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Efecto de la ingesta de cinc en los niveles séricos/plasmáticos, en el crecimiento y en el desarrollo neurológico en los lactantes: Metaanálisis.

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Que me dejaron ser…

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ABREVIATURAS

EURRECA	EUROpean micronutrient RECommendations Aligned
IZiNCG	International Zinc Nutrition Consultative Group
OMS	Organización Mundial de la Salud
NOAEL	*No Observed Adverse Effect Level* (Nivel sin efecto adverso observado)
IGF-1	*Insulin-like growth factor-1* (Factor de crecimiento insulínico tipo 1)
ADN	Ácido desoxirribonucleico
ARN	Ácido ribonucleico
IDR	Ingesta Diaria Recomendada
IC	Intervalo de Confianza
B	Beta
Zn	Cinc
MUAC	Circunferencia media del brazo
WAZ	Peso para la edad z-score
LAZ	Longitud para la edad z-score
WLZ	Peso para la Longitud z-score
MDI	Índice de Desarrollo Mental
PDI	Índice de Desarrollo Psicomotor
DE	Desviación estándar
EE	Error estándar
BPN	Bajo peso al nacer
MEP	Malnutrición energético - proteica

1. Introducción

1. INTRODUCCIÓN

Los micronutrientes, tales como las vitaminas y los minerales, son esenciales para un adecuado crecimiento y metabolismo en los seres humanos. Aunque sólo son necesarios en pequeñas cantidades, su falta puede tener consecuencias graves para la salud y el desarrollo de las poblaciones de todo el mundo (WHO/FAO, Génova 2004). La mayoría de los países de Europa, para evitar deficiencias en la población general aparentemente sana, realizan sus recomendaciones basándose en la composición de los micronutrientes de la dieta. Estas recomendaciones sirven de fundamento para desarrollar las políticas de nutrición nacional o regional, los programas de educación nutricional y la regulación alimentaria de cada país. Sin embargo, como cada país utiliza para establecer las recomendaciones, métodos y conceptos propios, hay una gran variación de la ingesta de micronutrientes recomendada entre países (King & Garza 2007; Pavlovic *et al.* 2007; Prentice *et al.* 2004). Estas diferentes recomendaciones pueden confundir a quienes evalúan y planifican la ingesta de micronutrientes, como son los consumidores, los productores y los responsables de las políticas de nutrición.

La Red de Excelencia EURRECA (European Micronutrient Recommendations Aligned) se estableció a principios de 2007 con el objetivo de armonizar el proceso de establecimiento de las recomendaciones de micronutrientes en toda Europa, con especial atención a los grupos de la población más vulnerables, incluidos lactantes, embarazadas, población inmigrante y personas de edad avanzada (Gibson 2006; Junior *et al.* 2011).

Esta tesis ha sido escrita en el marco de la red EURRECA e incluye una muestra de los métodos de revisión estandarizados, transparentes y objetivos que se utilizaron

para resumir y evaluar las evidencias existentes para el establecimiento de recomendaciones, utilizando el cinc (Zn) como un ejemplo de micronutriente.

1.1 RED DE EXCELENCIA EURRECA

El objetivo general de la red de excelencia EURRECA fue la producción armonizada de directrices científicas para el desarrollo de las recomendaciones de micronutrientes, con el fin de avanzar hacia un proceso uniforme, transparente y basado en la evidencia que derivará en las ingestas de micronutrientes recomendadas (Matthys *et al.* 2011).

Inicialmente se propuso un marco para describir el proceso para establecer las recomendaciones de micronutrientes, el cual poseía varios pasos. Los primeros pasos incluían la selección de los micronutrientes, la selección de los grupos de población y la definición de los objetivos de salud que requerían una revisión de las recomendaciones. Además, se formó un Comité de expertos cuya responsabilidad fue la de establecer las necesidades específicas de micronutrientes y/o recomendaciones. En el siguiente paso se debían recoger los datos científicos existentes sobre las necesidades de los micronutrientes específicos y grupos de población definidos, resumirlos e integrarlos de acuerdo con el mejor método predefinido.

La hoja de ruta EURRECA (*Roadmap*) grafica los pasos que permitirán a EURRECA alcanzar su objetivo general. (Figura 1)

En el contexto de la red EURRECA, se dio prioridad a varios micronutrientes para los cuales la evidencia disponible sobre los requerimientos fue revisada de forma sistemática. Estos micronutrientes fueron priorizados de acuerdo con:

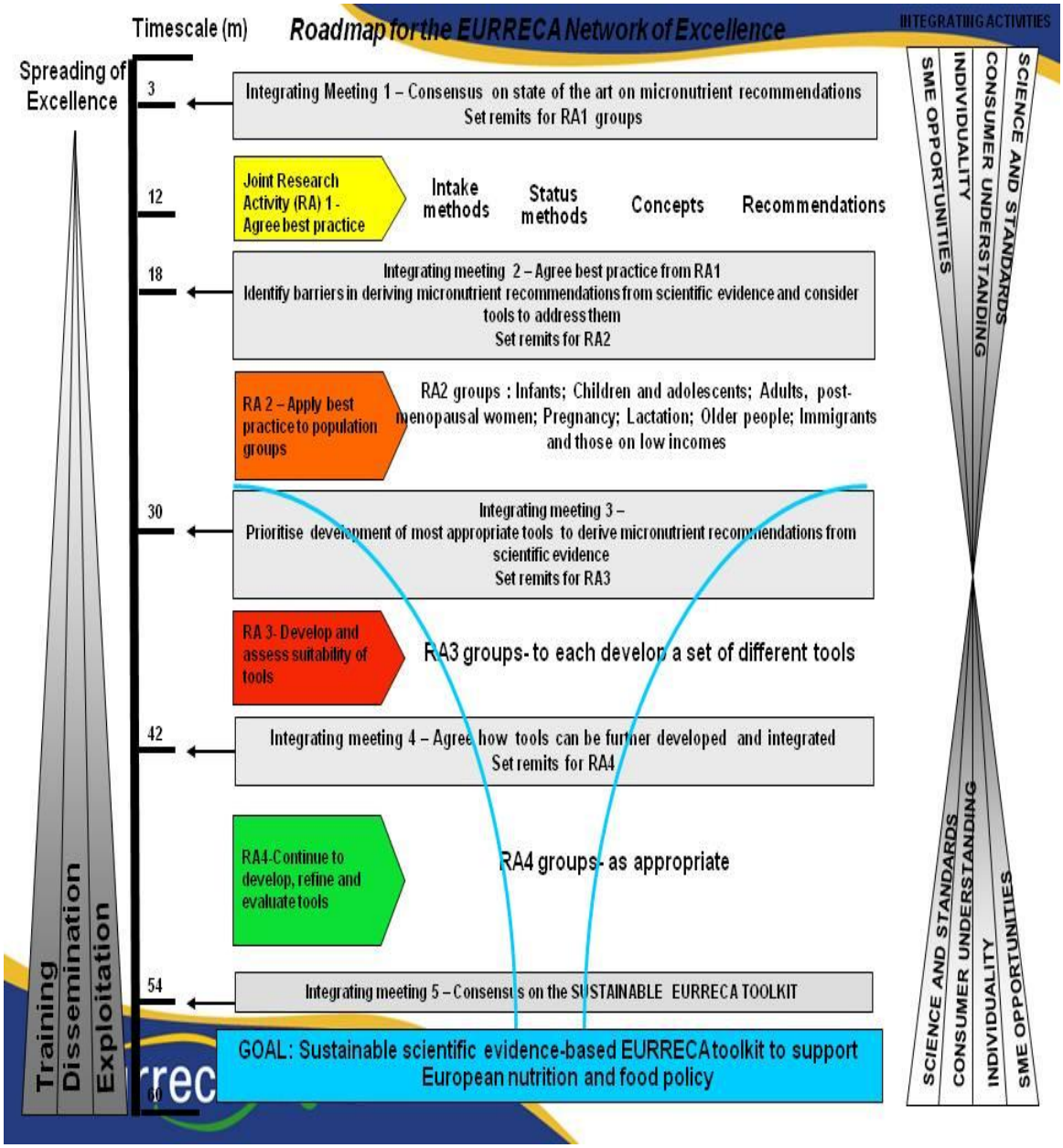
a) la cantidad de nuevos datos científicos de micronutrientes, disponibles para las diferentes etapas de vida, desde 2003, en particular, de ensayos aleatorizados controlados,

b) la relevancia de los micronutrientes en la salud pública, y

c) las variaciones en las actuales recomendaciones de micronutrientes (Cavelaars *et al.* 2010).

Uno de estos micronutrientes prioritarios fue el CINC.

Figura 1: Hoja de ruta (*Roadmap*) para la Red de Excelencia EURRECA



Visión general de las actividades de la Red de Excelencia EURRECA (Ashwell *et al*. 2008).

1.2 El CINC – GENERALIDADES

Nuestra percepción del Zn ha progresado en un tiempo extraordinariamente corto, desde la de ser un mineral de dudosa significación para la salud humana, a la de ser un micronutriente excepcional para la salud pública. Esto es más evidente en relación con el desarrollo prenatal y postnatal (Hambidge & Krebs 2007).

El Zn es un nutriente esencial, presente en todos los tejidos y fluidos corporales. La mayor proporción del Zn corporal está contenida en el músculo esquelético (50 – 60%), siendo apreciable también su contenido en el hueso (25 – 30% que puede llegar a 40% en el recién nacido a término). Sin embargo, hay otros órganos con concentraciones de Zn semejantes a los órganos mencionados (el hígado y el riñón, con 50 – 60 µg). Las mayores concentraciones de Zn se encuentran en coroides (250 – 280 µg/g) y secreciones prostáticas (300 – 400 µg/ml)(Olivares Grohnert *et al.* 2010). En el plasma representa alrededor del 0,1 por ciento del contenido total del Zn en el cuerpo. El Zn está presente en el organismo casi exclusivamente como Zn2 + unido a las proteínas celulares (Makonnen *et al.* 2003 b).

El papel biológico del Zn conocido hasta el momento es el de formar parte de estructuras y funciones de las proteínas, incluyendo más de 300 enzimas, factores de transcripción, sitios de receptores hormonales y membranas biológicas. El Zn tiene muchas funciones importantes en el ADN y en el metabolismo del ARN (MacDonald 2000), y está implicado en la transducción de señales, la expresión génica, y la apoptosis. Está involucrado en el metabolismo del ácido nucleico, en la proliferación celular, en la diferenciación y en el crecimiento (Hambidge & Krebs 2007).

1.3 METABOLISMO DEL CINC

La absorción y la excreción del Zn están controladas por mecanismos homeostáticos. Cuando la ingesta de Zn es pequeña, la absorción intestinal aumenta notablemente mientras que se reducen las pérdidas de este elemento por la orina y por el tracto intestinal.

La absorción del Zn es similar a la del calcio y tiene lugar mediante dos mecanismos: un mecanismo saturable, mediado por transportadores, que funciona más eficientemente cuando las concentraciones de Zn en la luz intestinal son bajas, y un mecanismo de difusión pasiva que depende de las concentraciones del mineral. Como el Zn se encuentra, por lo general, unido a aminoácidos y a pequeños péptidos, los iones tienen que ser liberados en las proximidades de las vellosidades para que puedan ser absorbidos.

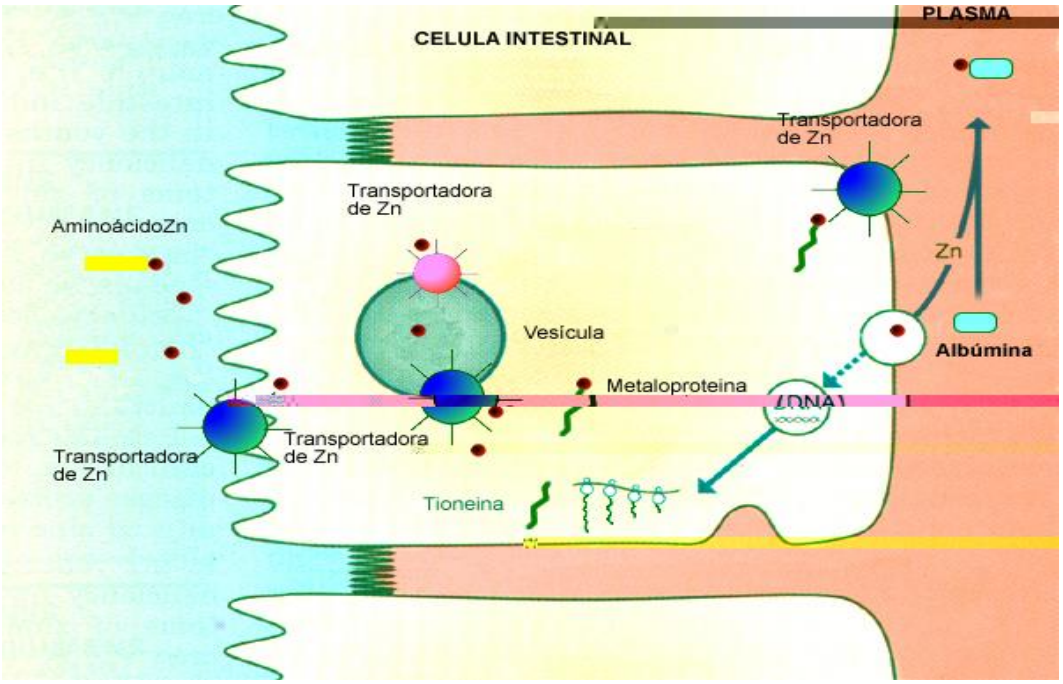
En el hombre, se conocen al menos 14 transportadores de Zn que regulan la entrada y la salida de este elemento en las células, agrupados en dos familias: ZnT y Zip. Estas proteínas parecen tener funciones opuestas: los transportadores ZnT reducen el Zn intracelular favoreciendo su salida de la célula o almacenándolo en vesículas intracelulares, mientras que las proteínas Zip aumentan las concentraciones intracelulares promoviendo la entrada del Zn extracelular o su liberación desde las vesículas almacén.

Una vez en el interior de la célula, el Zn se une a las metaloproteínas y otras proteínas del citoplasma celular. Mediante movimientos transcelulares, las metaloproteínas transportan el Zn hasta el extremo basolateral de las células enterohepáticas para su paso a la sangre. El paso a la sangre se realiza, también, mediante un mecanismo de transporte activo ya que las concentraciones de Zn plasmáticas son mayores que las concentraciones intracelulares. La cantidad de Zn que pasa a la sangre depende no

sólo de sus concentraciones intracelulares sino también de la albúmina disponible. La albúmina es la principal proteína transportadora del Zn, aunque también lo son, en menor medida, la transferrina y la a2-macroglobulina. Una parte importante del Zn de la sangre se localiza en los eritrocitos. El páncreas utiliza parte del Zn de la sangre para producir y excretar una serie de metaloproteínas necesarias para la digestión y la absorción de nutrientes.

La absorción digestiva del Zn depende de varios factores, en particular, del estado nutricional del individuo, de la integridad del intestino, de su concentración en la dieta y de la composición de la dieta (en cuanto a inhibidores y favorecedores de su absorción). Los fitatos y las dietas ricas en fibras secuestran el Zn impidiendo su absorción digestiva. El cobre y el cadmio utilizan los mismos transportadores compitiendo con el Zn y reduciendo su absorción. Elevadas concentraciones de calcio, también, reducen la absorción del Zn. Por el contrario, las dietas ricas en proteínas facilitan la absorción debido a que muchos aminoácidos y péptidos pequeños (sobre todo los sulfurados) forman quelatos que facilitan la absorción.

Figura 2: Absorción del Cinc



Fuente: *http://www.iqb.es/nutricion/zinc/zinc.htm#metabolismo*

1.4 BIODISPONIBILIDAD Y FUENTES ALIMENTARIAS DE CINC

El Zn está extensamente distribuido en alimentos y bebidas, pero tal como ocurre con otros elementos, los contenidos son muy variables y, en general, bajos. Son los productos de origen marino, especialmente los mariscos (ostras y crustáceos), los alimentos más ricos en Zn, seguidos de las carnes rojas, derivados lácteos, huevos y los cereales integrales. Los vegetales, con excepción de las leguminosas, no son alimentos que presentan altos contenidos en Zn. Por esto, las verduras, hortalizas y frutas, grasas, pescados y dulces son fuentes pobres de Zn (Cámara & Amaro 2003).

En los alimentos el Zn se halla asociado, particularmente, a las proteínas y ácidos nucleicos, lo que va a condicionar, en cierta medida, su biodisponibilidad. El Zn procedente de los alimentos vegetales es de menor biodisponibilidad debido a la presencia de ácido fítico que forma complejos insolubles poco absorbibles.

El procesado de alimentos es una de las principales causas de la pérdida de Zn. El ejemplo más representativo de este efecto lo constituyen los cereales, que pueden ver reducido su contenido desde un 20 a un 80% cuando son refinados (National Research Council 2001)

El Zn está en la mayoría de los multivitamínicos y suplementos minerales. Estos suplementos pueden contener gluconato, sulfato o acetato de Zn, pero no está claro si una forma es mejor que las otras. También se encuentra en algunos medicamentos de venta libre, como pastillas, aerosoles nasales y geles nasales para resfriados.

Tabla 1: **Fuentes alimenticias de Cinc**

Alimentos	Cinc (mg/100 g)
Ostras frescas	45 - 75
Almejas	21
Gérmen de trigo, salvado de trigo	13 - 16
Nueces de Brasil	7
Carnes	4,5 – 8,5
Queso parmesano	4
Guisantes secos	4
Avellanas	3,5
Yema de huevo	3,5
Cacahuetes	3
Sardinas	3
Pollo	2,85
Nueces	2,25
Pan integral	1,65
Garbanzos	1,4
Gambas o langostinos	1,15
Huevo entero	1,1
Leche	0,75

La biodisponibilidad biológica del Zn se relaciona con la eficiencia de su absorción intestinal. En general, el Zn que proviene de fuentes animales (especialmente de la carne) se absorbe mejor, y es más difícil de absorber cuando proviene de alimentos tales como legumbres y granos no refinados. Los oxalatos, los taninos y la fibra dietética presentes en las semillas son parte del problema, pero el principal inhibidor de la absorción de Zn es el ácido fítico (hexafosfato de inositol) (Solomons 2001). Esta sustancia puede enlazar al Zn y reducir su absorción (Lonnerdal 2000). Algunos ligandos como la histidina, metionina y cisteína, favorecen la captación del Zn.

Concentraciones altas de Zn pueden interferir la absorción de otros elementos traza, particularmente del cobre, y los efectos beneficiosos de la suplementación con Zn pueden ser suprimidos por completo por la inducción a la deficiencia del cobre, lo que puede conducir a una anemia grave y a la neutropenia (Prasad *et al.* 1978; Porter *et al*. 1977).

En la leche materna la concentración de Zn disminuye de manera gradual, desde un nivel de aproximadamente 0,23 mg/dl entre los 0 a 2 meses a 0,12 mg/dl durante los 6 a 8 meses de la lactancia, pero no disminuye su biodisponibilidad. La cantidad de Zn transferida de madre a hijo disminuye de 1,64 mg/día en lactantes de 0 a 2 meses a menos de 1 mg/día en lactantes de 6 a 8 meses, y se convierte aún en menos en las etapas posteriores de la lactancia. Si un bebé se alimenta exclusivamente con leche materna, una ingesta de 750 ml por día tiene una concentración de 2,67 mg de Zn/l, y proporciona la ingesta necesaria del nutriente. La Ingesta Diaria Recomendada (IDR) de los Estados Unidos y Canadá considera como valor de referencia 2,5 mg/l para la concentración de Zn en la leche humana (Krebs & Westcott 2002). La lactancia materna exclusiva puede ser considerada como una fuente adecuada para satisfacer las necesidades de Zn en los recién nacidos con un peso adecuado y nacidos a término hasta aproximadamente los 6 meses (Krebs & Hambidge 1986). En el caso de los sucedáneos de la leche, cuando estos se reconstituyen según las especificaciones del fabricante, proporcionan una concentración de 6 mg de Zn/l.

El período en el que se comienzan a introducir poco a poco los alimentos complementarios junto a la leche materna o a los sustitutos de la leche, es el período más crítico para la ingesta de Zn. Se cree que los alimentos complementarios a base de cereales, pueden interferir con la absorción de Zn obtenido de la leche. En general, se considera que los requerimientos de Zn en la infancia tardía y en el segundo año de vida no pueden ser cubiertos sin fortificación de los alimentos complementarios (Krebs & Hambidge 2007). Los niños de entre 9 y 11 meses de edad, deben obtener el 86% de la IDR de Zn a través de la alimentación complementaria (Dewey 2001). Sin embargo, las cantidades de alimentos de origen animal que pueden ser consumidas por los niños de entre 6 y 12 meses son, por lo general, insuficientes para cumplir con esos niveles recomendados de Zn (WHO/UNICEF 1998; Gibson *et al.* 1998). En los países industrializados, los alimentos complementarios fortificados con hierro han sido ampliamente consumidos durante décadas, y algunos productores también han

fortificado sus productos con Zn. Estos productos no están disponibles en todos los países en desarrollo (con la excepción de algunos programas sociales que llegan sólo a una pequeña parte de la población), a pesar de la creciente atención prestada a esta estrategia para asegurar una nutrición infantil adecuada (Lutter 2000; Lutter 2003). Una alternativa a la fortificación de alimentos es el uso de suplementos que se administran directamente al bebé en forma de gotas o mezclados con los alimentos complementarios.

1.5 RECOMENDACIONES EXISTENTES

En 1996, la Organización Mundial de la Salud (OMS) formuló recomendaciones sobre la ingesta de Zn en lactantes y en niños que formaban parte de una publicación de la OMS sobre las necesidades de vitaminas y minerales en humanos. En 2004, el Grupo Consultivo Internacional de Zinc (IZiNCG) también publicó recomendaciones de su ingesta.

En Europa, las recomendaciones para la ingesta de Zn en la infancia varían ampliamente, y las comparaciones son difíciles de establecer debido a las diferencias en la clasificación. Por ejemplo, se utilizan entre 4 y 6 categorías de edades para describir las recomendaciones de micronutrientes en la infancia con diferentes puntos de cortes utilizados por diferentes países europeos (Iglesia *et al.* 2010; Doets *et al.* 2008).

