deeper understanding of the mechanisms that occur during exposure;

- First study to combine the amount of nutrient intake and metabolomics data, which may fill the research gap and provide a direction for further research.

Limitations:

- This study only included men; thus, the results cannot be generalized to the entire population;
- The analysis solely concerned exposure to iAs, without considering the exposure to other compounds that could have influenced the results;
- The disadvantages related to 3-day dietary records hampered the acquirement of certain findings, and include: an underestimation of intake; the failure to account for the seasonality of intake; possible differences between the 3-day records and typical consumption; the fact that the analysis of the consumption of nutrients was based solely on diet, not including dietary supplements (48.3% of the respondents declared their use); and the consumption of rice, seafood, and fish, which may have interfered with the results due to their high As content (however, only 22.4% declared consuming fish in the last 48 h);
- Only one urine sample was taken from each participant (no multiple measurements/serial exposure data);
- Urinary concentrations of DMA and MMA were not determined.

Some of these limitations might have led to the underestimation of the relationship between the intake of nutrients and the putatively annotated metabolites. This research is a starting point and can serve as a groundwork for future studies.

5. Conclusions

Between the two groups of copper-smelting workers, namely, WN and WH, differences in their metabolic profiles were observed. Compared with the WN group, five pathways (the metabolism of amino acids, carbohydrates, glycans, vitamins, and nucleotides) with twenty-five putatively annotated metabolites were found to be increased in the WH group. In both study groups, negative correlations were observed between the intake of methionine; vitamins B2, B6, and B12; folate; and zinc and the signal intensity of the putatively annotated metabolites. Considering these correlations, it seems that a higher nutrient intake may reduce the severity of the adverse processes and disorders associated with iAs exposure. The findings of the present study indicate the need to educate the participants about the intake level of nutrients involved in the metabolism of iAs. These findings may contribute to further considerations during the development of dietary recommendations for people exposed to iAs. If the results of these analyses are confirmed in further studies, changes in diet could be the basis for reducing the adverse effects of exposure to iAs.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/metabo13010070/s1, Table S1: Dietary intake of selected nutrients involved in iAs metabolism regarding to the EAR norm.

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Oświadczenie o współautorstwie

Niniejszym oświadczam, że w pracy Sijko, M., Janasik, B., Wąsowicz, W., Kozłowska, L. (2023). Metabolic Changes and Their Associations with Selected Nutrients Intake in the Group of Workers Exposed to Arsenic. *Metabolites*, *13*(1), 70 mój indywidualny udział w jej powstaniu polegał na opracowaniu koncepcji treści i układu pracy, współudziale w opracowaniu metodyki badań metabolomicznych, wykonaniu analiz metabolomicznych, analizie kwestionariuszy 3-dniowego bieżącego notowania spożywanych produktów, potraw i napojów, wprowadzeniu danych do programu Dieta 6.0, opracowaniu statystycznym, napisaniu oryginalnego draftu artykułu, prowadzeniu korespondencji z wydawcą.

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Prośba o wypełnienie oświadczenia została przekazana współautorowi artykułu, jednak nie otrzymano zwrotu dokumentu. Zgodnie ze stanem mojej wiedzy w tym zakresie oraz z opisem wkładu autorów, załączonym do powyższego artykułu, indywidualny udział współautora obejmował w szczególności: współkoncepcję zastosowania metody analitycznej jaką jest metabolomika niecelowana do badań w tej grupie osób, opracowanie metodyki oznaczania stężenia arsenu w moczu i wykonanie oznaczeń, pozyskanie materiału biologicznego, akceptację merytoryczną artykułu.

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Sijko, M., Janasik, B., Wąsowicz, W., Kozłowska, L. (2023). Metabolic Changes and Their Associations with Selected Nutrients Intake in the Group of Workers Exposed to Arsenic. *Metabolites*, *13*(1), 70

mój indywidualny udział w jej powstaniu polegał na: Współudziale w kwalifikacji osób do badań, zebraniu prób materiału biologicznego i danych ankietowych od badanych, udział w części analitycznej (oznaczanie stężeń arsenu i form specjacyjnych), krytycznej ocenie uzyskanych wyników, współudziale w tworzeniu baz danych oraz na współudziale w redagowaniu manuskryptu.

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