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Polymorphisms Affecting Trace Element Bioavailability

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Abstract: This review outlines the nature of inter-individual variation in trace element bioavailability, focusing on genetic and epigenetic determinants. We note that pathogenic mutations responsible for dangerously high (or low) status for the micronutrient are unlikely to make large contributions to variability in bioavailability among the general population. Prospective genotyping (for variants in genes encoding selenoproteins) of participants in human studies illustrate one approach to understanding the complex interactions between genotype and trace element supply, which determine the functional bioavailability of selenium. Rapid advances in technological and bioinformatics tools; e. g., as used in Genome-Wide Association Studies, are opening new avenues for research on the genetic determinants of inter-individual variation in trace element bioavailability. This may include copy number variants in addition to the more widely studied polymorphisms. Future research on trace element bioavailability should encompass studies of epigenetic variants, including the role of non-coding (micro) RNA.

Key words: trace element, bioavailability, genotype, genome-wide association study, epigenetics, selenium

Introduction

The amount of any trace element available to carry out a specific function within a cell depends on many processes operating at several anatomical sites. Bioavailability depends initially on events in the gut lumen that determine how much of the element is released from food in a form which has potential to be absorbed by enterocytes in the small bowel, or by colonocytes in the large bowel (Figure 1). Within these epithelial cells, the micronutrient may be processed further, or subject to regulatory controls, before being released into the portal blood for transport to the liver. Events within the hepatocyte may determine how much of, and in what form, the trace element is released into the peripheral circulation. When supply of the trace element is less than optimal, tissues may be in competition, which may result in a hierarchy among tissues and, indeed, among proteins within tissues, for the available micronutrient.

Inter-individual variation in trace element bioavailability

Iron (Fe) bioavailability is influenced by many extrinsic (food-related) and intrinsic (consumer-related) factors [1] so that bioavailability varies widely between foods and diets and also between (and within) individuals. The magnitude of inter-individual variation on bioavailability of Fe is illustrated by a recent study of non-heme Fe absorption in 13 pre-menopausal women with marginal Fe status [2]. Despite identical Fe intakes, Fe absorption varied over an almost 20-fold range from 2.2 to 40.6 %. It is well established that Fe absorption is influenced by individual Fe status, but

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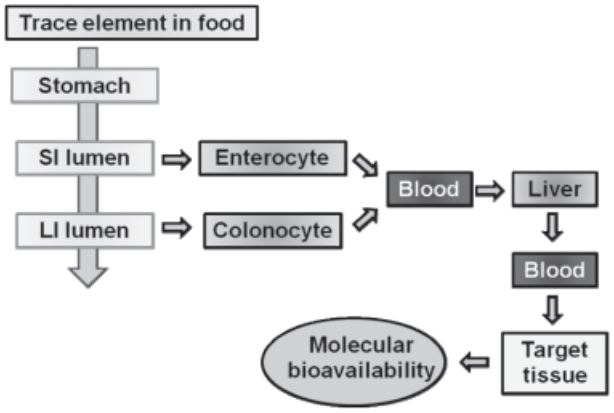

Figure 1: Overview of physiological steps which determine trace element bioavailability. Genetic and epigenetic variants in the genes encoding proteins and other regulatory molecules at each of these steps will contribute to inter-individual variability in trace element bioavailability. SI = small intestine; LI= large intestine

even after correcting absorption for a hypothetical mean serum ferritin concentration of 40 μg/L in one study, there was a greater than six-fold range in Fe absorption, and the authors suggested that additional physiological or genetic factors have strong influences on individual Fe absorption [2]. Zimmermann and colleagues quantified Fe absorption from standard noninhibitory test meals in 18 Mexican pre-school children and their mothers [3]. They observed a high correlation ($R^2 = 0.78$) between maternal, log-corrected fractional Fe absorption and that of their children [3]. Indeed, after adjustment for a serum ferritin concentration of 40 μg/L, 77 % of the variance in non-heme Fe absorption was explained by maternal-child relationships. The authors concluded that inheritance and/or shared environment explain most of the variance in dietary Fe absorption [3]. Such observations raise the question: "what are the genetic determinants of inter-individual differences in trace element absorption/ bioavailability?"