En algunos países, las recomendaciones de Zn para los lactantes se establecen generalmente sobre la base de la concentración de Zn en la leche materna, utilizando un enfoque factorial o la extrapolación de los valores obtenidos para las recomendaciones de los adultos (Gibson *et al.* 2008).

En general, las recomendaciones de Zn para los lactantes son:

Meses	Dosis
0-6 meses	2 mg/día
7-12 meses	3 mg/día

Fuente: http://www.nlm.nih.gov/medlineplus/spanish/ency/article/002416.htm

Hay muy poca información disponible sobre los efectos adversos de Zn en los recién nacidos. La mayoría de los países está utilizando el "NOAEL" (*No Observed Adverse Effect Level*), que es el nivel de ingesta de Zn en el cual no parecen ocurrir efectos adversos. De acuerdo con las recomendaciones del IZiNCG, los NOAEL para los niños son de 6 mg/día para los lactantes de 6 a 11 meses, de 8 mg/día para niños de 1 a 3 años, y de 14 mg/día para los niños 4 a 8 años. Es posible que los lactantes ingieran niveles más altos que los NOAEL sin ningún problema. Un estudio realizado en 2003 indicó que un alto porcentaje de niños en Estados Unidos consumían niveles más altos de Zn que los recomendados sin tener efectos secundarios (Arsenault & Brown 2003). Con lo cual, los NOAEL actuales parecen ser innecesariamente bajos.

1.6 VALORACIÓN DE LOS NIVELES DE CINC

La evaluación de los niveles de Zn en el hombre es compleja.

Los valores normales de Zn plasmático para adultos se mantienen entre 11 y 17,6 µmol/l (70 – 80 a 115 µg/dl), existiendo pequeñas diferencias entre hombre y mujer. Sin embargo, la determinación de la concentración de Zn plasmático es un indicador insuficiente de los niveles de Zn, puesto que el Zn plasmático sólo se ve alterado cuando los depósitos de Zn se encuentran considerablemente disminuidos.

A pesar de ello, una revisión sistemática reciente concluyó que la concentración de Zn en suero o plasma era sensible a los suplementos de Zn. Por ello, es el biomarcador más utilizado para evaluar los niveles de este mineral (Lowe *et al.* 2009).

Para los lactantes y los niños, no existe ningún método que sea completamente válido y fiable para diagnosticar los niveles de Zn:

- Zn en suero o plasma es el método más común para diagnosticar los niveles de Zn. Se han llegado a definir los valores normales por encima de los 70 microgramos/dl (> 10,7 milimoles por litro).

Sin embargo, para la determinación de Zn en la circulación es necesario obtener al menos 0,5 ml de plasma o suero libre de minerales, lo cual tiene implicaciones tanto para los recién nacidos como para los lactantes. El Zn no se puede determinar en muestras de sangre recogidas a través de los capilares, lo que limita su medición en los estudios de campo con niños. El Zn en suero y en plasma es un parámetro adecuado para el diagnóstico de deficiencia clínica severa, pero no para la identificación de la deficiencia marginal de Zn, lo que sería la aplicación principal en los estudios de nutrición a nivel poblacional (Aggett 1991; Haase *et al*. 2006).

Además, existen varios factores que interfieren con la medición exacta de Zn en el plasma. La hemólisis de la muestra de sangre o la contaminación de la muestra pueden sobreestimar el valor real. Los niveles bajos de albúmina sérica, o la presencia de infección pueden disminuir, aunque solo en apariencia, los verdaderos valores de Zn. Por otra parte, a nivel individual, las concentraciones circulantes de Zn tienen una baja correlación con el Zn depositado en los tejidos como el hígado, el hueso o el músculo.

- Otros elementos que circulan a nivel celular incluyendo glóbulos rojos, glóbulos blancos y plaquetas, también se han utilizado para evaluar la concentración de Zn en el organismo. Sin embargo, para esta medición, es necesario obtener muestras

adecuadas, y, por lo tanto, el alto volumen de sangre necesario limita su uso a nivel de población.

- Zn en cabello: El cabello es un material atractivo para medir, en especial en niños, debido a la naturaleza no invasiva de la colección. Aunque no existen valores de corte universalmente aceptados para definir las concentraciones normales o anormales de Zn en el cabello humano, podría ser un método válido para determinar niveles de Zn a nivel de población. Sin embargo, a nivel individual, la concentración de Zn en pelo no refleja de manera fiel el estado nutricional de Zn, y por lo tanto este método no debe ser utilizado en un entorno clínico.

Los avances más recientes en la tecnología biomédica ofrecen algunas perspectivas interesantes para mejorar la evaluación de los niveles del Zn a nivel individual:

- Biología molecular: la disponibilidad del Zn puede ser evaluada a través de la regulación homeostática de la célula. En la actualidad se están desarrollando pruebas para medir la expresión génica de las proteínas que regulan el Zn en los monocitos circulantes.

- Estudio del metabolismo del Zn: en este campo se ha realizado un progreso significativo. La absorción de Zn, su excreción y el total de Zn orgánico, se puede determinar en niños a través de la medición de isótopos no radiactivos estables.

1.7 EFECTOS DEL CINC SOBRE LA SALUD:

1.7.1 DEFICIT DE CINC:

La prevalencia real de la deficiencia de Zn no se conoce con exactitud, ya que, a diferencia de lo que ocurre con el hierro, no se cuenta con indicadores de laboratorio de alta sensibilidad y fiabilidad. Sin embargo, la prevalencia estimada en población general es alta, con miles de millones de personas en riesgo, en particular en los

países en desarrollo (Maret & Sandstead 2006). Niveles bajos de Zn tal vez afecten a un 50% de la población mundial, que posean un estado nutricional deficiente (Alnwick *et al.* 1993; Gibson 1994; Brown *et al.* 2001). Aunque la deficiencia de Zn severa es poco común en las poblaciones europeas, la deficiencia marginal es probable que sea mucho más frecuente (Gibson 2008).

Los grupos de población más vulnerables a la deficiencia de Zn son los lactantes, los preescolares, las mujeres embarazadas y las mujeres lactantes, debido a sus requisitos elevados de este nutriente. Desafortunadamente, se han llevado a cabo pocos estudios para determinar directamente el grado de deficiencia de Zn en las poblaciones mediante el uso de indicadores biológicos.

Un factor importante asociado con el desarrollo de la deficiencia de Zn en las poblaciones es la ingesta inadecuada de Zn en la dieta. Sin embargo, existen otros factores agravantes tales como los depósitos reducidos al nacer (por prematuridad o por bajo peso al nacer), los altos requerimientos fisiológicos de Zn, por ejemplo, durante la infancia, la adolescencia, el embarazo y la lactancia (Black 1998), y / o por pérdidas excesivas como consecuencia de la diarrea o de enfermedades específicas como la enfermedad celíaca, la enfermedad de Crohn, el síndrome del intestino corto o la fibrosis quística. También, el tratamiento con ciertos medicamentos (por ejemplo, penicilamina, tiazidas y glucagón) pueden aumentar su deficiencia (Gibson 2005). A diferencia de muchos otros nutrientes, no hay reserva funcional de Zn disponible en el organismo. Sin embargo, los niños pueden ser capaces de aprovechar el Zn hepático acumulado durante la gestación (Zlotkin & Cherian 1988).

Las consecuencias y manifestaciones de la deficiencia grave de Zn en niños puede ser retraso importante del crecimiento lineal y del desarrollo, falta de apetito, retraso en la maduración sexual e hipogonadismo, alopecia, dermatitis, retraso en la cicatrización de las heridas, diarrea, neumonía y malaria (Fischer Walker 2004), limitación en los sentidos del gusto y el olfato y ceguera nocturna. Además, la deficiencia de Zn puede disminuir la función inmune, dando lugar a una mayor susceptibilidad a las infecciones

(Maret & Sandstead 2006; Fraker & King 2004; Shankar & Prasad 1998; Wellinghausen *et al.* 1997).

Diferentes mecanismos pueden explicar las consecuencias para la salud anteriormente mencionadas debidas a la deficiencia de Zn. Por ejemplo, la deficiencia de Zn eleva una hormona anorexígena, el neuropéptido Y, lo que explica la disminución del apetito. La ceguera nocturna es probablemente debida a la dependencia de una enzima que contiene Zn en el metabolismo del retinol en las varillas de la retina. El agotamiento de Zn metaloproteína en la lengua puede explicar la pérdida de la agudeza del gusto. Un estudio en India encontró una disminución en la mortalidad por enfermedades infecciosas en los niños nacidos a término y niños pequeños para la edad gestacional que recibieron suplementación con Zn entre los primeros meses y el noveno mes de edad (Sazawal *et al.* 2001). Resultados de varios estudios indican que la suplementación con Zn puede reducir significativamente las tasas de diarrea y de neumonía en niños pequeños (Bhutta et al. 1999; Brooks *et al.* 2005), y aumentar la tasa de crecimiento de los niños con retraso del crecimiento (Brown 2002).

Durante la diarrea aguda, la suplementación con Zn reduce la duración y la severidad de la enfermedad, por lo que ahora la OMS recomienda la suplementación con Zn como un complemento a la terapia de rehidratación, para reemplazar las pérdidas excesivas de Zn durante los períodos de diarrea (WHO/Unicef 2004). La dosis recomendada para los suplementos en el tratamiento de la diarrea aguda es de 20 mg/día durante 10 a 14 días para los niños mayores de 12 meses, y la mitad de esta dosis (10 mg/d) para los niños de <12 meses.

Específicamente, analizaremos cómo influye y cuál es la relación entre la ingesta de Zn y los niveles de Zn en suero o plasma, en el crecimiento y en el desarrollo neurológico:

1.7.2 El Cinc en los niveles de suero/plasma

Tal y como hemos comentando, la evaluación de la ingesta de Zn en los individuos se complica por el hecho de que no existe ningún biomarcador aceptado, sensible y específico de los niveles de Zn existentes (King 1990). Si bien es cierto que el suero o plasma disminuyen las concentraciones de Zn después de varias semanas de introducción de una dieta que contenga una cantidad muy restrictiva de Zn (Baer *et al*. 1985), las concentraciones séricas de este mineral generalmente se mantienen dentro del rango normal cuando se producen reducciones pequeñas o moderadas en la ingesta de Zn.

Con respecto a las evidencias disponibles de la relación ingesta – nivel sérico/plasmático de Zn, la mayoría de los estudios individuales en lactantes se han diseñado con otros objetivos distintos de la determinación en suero o plasma de este micronutriente. Por ejemplo, el estudio de Sazawal *et al.* (1996) en la India obtuvo un incremento en los niveles de Zn plasmático después de la suplementación con Zn, pero este estudio se centró básicamente en la evaluación del uso de suplementos de Zn para la reducción de la incidencia de la diarrea. En otros casos, no se analizó la contribución única del Zn, sino su posible interacción con otros nutrientes. Este es el caso de los estudios realizados en Thailandia por el grupo de Wasantwisut *et al.* (2006), en Bangladesh por Chang *et al*. (2010) y en Indonesia por Lind *et al*. (2003), los que mostraron resultados positivos de la suplementación con Zn en los niveles de Zn en suero, pero que referían a la comparación del efecto de la suplementación del Zn combinado o no con hierro, en relación con el crecimiento ponderal, con la reducción de la cantidad de hospitalizaciones de los niños, o simplemente con los niveles de hemoglobina o transferrina en suero.

Además, las poblaciones de los estudios son variables. Algunos se llevaron a cabo en lactantes sanos de población rural como es el caso de Wasantwisut (2006), de Lind (2003), de Bates (1993) o de Umeta *et al.* (2000). Otros fueron realizados en poblaciones con malnutrición energético - proteica (MEP). Tal es el caso del estudio de

Makonnen *et al.* (2003) realizado en Lesotho. En este estudio se identificó la deficiencia de Zn como co-factor de la MEP pero, además, se observó que los incrementos en los niveles de Zn en suero recién se hicieron evidentes a los 60 días de la suplementación, lo que habla de un efecto del Zn a largo plazo. Umeta *et al*. (2000) en Etiopia incluyeron en su estudio niños con déficit de crecimiento. Estos, también encontraron un efecto positivo en los niveles de Zn en suero tras la suplementación. Concluyeron, además, que la suplementación con Zn es capaz de revertir la detención del crecimiento en estos niños debido, aparentemente, a la mejora del apetito y de la inmunidad, lo que repercute en la disminución de la morbilidad. En estos niños la suplementación con Zn es esencial para un adecuado "catch-up" en el crecimiento. Sin embargo dejaron claro que no es el único micronutriente necesario.

El estudio de Osendrap *et al.* (2002) también realizado en Bangladesh se llevó a cabo en barrios marginales de la cuidad de Dhaka. Si bien en este estudio el objetivo principal fue evaluar la suplementación con Zn en el crecimiento, se observó un incremento en los niveles de Zn séricos solamente en aquellos lactantes con niveles de Zn basales bajos (>9,18 µmol/L), en los que, además, se observó un incremento de peso significativo. La misma línea siguió el estudio de Walravens *et al*. (1989), realizado en lactantes provenientes de familias pobres de la ciudad de Denver, USA. El enfoque principal de este estudio fue la evaluación del crecimiento, pero la evaluación de los niveles de Zn en plasma no encontró diferencias significativas entre los grupos suplementados y los controles.

Está claro que todos los estudios incluidos son en países subdesarrollados o en población pobre y de bajos recursos, donde, seguramente, la dieta fue pobre en proteína animal, rica en fitatos; y donde las condiciones socio sanitarias colaboraron en el desarrollo de deficiencia de micronutrientes.

A pesar de la existencia de todos estos estudios, hasta donde conocemos, no se ha realizado aún ningún metaanálisis que evalúe los niveles de Zn en suero o plasma en

función de los niveles de ingesta de Zn y que, además, lo evalúe en lactantes. Sólo existe un metaanálisis realizado en 2002 (Brown *et al*.) en 15 estudios que incluían niños prepúberes y que revelaron efectos positivos y estadísticamente significativos de la suplementación con Zn en las concentraciones séricas del micronutriente. Sin embargo, hubo gran heterogeneidad en los resultados.

Comprender la relación entre la ingesta alimentaria de micronutrientes y sus niveles en suero o plasma en lactantes es esencial para derivar, entonces, las recomendaciones dietéticas.

1.7.3 El Cinc en el Crecimiento

El Zn es un micronutriente esencial para el crecimiento normal durante la infancia. Los lactantes tienen un requerimiento relativamente alto de Zn por unidad de peso corporal durante un período sensible de rápido crecimiento y desarrollo (Hermoso *et al.* 2010).

Consecuencias fisiológicas funcionales de la deficiencia de Zn leve (por ejemplo, retraso del crecimiento), a menudo, son evidentes antes de que las concentraciones de Zn en el plasma y / o en los tejidos se reduzcan significativamente (Gibson *et al.* 1989; Ruz *et al.* 1991). Una ingesta inadecuada de Zn es probable que sea un factor importante que contribuya a la falta de crecimiento en los niños que están desnutridos, ya que las dietas bajas en proteínas tienden a ser bajas en Zn (Golden & Golden 1981). El retraso del crecimiento comienza a los 6 meses de edad en los países menos desarrollados con una rápida progresión (The World Bank 2006) y coincide con un momento crítico en el suministro de Zn en la dieta, conocido por la OMS como “un problema en la alimentación complementaria” (Dewey & Brown 2003).

Los estudios de suplementación con Zn en lactantes han obtenido diferentes efectos sobre el crecimiento. Algunos estudios no han encontrado diferencias significativas entre los grupos suplementados con Zn y los controles. Este es el caso del estudio de

Dijkhuizen *et al*. (2001), en el que se argumentó que suplementar con Zn no es suficiente para permitir un crecimiento óptimo en los lactantes que poseen un estado nutricional deficiente, y que existen otros factores subyacentes en la dieta o en las circunstancias de estos niños que efectivamente son los que afectan el crecimiento. Misma conclusión obtuvo Hamadani *et al.* (2001) quienes señalaron que el poco efecto hallado en su estudio en el grupo tratado con Zn se debió al desequilibrio de micronutrientes presente en los niños desnutridos que requieren otros nutrientes además de éste. Muller *et al*. (2003) también encontraron poco efecto en sus resultados, y, nuevamente, los niños incluidos en su estudio tenían un estado nutricional deficiente. Meeks Gardner *et al.* (1998) también mencionaron que la insuficiente ingesta energética y proteica podría afectar la falta de respuesta positiva observada en los parámetros de crecimiento, pero estos autores también argumentaron que el tiempo de suplementación fue insuficiente como para observar cambios en el crecimiento.

Estos resultados van en concordancia con la evidencia existente de que el Zn es más efectivo en niños con relativamente mejor estado nutricional, mientras que entre aquellos cuyo estado nutricional es deficiente, la respuesta a dicha suplementación parece ser limitada (Kikafunda *et al.* 1998).

Sin embargo, existen estudios en los que el efecto de la suplementación con Zn fue beneficioso, a pesar del estado nutricional deficiente del lactante. Por ejemplo, Rivera *et al.* (1998) en un estudio controlado doble ciego llevado a cabo en lactantes guatemaltecos con bajo peso y deficiencia de este micronutriente, confirmaron un efecto antropométrico positivo para la suplementación con Zn. Igualmente, Sur *et al.* (2003), encontraron diferencias significativas en el crecimiento lineal en niños con bajo peso al nacer suplementados con sulfato de Zn y no suplementados, y plantearon que las diferencias pueden hacerse más evidentes después del primer año de vida. Mismo resultado obtuvieron Ninh *et al.* (1996) los que demostraron que la suplementación con Zn de los niños vietnamitas con retraso del crecimiento, aumentaba las

concentraciones de IGF-1 (*insulin-like growth factor-1)* en plasma, así como el crecimiento propiamente dicho. Los efectos beneficiosos de la suplementación con Zn en el estudio de Osendrap *et al.* (2002) fueron significativamente mayores en los niños que tenían concentraciones séricas de Zn bajas al inicio del estudio. Por último, el estudio de Lind *et al.* (2004) también encontró efecto positivo de la suplementación con Zn en el crecimiento. Sin embargo, en este caso, los niños estudiados eran en su mayoría niños saludables que aún estaban siendo alimentados a pecho, que poseían acceso al agua potable, cuyas madres poseían un alto nivel educativo, lo que podría haber añadido un efecto protector.

Con respecto a los metaanálisis llevados a cabo sobre esta asociación, destaca la aportación de Brown *et al.* (1998) que publicaron un metaanálisis de 25 ensayos clínicos aleatorizados, que investigaron el efecto de los suplementos de Zn en los niveles de Zn en plasma y en el crecimiento, en niños de entre 3,6 a 13 años en países en desarrollo. Los estudios incluidos fueron bastante heterogéneos (algunos incluían niños hospitalizados, con malnutrición y con retraso del crecimiento). Los resultados de su análisis indicaron que la suplementación con Zn mejoraba significativamente el peso para la edad (WAZ) y la talla para la edad z-score (LAZ) de los niños incluidos en los estudios, en especial en los niños con retraso del crecimiento o con desnutrición al inicio del estudio.

En 2002, este mismo autor realizó una versión actualizada de ese mismo metaanálisis con 33 estudios que evaluaban nuevamente la suplementación con Zn en el crecimiento. Este nuevo estudio también se desarrollo en niños prepúberes y en países en desarrollo. Los resultados encontraron en este caso efectos significativos en el crecimiento tras la suplementación con Zn para el peso, aunque no hubo efecto positivo en el metaanálisis del WLZ.

Si bien los metaanálisis de Brown se realizaron en niños prepúberes, sirven como antecedente y sientan las bases para la realización del presente metaanálisis realizado en lactantes. Hasta el momento, la información disponible no permite establecer

claramente cuál es la situación nutricional más beneficiosa para suplementar con Zn y obtener un efecto positivo en el crecimiento en este segmento etario.

1.7.4 El Cinc en el Desarrollo Neurológico

El Zn está también presente en el cerebro y contribuye a su estructura y función. Las pruebas existentes de estudios en animales y en humanos sugieren que su deficiencia puede llevar a retrasos en el desarrollo cognitivo. La grave deficiencia de Zn en animales se ha asociado con malformaciones estructurales del cerebro, tales como la anencefalia, microcefalia e hidrocefalia, con problemas de conducta, como la reducción de la actividad y el déficit en la memoria a corto plazo y el aprendizaje espacial. En los seres humanos, la deficiencia severa de Zn puede causar función anormal del cerebelo y disminución de respuestas conductuales y emocionales (Black 1998).

Aunque los mecanismos que vinculan la deficiencia de Zn con el desarrollo cognitivo no están claros, parece que ésta puede conducir a un déficit del funcionamiento neuropsicológico infantil, de la actividad, o del desarrollo motor, y por lo tanto interferir con el rendimiento cognitivo (Black 1998).

Relativamente pocos estudios han examinado el efecto de la suplementación con Zn sobre el comportamiento y el desarrollo en los lactantes.

Muchos de los estudios han sido realizados en niños con bajo peso al nacer. Tal es el caso del estudio de Friel *et al*. (2001) realizado en Canadá, en el que se observó que la suplementación con Zn mejoró el desarrollo motor en los recién nacidos. El estudio de Jiménez *et al*. (2007) también en niños con bajo peso al nacer concluyó que la suplementación con Zn influía de forma positiva en el desarrollo motor, pero no en el desarrollo mental de los niños. Uno de los motivos de este hallazgo podría ser el aporte de Zn por parte de la alimentación complementaria, sobre todo del tercero al

sexto mes. Estos resultados coinciden con los de Bhatnagar *et al.* (2001), quienes también plantearon que la suplementación con Zn en este grupo de niños resulta en un mejor desarrollo motor. En cambio, los estudios realizados por Black *et al.* (2004) en lactantes de entre 6 y 10 meses de edad también con bajo peso al nacer y suplementados durante 9 meses con 5 mg/día de Zn, y el realizado en lactantes sanos pero también con bajo peso al nacer en Brasil por el grupo de Ashworth *et al*. (1998) no obtuvieron ningún efecto sobre el desarrollo.

Otros estudios han sido llevados a cabo en poblaciones de niños desnutridos. Las conclusiones a las que llega Hamadani *et al*. (2001) en niños desnutridos de Bangladesh indicaron que las calificaciones del índice de desarrollo mental del grupo tratado con Zn fueron significativamente inferiores a las del grupo placebo, aunque aclararon que este resultado pudo haberse obtenido por desequilibrios de micronutrientes.

Sin embargo, los resultados obtenidos para lactantes sanos y nacidos a término por los diferentes estudios son contradictorios. Por ejemplo, Castillo Duran *et al.* (2001) encontraron que los suplementos con Zn pueden tener un efecto beneficioso sobre el desarrollo mental y el comportamiento motor. Pero Lind *et al*. (2004) en Indonesia no obtuvieron ningún efecto sobre el desarrollo.

Como observamos, existe gran controversia entre los resultados hallados por los diferentes estudios con situaciones nutricionales diferentes entre las distintas poblaciones analizadas. Además, hasta donde sabemos, no se ha publicado ningún metaanálisis que haya integrado las evidencias disponibles sobre la influencia de la ingesta de Zn en el desarrollo neurológico en lactantes.

1.8 ESTRATEGIAS PARA PREVENIR DEFICIENCIA DE CINC

Hay tres estrategias generales para prevenir la deficiencia de Zn en los lactantes y en los niños:

1.8.1 La suplementación con Cinc

El IZiNCG sugiere que las dosis de los suplementos de Zn debe ser de 5 mg/d en los niños de entre 7 meses y 3 años, y de 10 mg/día para niños mayores (IZiNCG 2009).

1.8.2 Los cambios en la dieta

- Aumentar la producción y el consumo de alimentos con alto contenido de Zn y la biodisponibilidad (alimentos de origen animal).

- Reducir el contenido de fitatos de los cereales y legumbres de la dieta.

- Promover la alimentación con leche materna durante los primeros 6 meses de vida, para asegurarse una ingesta suficiente de Zn altamente biodisponible.

- Reducir el riesgo de diarrea, que causa pérdidas excesivas de este mineral.

1.8.3 La fortificación de los alimentos

Es la adición de micronutrientes a los alimentos procesados (WHO /FAO 2006). El óxido de Zn y el sulfato de Zn son compuestos que generalmente se consideran seguros para el consumo humano y por lo tanto pueden ser utilizados en la fortificación de alimentos. Ambos compuestos de Zn parecen absorberse igual de bien en los alimentos fortificados (Brown et al. 2007).

Algunos estudios han demostrado que el enriquecimiento con Zn puede aumentar su absorción diaria. Sin embargo, hay poca evidencia disponible sobre el efecto positivo de la fortificación de Zn en el crecimiento de niños o de alguna otra respuesta relacionada con el metabolismo del Zn, excepto entre los niños con bajo peso al nacer o en aquellos con desnutrición severa. Por lo tanto, la suplementación con Zn debe ser considerada el método más fiable para prevenir o tratar la deficiencia de Zn en las poblaciones que se encuentran con alto riesgo de esta deficiencia, hasta que tengamos una mejor información disponible acerca de otras estrategias alternativas.

En resumen, y dada la clara necesidad de integrar tan diversa información existente, y con el objetivo fundamental de evitar el déficit del Zn y de promover la salud y el bienestar de los lactantes, es que hemos realizado este trabajo, el cual presenta una revisión sistemática de los datos existentes de todos los ensayos controlados aleatorizados que cumplen con los estándares de calidad de EURRECA (Matthys *et al.* 2011), que investigaron el efecto de la ingesta de Zn sobre la salud, analizando su efecto en los niveles de Zn en suero o plasma, en el crecimiento y en los parámetros de desarrollo neurológico en lactantes, combinando estos estudios en forma de metaanálisis, es decir, utilizando técnicas estadísticas de combinación de resultados.

2. Objetivos

2. OBJETIVOS

2.1 Objetivo General

Desarrollar una revisión sistemática y metaanálisis para valorar el efecto de la ingesta de Zn en los niveles de Zn séricos/plasmáticos, en el crecimiento y en el desarrollo neurológico de los lactantes, y establecer la relación dosis-respuesta posible para cada resultado.

2.2. Objetivos Específicos

2.2.1. Evaluar a través de un metaanálisis el efecto de la ingesta de Zn en los niveles de Zn en suero/plasma en lactantes.

2.2.2. Evaluar a través de un metaanálisis el efecto de la ingesta de Zn en el crecimiento en lactantes.

2.2.3. Evaluar a través de un metaanálisis el efecto de la ingesta de Zn en el desarrollo neurológico en lactantes.

3. Material y métodos

3. MATERIALES Y MÉTODOS

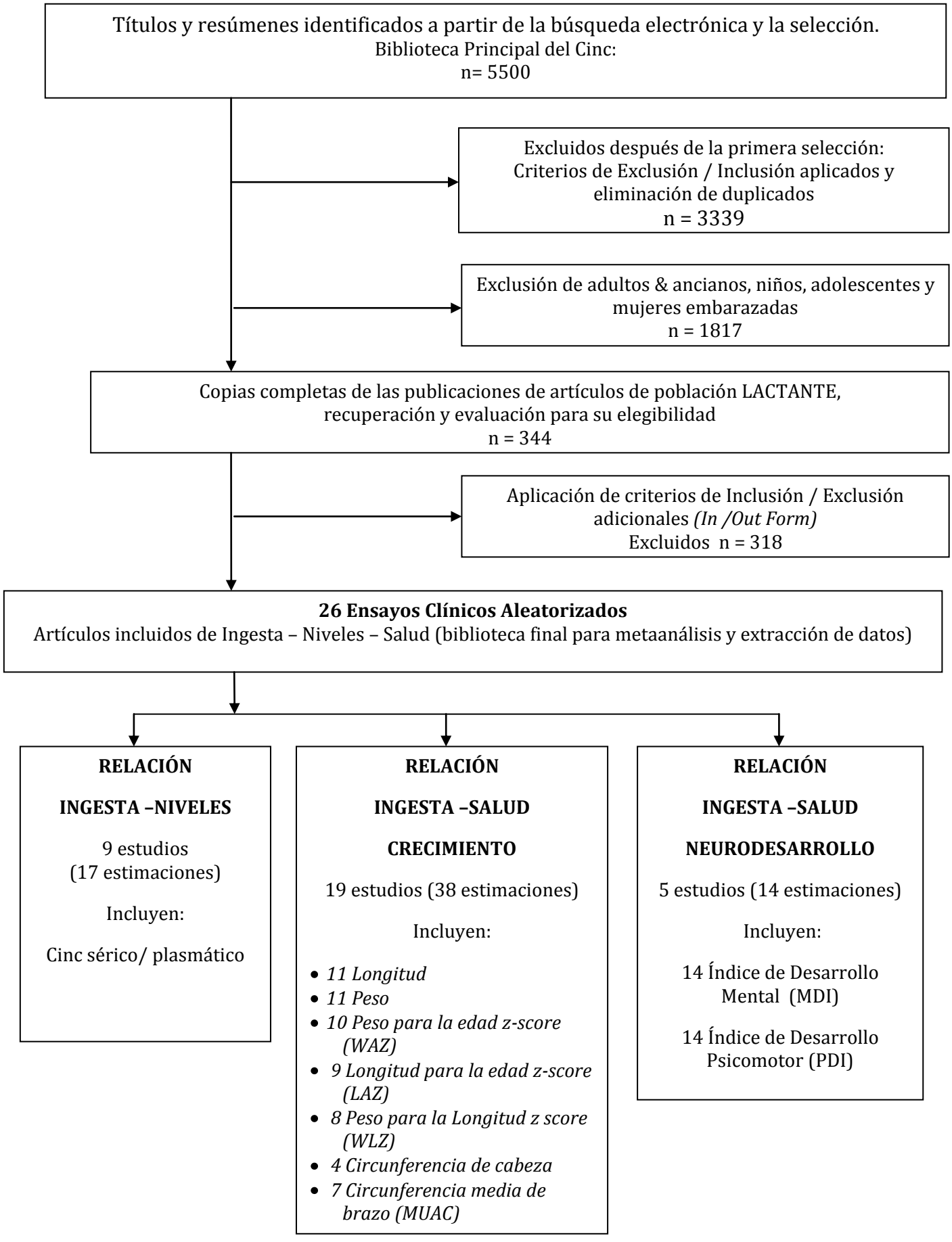
Esta investigación se llevó a cabo en el marco de EURRECA, Red de Excelencia cuyo objetivo fue identificar las necesidades de micronutrientes para la salud óptima en las poblaciones europeas y desarrollar metodologías para estandarizar el proceso de establecimiento de las recomendaciones de micronutrientes *(www.eurreca.org).*

3.1 Estrategia de búsqueda

Se realizó una revisión sistemática de la bibliografía disponible. La búsqueda bibliográfica se llevó a cabo a través de una búsqueda electrónica realizada en Medline, Embase y Cochrane hasta febrero de 2010. (Anexos 1, 2 y 3)

El procedimiento para la identificación, la selección de los artículos y la extracción de datos se ilustra en la Figura 3.

Figura 3: Diagrama de flujo para la revisión sistemática.



Para identificar los estudios más relevantes en las bases de datos, se estableció una estrategia de búsqueda utilizando términos de texto con truncamiento apropiado e indexación de términos relevantes.

Se utilizaron las siguientes palabras: *"randomized controlled trial", "double-blind procedure", "human", "zinc or zn or zinc sulphate or zinc gluconate or zinc acetate or methionine or zinc isotope", "intake or diet or supplement or deplete or status or serum or plasma or leukocyte or concentration or fortify", "Nutritional Support or Dietary Supplements or nutritional requirements or breast feeding or infant food or bottle feeding or infant formula", "Nutritional Status or Deficiency Diseases or supplementation or diet supplementation or dietary intake or diet restriction or mineral intake or Diet or Food, Fortified or nutrition assessment or Nutritive Value".*

Los idiomas incluidos se limitaron a los hablados en la Red EURRECA (inglés, holandés, francés, alemán, húngaro, italiano, noruego, polaco, español, griego y serbio.). Además, se comprobaron las listas de referencias de los artículos seleccionados y de revisiones relacionadas publicadas en la literatura.

3.2 Selección de artículos

Los títulos de los artículos identificados en las búsquedas fueron introducidos en una biblioteca de EndNote versión X1, que fue el programa establecido por la red de excelencia EURRECA para crear la base de datos general con la que se trabajaría (*www.endnote.com).*

3.2.1 Criterios de inclusión y exclusión

Los criterios de inclusión y exclusión fueron establecidos por el grupo de Eurreca y se resumen a continuación:

Criterios de inclusión

1. Se incluyeron solamente ensayos aletorizados controlados.

2. Se incluyeron estudios realizados en lactantes humanos (0-12 meses).

3. Se incluyeron aquellos estudios que evaluaran el efecto de la ingesta de Zn en las concentraciones de Zn en el suero/plasma, en el crecimiento y en el desarrollo neurológico.

4. Se incluyeron aquellos estudios que señalaran la ingesta de Zn realizada a partir de la ingesta de alimentos naturales (leche materna, fórmula infantil), de alimentos fortificados (por ejemplo fórmula fortificada y cereales) o de suplementos.

Criterios de exclusión

1. Se excluyeron los estudios realizados en animales.

2. Se excluyeron los estudios que realizaran intervenciones combinadas (> de 1 micronutriente o micronutrientes + intervención del estilo de vida que no estudiara el efecto de forma separada del micronutriente).

3. Se excluyeron los estudios que no fueran primarios (por ejemplo cartas y comentarios narrativos), y las publicaciones duplicadas.

4. Se excluyeron los estudios donde la relación ingesta de Zn - niveles de Zn en suero/plasma o ingesta de Zn – crecimiento o entre ingesta de Zn - desarrollo neurológico no fuera reportada o evaluada.

Se seleccionaron los títulos y resúmenes del 10% del total de publicaciones de la biblioteca EndNote construida. Se eliminaron las publicaciones duplicadas y se verificaron las restantes por dos revisores. Sólo cuando ambos evaluadores coincidieron en que los títulos y resúmenes cumplían con los criterios de inclusión, los artículos fueron incluidos. Cuando un título y su resumen no permitían su inclusión con certeza, se obtenía el texto completo del artículo para su evaluación. El 90% restante de la biblioteca EndNote construida se distribuyó en partes iguales entre los revisores, para su evaluación y verificación.

Tras el proceso inicial de selección, se realizó la agrupación de los artículos según los diferentes grupos de edad (adultos & ancianos, lactantes, niños, adolescentes y mujeres embarazadas). En este estudio solamente se trabajó con lactantes. Se obtuvieron los textos de los artículos completos para la aplicación de los criterios de selección adicionales (*In/Out Form*).

3.2.2 Criterios de inclusión y exclusión adicionales *(In/Out Form)*

El grupo Eurreca estableció nuevos criterios de inclusión y exclusión adicionales (Anexo 4).

Estos criterios fueron:

Criterios de inclusión adicionales

1. Se incluyeron estudios en los que la duración de la intervención fuera de al menos 2 semanas.

2. Se incluyeron estudios que informaran datos iniciales o basales de todas las medidas analizadas.

Criterios de exclusión adicionales

1. Se excluyeron los ensayos controlados no aleatorizados, los ensayos no controlados y los ensayos con datos insuficientes o poco claros.

Los datos de cada estudio seleccionado fueron extraídos y organizados en una base de datos de Microsoft Access (Microsoft Corp, Redmond, WA).

3.3 Exposición, desenlace y evaluación de otras variables

3.3.1 Exposición

Como exposición se consideró la influencia de la ingesta de Zn sobre los niveles de Zn en suero/plasma, los parámetros de crecimiento infantil y las medidas de desarrollo neurológico infantil.

La ingesta de Zn debía ser realizada a partir de la ingesta de alimentos naturales (leche materna, fórmula infantil), de alimentos fortificados (por ejemplo fórmula fortificada y cereales) o de suplementos.

Si los ensayos clínicos seleccionados no informaban sobre la ingesta de Zn de la dieta (además del de la suplementación), se les imputaba un valor de 1,3 mg/día que resultaba de promediar la ingesta alimentaria de Zn de los ensayos clínicos que sí informaban de dicha ingesta (según protocolo Eurreca: *www.eurreca.org).*

3.3.2 Desenlace

Se midieron 3 posibles desenlaces:

1. **Niveles séricos/plasmáticos de Zn**:

Los niveles de Zn en los estudios incluidos en el metaanálisis se evaluaron en suero o plasma. Inicialmente, se intentó incluir estudios que utilizaran Zn urinario o Zn en pelo como biomarcadores. Sin embargo, debido a la escasez de datos obtenidos, fueron excluidos de este estudio.

2. **Parámetros de Crecimiento**:

Los parámetros de crecimiento evaluados en los estudios incluidos en el metaanálisis fueron: peso, longitud, perímetro braquial (circunferencia media del brazo - MUAC), perímetro cefálico, WAZ (peso para la edad z-score), LAZ (longitud para la edad z-score) y WLZ (peso para la longitud z-score).

En todos los estudios incluidos, los pesos, longitudes y circunferencias se midieron utilizando técnicas estándar de medición. Las puntuaciones z-score se calcularon mediante el uso de datos de referencia del NCHS (Centro Nacional de Estadísticas Sanitarias) (Hamill *et al.* 1977.) Éstas representan la distancia de un valor por encima o por debajo de la media de peso o longitud de los lactantes nacidos a término de cada edad expresado en unidades de desviación. Una puntuación z por debajo de - 2 se considera un valor bajo y por debajo - 3 como muy bajo.

3. **Parámetros de Neurodesarrollo**:

Los parámetros evaluados en el desarrollo neurológico en los estudios incluidos en el metaanálisis fueron MDI (Índice de Desarrollo Mental) y PDI (Índice de Desarrollo Psicomotor). Ambos índices fueron medidos por la escala de Bayley de Desarrollo Infantil II en todos los estudios.

La Escala de Desarrollo Infantil de Bayley fue diseñada y validada por Nancy Bayley de la Universidad de Berckeley en 1969 para evaluar niños de edades comprendidas entre el nacimiento y los 30 meses. En 1993 su rango de aplicación se extendió hasta los 42 meses. Es el instrumento más utilizado para evaluar el desarrollo mental y psicomotor de los bebés.

Los diferentes parámetros que mide esta escala van de lo simple a lo más complejo, teniendo en cuenta la evolución y el desarrollo de los niños comprendidos en estas edades. Otra aportación importante de esta escala es precisamente el índice de

desarrollo que ubica al niño, sin tener en cuenta su edad cronológica, en una edad madurativa, siendo esto muy importante para comenzar una estimulación adecuada.

3.3.3 Otras variables

Además, otras variables se tuvieron en cuenta como posibles modificadoras del efecto. Se consideraron:

- las dosis diaria de ingesta de Zn (1 a 4 mg; 4,1 a 8 mg; 8,1 a 12 mg y > 12.1 mg).
- la duración de la intervención (de 1 a 3 semanas, 4 a 20 semanas y > 20 semanas).
- la situación nutricional (saludable, con riesgo nutricional y con estado nutricional deficiente).
- el riesgo de sesgo (bajo, moderado o alto).

En el caso de la dosis y de la duración de la intervención, la creación de los diferentes grupos fue arbitraria, asegurando un adecuado número de estudios en cada grupo.

En el caso de la situación nutricional, esta se clasificó como:

- Saludable
- Con riesgo nutricional: los niños que vivían en familias de bajos ingresos con nivel socioeconómico bajo.
- Con estado nutricional deficiente: niños con malnutrición proteico-energética (PEM), pero sin anomalías congénitas, ni parálisis cerebral, ni enfermedad del corazón, o lactantes con bajo peso al nacer mantenido durante el primer año, con retraso del crecimiento o con desnutrición evidenciado por puntuaciones WAZ y LAZ por debajo de - 2.

El riesgo de sesgo se desarrolló con el fin de evaluar la calidad de los estudios incluidos. Durante la extracción de datos de los estudios seleccionados, se recogieron los siguientes indicadores de validez interna que son específicos para estudios aleatorizados controlados:

1) Método adecuado de generación de la secuencia,

2) Método de asignación adecuado,

3) Enmascaramiento adecuado,

4) Número de participantes al inicio, abandonos y razones de abandono,

5) Adecuada financiación,

6) Otras posibles fuentes de sesgo.

Sobre la base de estos indicadores, dos revisores evaluaron el riesgo de sesgo general. Los desacuerdos se resolvieron mediante discusión. Los criterios para juzgar estos indicadores fueron adaptados del Manual Cochrane (Higgins JPT & Green S 2009) y publicados en una guía por el grupo Eurreca. (Anexo 5)

La evaluación para cada objetivo se muestra en las siguientes tablas: (Tablas 2, 3 y 4)

Tabla 2. Evaluación de la validez de los estudios aleatorizados controlados incluidos en el metaanálisis de niveles de Zn en suero / plasma de lactantes.

Autor, Año	Adecuada Generación de la Secuencia	Adecuada Asignación	Adecuado Enmascaramiento	Abandonos registrados y Adecuada información de los desenlaces	Adecuada Financiación	Otras posibles fuentes de sesgo	Total Riesgo de sesgo
Bates 1993	Si	No	Poco claro	Si	No	No	Alto
Chang 2010	Si	Si	Si	Si	Si	Si	Bajo
Lind 2003	Si	Si	Si	Si	Si	Si	Bajo
Makonnen 2003	Si	Poco claro	Si	Si	Poco claro	Si	Moderado
Osendarp 2002	Poco claro	Poco claro	Si	Si	Si	Si	Moderado
Sazawal 1996-2004	Poco claro	Poco claro	Si	Poco claro	Si	Si	Moderado
Umeta 2000	Poco claro	Poco claro	Si	Poco claro	Poco claro	Si	Alto
Walravens 1989	Si	Poco claro	Si	Si	Si	Si	Bajo
Wasantwisut 2006	Si	Si	Si	Si	Si	Si	Bajo

Tabla 3. Evaluación de la validez de los estudios aleatorizados controlados incluidos en el metaanálisis del crecimiento de lactantes.

Autor, Año	Adecuada Generación de la Secuencia	Adecuada Asignación	Adecuado Enmascaramiento	Abandonos registrados y Adecuada información de los desenlaces	Adecuada Financiación	Otras posibles fuentes de sesgo	Total Riesgo de sesgo
Arsenault 2008	No	No	Si	No	Si	Si	Alto
Bates 1993	Si	No	Poco claro	Si	No	No	Alto
Berger 2006	Poco claro	Poco claro	Si	Si	Si	Si	Moderado
Dijkhuizen 2001	Poco claro	No	Si	Si	Si	Si	Moderado
Fischer Walker 2009	Poco claro	Poco claro	Poco claro	Si	Si	Si	Moderado
Gardner 2005	Poco claro	Poco claro	Si	Si	Si	Si	Moderado
Hamadani 2001	Poco claro	Poco claro	Si	No	Si	Si	Alto
Heinig 2006	Poco claro	Si	Si	Si	Si	Si	Bajo
Lind 2004	Si	Si	Si	Si	Si	Si	Bajo
Meeks Gardner 1998	Poco claro	Poco claro	Si	Si	Si	Si	Moderado
Muller 2003	Si	Poco claro	Si	Si	Si	Si	Bajo
Ninh 1996	Si	Si	Si	Poco claro	Si	Si	Bajo
Olney 2006	No	No	Poco claro	Poco claro	Si	Si	Alto
Osendarp 2002	Poco claro	Poco claro	Si	Si	Si	Si	Moderado
Rivera 1998	Poco claro	Poco claro	Si	Si	Si	Si	Alto
Sur 2003	Si	Si	Si	No	Si	Si	Moderado
Umeta 2000	Poco claro	Poco claro	Si	Poco claro	Poco claro	Si	Alto
Walravens 1989	Si	Poco claro	Si	Si	Si	Si	Bajo
Wasantwisut 2006	Si	Si	Si	Si	Si	Si	Bajo

Tabla 4. Evaluación de la validez de los estudios aleatorizados controlados incluidos en el metaanálisis del neurodesarrollo de lactantes.