Genetic influences on trace element bioavailability

Well-known, but relatively rare, diseases and disorders characterized by unusually high or low trace element status are due to pathogenic mutations in the genes encoding proteins required for transport or other metabolic processing. For example, mutations in *SCL39 A4* (*ZIP4*), which encodes a zinc transporter protein, cause acrodermatitis enteropathica [4], whilst mutations in *HAMP* gene (which encodes the 25aa peptide hepcidin) are associated with juvenile hemochromatosis [5]. Hepcidin is a key regulator of Fe absorption through degradation of ferroportin, a transporter which facilitates Fe egress from enterocytes [6]. The possible impact of such pathogenic mutations in influencing trace element bioavailability is discussed further below.

Genotypic effects on trace element functional bioavailability – selenium as an exemplar

The biological functions of selenium (Se) are due largely to the conversion of dietary Se into the amino-acid selenocysteine (Sec) found in the so-called selenoproteins, of which there are ≈25 in mammals [7]. Dietary Se is converted to selenide, which then reacts with seryl-tRNA to form selenocysteyl tRNA. Sec is incorporated into the amino acid sequence of selenoproteins during translation using the codon UGA for selenocysteyl tRNA. In most mRNA, UGA is a stop codon but, in selenoprotein mRNA, recoding of the UGA codon to incorporate Sec occurs and this requires a specific stem-loop structure (Sec-insertion sequence: SECIS) within the mRNA. In eukaryotic selenoprotein mRNAs, this is found within the 3'untranslated region (3'UTR). The functional bioavailability of dietary Se is determined by the extent to which the ingested Se supports synthesis of selenoproteins to carry out antioxidant and other functions [8]. The inter-individual variation in functional bioavailability of Se from any particular dietary intake will depend on each individual's ability to release Se from foods, to transport Se across the gut epithelium, to synthesize selenocysteyl-tRNA from selenide, to transport Se around the body as selenoprotein P (SePP) (and, possibly, other transport proteins), and to deliver Sec to specific tissues to support the synthesis of other selenoproteins. Genetic variations in the selenoprotein genes, or in the machinery of Sec incorporation, can influence selenoprotein function and thus Se functional bioavailability [8].

SePP, which in humans contains 10 selenocysteines, is the major plasma selenoprotein and it accounts for ≈65 % of plasma Se. SePP is synthesized in the liver and then secreted into the plasma, where it transports and delivers Se to other tissues [9]. Functional variants in *SEPP1* would affect Se metabolism and thus potentially Se bioavailability. Three variants in SePP appear to be functionally important – a TC repeat sequence variant in the promoter region, a G/A variant within the 3'UTR (rs7579), and a G/A variant that causes

an Ala to Thr change at codon 234 (rs3877899) in the coding region. In the SELGEN Study, healthy human volunteers were recruited prospectively according to their genotype for rs7579 and rs3877899 and supplemented with 100 μg Se (as sodium selenite) per day for 6 weeks. Both SNPs in *SEPP1* affected the pattern of SePP isoforms in plasma [10] and the synthesis, and response to supplementation of other selenoproteins, including lymphocyte GPx4 and GPx1, plasma GPx3, and thioredoxin reductase 1 (TR1) and erythrocyte TR1 [11] [10]. The SELGEN Study illustrates how single nucleotide polymorphisms (SNPs) in the *SEPP1* gene can affect Se bioavailability by modulating the capacity of SePP to deliver Se to target selenoproteins.

Potentially, selenoprotein expression and activity, and thus functional Se bioavailability, will be affected by genetic variants in not only selenoprotein genes (promoter, coding region, or 3'UTR) *per se* but also in genes encoding components of the Se incorporation machinery (e. g., SBP2 or eF-Sec), in tRNA-Sec, or Se transport [8]. Therefore, in future work it will be important to consider how SNPs in the whole selenoprotein metabolic pathway, or selenome, affect functional bioavailability and to assess the impact of both interactions between SNPs in multiple genes within the pathway and their additive effects. Consequences of one SNP may be magnified or counterbalanced by variants in other genes and this net 'pathway effect' is likely to determine the overall physiological interaction between individual genotype and ingested Se and, hence, Se bioavailability.