Autor, Año	Adecuada Generación de la Secuencia	Adecuada Asignación	Adecuado Enmascaramiento	Abandonos registrados y Adecuada información de los desenlaces	Adecuada Financiación	Otras posibles fuentes de sesgo	Total Riesgo de sesgo
Ashworth 1998	Poco claro	Poco claro	Poco claro	Si	Si	Si	Alto
Castillo Duran 2001	Poco claro	Poco claro	Si	Si	Si	Si	Moderado
Hamadani 2001	Poco claro	Poco claro	Si	No	Si	Si	Alto
Jiménez 2007	Si	Si	Si	Poco claro	Si	Si	Bajo
Lind 2004	Si	Si	SI	Si	Si	Si	Bajo

3.4 Recogida y extracción de información

3.4.1 En los niveles de Zn en suero/plasma

Cuando los niveles de Zn se midieron en diferentes momentos dentro de la misma población estudiada, esas medidas se consideraron como diferentes estimaciones (Bates *et al.* 1993, Makonnen *et al.* 2003 a y Makonnen *et al.* 2003 b).

Uno de los estudios refirió los datos del total de niños incluidos, por separado entre niños y niñas, y según la edad: <11 meses y > 11 meses (Sazawal *et al.* 1996). Las mediciones de este estudio se consideraron entonces como cinco estimaciones diferentes en el metaanálisis.

Un estudio clasificó a los lactantes en dos grupos: con retraso del crecimiento y sin retraso del crecimiento, y también, en este caso, ambos grupos fueron tratados como dos estimaciones diferentes (Umeta *et al.* 2000).

3.4.2 En el crecimiento

Cuando el crecimiento se midió en diferentes momentos dentro del mismo estudio, las medidas se utilizaron como diferentes estimaciones (Hamadani *et al.* 2001, Heinig *et al.* 2006, Sur *et al.* 2003).

Un estudio analizó a los lactantes separados por género (niños y niñas) (Walravens *et al.* 1989).

Un estudio clasificó a los lactantes en dos grupos: con retraso del crecimiento y sin retraso en el crecimiento, y también, en este caso, ambos grupos fueron tratados como dos estimaciones diferentes dentro del metaanálisis (Umeta *et al.* 2000).

El estudio de Osendrap *et al.* (2002) dividió la muestra en tres grupos (todos los lactantes, lactantes con bajos niveles séricos de Zn < 9,18 mmol/L, y lactantes con niveles normales de Zn sérico > 9,18 mmol/L).

El estudio de Arsenault *et al*. (2008) evaluó la ingesta de Zn utilizando un suplemento líquido y una papilla fortificada con el micronutriente.

3.4.3 En el neurodesarrollo

Cuando el desarrollo neurológico se midió en diferentes momentos en la misma población, cada medición se utilizó como un análisis o estimación diferente (Ashworth *et al.* 1998; Castillo-Durán *et al.* 2001; Hamadani *et al.* 2001; Jiménez *et al*. de 2007).

En un estudio (Ashworth *et al.* 1998) las dosis de Zn y la duración de la intervención fueron diferentes, por lo que se dividió la muestra en dos grupos.

3.5 Análisis estadístico

De cada estudio seleccionado se evaluaron las medidas finales de Zn en suero o plasma; las medidas finales del peso, longitud, perímetro braquial, perímetro cefálico, WAZ, LAZ y WLZ para el crecimiento y las medidas finales de los índices MDI y PDI para el neurodesarrollo, todas recogidas como media y desviación estándar (DE) o error estándar (EE). (ANEXO 6)

A partir de la media y la DE de cada estudio se calcularon los coeficientes beta (β) y sus EE, debido a que el modelo estadístico utilizado para estimar la relación entre la ingesta de Zn (variable x) y el nivel de Zn en suero/plasma o el crecimiento o el neurodesarrollo (variable y) se basa en la suposición de que esta relación lineal (ingesta/niveles de Zn séricos/plasmáticos o ingesta/crecimiento o ingesta/desarrollo

neurológico) es una función logarítmica y que tanto la ingesta como el nivel sérico/plasmático o el crecimiento o el desarrollo neurológico siguen una distribución logarítmica normal (es decir que el logaritmo natural de la ingesta y el Zn sérico/plasmático o el crecimiento o el desarrollo neurológico tienen una distribución normal). Así, el valor esperado para la puntuación niveles séricos/plasmáticos o crecimiento o neurodesarrollo se expresa como:

$\mu y = \beta * \mu x + \text{intercepción}$

donde μy representa la media del logaritmo natural de la variable y (= puntuación niveles séricos/plasmáticos o puntuación crecimiento o puntuación desarrollo neurológico), β representa el coeficiente de regresión, y μx representa la media del logaritmo natural de la variable x (= ingesta de Zn).

Esta forma de relación lineal de escala $\log_e$–$\log_e$ corresponde a una función monótona cóncava en la escala original para $\beta < 1$. Esta forma se supone que es realista para la relación biológica entre la ingesta de Zn y los diferentes parámetros. Debido a que la verdadera curva dosis-respuesta es desconocida, esta aproximación proporciona una metodología práctica para estimar la relación dosis-respuesta.

Se aplicaron procedimientos de metaanálisis formal para combinar los resultados de los estudios seleccionados, y así revisar sistemáticamente las diferencias en los parámetros medidos según la ingesta de Zn (Dickersin 2002, Greenland 1998).

Se consideró un modelo de efectos aleatorios por ser más apropiado que un modelo de efectos fijos. Se utilizó el método de DerSimonian y Laird para combinar los resultados (β) de los diferentes estudios (DerSimonian & Laird 1986).

Todos los coeficientes β proporcionados por los estudios, fueron combinados utilizando un criterio de ponderación wi. El inverso de la varianza fue el criterio de ponderación o peso aplicado.

La fórmula utilizada para estimar el efecto ponderado fue (Hedges 1982):

$$\beta \text{ ponderada} = \Sigma \beta i \, wi / \Sigma wi$$

donde β ponderada es la media ponderada de los coeficientes β de los niveles de Zn en suero/plasma, de los parámetros de crecimiento y de los parámetros de neurodesarrollo.

el peso (wi) de cada estudio se calculó como:

$$wi = 1 / Vi + \tau^2$$

donde V es la varianza de cada estudio y τ^2 es la varianza entre estudios. Además de esto, se calculó un intervalo de confianza del 95% (IC95%) para la estimación del tamaño del efecto combinado:

$$IC\ 95\% = \beta \text{ ponderada} \pm (1{,}96 \times EE \text{ ponderado})$$

donde EE es el error estándar de la estimación ponderada (Greenland 1998).

Se realizó además una prueba de heterogeneidad, estimando el valor del estadístico Q que sigue una distribución chi-cuadrado con n-1 grados de libertad, siendo n el número de estudios incluidos en el análisis. El índice I^2 expresa el grado de heterogeneidad, pues describe el porcentaje de la variación (de 0 a 100%) entre los estudios que es debida a la heterogeneidad y no al azar. El punto de corte para detectar heterogeneidad se sitúa en el 5% (p=0,05). Un valor de p por debajo de 0,05 para este índice, indica la presencia de heterogeneidad, y, de alguna manera, compromete la validez de las estimaciones combinadas (Takkouche *et al.* 1999).

Cuando los estudios combinados en el metaanálisis fueron heterogéneos, se evaluaron posibles fuentes de heterogeneidad mediante meta-regresiones lineales (Greenland 1998).

Se realizaron meta-regresiones utilizando la duración de la intervención, las dosis de ingesta de Zn, la situación nutricional y el riesgo de sesgo como variables independientes. Como variables dependientes se utilizaron los coeficientes beta obtenidos en cada estudio de niveles de Zn en suero/plasma, de los diferentes parámetros de crecimiento y de los diferentes parámetros de neurodesarrollo de acuerdo con la ingesta de Zn.

La existencia de diferencias estadísticamente significativas en la media ajustada de los coeficientes β entre las diferentes categorías de cada variable modificadora del efecto fue valorada a través de pruebas de ANCOVA.

Además se llevaron a cabo metaanálisis adicionales por subgrupos teniendo en cuenta sólo aquellos grupos que proporcionaron valores significativos en la meta-regresión.

También se realizaron análisis de sensibilidad. En ellos se excluyeron los estudios considerados atípicos o con valores extremos y se recalcularon las estimaciones de las betas ponderadas en cada parámetro del crecimiento y del desarrollo neurológico. En los niveles de Zn en suero o plasma, el análisis de sensibilidad no se realizó debido a que ningún valor se consideró atípico o extremo.

Por último, se estimó la relación dosis-respuesta en aquellos casos en los que la asociación entre la ingesta de Zn y los diferentes parámetros analizados resultara estadísticamente significativa y no se encontrara heterogeneidad en el metaanálisis.

Para llevar a cabo los análisis estadísticos se utilizó la versión (7,0) de Microsoft Excel, SPSS 10.0 para Windows y Review Manager 5.1.

4. Resultados

4. RESULTADOS

Cinco mil quinientos artículos fueron identificados inicialmente: 5126 gracias a la estrategia de búsqueda electrónica y 374 mediante búsqueda manual y revisión de referencias. Después de aplicar los criterios de inclusión/exclusión, 344 artículos parecían ser potencialmente relevantes. Después de aplicar los criterios de inclusión/exclusión adicionales *(In/Out Form)* y de agrupar los estudios por desenlace, 9 ensayos controlados aleatorizados (17 estimaciones), se seleccionaron para evaluar los niveles de Zn en suero/plasma (Bates *et al.* 1993; Chang *et al.* 2010; Lind *et al.* 2003; Makonnen *et al.* 2003a; Makonnen *et al.* 2003b; Osendarp *et al.* 2002; Sazawal *et al.* 1996; Sazawal *et al.* 2004; Umeta *et al.* 2000; Walravens *et al.* 1989; Wasantwisut *et al.* 2006); 19 ensayos controlados aleatorizados (38 estimaciones) fueron seleccionados para evaluar el crecimiento (Arsenault *et al.* 2008; Bates *et al.* 1993; Berger *et al.* 2006; Dijkhuizen *et al.* 2001; Fischer Walker *et al.* 2009; Gardner *et al.* 2005; Hamadani *et al.* 2001; Heinig *et al.* 2006; Lind *et al.* 2004; Meeks Gardner *et al.* 1998; Muller *et al.* 2003; Ninh *et al.* 1996; Olney *et al.* 2006; Osendrap *et al.* 2002; Rivera *et al.* 1998; Sur *et al.* 2003; Umeta *et al.* 2000; Walravens *et al.* 1989; Wasantwisut *et al.* 2006) y 5 ensayos controlados aleatorizados (9 estimaciones) fueron seleccionados para el metaanálisis de desarrollo neurológico (Ashworth *et al.* 1998; Castillo-Duran *et al.* 2001; Hamadani *et al.* 2001; Jiménez *et al.* 2007; Lind *et al.* 2004). (Figura 1)

Las características descriptivas de los estudios incluidos en los metaanálisis se presentan en las siguientes tablas: (Tablas 5, 6, 7)

Tabla 5: Características de los 9 estudios (17 estimaciones) del nivel de cinc en suero o plasma incluidos en el metaanálisis

Autor	Año del estudio	País	Muestra (rango de edad o media)	Número de lactantes (n)		Dosis de Cinc (mg)	Semanas de intervención	Desenlace	Situación Nutricional	Riesgo de sesgo
				Zn [1]	C[1]					
Bates (a) (b)	1993	Gambia	5,7 a 27 meses	30 46	28 44	20	2 8	Nivel en plasma	Saludable	Riesgo alto
Chang	2010	Bangladesh	6 a 18 meses	85	89	2,5	24	Nivel en suero	Con riesgo nutricional	Riesgo bajo
Lind	2003	Indonesia	6,1 (0,5) meses	134	143	10	24	Nivel en suero	Saludable	Riesgo bajo
(a) Makonnen (b) (c)	2003	Lesotho	6 a 60 meses	142 141 138	121 119 116	10	4 8 12	Nivel en suero	Estado nutricional deficiente	Riesgo moderado
Osendarp	2002	Bangladesh	3 a 5 semanas	138	133	5	20	Nivel en suero	Saludable	Riesgo moderado
Sazawal (a) (b) (c) (d) (e)	1996-2004	India	6 a 35 meses 6 a 11 meses > 11 meses Niñas Niños	223 78 69 115 108	224 78 73 106 118	10	16	Nivel en plasma	Con riesgo nutricional	Riesgo moderado
(a) Umeta (b)	2000	Etiopia	Zn retraso crecim. 9,5 (2,0) me Control retraso crecim. 9,7 (2,0) me Zn s/ retraso crecim. 9,3 (2,1) me Control s/retraso crecim. 9,2(2,0) me	25 25	 25 25	8,57	24	Nivel en suero	Saludable	Riesgo alto
Walravens	1989	USA	8 a 27 meses	16	25	5,7	24	Nivel en plasma	Con riesgo nutricional	Riesgo bajo
Wasantwisut	2006	Thailandia	4 a 6 meses	58	66	10	24	Nivel en suero	Saludable	Riesgo bajo

[1]Zn: Grupo Intervención / C[1]: Grupo Control
(a - e): Estimaciones

Tabla 6: Características de los 19 estudios (38 estimaciones) de crecimiento incluidos en el metaanálisis

Autor	Año del estudio	País	Muestra (rango de edad o media)	Número de lactantes (n)		Dosis de Cinc (mg)	Semanas de intervención	Desenlace (Crecimiento)	Situación Nutricional	Riesgo de sesgo
				Zn[1]	C[1]					
Arsenault (a) (b)	2008	Perú	6 to 8 meses	44 44	36 38	3 en suplemento líquido 3 en papilla fortificada	24	MUAC	Con riesgo nutricional	Riesgo alto
Bates	1993	Gambia	5,7 to 27 meses	50	53	20	60	Peso – Longitud - MUAC	Saludable	Riesgo alto
Berger	2006	Vietnam	4 to 7 meses	195	191	10	24	Peso - Longitud - WAZ – LAZ - WLZ	Con riesgo nutricional	Riesgo moderado
Dijkhuizen	2001	Indonesia	Mean 4,2 meses	90	98	7	24	Peso - Longitud - WAZ – LAZ – WLZ	Estado nutricional deficiente	Riesgo moderado
Fischer Walker	2009	Bangladesh	6,3 ± 0,3 meses	140	141	2,8	24	Peso - Longitud	Con riesgo nutricional	Riesgo moderado
Gardner	2005	Jamaica	9 to 30 meses	59	55	10	24	Peso - Longitud - WAZ – LAZ - WLZ	Estado nutricional deficiente	Riesgo moderado
Hamadani (a) (b)	2001	Bangladesh	1 to 13 meses	109 101	103 97	5	28 52	WAZ – LAZ WLZ	Estado nutricional deficiente	Riesgo alto
Heinig (a) (b) Para Perim.Cefálico	2006	USA	3 to 10 meses	37 37 37	33 33 33	5	16 40 24	Peso - Longitud - MUAC - Perímetro cefálico	Saludable	Riesgo bajo
Lind	2004	Indonesia	6 to 12 meses	164	163	10	8	WAZ - LAZ-WLZ	Saludable	Riesgo bajo
Meeks Gardner	1998	Jamaica	6 to 24 meses	24	31	5	12	Perímetro cefálico	Estado nutricional deficiente	Riesgo moderado
Muller	2003	Burkina Faso	Groupo Zn 18,7 ± 7,0 me Grupo Placebo: 17,6 ± 6,5 me	329	332	1,78	24	MUAC	Con riesgo nutricional	Riesgo bajo

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Ninh	1996	Vietnam	4 a 36 meses	73	73	10	20	Peso - Longitud - WAZ – LAZ - WLZ	Estado nutricional deficiente	Riesgo bajo
Olney	2006	Zanzibar	5 a 12 meses	58	44	10	24	WAZ – LAZ	Estado nutricional deficiente	Riesgo alto
Osendarp (a) (b) Bajo nivel de Zn < 9.18 µmol/L (c) Nivel Zn normal > 9.18 µmol/L	2002	Bangladesh	3 a 5 semanas	 133 16 115	 138 21 117	5	20	Peso - Longitud - MUAC Perímetro cefálico	Saludable	Riesgo moderado
Rivera	1998	Guatemala	6 a 9 meses	44	45	10	28	Peso - Longitud - WAZ – LAZ - WLZ- MUAC - Perímetro cefálico	Con riesgo nutricional	Riesgo alto
(Sur) (a) (b) (c) (d) (e) (f) (g) (h) (i) (j) (k) (l)	2003	India	0 a 12 meses	50 50 50 50 50 50 50 50 50 50 50 50	50 50 50 50 50 50 50 50 50 50 50 50	3,57 3,57 3,57 3,57 3,57 3,57 3,57 3,57 3,57 3,57 3,57 3,57	1 2 3 4 5 6 7 8 9 10 11 12	WAZ	Estado nutricional deficiente	Riesgo moderado
Umeta (a) (b)	2000	Etiopia	Zn retraso crecim. 9,5 ± 2,0 me Control retraso crecim 9,7 ± 2,0 me Zn s/ retraso crecim. 9,3 ± 2,1me Control s/retr. crecim 9,2 ± 2,0 me	47 45 	 47 45	8,57	24	MUAC	Saludable (con o sin retraso del crecim.)	Riesgo alto
Walravens (a) Niños (b) Niñas	1989	USA	8 a 27 meses	 13 12	 13 12	 5,7 5,7	24	Peso - Longitud	Con riesgo nutricional	Riesgo bajo
Wasantwisut	2006	Thailandia	4 a 6 meses	153	151	10	24	Peso – Longitud - WAZ – LAZ - WLZ	Saludable	Riesgo bajo

[1]Zn: Grupo Intervención / C[1]: Grupo Control
(a - l): Estimaciones
MUAC: Mid upper arm circumference (Circunferencia de brazo media)
WAZ: Weight for age z – score (Peso para la edad para la puntuación z)
LAZ: Length for age z- score (Longitud para la edad para la puntuación z)
WLZ: Weight for length z-score (Peso para la longitud para la puntuación z)

Tabla 7: Características de los 5 estudios (14 estimaciones) de desarrollo neurológico incluidos en el metaanálisis

Autor	Año del estudio	País	Muestra (rango de edad o media)	Número de lactantes (n)		Dosis de Cinc (mg)	Semanas de intervención	Desenlace	Situación Nutricional	Riesgo de sesgo
				Zn[1]	C[1]					
Ashworth (a)	1998	Brasil	6 a 12 meses	53	56	1	24	MDI-PDI	Estado nutricional deficiente	Riesgo alto
(b)				44	48		48			
(c)				53	54	5	24			
(d)				44	46		48			
Castillo Duran (a)	2001	Chile	Media 6 meses	45	57	5	24	MDI-PDI	Saludable	Riesgo moderado
(b)				45	57		48			
Hamadani (a)	2001	Bangladesh	1 a 13 meses	109	103	5	28	MDI-PDI	Estado nutricional deficiente	Riesgo alto
(b)				101	97		52			
(a)	2007	Cuba	1 a 12 meses	76	87	10	4	MDI-PDI	Estado nutricional deficiente	Bajo riesgo
Jiménez (b)				76	87		13			
(c)				76	87		26			
(d)				76	87		39			
(e)				76	87		52			
Lind	2004	Indonesia	6 a 12 meses	162	161	10	8	MDI-PDI	Saludable	Bajo riesgo

[1]Zn: Grupo Intervención / C: Grupo Control
(a - e): Estimaciones
MDI: Índice Desarrollo Mental
PDI: Índice Desarrollo Psicomotor

4.1 Resultados para niveles de Zn en suero/plasma

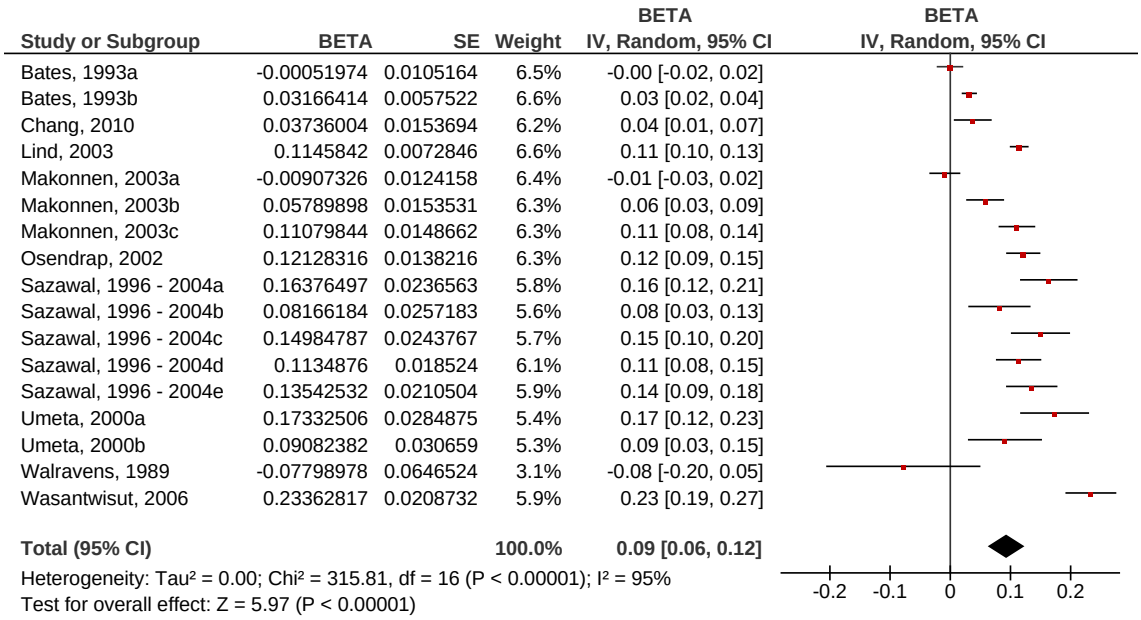
De los 9 estudios incluidos para evaluar el nivel de Zn en el suero/plasma, sólo 4 cumplieron estrictamente con la edad de los lactantes (0 a 12 meses) (Lind *et al.* 2003; Osendarp *et al.* 2002; Umeta *et al.* 2000; Wasantwisut *et al.* 2006). Los otros 5 estudios, también incluyeron este rango de edad entre los niños, pero no detallaron cuántos fueron en realidad de 0 a 12 meses (Bates *et al.* 1993; Chang *et al.* 2010; Makonnen *et al.* 2003a; Makonnen *et al.* 2003b; Sazawal *et al.* 1996; Sazawal *et al.* 2004; Walravens *et al.* 1989). En ningún caso, la edad de los niños se extendió más allá de 27 meses, a excepción del estudio de Makonen *et al*. (2003 a, 2003 b), que incluyó a niños de hasta 5 años. Sin embargo, éste aclara que casi el 45% estuvieron dentro del rango de 12 a 23 meses, por lo que se calcula que el 55% restante fue menor o mayor que el rango utilizado en este metaanálisis.

Cinco estudios fueron de Asia, 1 de América del Norte y 3 de África. La duración de las intervenciones fue de 2 a 24 semanas. Las dosis de la ingesta de Zn variaron de 2,5 a 20 mg por día. El rango de edad de los estudios incluidos fue de 3 semanas a 60 meses. La situación nutricional de los lactantes también varió entre los estudios: 5 estudios se realizaron en niños sanos (Bates *et al.* 1993; Lind *et al.* 2003; Osendarp *et al.* 2002; Umeta *et al.* 2000; Wasantwisut *et al.* 2006), 3 en niños con riesgo nutricional (Chang *et al.* 2010; Sazawal *et al.* 1996; Sazawal *et al.* 2004; Walravens *et al.* 1989), y 1 estudio se llevó a cabo en niños con estado nutricional deficiente (Makonnen *et al.* 2003a; Makonnen *et al.* 2003b). El riesgo de sesgo también varió entre los estudios: 2 estudios tuvieron un alto riesgo de sesgo, 3 tuvieron un riesgo de sesgo moderado y 4 tuvieron un riesgo de sesgo bajo.

La mayoría de los ensayos clínicos aleatorizados no encontró ninguna diferencia entre las concentraciones de Zn basales medidas en suero o plasma (n = 8). Sólo el estudio de Bates *et al.* (1993) no mencionó nada al respecto.

Las diferencias entre los niveles de Zn en suero/plasma medidas de acuerdo con el grupo de intervención en cada estudio en particular y en el análisis conjunto de todos los estudios incluidos se muestran en la Figura 4.

Figura 4: Niveles de Cinc en suero/plasma



El coeficiente β ponderado fue de 0,09 (IC 95%: 0,06 a 0,12). Sin embargo, en los análisis existió una heterogeneidad significativa (I^2 = 95%, p= <0,00001).

Con el fin de investigar aquellas variables posibles modificadoras del efecto, se realizó una meta-regresión (Tabla 8).

Tabla 8: Meta-regresión. Efecto de la ingesta de Zn sobre los niveles de Zn en suero o plasma de acuerdo con diferentes características de los estudios incluidos en el metaanálisis (Coeficiente β medio e IC 95%)

Nivel de Zn en suero /plasma	n	Coeficiente β medio	IC(95%)	P Ancova*
Por duración de la intervención				
1 a 3 semanas	1	0,0390	-0,0556 a 0,1337	
4 a 20 semanas	10	0,0712	0,0318 a 0,1107	
> 20 semanas	6	-0,0535	-0,1137 a 0,0068	
				0,005
Por Dosis				
1 a 4 mg	1	0,1011	0,0074 a 0,1948	
4,1 a 8 mg	2	-0,0143	-0,0763 a 0,0477	
8,1 a12 mg	12	0,1070	0,0663 a 0,1478	
> 12 mg	2	-0,1181	-0,1921 a -0,0440	
				<0,001
Por Situación Nutricional				
Saludable	7	0,1304	0,0819 a 0,1789	
Con riesgo nutricional	7	-0,0004	-0,0408 a 0,0400	
Estado nutricional deficiente	3	-0,0732	-0,1312 a -0,0152	
				<0,001
Por Riesgo de Sesgo				
Bajo	4	0,0470	-0,0104 a 0,1043	
Moderado	9	0,0049	-0,0381 a 0,0480	
Alto	4	0,0049	-0,0381 a 0,0480	
				0,241

* Valor p obtenido a través de ANCOVA

El efecto de la ingesta de Zn en los niveles de Zn en suero/plasma cambió dependiendo de la duración de la intervención, la dosis de ingesta y la situación nutricional (p = 0,005, <0,001 y <0,001), respectivamente.

Después de estratificar la muestra según las variables modificadoras del efecto señaladas en la meta-regresión, los resultados, según la duración de la intervención mostraron un efecto positivo cuando la ingesta de Zn se proporcionó durante un período de tiempo medio (de 4 a 20 semanas) (β = 0,09, IC del 95% 0,06 a 0,13) y largo (más de 20 semanas) (β = 0,11, IC 95%: 0,05 a 0,17). Sin embargo, estos coeficientes β ponderados aún mostraron evidencia importante de heterogeneidad (I^2 = 94 %, p=< 0,00001 y 93%, p=< 0,00001) respectivamente (Tabla 9).

Tabla 9: Análisis de subgrupos. Efecto de la ingesta de Zn sobre los niveles de Zn en suero /plasma en cada categoría de las variables modificadoras del efecto (coeficiente β ponderado e IC 95%)

Nivel de Zn en suero /plasma	Coeficiente β ponderado	Chi² (df, P)	I²
Todos los estudios (n=17)	0,09 (0,06 a 0,12)	315,81 (16, < 0,00001)	95%
Por duración de la Intervención			
1 a 3 semanas (n=1)	0 (-0,02 a 0,02)		
4 a 20 semanas (n=10)	0,09 (0.06 a 0,13)	141,21 (9, < 0,00001)	94%
> 20 semanas (n=6)	0,11 (0,05 a 0,17)	71,40 (5, < 0,00001)	93%
Por dosis			
1 a 4 mg (n=1)	0,04 (0,01 a 0,07)		
4,1 a 8 mg (n=2)	0,04 (-0,17 a 0,25)	9,85 (1, 0,002)	90%
8,1 a 12 mg (n=12)	0,12 (0,08 a 0,15)	151,38 (11, < 0,00001)	93%
> 12 mg (n=2)	0,02 (-0,01 a 0,05)	7,21 (1, 0,007)	86%
Por Situación Nutricional			
Saludable (n=7)	0,11 (0,06 a 0,16)	215,29 (6, < 0,00001)	97%
Con riesgo nutricional (n=7)	0,10 (0,05 a 0,14)	39,48 (6, < 0,00001)	85%
Estado nutricional deficiente (n=3)	0,05 (-0,02 a 0,12)	39,26 (2, < 0,00001)	95%

* I² Índice que mide el grado de heterogeneidad

Cuando las dosis de Zn variaron de 4,1 a 8 mg/día, no hubo efecto significativo de la ingesta de Zn en los niveles de Zn en suero/plasma, mientras que se observó un efecto positivo cuando las dosis oscilaron entre 8,1 a 12 mg/día (β = 0,12, IC 95%: 0,08 a 0,15). Para dosis superiores a 12 mg/día no se registró ningún efecto. Sin embargo, para dosis por encima de 8 mg/día se observó gran evidencia de heterogeneidad.

Cuando los estudios se clasificaron según la situación nutricional, los estudios realizados en lactantes sanos y con riesgo nutricional, registraron una asociación positiva entre la ingesta de Zn en los niveles de Zn en suero/plasma (β = 0,11, IC 95%: 0,06 a 0,16 y β = 0,10; IC 95%: 0,05 a 0,14), respectivamente. Sin embargo, no se encontró asociación cuando la situación nutricional de los niños fue deficiente (β = 0,05, IC 95% -0,02 a 0,12). Una vez más, el coeficiente β ponderado continuó mostrando gran evidencia de heterogeneidad (I^2 = 85 %, p= < 0,00001 a 97%, p= < 0,00001).

Debido a la gran heterogeneidad encontrada en todos los análisis, se decidió no realizar el cálculo de la relación dosis-respuesta entre la ingesta de Zn y los niveles de Zn en suero o plasma.

4.2 Resultados para Crecimiento

De los 19 estudios incluidos en el metaanálisis de crecimiento, sólo 12 cumplieron estrictamente con el rango de edad (0 a 12 meses) (Arsenault *et al.* 2008; Berger *et al.* 2006; Dijkhuizen *et al.* 2001; Fischer Walker *et al.* 2009; Heinig *et al.* 2006; Lind *et al.* 2004; Olney *et al.* 2006; Osendrap *et al.* 2002; Rivera *et al.* 1998; Sur *et al.* 2003; Umeta *et al.* 2000; Wasantwisut *et al.* 2006). Los otros 7 estudios incluían esta edad entre los niños, pero no detallaban cuántos eran en realidad de 0 a 12 meses (Bates *et al.* 1993; Gardner *et al.* 2005; Hamadani *et al.* 2001; Meeks Gardner *et al.* 1998; Muller *et al.* 2003; Ninh *et al.* 1996; Walravens *et al.* 1989). En ningún caso, las edades se extendieron más allá de los 27 meses, a excepción de Gardner y Ninh *et al.* (2005, 1996), que incluyeron niños de hasta 30 y 36 meses, respectivamente.

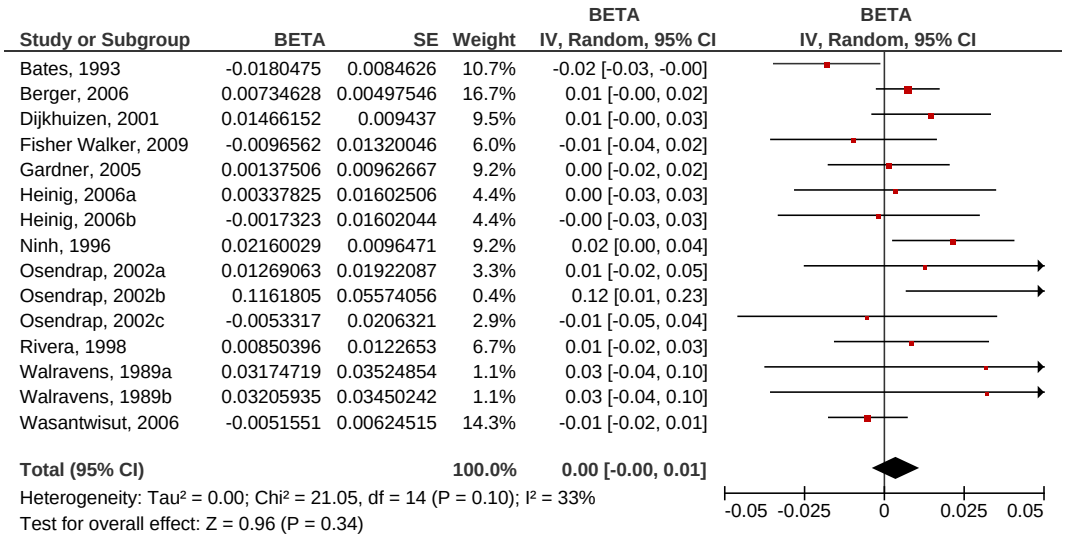
Cuatro estudios fueron de América Latina y el Caribe, 2 de América del Norte, 9 de Asia y 4 de África. La duración de las intervenciones varió de 1 a 60 semanas. Las dosis de ingesta de Zn variaron desde 1,78 hasta 20 mg por día. La situación nutricional de los lactantes también varió: 6 estudios se realizaron en lactantes sanos (Bates *et al.* 1993; Heinig *et al.* 2006; Lind *et al.* 2004; Osendrap *et al.* 2002; Umeta *et al.* 2000; Wasantwisut *et al.* 2006), 6 estudios en lactantes con riesgo nutricional (Arsenault *et al.* 2008; Berger *et al.* 2006; Fischer Walker *et al.* 2009; Muller *et al.* 2003; Rivera *et al.* 1998; Walravens *et al.* 1989), y 7 estudios se llevaron a cabo en lactantes con estado nutricional deficiente (Dijkhuizen *et al.* 2001; Gardner *et al.* 2005; Hamadani *et al.* 2001; Meeks Gardner *et al.* 1998; Ninh *et al.* 1996; Olney *et al.* 2006; Sur *et al.* 2003). El riesgo de sesgo también varió entre los estudios: 6 tuvieron un alto riesgo de sesgo, 7 tuvieron un riesgo de sesgo moderado y 6 mostraron un riesgo de sesgo bajo.

La mayoría de los ensayos clínicos no refirieron ninguna diferencia basal en las diferentes medidas de crecimiento (n = 16). Sin embargo, Arsenault y Rivera *et al*.

(2008; 1998) realizaron un ajuste por diferencias iniciales. Y sólo el estudio de Bates *et al.* (1993) no mencionó nada respecto de este asunto.

Las diferencias entre los resultados de los diferentes parámetros de crecimiento evaluados (peso, talla, perímetro braquial, perímetro cefálico, WAZ, LAZ y WLZ) de acuerdo con la ingesta de Zn en cada estudio particular y en los análisis conjunto de todos los estudios incluidos se muestran en las figuras 5 a 11.

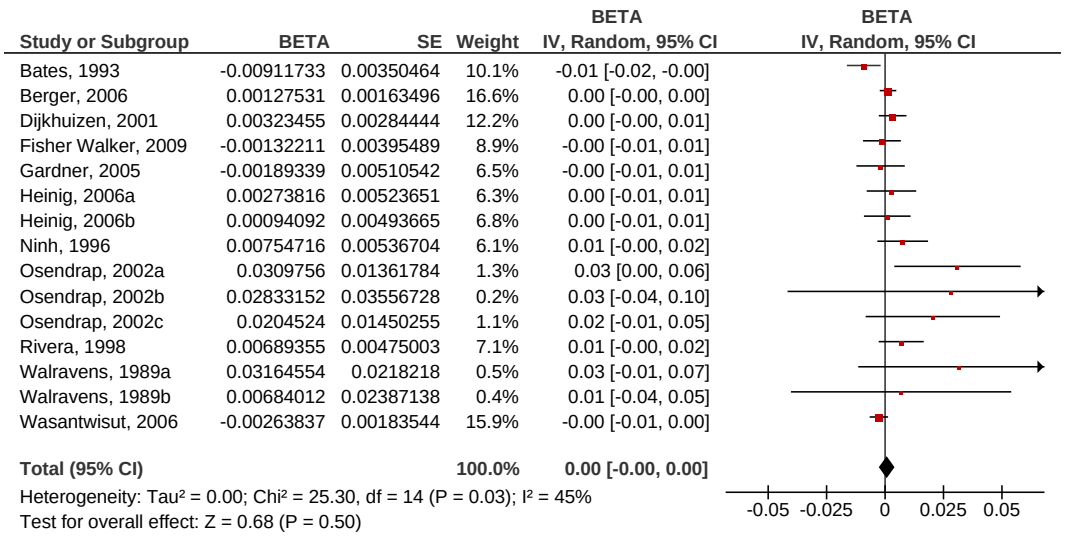
Figura 5: Crecimiento – Peso –



Study or Subgroup	BETA	SE	Weight	BETA IV, Random, 95% CI
Bates, 1993	-0.0180475	0.0084626	10.7%	-0.02 [-0.03, -0.00]
Berger, 2006	0.00734628	0.00497546	16.7%	0.01 [-0.00, 0.02]
Dijkhuizen, 2001	0.01466152	0.009437	9.5%	0.01 [-0.00, 0.03]
Fisher Walker, 2009	-0.0096562	0.01320046	6.0%	-0.01 [-0.04, 0.02]
Gardner, 2005	0.00137506	0.00962667	9.2%	0.00 [-0.02, 0.02]
Heinig, 2006a	0.00337825	0.01602506	4.4%	0.00 [-0.03, 0.03]
Heinig, 2006b	-0.0017323	0.01602044	4.4%	-0.00 [-0.03, 0.03]
Ninh, 1996	0.02160029	0.0096471	9.2%	0.02 [0.00, 0.04]
Osendrap, 2002a	0.01269063	0.01922087	3.3%	0.01 [-0.02, 0.05]
Osendrap, 2002b	0.1161805	0.05574056	0.4%	0.12 [0.01, 0.23]
Osendrap, 2002c	-0.0053317	0.0206321	2.9%	-0.01 [-0.05, 0.04]
Rivera, 1998	0.00850396	0.0122653	6.7%	0.01 [-0.02, 0.03]
Walravens, 1989a	0.03174719	0.03524854	1.1%	0.03 [-0.04, 0.10]
Walravens, 1989b	0.03205935	0.03450242	1.1%	0.03 [-0.04, 0.10]
Wasantwisut, 2006	-0.0051551	0.00624515	14.3%	-0.01 [-0.02, 0.01]
Total (95% CI)			**100.0%**	**0.00 [-0.00, 0.01]**

Heterogeneity: Tau² = 0.00; Chi² = 21.05, df = 14 (P = 0.10); I² = 33%
Test for overall effect: Z = 0.96 (P = 0.34)

Figura 6: Crecimiento – Longitud –



Study or Subgroup	BETA	SE	Weight	BETA IV, Random, 95% CI
Bates, 1993	-0.00911733	0.00350464	10.1%	-0.01 [-0.02, -0.00]
Berger, 2006	0.00127531	0.00163496	16.6%	0.00 [-0.00, 0.00]
Dijkhuizen, 2001	0.00323455	0.00284444	12.2%	0.00 [-0.00, 0.01]
Fisher Walker, 2009	-0.00132211	0.00395489	8.9%	-0.00 [-0.01, 0.01]
Gardner, 2005	-0.00189339	0.00510542	6.5%	-0.00 [-0.01, 0.01]
Heinig, 2006a	0.00273816	0.00523651	6.3%	0.00 [-0.01, 0.01]
Heinig, 2006b	0.00094092	0.00493665	6.8%	0.00 [-0.01, 0.01]
Ninh, 1996	0.00754716	0.00536704	6.1%	0.01 [-0.00, 0.02]
Osendrap, 2002a	0.0309756	0.01361784	1.3%	0.03 [0.00, 0.06]
Osendrap, 2002b	0.02833152	0.03556728	0.2%	0.03 [-0.04, 0.10]
Osendrap, 2002c	0.0204524	0.01450255	1.1%	0.02 [-0.01, 0.05]
Rivera, 1998	0.00689355	0.00475003	7.1%	0.01 [-0.00, 0.02]
Walravens, 1989a	0.03164554	0.0218218	0.5%	0.03 [-0.01, 0.07]
Walravens, 1989b	0.00684012	0.02387138	0.4%	0.01 [-0.04, 0.05]
Wasantwisut, 2006	-0.00263837	0.00183544	15.9%	-0.00 [-0.01, 0.00]
Total (95% CI)			**100.0%**	**0.00 [-0.00, 0.00]**

Heterogeneity: Tau² = 0.00; Chi² = 25.30, df = 14 (P = 0.03); I² = 45%
Test for overall effect: Z = 0.68 (P = 0.50)

Figura 7: Crecimiento – Perímetro cefálico –

Study or Subgroup	BETA	SE	Weight	BETA IV, Random, 95% CI
Heinig, 2006	0.00126049	0.00391991	22.1%	0.00 [-0.01, 0.01]
Meeks Gardner, 1998	0.00590717	0.00760938	5.9%	0.01 [-0.01, 0.02]
Osendrap, 2002	-0.0016081	0.00255384	52.0%	-0.00 [-0.01, 0.00]
Rivera, 1998	0.0028015	0.00411737	20.0%	0.00 [-0.01, 0.01]
Total (95% CI)			**100.0%**	**0.00 [-0.00, 0.00]**

Heterogeneity: Tau² = 0.00; Chi² = 1.53, df = 3 (P = 0.68); I² = 0%
Test for overall effect: Z = 0.19 (P = 0.85)

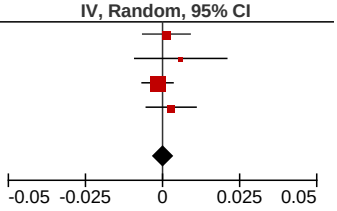


Figura 8: Crecimiento – MUAC (Circunferencia Superior del Brazo) –

Study or Subgroup	BETA	SE	Weight	BETA IV, Random, 95% CI
Arsenault, 2008 a	-0.0065066	0.01271913	6.2%	-0.01 [-0.03, 0.02]
Arsenault, 2008 b	0.00743958	0.01260497	6.3%	0.01 [-0.02, 0.03]
Bates, 1993	-0.0277362	0.04472781	0.5%	-0.03 [-0.12, 0.06]
Heinig, 2006	0.0239698	0.01905093	2.8%	0.02 [-0.01, 0.06]
Muller, 2003	0.0110113	0.00982811	10.4%	0.01 [-0.01, 0.03]
Osendrap, 2002	-0.0049165	0.00534505	35.3%	-0.00 [-0.02, 0.01]
Rivera, 1998	0.00328778	0.00761011	17.4%	0.00 [-0.01, 0.02]
Umeta, 2000 a	0.01146116	0.00859427	13.6%	0.01 [-0.01, 0.03]
Umeta, 2000 b	0.01285006	0.01165681	7.4%	0.01 [-0.01, 0.04]
Total (95% CI)			**100.0%**	**0.00 [-0.00, 0.01]**

Heterogeneity: Tau² = 0.00; Chi² = 6.91, df = 8 (P = 0.55); I² = 0%
Test for overall effect: Z = 0.98 (P = 0.33)

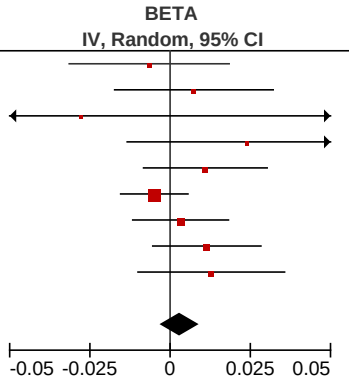


Figura 9: Crecimiento – WAZ (Peso para la edad Z – score) –

Study or Subgroup	BETA	SE	Weight	BETA IV, Random, 95% CI
Berger, 2006	0.0647417	0.0377112	10.6%	0.06 [-0.01, 0.14]
Dijkhuizen, 2001	0.0593347	0.0692402	5.7%	0.06 [-0.08, 0.20]
Gardner, 2005	-0.018498	0.0494441	8.4%	-0.02 [-0.12, 0.08]
Hamadani, 2001a	0	0.0829569	4.5%	0.00 [-0.16, 0.16]
Hamadani, 2001b	0.0633639	0.0810719	4.6%	0.06 [-0.10, 0.22]
Lind, 2004	0.1202346	0.0532252	7.8%	0.12 [0.02, 0.22]
Ninh, 1996	0.1387323	0.0441472	9.4%	0.14 [0.05, 0.23]
Olney, 2006	-0.032371	0.041484	9.9%	-0.03 [-0.11, 0.05]
Rivera, 1998	-0.018498	0.0843635	4.4%	-0.02 [-0.18, 0.15]
Sur, 2003a	-0.045933	0.0859668	4.2%	-0.05 [-0.21, 0.12]
Sur, 2003b	-0.053588	0.127849	2.2%	-0.05 [-0.30, 0.20]
Sur, 2003c	-0.038277	0.1122829	2.8%	-0.04 [-0.26, 0.18]
Sur, 2003d	0.1071769	0.1209568	2.5%	0.11 [-0.13, 0.34]
Sur, 2003e	0.1454544	0.1256994	2.3%	0.15 [-0.10, 0.39]
Sur, 2003f	0.0535884	0.1405654	1.9%	0.05 [-0.22, 0.33]
Sur, 2003g	0.0688994	0.1492998	1.7%	0.07 [-0.22, 0.36]
Sur, 2003h	0.1913873	0.1485245	1.7%	0.19 [-0.10, 0.48]
Sur, 2003i	0.1531099	0.1447394	1.8%	0.15 [-0.13, 0.44]
Sur, 2003j	0.1531099	0.1441267	1.8%	0.15 [-0.13, 0.44]
Sur, 2003k	0.2526313	0.1569395	1.6%	0.25 [-0.05, 0.56]
Sur, 2003l	0.5511955	0.1565394	1.6%	0.55 [0.24, 0.86]
Wasantwisut, 2006	0	0.0477421	8.7%	0.00 [-0.09, 0.09]
Total (95% CI)			**100.0%**	**0.06 [0.02, 0.10]**