Genome-wide association studies (GWAS) of iron status

It is estimated that 25–50 % of the variation in markers of Fe status can be explained by genetic factors, but pathogenic mutations in genes such as *HFE* (responsible for hemochromatosis) explain only a small proportion (≈5 %) of the heritability of Fe status. At present, genome-wide association studies (GWAS) are one of the most popular approaches for identifying genetic variants associated with phenotype. In the GWAS approach, the frequency of genetic variants (often involving hundreds of thousands of variants studied simultaneously) is investigated in large numbers of phenotypically distinct individuals (again often using DNA from thousands or tens of thousands of subjects). A recent GWAS using 2 sets of Australians of European descent found that 3 variants in the *TF* gene (encodes transferrin) plus the *HFE* C282Y mutation explained ≈40 % of the genetic variance in serum transferrin [12]. Similarly, a GWAS carried out using participants in the InCHIANTI and Baltimore Longitudinal Study of Aging, with replication in the Women's Health and Aging Study, reported that 2 polymorphisms in the *TMPRSS6* gene (encodes the transmembrane serine protease 6 (also known as matriptase-2)) were most strongly associated with serum Fe concentration [13]. TMPRSS6 regulates Fe absorption through suppression of *HAMP* expression [6]. However, each polymorphism in *TMPRSS6* explained only ≈1 % of the variance in serum Fe concentration and other candidate genes; e. g., *HFE, CYBRD1, SLCIIA1, TF,* and *TFRC* were not significant in this analysis [13]. These results indicate that many other genetic variants which modulate Fe status and Fe bioavailability remain to be discovered.

Where is the missing genetic information which explains inter-individual variation in trace element bioavailability?

It is possible that the heritable component of trace element bioavailability is due to the sum of many small effects due to variants in a multitude of genes. There may be an analogy with human height (which has a very high heritability of ≈80 %), where the GWAS approach has identified about 50 gene variants which, in total, explain only ≈5 % of the phenotypic variance and where 45 % of the variance could be explained by mathematical summation of the effects of nearly 300,000 polymorphisms [14]. However, other genetic and epigenetic variants may also contribute to inter-individual variation in trace element bioavailability. For example, copy number variants (CNVs) occur widely in the human genome [15]. To date, there do not appear to be any published studies of CNVs and trace element bioavailability but it is reasonable to anticipate that such variants will be responsible for inter-individual variation in the activities of proteins responsible, mechanistically, for the phenotype of trace element bioavailability. The gene encoding salivary amylase is highly polymorphic, and CNVs in this gene correlate strongly with protein expression and characterize different populations world-wide [16]. It will be important to investigate CNVs in pancreatic proteases and in the genes encoding epithelial transporter or other regulatory proteins as part of the strategy for explaining the variation in trace element bioavailability.

The recent advances in epigenetics research "…*provide hope that we are more than just the sequence of our genes*…" [17] and emphasize the importance of

considering the epigenetic (and other) mechanisms which regulate gene expression and, therefore, all cellular functions. It is now apparent that the integration of DNA methylation marks, patterns of post-translational modifications of histones, and chromatin structure with the activities of very small (non-coding) RNAs (miRNA), play a major role in controlling gene expression not only during embryonic and fetal development but throughout the life course. To date there appears to have been little attempt to apply epigenetics-based approaches to research on trace element bioavailability and this is an area ripe for exploitation. Recently, Andolfo and colleagues demonstrated that over-expression of the miRNA *Let-7d* in two different human cell lines reduced expression of the Fe transporter DMT1 [18]. Similarly, Liao and Lönnerdal (2010) reported that *miR-584* lowered expression of the lactoferrin receptor in Caco-2 cells, which suggests that *miR-584* may regulate expression of this receptor in the perinatal period [19].The lactoferrin receptor appears to play a role in absorption of lactoferrin-bound Fe from breast milk. Given that miRNA regulates expression of about one third of all protein-encoding genes, across all cellular processes investigated [20], there is a high likelihood that they will play a significant role in trace element bioavailability. In common with other regions of the genome, the domains encoding miRNA are also variable [21], which adds a further layer of genetic complexity.

Summary

Rapid advances in technological and bioinformatics tools are opening new avenues for research on the molecular determinants of inter-individual variation in trace element bioavailability. It is probable that such research will need to encompass both genetic and epigenetic variants. The application of a systems biology approach in research on trace element bioavailability may be helpful not only in advancing understanding of a complex aspect of biology but also as a route to quantitative models that are capable of predicting functional bioavailability in different dietary and genetic settings.

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