Heterogeneity: Tau² = 0.00; Chi² = 31.14, df = 21 (P = 0.07); I² = 33%
Test for overall effect: Z = 2.69 (P = 0.007)

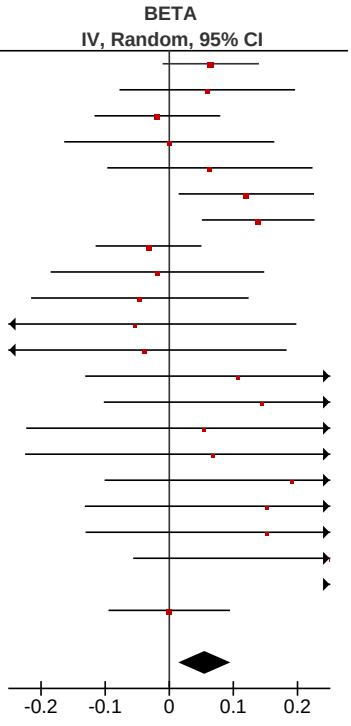


Figura 10: Crecimiento – LAZ (Longitud para la edad Z- score) –

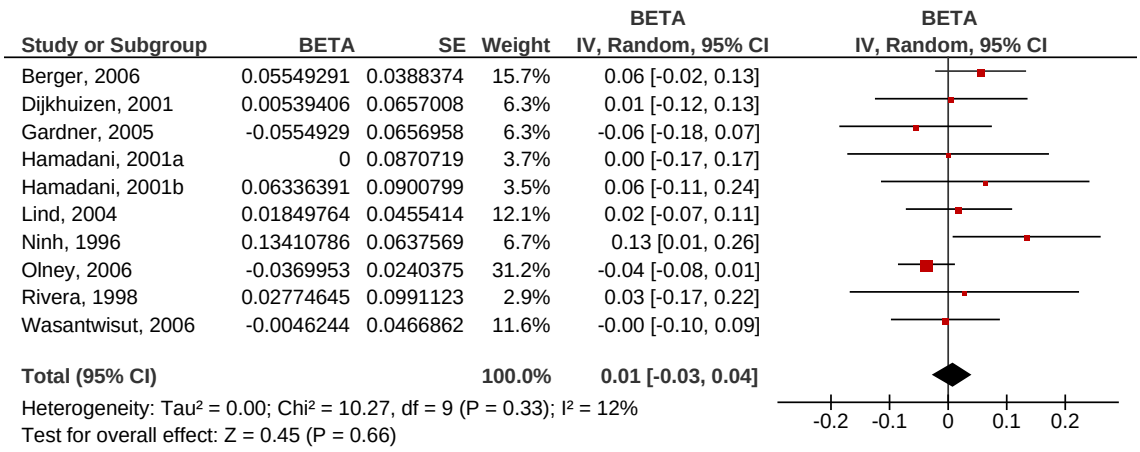
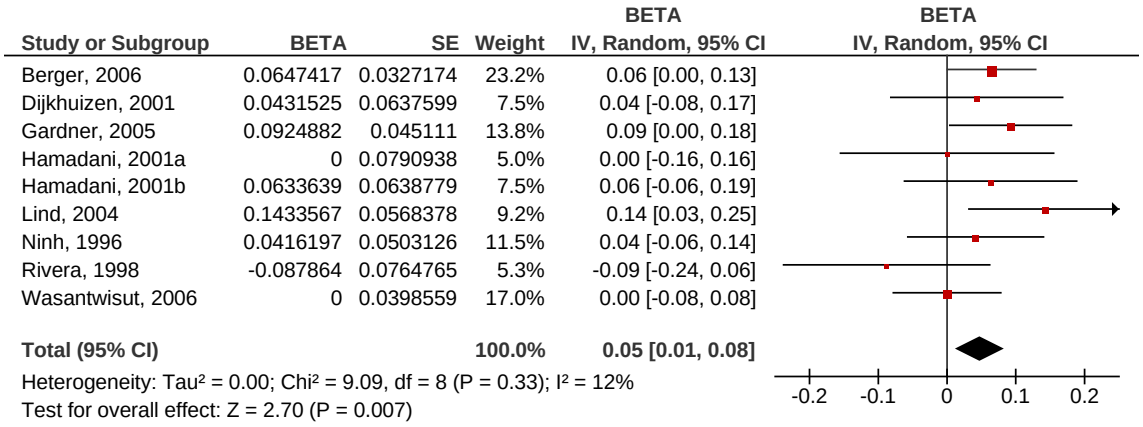


Figura 11: Crecimiento – WLZ (Peso para la Longitud Z- score) –



En los análisis combinados, la ingesta de Zn no se asoció con el peso, con la longitud, con el perímetro braquial, con el perímetro cefálico y con LAZ. Sin embargo, la ingesta de Zn tuvo un efecto positivo y estadísticamente significativo en WAZ (β = 0,06, IC 95%: 0,02 a 0,10) y en WLZ (β = 0,05, IC 95%: 0,01 a 0,08).

Dado que se aplicó una transformación logarítmica de base-e en la ingesta de Zn y los parámetros de crecimiento antes del cálculo de los coeficientes β de cada estudio específico, el coeficiente β ponderado representa la diferencia en el valor pronosticado de WAZ y WLZ para cada diferencia de una unidad en la ingesta de Zn (en escala logarítmica de base e). Por lo tanto, un coeficiente β ponderado de 0,06 significa que si se dobla la ingesta de Zn (x2), la diferencia en WAZ es $2^{\hat{\beta}}$ ($2^{0,06}$ = 1,04). Para un

coeficiente β ponderado de 0,05, la diferencia en WLZ es de 1,035. Esto significa que una persona con una doble ingesta de Zn tiene, aproximadamente, un 4% mayor valor en WAZ y en WLZ que una persona que ingiere la mitad. (Figura 12, 13)

Figura 12: WAZ (Kg/edad z-score) en función de la ingesta de cinc en la dieta (mg/día), estimado en el metaanálisis.

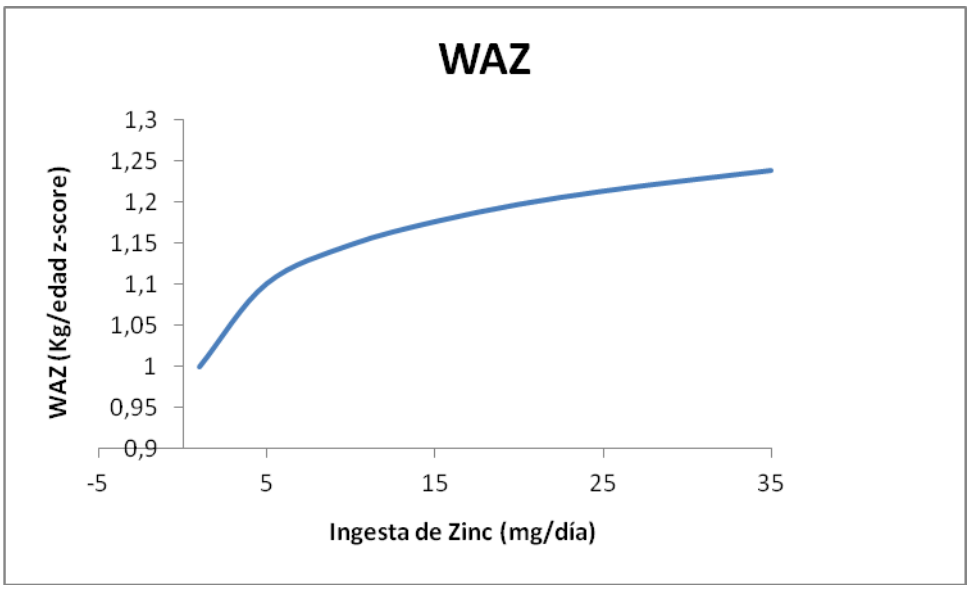
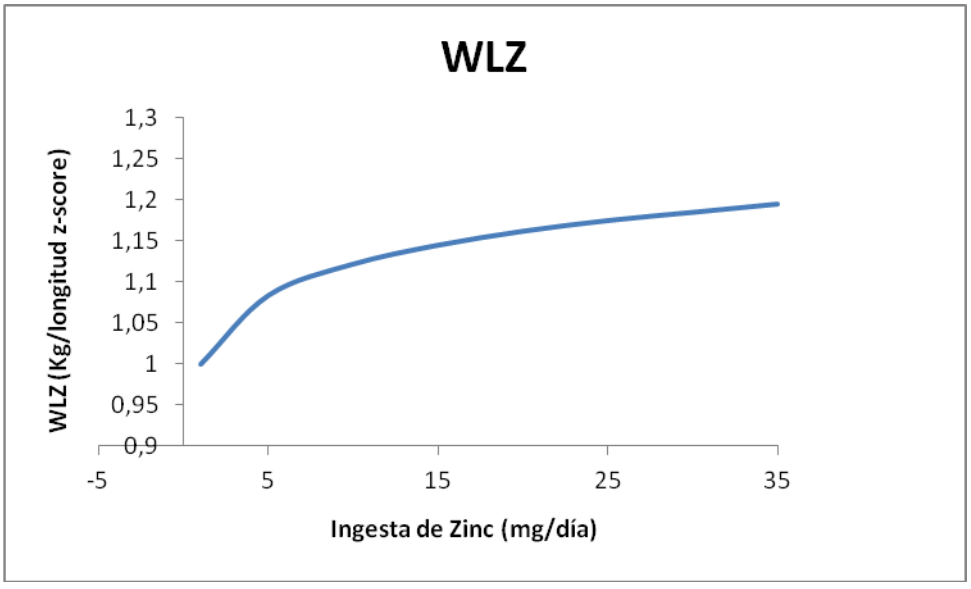


Figura 13: WLZ (Kg/Longitud z-score) en función de la ingesta de cinc en la dieta (mg/día), estimado en el metaanálisis.



Con excepción de la longitud (I^2 = 45%, p = 0,03), no hubo heterogeneidad en ningún otro análisis. Con el fin de investigar aquellas variables que pueden ser posibles modificadoras del efecto sobre la longitud, se realizó una meta-regresión (Tabla 10).

El efecto de la ingesta de Zn en la longitud cambió dependiendo de la duración de la intervención y de la dosis (p ANCOVA = 0,008 y 0,023), respectivamente.

Tabla 10: Meta-regresión. Efecto de la ingesta de Zn sobre el crecimiento de acuerdo con diferentes características de los estudios incluidos en el metaanálisis (Coeficiente β medio e IC 95%)

Crecimiento: Longitud	**n**	**Coeficiente β medio**	**IC (95%)**	**P Ancova***
Por Tiempo				
4 a 20 semanas	5	0,0113	0,008 a 0,0219	
> 20 semanas	10	-0,0026	-0,0089 a 0,0037	
				0,008
Por Dosis				
1 a 4 mg	1	-0,0058	-0,0245 a 0,0130	
4,1 a 8 mg	8	0,0162	0,0078 a 0,0245	
8,1 a 12 mg	5	0,0057	-0,0016 a 0,0130	
> 12 mg	1	0,0014	-0,0200 a 0,0229	
				0,023
Por Situación Nutricional				
Saludable	7	0,0010	-0,0066 a 0,0086	
Con riesgo nutricional	5	0,0128	0,0025 a 0,0230	
Estado nutricional deficiente	3	-0,0006	-0,0122 a 0,0110	
				0,083
Por Riesgo de Sesgo				
Bajo	6	0,0016	-0,0084 a 0,0116	
Moderado	7	0,0074	-0,0013 a 0,0161	
Alto	2	0,0042	-0,0105 a 0,0189	
				0,409

* Valor p obtenido a través de ANCOVA

La Tabla 11 muestra los resultados de los análisis de longitud después de estratificar los estudios según las variables modificadoras del efecto señaladas en la meta-regresión. Después de estratificar por la duración de la intervención y por la dosis, la heterogeneidad se atenuó.

A corto plazo (de 4 a 20 semanas), la ingesta de Zn se asoció positivamente con los valores de longitud (β = 0,01, IC 95% 0 a 0,02). Sin embargo, no se encontró efecto cuando la suplementación se prolongó durante más de 20 semanas (β = -0,001; IC 95% -0,003 a 0,002).

A dosis de Zn medias (4,1 a 8 mg/día), la ingesta de Zn se asoció positivamente con los valores de longitud (β = 0,003, IC 95% 0 a 0,01). No obstante, la magnitud del efecto fue pequeña. No se encontró efecto a dosis de Zn bajas o altas (1 a 4 o > 12 mg/día) (β = 0; IC 95% -0,01 a 0,004 y β = 0,01, IC 95% -0,02 a 0), respectivamente.

Tabla 11: Análisis de subgrupos. Efecto de la ingesta de Zn sobre el crecimiento en cada categoría de las variables modificadoras del efecto (coeficiente β ponderado e IC 95%)

Crecimiento: Longitud	**Coeficiente β ponderado**	**Chi² (df, P)**	**I² ***
Todos los estudios (n=15)	0,001 (-0,002 a 0,004)	25,30 (14, 0,03)	45%
Por Tiempo			
4 a 20 semanas (n=5)	0,01 (0 a 0,02)	4,93 (4, 0,29)	19%
> 20 semanas (n=10)	-0,001 (-0,003 a 0,002)	15,18 (9, 0,09)	41%
Por dosis			
1 a 4 mg (n=1)	0 (-0,01 a 0,01)		
4,1 a 8 mg (n=8)	0,003 (0 a 0,01)	7,81 (7, 0,35)	10%
8,1 a 12 mg (n=5)	0 (-0,002 a 0,004)	6,85 (4, 0,14)	42%
> 12 mg (n=1)	0,01 (-0,02 a 0)		

* I^2 Índice que mide el grado de heterogeneidad

Los resultados de los análisis de sensibilidad se muestran en la Tabla 12. Las estimasiones de Osendrap *et al*. (b) (2002), Walravens *et al*. (a) y Walravens *et al*. (b) (1989) se consideraron como valores extremos en el análisis del Peso debido a que los límites del coeficiente beta fueron muy amplios (de IC 95 %: 0,01 a 0,23; IC 95% -0,04 a 0,10 e IC 95% -0,04 a 0,10), respectivamente. Cuando estas estimaciones se excluyeron, la asociación nula vista anteriormente se mantuvo.

En el análisis de WAZ, las diferentes estimaciones obtenidas en el estudio de Sur *et al*. (2003) (d, e, f, g, h, i, j, k, l) se consideraron como valores extremos por las mismas razones. Cuando se excluyeron, se observó una atenuación del efecto positivo de la ingesta de Zn sobre el parámetro WAZ (β = 0,03, IC 95% 0 a 0,07).

La estimación de Osendrap *et al*. (b) (2002) se consideró también como atípico en el análisis de la longitud. Cuando se excluyó, la asociación nula obtenida anteriormente persistió al igual que se mantuvo la heterogeneidad (I^2 = 47%, p = 0,03).

Tabla 12: Análisis de sensibilidad. Efecto de la ingesta de Zn sobre el crecimiento, eliminando valores extremos (coeficiente β ponderado e IC 95%)

	Coeficiente β ponderado	Chi² (dif, P)	I²
Crecimiento: Peso			
Todos los estudios (n=15)	0,004 (-0,004 a 0,01)	21,05 (14, 0,10)	33%
Todos los estudios excluidos (n=3)	0 (-0,005 a 0,01)	15,53 (11, 0,16)	29%
Osendrap et al. 2002 b	0,12 (0,01 a 0,23)		
Walravens et al. 1989 a	0,03 (-0,04 a 0,10)		
Walravens et al. 1989 b	0,03 (-0,04 a 0,10)		
Crecimiento: MUAC			
Todos los estudios (n=9)	0,003 (-0,003 a 0,01)	6,91 (8, 0,55)	0%
Todos los estudios excluidos (n=1)	0 (-0,003 a 0,01)	6,43 (7, 0,49)	0%
Bates et al.1993	-0,03 (-0,12 a 0,06)		
Crecimiento: WAZ			
Todos los estudios (n=22)	0,06 (0,02 a 0,10)	31,14 (21, 0,07)	33%
Todos los estudios excluidos (n=9)	0,03 (0 a 0,07)	15,67 (12, 0,21)	23%
Sur et al. 2003 d	0,11 (-0,13 a 0,34)		
Sur et al. 2003 e	0,15 (-0,10 a 0,39)		
Sur et al. 2003 f	0,05 (-0,22 a 0,33)		
Sur et al. 2003 g	0,07 (-0,22 a 0,36)		
Sur et al. 2003 h	0,19 (-0,10 a 0,48)		
Sur et al. 2003 i	0,15 (-0,13 a 0,44)		
Sur et al. 2003 j	0,15 (-0,13 a 0,44)		
Sur et al. 2003 k	0,25 (-0,05 a 0,56)		
Sur et al. 2003 l	0,55 (0,24 a 0,86)		
Crecimiento: Talla			
Todos los estudios (n=15)	0,001 (-0,002 a 0,004)	25,30 (14, 0,03)	45%
Todos los estudios excluidos (n=1)	0 (-0,002 a 0,004)	24,67 (13, 0,03)	47%
Osendrap et al. 2002 b	0,03 (-0,04 a 0,10)		

* I² Índice que mide el grado de heterogeneidad

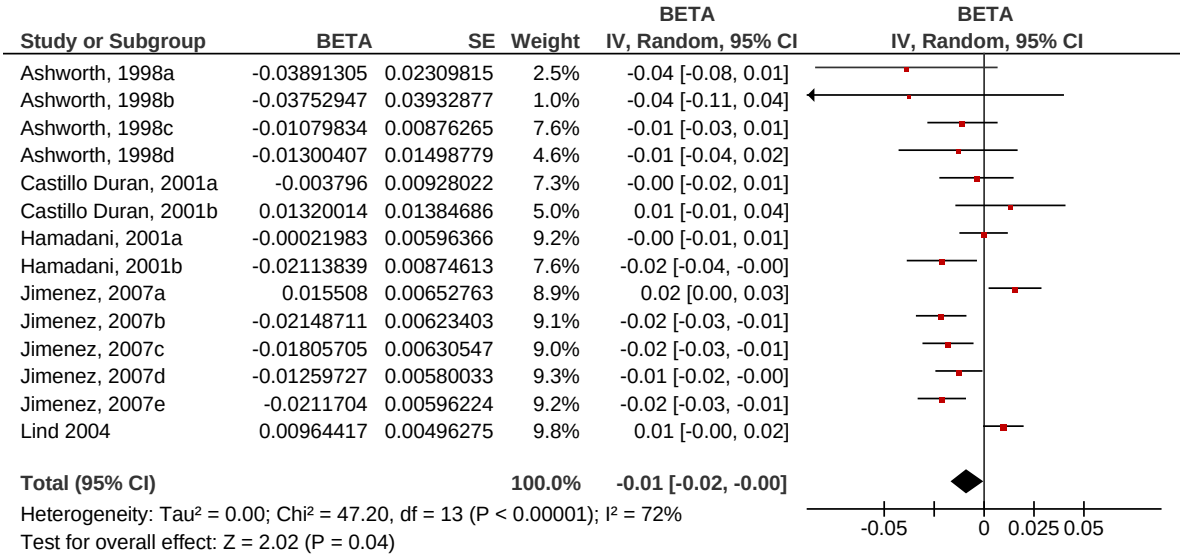
4.3 Resultados para Neurodesarrollo

Dentro de los estudios seleccionados para neurodesarrollo tres de ellos fueron de América Latina y el Caribe y dos de Asia. La duración de las intervenciones varió de 4 a 52 semanas. Las dosis de Zn fueron de 1 a 10 mg por día. La edad de los lactantes fue entre 1 a 12 meses. Sin embargo, se incluyó un estudio (Hamadani *et al.* 2001) con niños de 13 meses. La situación nutricional también fue diferente entre los estudios: dos estudios se realizaron en lactantes sanos (Castillo-Durán C *et al*. 2001; Lind *et al*. 2004), y tres en lactantes con estado nutricional deficiente (Ashworth *et al.* 1998; Hamadani *et al*. 2001; Jiménez *et al.* 2007). Ningún estudio seleccionado incluyó lactantes en riesgo nutricional. El riesgo de sesgo también varió entre los estudios: dos tuvieron un alto riesgo de sesgo (Ashworth *et al.* 1998; Hamadani *et al.* 2001), uno tuvo un riesgo moderado (Castillo-Durán *et al*. 2001) y dos tuvieron riesgo de sesgo bajo (Jiménez *et al*. 2007; Lind *et al*. 2004).

La mayoría de los ensayos clínicos no refirieron ninguna diferencia basal en las medidas de MDI o PDI (n = 3). Sin embargo, Ashworth *et al.* 2008 realizaron un ajuste por diferencias iniciales. Sólo el estudio de Jiménez *et al.* no mencionó nada respecto de este asunto.

Las diferencias en los parámetros de desarrollo neurológico (MDI y PDI) de acuerdo con la ingesta de Zn en cada estudio en particular y en el análisis en conjunto se muestran en las figuras 14 y 15.

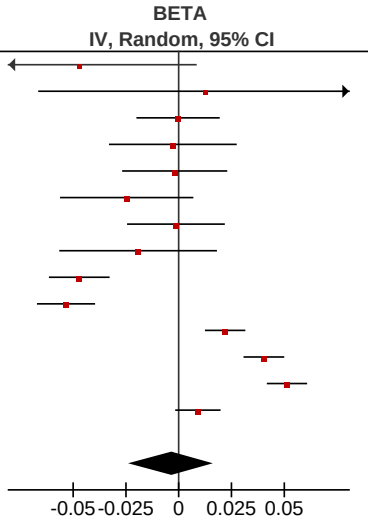
Figura 14: Neurodesarrollo: MDI



Study or Subgroup	BETA	SE	Weight	BETA IV, Random, 95% CI
Ashworth, 1998a	-0.03891305	0.02309815	2.5%	-0.04 [-0.08, 0.01]
Ashworth, 1998b	-0.03752947	0.03932877	1.0%	-0.04 [-0.11, 0.04]
Ashworth, 1998c	-0.01079834	0.00876265	7.6%	-0.01 [-0.03, 0.01]
Ashworth, 1998d	-0.01300407	0.01498779	4.6%	-0.01 [-0.04, 0.02]
Castillo Duran, 2001a	-0.003796	0.00928022	7.3%	-0.00 [-0.02, 0.01]
Castillo Duran, 2001b	0.01320014	0.01384686	5.0%	0.01 [-0.01, 0.04]
Hamadani, 2001a	-0.00021983	0.00596366	9.2%	-0.00 [-0.01, 0.01]
Hamadani, 2001b	-0.02113839	0.00874613	7.6%	-0.02 [-0.04, -0.00]
Jimenez, 2007a	0.015508	0.00652763	8.9%	0.02 [0.00, 0.03]
Jimenez, 2007b	-0.02148711	0.00623403	9.1%	-0.02 [-0.03, -0.01]
Jimenez, 2007c	-0.01805705	0.00630547	9.0%	-0.02 [-0.03, -0.01]
Jimenez, 2007d	-0.01259727	0.00580033	9.3%	-0.01 [-0.02, -0.00]
Jimenez, 2007e	-0.0211704	0.00596224	9.2%	-0.02 [-0.03, -0.01]
Lind 2004	0.00964417	0.00496275	9.8%	0.01 [-0.00, 0.02]
Total (95% CI)			**100.0%**	**-0.01 [-0.02, -0.00]**

Heterogeneity: Tau² = 0.00; Chi² = 47.20, df = 13 (P < 0.00001); I² = 72%
Test for overall effect: Z = 2.02 (P = 0.04)

Figura 15: Neurodesarrollo: PDI



Study or Subgroup	BETA	SE	Weight	BETA IV, Random, 95% CI
Ashworth, 1998a	-0.04718184	0.02821577	5.1%	-0.05 [-0.10, 0.01]
Ashworth, 1998b	0.01289568	0.04033897	3.6%	0.01 [-0.07, 0.09]
Ashworth, 1998c	-0.00028807	0.00987517	7.7%	-0.00 [-0.02, 0.02]
Ashworth, 1998d	-0.00277611	0.0152263	7.0%	-0.00 [-0.03, 0.03]
Castillo Duran, 2001a	-0.00190761	0.01249592	7.4%	-0.00 [-0.03, 0.02]
Castillo Duran, 2001b	-0.02462323	0.01591364	6.9%	-0.02 [-0.06, 0.01]
Hamadani, 2001a	-0.00132517	0.01161701	7.5%	-0.00 [-0.02, 0.02]
Hamadani, 2001b	-0.0192412	0.01885681	6.5%	-0.02 [-0.06, 0.02]
Jimenez, 2007a	-0.04710609	0.00713651	7.9%	-0.05 [-0.06, -0.03]
Jimenez, 2007b	-0.05338904	0.00682379	8.0%	-0.05 [-0.07, -0.04]
Jimenez, 2007c	0.02199735	0.00468899	8.1%	0.02 [0.01, 0.03]
Jimenez, 2007d	0.04034296	0.0047288	8.1%	0.04 [0.03, 0.05]
Jimenez, 2007e	0.05126824	0.00466531	8.1%	0.05 [0.04, 0.06]
Lind 2004	0.0090771	0.00528291	8.1%	0.01 [-0.00, 0.02]
Total (95% CI)			**100.0%**	**-0.00 [-0.02, 0.02]**

Heterogeneity: Tau² = 0.00; Chi² = 289.41, df = 13 (P < 0.00001); I² = 96%
Test for overall effect: Z = 0.33 (P = 0.74)

El coeficiente β ponderado fue de -0,01 (IC del 95%: -0,2 a 0) para el MDI y de 0 (IC 95%: -0,02 a 0,02) para el PDI. Sin embargo, se encontró heterogeneidad en ambos análisis (I ² para MDI = 72%, p= <0,00001 y I ² para PDI = 96%, p= <0,00001).

A fin de explorar aquellas variables que pudieran ser posibles modificadoras del efecto, se realizó una meta-regresión (Tabla 13).

Tabla 13: Meta-regresión. Efecto de la ingesta de Zn sobre el neurodesarrollo (MDI, PDI) de acuerdo con diferentes características de los estudios incluidos en el metaanálisis (Coeficiente β medio e IC 95%)

	n	Coeficiente β medio	IC (95%)	P Ancova *
MDI				
Por duración de la intervención				
4 a 20 semanas	3	-0,0005	-0,0143 a 0,0132	
> 20 semanas	11	-0,0148	-0,0224 a -0,0073	
				0,076
Por dosis				
1 a 4 mg	2	-0,0236	-0,0386 a -0,0087	
4,1 a 8 mg	6	0,0033	-0,0067 a 0,0133	
8,1 a 12 mg	6	-0,0027	-0,0113 a 0,0060	
				0,002
Por Situación Nutricional				
Saludable	3	-0,0014	-0,0145 a 0,0118	
Estado nutricional deficiente	11	-0,0140	-0,0272 a -0,0008	
				0,242
Por Riesgo de Sesgo				
Bajo	6	-0,0088	-0,0189 a 0,0013	
Moderado	2	-0,0054	-0,0261 a 0,0152	
Alto	6	-0,0088	-0,0189 a 0,0013	
				0,800
PDI				
Por duración de la intervención				
4 a 20 semanas	3	-0,0757	-0,0977 a -0,0538	
> 20 semanas	11	0,0124	0,0004 a 0,0244	
				<0,001
Por Dosis				
1 a 4 mg	2	-0,0538	-0,0776 a -0,0299	
4,1 a 8 mg	6	-0,0425	-0,0584 a -0,0266	
8,1 a 12 mg	6	0,0012	-0,0125 a 0,0150	
				<0,001
Por Situación Nutricional				
Saludable	3	-0,0020	-0,0230 a 0,0189	
Estado nutricional deficiente	11	-0,0613	-0,0823 a -0,0404	
				<0,001
Por Riesgo de Sesgo				
Bajo	6	-0,0095	-0,0256 a 0,0067	
Moderado	2	-0,0761	-0,1090 a -0,0433	
Alto	6	-0,0095	-0,0256 a 0,0067	
				0,002

* Valor p obtenido a través de ANCOVA

El efecto de la ingesta de Zn en el MDI se modificó dependiendo de la dosis (p ANCOVA = 0,002). En cuanto al PDI, hubo un efecto diferencial de la ingesta de Zn en función de la duración de la intervención, la dosis, el estado nutricional y el riesgo de sesgo (p = ANCOVA <0,001, <0,001, <0,001, <0,002), respectivamente.

La Tabla 14 muestra los resultados después de la estratificación de la muestra de acuerdo con los modificadores del efecto identificados en la meta-regresión.

Después de estratificar por la dosis, el coeficiente β ponderado para el MDI todavía mostró heterogeneidad (I^2 = 87%, p= <0,00001) para dosis de Zn altas (8,1 a 12 mg/día), pero esta heterogeneidad desapareció en algunos estratos tales como dosis de Zn bajas y medias (de 1 a 4 mg/día y de 4,1 a 8 mg/día). Sin embargo, no hubo ningún efecto significativo en la ingesta de Zn en estas dosificaciones.

En el caso del coeficiente β ponderado para el PDI, la heterogeneidad desapareció cuando los estudios se agruparon en un período de intervención de 4 a 20 semanas, con dosis bajas y medias de Zn (de 1 a 4 mg/día y de 4,1 a 8 mg/día) y en los estudios con riesgo de sesgo moderado o alto. La heterogeneidad se mantuvo en los estudios que tuvieron más de 20 semanas de intervención (I^2 = 88%, p= <0,00001), altas dosis de Zn (8,1 a 12 mg/día) (I^2 = 98%, p= <0,00001), lactantes con estado de nutrición saludable o deficiente (I^2 = 54%, p= 0,11 y 96 %, p= <0,00001) y para el riesgo de sesgo bajo (I^2 = 98%, p= <0,00001).

La suplementación con Zn mostró un efecto negativo, débil y significativo en la puntuación PDI en los estudios con una duración de 4 a 20 semanas (β = -0,05, IC 95%: -0,06 a -0,04).

Tabla 14: Análisis de subgrupos. Efecto de la ingesta de Zn sobre el neurodesarrollo (MDI, PDI) en cada categoría de las variables modificadoras del efecto (coeficiente β ponderado e IC 95%)

	Coeficiente β ponderado	Chi² (df, P)	I² *
MDI			
Todos los estudios (n=14)	-0,01 (-0,02 a 0)	47,20 (13, < 0,00001)	72%
Por Dosis			
1 a 4 mg (n=2)	-0,04 (-0,08 a 0)	0 (1, 0,98)	0%
4,1 a 8 mg (n=6)	-0,01 (-0,01 a 0)	6,43 (5, 0,27)	22%
8,1 a 12 mg (n=6)	-0,01 (-0,02 a 0,01)	38,19 (5, <0,00001)	87%
PDI			
Todos los estudios (n=14)	0 (-0,02 a 0,02)	289,41 (13, < 0,00001)	96%
Por duración de la Intervención			
4 a 20 semanas (n=2)	-0,05 (-0,06 a -0,04)	0,40 (1, 0,52)	0%
> 20 semanas (n=12)	0,01 (-0,01 a 0,02)	93,24 (11, < 0,00001)	88%
Por Dosis			
1 a 4 mg (n=2)	-0,02 (-0,08 a 0,03)	1,49 (1, 0,22)	33%
4,1 a 8 mg (n=6)	-0,01 (-0,02 a 0,01)	2,50 (5, 0,78)	0%
8,1 a 12 mg (n=6)	0 (-0,03 a 0,04)	268,60 (5, < 0,00001)	98%
Por Situación Nutricional			
Saludable (n=3)	0 (-0,02 a 0,02)	4,36 (2, 0,11)	54%
Estado nutricional deficiente (n=11)	0 (-0,03 a 0,02)	281,40 (10, < 0,00001)	96%
Por Riesgo de Sesgo			
Bajo (n=6)	0 (-0,03 a 0,04)	268,60 (5, < 0,00001)	98%
Moderado (n=2)	-0.01 (-0,03 a 0,01)	1,26 (1, 0,26)	21%
Alto (n=6)	0 (-0,02 a 0,01)	3,35 (5, 0,65)	0%

* I^2 Índice que mide el grado de heterogeneidad

Los resultados de los análisis de sensibilidad se muestran en la Tabla 15.

No se encontró una asociación significativa entre la ingesta de Zn y los índices MDI y PDI.

La estimación de Ashworth *et al.* (b) (1998) se consideró como un valor atípico en el análisis de MDI porque los límites del coeficiente beta eran muy anchos (IC 95%= -0,11 a 0,04). Cuando esta estimación se excluyó, la asociación persistió al igual que el grado de heterogeneidad. Lo mismo ocurrió cuando se excluyeron las estimaciones de Ashworth *et al.* (1998) (a) y (b) del metaanálisis del índice PDI.

Debido a la gran heterogeneidad encontrada en la mayoría de los análisis del desarrollo neurológico, se decidió no realizar el cálculo de la relación dosis-respuesta.

Tabla 15: Análisis de sensibilidad. Efecto de la ingesta de Zn sobre el neurodesarrollo (MDI, PDI), eliminando valores extremos (coeficiente β ponderado e IC 95%)

	Coeficiente β ponderado	Chi² (dif, P)	I²
MDI			
Todos los estudios (n=14)	-0,01 (-0,02 a 0)	47,20 (13, <0,00001)	72%
Todos los estudios excluidos (n=1)	-0,01 (-0,02 a 0)	46,60 (12, <0,00001)	74%
Ashworth et al. 1998 b	-0,04 (-0,11 a 0,04)		
PDI			
Todos los estudios (n=14)	0 (-0,02 a 0,02)	289,41 (13, <0,00001)	96%
Todos los estudios excluidos (n=2)	0 (-0,02 a 0,02)	284,88 (11, <0,00001)	96%
Ashworth et al. 1998 a	-0,05 (-0,10 a 0,01)		
Ashworth et al. 1998 b	0,01 (-0,07 a 0,09)		

* I² Índice que mide el grado de heterogeneidad

Resultados

5. Discusión

5. DISCUSIÓN

Algunas consideraciones básicas e indispensables serán realizadas antes de centrarnos en la discusión formal de los resultados obtenidos para cada uno de los objetivos planteados en la presente memoria:

5.1 La heterogeneidad encontrada

En la realización de este trabajo, la heterogeneidad encontrada en los estudios incluidos y su interpretación provocó un gran debate entre los integrantes del grupo EURRECA.

Los estudios realizados en diferentes lugares y tiempos, sobre distintas poblaciones y con diseños diferentes, con frecuencia dan lugar a resultados que son diferentes entre sí. Esta heterogeneidad no puede ignorarse. Se debe indagar qué explicación pueden tener estas diferencias (fuentes de heterogeneidad). Más aún, a veces, la misión más importante de un metaanálsis no es hacer una síntesis unificadora, sino subrayar las diferencias entre los resultados de los distintos estudios disponibles. El metaanálisis sólo debe extraer un estimador común cuando de verdad pueda asumirse que existe un parámetro común, de lo contrario es preferible que sirva para dejar claro que no se puede combinar lo que es tan dispar (Delgado-Rodríguez & Sillero Arenas (en prensa) 2012). Esta situación fue la encontrada en gran parte de este trabajo. Y, es por esta razón también, por la que no se realizó el cálculo de la relación dosis – respuesta en aquellos metaanálisis cuyos índices de heterogeneidad tuvieron una p menor a 0,05 para ese índice y sólo se calculó en los metaanálisis de WAZ y WLZ medidos en los estudios de crecimiento.

Sin embargo, otros grupos de EURRECA argumentaron que la heterogeneidad se justifica con la realización de un metaanálisis que asume un modelo de efectos aleatorios y no fijos. Su razonamiento se basó en que, en un modelo de efectos

aleatorios la inferencia asume que los diferentes estudios incluidos en el metaanálisis son una muestra aleatoria de varias poblaciones hipotéticas de estudios, asunción más realista que tener todo el universo de estudios como subyace en el modelo de efectos fijos. Sin embargo, esta aproximación debe emplearse cuando hay heterogeneidad pero no es la solución frente a ella. Además, el modelo de efectos aleatorios incluye una variabilidad adicional (tau) y, por lo tanto, produce un intervalo de confianza más amplio y de menor potencia.

El presente trabajo también se realizó utilizando un modelo de efectos aleatorios pero hemos preferido no integrar los resultados. Los modelos de efectos aleatorios en los metaanálisis son temas muy discutidos en la comunidad científica que exceden los objetivos del presente trabajo.

5.2 Nuestros resultados

Nuestros resultados indicaron que los suplementos de Zn aumentan los niveles de Zn en suero o plasma, favorecen algunos parámetros de crecimiento, pero no parecen influir en el desarrollo neurológico de los lactantes.

5.2.1 Niveles de Zn en suero/plasma:

Tanto las concentraciones de Zn plasmáticas como séricas son los indicadores bioquímicos más utilizados para medir niveles de Zn, pero sus valores no son necesariamente idénticos. Estudios diseñados para comparar las concentraciones de Zn observaron niveles más altos de Zn en suero que en el plasma (Kasperek et al. 1981; English & Hambidge 1988). Estas diferencias pueden haber ocurrido debido a que las muestras de suero se separaron de las células de la sangre después de un período de tiempo mayor que las muestras de plasma. Al controlar tanto la cantidad de sangre recogida como el tiempo de separación de las células, no se encontraron diferencias en las concentraciones de Zn entre suero y plasma (English & Hambidge

1988). A fin de simplificar nuestro resultado, siempre nos hemos referido a " Zn en suero o plasma ", sin hacer distinción alguna entre ellos.

Nuestros resultados indicaron que los suplementos de Zn aumentaban ligeramente los niveles de Zn en suero o plasma de los lactantes.

Estos resultados coinciden con aquellos obtenidos en algunos estudios individuales. El estudio de Lind *et al.* 2003 por ejemplo, sugiere que tal vez sea necesario suplementar el Zn cada día o por un periodo más largo de tiempo para encontrar un efecto más significativo debido a que en el organismo no tenemos reservas de Zn y éste, además, tiene una circulación (*turnover*) muy rápida. Ellos observaron que en su curva dosis - respuesta nunca se logró generar un efecto meseta como resultado de la suplementación. Otra explicación posible de este ligero aumento en los niveles de Zn encontrado en nuestro análisis es la de una dosis insuficiente, tal y como menciona el estudio de Osendrap *et al.* (2002)

Un metaanálisis similar a éste fue realizado por el grupo de Hall Moran *et al*. (2012) en Reino Unido pero en niños de 1 a 17 años. El coeficiente beta ponderado obtenido después del análisis de 18 ensayos aleatorizados fue de 0,12 (95% CI 0,04 a 0,20). Sin embargo obtuvo un $I^2 =$ 97.6% con una $p = < 0.005$, lo que indica heterogeneidad significativa. No obstante, este grupo propuso una relación dosis – respuesta, aunque aclara que debe considerársela cuidadosamente.

En nuestro metaanálisis también se encontró gran evidencia de heterogeneidad. Después de realizar varios análisis de subgrupos, el coeficiente β ponderado para cada sub-análisis continuó mostrando la misma circunstancia. Y por esta razón se decidió no calcular la relación dosis – respuesta.

Varios factores podrían explicar la presencia de heterogeneidad en nuestro análisis.

Algunos autores señalan en sus estudios, el hecho de que las concentraciones séricas de Zn varían de acuerdo con la hora del día, con la proximidad de las comidas consumidas con anterioridad, y con la reciente actividad física u otras formas de estrés. Indican que estos factores podrían producir fluctuaciones de hasta un 20%

durante un período de 24 horas (Hambidge *et al.* 1989). Existe una variación diurna de la concentración de Zn que se produce generalmente como resultado de los cambios metabólicos después del consumo de la comida, incluso algunas variaciones pueden ocurrir como resultado de la variación del ritmo circadiano normal en el metabolismo (Guillard *et al.* 1979; Wallock *et al.* 1993). Existen estudios cuyos resultados mostraron una disminución de las concentraciones séricas de Zn luego del consumo de comidas (Wallock *et al.* 1993; Goode *et al.* 1991), mientras que el resultado del ayuno nocturno mostró un aumento de las concentraciones circulantes de Zn (Wallock *et al.* 1993). Este hecho podría considerarse como un posible modificador del efecto capaz de explicar la inconsistencia de nuestros hallazgos. De los estudios incluidos en nuestro metaanálisis, sólo los realizados por Osendrap *et al*. (2002), Umeta *et al.* (2000), Walravens *et al.* (1989) y Wasantwisut *et al*. (2006) informaron a cerca de la hora del día en que se tomaron las muestras de sangre: “las muestras fueron recogidas en horas de la mañana”.

Otros factores como son la infección y la inflamación también pueden haber disminuido los valores séricos de Zn. La magnitud de esta disminución dependerá de la gravedad y de la etapa de la infección (Brown 1998). Algunos estudios mostraron reducciones de aproximadamente un 10% a un 12% en la concentración de Zn en el suero debido a la infección, en comparación con grupos de referencia sanos (Thurnham *et al.* 2005). Otros factores, tales como los niveles séricos bajos de albúmina, el recuento elevado de glóbulos blancos o el uso de hormonas, también pueden afectar los niveles séricos de Zn, y deben ser considerados en la interpretación de los resultados de laboratorio (IZiNCG 2004). En este metaanálisis, todos los estudios incluidos tuvieron en cuenta la presencia de enfermedad durante la intervención y han considerado si los niveles de Zn fueron afectados por estos hechos.

Por último, un punto muy importante para considerar es el hecho de que los lactantes que sufren malnutrición proteico-energética tienen concentraciones de Zn bajas en plasma, músculo e hígado (Hansen & Lehman 1969; Cheek et al. 1970). Debido a que

el Zn es necesario para la síntesis de tejido durante la rehabilitación nutricional, la cantidad requerida será superior a la oferta alimentaria (Castillo-Duran *et al.* 1987; Prasad 1991; Gibson *et al.* 1998). El estudio de Makonnen *et al.* (2003) fue el único en este metaanálisis que incluyó niños con desnutrición proteico - energética. En él, la mejora en los niveles de Zn se hizo evidente sólo después de 60 días. Estos niños demoraron más de un mes en aumentar los niveles séricos de Zn de manera significativa. Este hecho podría explicar el efecto limitado que la suplementación de Zn ha tenido en los niveles séricos de Zn después de 30 días. La inclusión de estudios realizados en niños desnutridos puede haber contribuido a la falta de significación estadística obtenida en este metaanálisis.

5.2.2 Crecimiento:

Nuestros resultados indicaron que los suplementos de Zn aumentaban algunos parámetros de crecimiento en los niños estudiados. La ingesta de Zn tuvo un efecto directo y estadísticamente significativo en WAZ (β = 0,06, IC 95% 0,02 a 0,10) y en WLZ (β = 0,05, IC 95% 0,01 a 0,08). Sólo se encontró heterogeneidad significativa en el análisis de la longitud (I^2 = 45%, p = 0,03). Sin embargo, después de estratificar por varios factores, la heterogeneidad se atenuó.

A nuestro entender, este metaanálisis es el único de los realizados en esta tesis, que puede presentar una estimación de la relación dosis-respuesta entre la ingesta de Zn y los parámetros de crecimiento en los lactantes. Esta estimación de la relación dosis-respuesta indica que un lactante con una ingesta de Zn de 10 mg/día tiene un WAZ y un WLZ un 4% más alto que los de un lactante que tiene un consumo de Zn de 5 mg/día.

En algunos estudios el tiempo de la intervención puede haber sido demasiado corto para obtener un impacto positivo en el crecimiento. Este es el caso de los estudios de Heinig *et al*., Lind *et al*., Meeks Gardner *et al*. y Sur *et al.* (2006; 2004; 1998; 2003).

Lind *et al.* (2004) informaron mejoras en el crecimiento en un período de 3 meses. Sin embargo, en los resultados obtenidos por Bates *et al.* y Heinig *et al.* (1993; 2006) no se pudieron observar efectos positivos en períodos de suplementación más largos. También Rivera *et al.* y Umeta *et al*. (1998; 2000) encontraron que el MUAC no se modificaba debido a la extensión del período de suplementación, el cual, aparentemente, era demasiado corto para encontrar algún efecto medible. La influencia del tiempo de intervención se hace más relevante cuando los estudios se llevan a cabo en los recién nacidos con bajo peso debido justamente a su bajo peso, junto a la falta de madurez asociada a los bebés prematuros, los que requieren un ajuste de la edad gestacional con la edad cronológica para la evaluación correcta de la recuperación del crecimiento (Prasad 1991). Este es el caso de los estudios de Meeks Gardner *et al.* y de Sur *et al.* (1998; 2003), que se llevaron a cabo en lactantes con un estado nutricional deficiente. Por lo tanto, son necesarios más ensayos clínicos para analizar los efectos de la suplementación con Zn sobre el crecimiento a largo plazo antes de llegar a conclusiones significativas.

Además, es importante tener en cuenta, que los datos de nuestro metaanálisis se obtuvieron, en general, de países en desarrollo y que se incluyeron datos de lactantes con bajo peso y con desnutrición, lo que podría haber dado lugar al pobre efecto que encontramos. Varios estudios al respecto se han llevado a cabo, y en muchos de ellos se han observado efectos positivos de la suplementación de Zn en el crecimiento entre grupos de lactantes nutricionalmente desfavorecidos, incluyendo lactantes con retraso del crecimiento (Arsenault *et al.* 2008; Rivera et al. 1998; Umeta *et al.* 2000; Walravens *et al.* 1989), y, en particular, entre lactantes desnutridos (Ninh *et al.* 1996). Por otro lado, no se observó respuesta a la suplementación con Zn en el crecimiento en lactantes sanos gambianos, ni en lactantes sanos de USA (Bates *et al.* 1993; Heinig *et al.* 2006). Por lo tanto, no está claro si los lactantes sanos en los países industrializados se beneficiarían con el aumento en la ingesta de Zn.

5.2.3 Neurodesarrollo:

En el caso del metaanálisis que evaluó el neurodesarrollo de los lactantes, los resultados obtenidos indicaron que la ingesta de Zn no parece influir en el desarrollo neurológico de los bebés, tanto a nivel mental como a nivel psicomotor. Inclusive, se encontró un efecto negativo en el desarrollo psicomotor infantil (PDI) cuando la suplementación con Zn osciló entre 4 a 20 semanas.

La interpretación de los resultados obtenidos debe ser considerada cuidadosamente por un número de razones. En primer lugar, la magnitud del efecto fue bastante pequeña. Por otra parte, en los estudios agrupados hubo heterogeneidad y, en general, después de estratificar la muestra de acuerdo con aquellas variables posibles modificadoras del efecto, la heterogeneidad persistió a través de los diversos análisis.

Todos los estudios incluidos en este metaanálisis utilizaron para la medición del desarrollo neurológico las escalas de Bayley. Este es el instrumento más utilizado para poner a prueba el desarrollo mental y psicomotor. A pesar de que las Escalas Bayley de Desarrollo Infantil fueron estandarizadas en los Estados Unidos, se han utilizado en muchos otros países, sobre todo en estudios enfocados a la nutrición y al desarrollo. Es posible que entre los diferentes países se produzcan ligeras diferencias en la administración de la prueba. Además, los niños en las diferentes culturas están expuestos a ambientes muy diferentes, por lo que se espera que afecte, también, su desarrollo. Por lo tanto, es difícil interpretar las diferencias de los resultados obtenidos entre las diferentes culturas (Black & Matula 2000). Es decir que, aunque todos los estudios incluidos usaron el mismo instrumento para medir el desarrollo neurológico, éste podría ser una fuente de heterogeneidad en sí mismo y otro factor que contribuya a la falta de efecto encontrada en el análisis de la suplementación con Zn en el neurodesarrollo.

En algunos estudios, las puntuaciones de neurodesarrollo para la ingesta de Zn fueron más bien pequeñas (Hamadani *et al.* 2001). Sin embargo, los recién nacidos incluidos

en ese estudio estaban desnutridos y, por lo tanto, requerían más nutrientes y no solamente Zn. Esta misma observación puede hacerse para los estudios de Ashworth *et al*. y Jiménez *et al*. (1998; 2007), quienes evaluaron a lactantes con bajo peso al nacer (BPN). Ashworth *et al.* (1998) concluyeron que la duración de su intervención fue insuficiente, mientras que el estudio realizado por Jiménez *et al*. (2007), mostró un incremento en el desarrollo después de la suplementación con Zn. Este incremento fue más evidente en el desarrollo motor (PDI) que en el desarrollo mental (MDI), especialmente, entre los 3 a 6 meses de edad. Además, este autor no asegura que el tiempo de suplementación pueda ser responsable de los efectos informados. Sin embargo, tal y como ya se ha mencionado, en estudios realizados en bebés con bajo peso, la duración de la intervención es muy importante, justamente debido a su bajo peso y a la falta de madurez asociada a los bebés prematuros, ya que estos requieren un ajuste de la edad gestacional a la edad cronológica para la evaluación correcta de la recuperación del crecimiento (Rugolo 2005). Se requieren nuevos estudios para analizar los efectos de la deficiencia de Zn a largo plazo en el neurodesarrollo de lactantes.

Otros factores que se deben considerar son la influencia del contexto social, el entorno de cuidadores y la estimulación del desarrollo, factores todos que desempeñan un papel muy importante en la evolución del desarrollo cognitivo en los niños (Black 1998). Es evidente que todos estos factores juegan un papel relevante para el funcionamiento neuropsicológico, la actividad y el desarrollo motor y todos los estudios incluidos en nuestro metaanálisis dan cuenta de estas variables, a excepción de Jiménez *et al*. 2007.

En los lactantes de 6 a 8 meses de edad, se deben tener en cuenta algunas consideraciones especiales, ya que este grupo es especialmente vulnerable a sufrir deficiencia de Zn. Se trata de niños que se encuentran en el período de transición entre la lactancia exclusiva y la introducción gradual de los alimentos sólidos. La deficiencia de Zn es un problema, particularmente relevante en los países

subdesarrollados, pero hay una creciente evidencia que sugiere que esto está presente también en las poblaciones con una nutrición adecuada (Skinner *et al.* 1997). Se necesitan más estudios que examinen la respuesta a la suplementación con Zn o a la fortificación en el desarrollo neurológico de las poblaciones que son deficientes en Zn, y en aquellas en las que hay ausencia de pobreza. La deficiencia de Zn constituye un grave problema de salud pública que pone en peligro el adecuado desarrollo de millones de niños en todo el mundo en los países desarrollados y en desarrollo (Sandstead 1996).

5.3 Limitaciones de los metaanálisis

En estos metaanálisis hemos tenido en cuenta algunas limitaciones, gracias a las cuales consideramos que la interpretación de nuestros resultados debe ser considerada cuidadosamente.

En primer lugar, el número de estudios que se seleccionaron para incluir en los metaanálisis fue pequeño en el caso de los metaanálisis de los niveles de Zn en suero o plasma y en el de neurodesarrollo. La información sobre la ingesta de Zn y los niveles sérico / plasmáticos se llevó a cabo en 9 estudios; el desarrollo neurológico sólo se evaluó en 5 estudios. En el caso del metaanálisis de crecimiento, a pesar de que se incluyeron en total 19 estudios de intervención para medir el efecto de la suplementación con Zn, por ejemplo, en el caso de la asociación entre la ingesta de Zn y el perímetro cefálico sólo se incluyeron 4 estudios. Por lo tanto, el poder estadístico de los análisis es limitado y puede haber conducido a una falta de significación estadística en algunos análisis. Deberían realizarse un mayor número de estudios individuales para analizar el efecto de la ingesta de Zn que posteriormente podrían integrarse en futuros metaanálisis.

Además, es bien sabido que cuando se llevan a cabo muchas comparaciones estadísticas (por ejemplo: diferentes análisis de subgrupos, diferentes análisis de

sensibilidad), una o más pueden alcanzar significación debido sólo al azar (Bland & Altman 1995).

También es importante tener en cuenta la calidad científica de los estudios incluidos. A pesar de que los metaanálisis se utilizan cada vez más para consolidar los resultados de múltiples estudios sobre un mismo tema para poder desarrollar políticas basadas en la evidencia clínica y programas de salud pública; la fiabilidad de las conclusiones depende de la calidad metodológica de los estudios originales, de la conveniencia de los criterios de inclusión utilizados y de la minuciosidad de la revisión y de la síntesis de información (Brown *et al.* 2002). Si bien este metaanálisis se llevó a cabo siguiendo estrictos protocolos de revisión sistemática y adhiriéndose a los estándares de calidad desarrollados por EURRECA (Matthys *et al.* 2011), la evaluación del riesgo de sesgo de los estudios incluidos reveló que la mayoría (n = 5 para los niveles de Zn en suero o plasma; n= 13 para el crecimiento y n=3 para el neurodesarrollo) tenían un riesgo de sesgo moderado y alto.

Algunas variables que podrían actuar como modificadores del efecto, factores de confusión o como ambas, también deben ser consideradas: el bajo peso al nacer, la lactancia materna, la pobreza y la privación social, y/o el contenido y la biodisponibilidad del Zn en la dieta local. Todos estos han sido factores importantes que contemplaron la mayoría de los estudios incluidos en nuestros metaanálisis. No obstante, es poco probable que los factores de confusión puedan haber afectado los resultados, ya que todos los estudios incluidos en los metaanálisis son ensayos clínicos aleatorizados. Sin embargo, solo en algunos estudios en los que se observó alguna diferencia inicial o existió algún fallo en el proceso de aleatorización, se realizó un ajuste de las diferencias iniciales. Como ya se mencionó anteriormente, para el metaanálisis de los niveles de Zn en suero o plasma todos los estudios incluidos asumieron que no había diferencias iniciales o basales en los niveles de Zn en suero o plasma. La única excepción fue Bates *et al.* (1993) quienes no mencionan nada respecto de este asunto. En el metaanálisis de crecimiento, Arsenault *et al*. y Rivera *et*

al. (2008; 1998) realizaron un ajuste de las diferencias iniciales. Los otros autores no encontraron diferencias iniciales. La única excepción fue Bates *et al.* (1993), quienes nuevamente en este caso no mencionan nada al respecto. Por último, para el metaanálisis de neurodesarrollo, Ashworth *et al.* (1998) realizaron un ajuste de las diferencias iniciales, mientras que Castillo Durán *et al*. 2001, Hamadani *et al.* 2001, Lind *et al.* 2004 no encontraron diferencias iniciales. Jiménez *et al.* 2007 no mencionaron nada con respecto a este tema.

Algunos aspectos metodológicos de los estudios incluidos, tales como las variaciones en la dosis, la forma química del Zn utilizado, el método de administración de Zn y la duración de la suplementación también pueden haber influido en los resultados (Brown *et al.* 2002). Sin embargo, muchos de estos aspectos como la dosis o la duración de la suplementación son descritos al llevar a cabo análisis de subgrupos y/o meta regresiones.

La edad de los niños es otro punto importante que se debe tener en cuenta. En nuestro metaanálisis se incluyeron, como se mencionó anteriormente, niños con edades comprendidas entre los 0 y los 60 meses (5 años) en el caso de niveles de Zn en suero o plasma; de 0 a 36 meses en el metaanálisis de crecimiento y de 1 a 13 meses en el de neurodesarrollo. Consideramos que no había razón para excluir del análisis a ningún estudio que no se hubiera ceñido exclusivamente al grupo de 0 a 12 meses de edad (lactantes), ya que, en el caso de los niveles de Zn en suero o plasma, de los 9 artículos incluidos, solo 4 cumplían estrictamente con la edad de 0 a 12 meses. Los otros 5 artículos incluían esta edad entre sus niños, pero no detallaban cuántos eran en realidad del grupo de 0 a 12 meses. Además, en una reciente publicación realizada por Hall Moran *et al.* 2012 en el marco del grupo EURRECA, y que se adjunta al final de esta memoria, se realizó un metaanálisis que evaluó los niveles de Zn en suero o plasma de niños y adolescentes. El rango de edad evaluado fue de 1 a 17 años. El coeficiente β ponderado obtenido en este trabajo fue de 0.12

(95% CI 0.04, 0.20; $p < 0.005$; I^2 97.6%). A la vista de este resultado, y dada la similitud de este valor con el hallado por nosotros, es poco probable que la edad en nuestro trabajo haya sesgado los resultados, ya que la magnitud del efecto encontrada para la ingesta de Zn fue similar, independientemente de la edad de los niños.

Para el metaanálisis de crecimiento, de los 19 estudios incluidos, sólo 5 no se ciñen estrictamente a 0 a 12 meses de edad, aunque los incluyen en la edad de los niños estudiados. En este grupo se aplicó el mismo criterio que el considerado en la evaluación de los niveles de Zn en suero/plasma. En el metaanálisis de neurodesarrollo solo el estudio de Hamadani *et al.* incluyó niños de hasta 13 meses.

5.4 La finalidad de este estudio en España

Encontrar información actualizada acerca de la ingesta de Zn en población española resulta tarea difícil. Algunos estudios indican deficiencias en los niveles de ingesta de este micronutriente. Por ejemplo, los datos de ingesta de Zn recogidos en la Encuesta Nacional de Ingesta Dietética (ENIDE) que se realizó entre 2009 – 2010 y que evaluó la situación nutricional de la dieta española, reveló que la ingesta de este mineral está por debajo del 80% de las IDRs en todos los grupos (por género y rango de edad), lo cual indica deficiencia. Con respecto a la proporción de la población con riesgo de ingesta inadecuada de Zn, en relación con los requerimientos medios estimados, se observó que era del 1,5% al 2,7% en mujeres y del 15 al 30% en hombres. Estos datos son acordes con los encontrados en otras poblaciones (Van Rossum *et al*., 2011).

Si la búsqueda de información sobre ingesta de Zn se realiza en lactantes sanos, es decir, en niños de 0 a 12 meses, los datos encontrados todavía son más escasos. Salvo excepciones, desafortunadamente, no existen estudios epidemiológicos actualizados que informen sobre la situación nutricional o los niveles de ingesta de los lactantes, mucho menos que informen sobre este micronutriente en concreto.

Sabemos, además, que la población infantil española se ha modificado en los últimos años gracias a la inmigración existente y que la globalización ha contribuido a este hecho. Por lo tanto, pensar en una dieta y en una ingesta determinada basándonos en la típica dieta española sería una visión reduccionista de la cuestión.

Una de estas excepciones es el estudio realizado en la Universidad de La Laguna en 2000 por Rodríguez *et al.,* que evaluó la concentración media de Zn de fórmulas en polvo para lactantes y las comparó con las concentraciones de Zn encontradas en la leche materna. Además de observar que la fórmula tenía concentraciones de Zn significativamente mayores que las de la leche humana, observó una disminución progresiva de la concentración de Zn en la leche materna durante la lactancia. Además, encontró que la leche humana obtenida en la primavera tenía concentraciones de Zn más bajas que en otoño, lo que podría deberse a cambios estacionales en los hábitos nutricionales de las madres.

Por otro lado, pocas son las evidencias que han relacionado la ingesta/niveles plasmáticos o séricos de Zn con diferentes parámetros de salud. En otro estudio realizado por Domenech *et al*. (2001) igualmente en la Universidad de La Laguna, que analizó la relación entre el Zn y algunos factores de crecimiento en recién nacidos, llegó a la conclusión de que durante las primeras semanas de vida había una disminución progresiva en los niveles séricos de Zn que afectaban evidentemente al crecimiento de los lactantes. En el estudio de Díaz-Gómez *et al.* 2003, también realizado en Canarias, se encontró un efecto positivo sobre el crecimiento lineal en niños prematuros suplementados con Zn.

Evidentemente, el Zn aparece en forma discreta en la literatura científica española y europea. Se sabe que, en efecto, su ingesta y su suplementación producen determinados beneficios en los lactantes, pero es evidente que la gran mayoría de estudios existentes están realizados en contextos totalmente diferentes del europeo, en poblaciones con grandes carencias sociales y nutricionales como ya hemos puesto

de manifiesto a lo largo de esta memoria. Está claro que existe una gran dificultad en la extrapolación de esta información a poblaciones occidentales. También, es sabido que un gran porcentaje de la población no cubre los requerimientos de Zn. Estudios que examinen la respuesta a la suplementación o fortificación con Zn en poblaciones que son también deficientes en Zn, pero en ausencia de pobreza podrían ayudar a clarificar en gran manera los beneficios aportados por este micronutriente. Este hecho ha sido la esencia del grupo EURRECA y el objetivo final del presente trabajo.

6. Conclusiones

6. CONCLUSIONES

PRIMERA: La asociación entre la ingesta de Zn en los lactantes y sus niveles de Zn en suero o plasma fue directa y estadísticamente significativa. La magnitud del efecto fue pequeña. Sobre la base de este grupo limitado de estudios y de su heterogeneidad, no se encontró información suficiente para sugerir que los suplementos de Zn tengan un efecto positivo en los niveles de Zn en suero o plasma de los lactantes.

SEGUNDA: En el caso del crecimiento, se ha establecido una relación dosis-respuesta entre la ingesta de Zn y algunos parámetros de crecimiento (WAZ y WLZ) en lactantes. Para los demás parámetros de crecimiento, no se encontró efecto significativo. Estos datos pueden ser utilizados como prueba complementaria para respaldar los valores de referencia de ingesta de Zn, a pesar de que hay que reconocer las restricciones de la extrapolación de nuestros resultados a otras poblaciones, especialmente a poblaciones desarrolladas.

TERCERA: No se encontró asociación estadísticamente significativa entre la ingesta de Zn y el desarrollo neurológico en los lactantes. Sobre la base de este grupo limitado de estudios y su heterogeneidad, no se encontró información suficiente para sugerir que los suplementos de Zn tienen un efecto positivo sobre el desarrollo neurológico de los lactantes.

CUARTA: Esta tesis ilustra que, hasta ahora, la evidencia disponible sobre los efectos de la ingesta de Zn en los lactantes es inconsistente, y que los efectos en los niveles de suero o plasma, en el crecimiento y en el desarrollo neurológico deben ser aún revisados. Se necesita más investigación estandarizada, de manera urgente, que permita obtener conclusiones con bases científicas para aclarar el papel de la suplementación con Zn en los lactantes, particularmente, en Europa y en otras sociedades desarrolladas.

7. Referencias

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8. Anexos

Anexos I, II y III:

Búsquedas electrónicas

ANEXO I: Búsqueda electrónica EMBASE

ANEXO II: Búsqueda electrónica MEDLINE

ANEXO III: Búsqueda electrónica COCHRANE

ANEXO I: Búsqueda electronica EMBASE

No.	*Search term*	*Results*
1	random$.ti,ab.	389782
2	factorial$.ti,ab.	8131
3	(crossover$ or cross over$ or cross-over$).ti,ab.	39188
4	placebo$.ti,ab.	109036
5	(doubl$ adj blind$).ti,ab.	84173
6	(singl$ adj blind$).ti,ab.	7399
7	assign$.ti,ab.	107626
8	allocat$.ti,ab.	34034
9	volunteer$.ti,ab.	98427
10	crossover procedure.sh.	20937
11	double-blind procedure.sh.	71265
12	randomized controlled trial.sh.	165330
13	single blind procedure.sh.	7943
14	or/1-13	653981
15	animal/ or nonhuman/ or animal experiment/	3415144
16	human/	6387488
17	16 and 15	527284
18	15 not 17	2887860
19	14 not 18	569709
20	(cohort* or ''case control*'' or cross-sectional* or ''cross sectional'' or case-control* or prospective or ''systematic review*'').mp.	456251
21	epidemiologic studies/ or case-control studies/ or cohort studies/ or longitudinal studies/ or follow -up studies/ or prospective studies/ or cross-sectional studies/ or clinical trials as topic/ or intervention studies/ or pilot projects/ or Meta Analysis/	521189
22	21 or 19 or 20	1226072
23	22 not 18	1202709
24	((zinc or zn or zinc sulphate or zinc gluconate or zinc acetate or methionine or zinc isotope*) adj3 (intake* or diet* or supplement* or deplet* or status or serum or plasma or leukocyte or concentration* or expos* or fortif* or urine or hair)).mp.	20843
25	*zinc/	19319
26	zinc blood level/	2364
27	zinc deficiency/	3138
28	supplementation/ or diet supplementation/ or dietary intake/ or exp diet restriction/ or exp mineral intake/ or Infant nutrition/ or artificial milk/ or breast milk/ or bottle feeding/ or breast feeding/ or lactation/	128229
29	exp nutritional status/ or nutritional deficiency/ or exp zinc deficiency/ or trace metal blood level/ or exp zinc blood level/	25790

30	(intake* or diet* or supplement* or deplet* or status or serum or plasma or leukocyte or concentration* or expos* or fortif* or urine or hair).mp.	2745875
31	28 or 30 or 29	2766626
32	25 and 31	12399
33	27 or 32 or 24 or 26	26275
34	33 and 23	2546

ANEXO II: Búsqueda electronica MEDLINE

No.	*Search term*	*Results*
1	randomized controlled trial.pt.	280821
2	controlled clinical trial.pt.	79998
3	randomized.ab.	196604
4	placebo.ab.	117891
5	clinical trials as topic.sh.	146242
6	randomly.ab.	145491
7	trial.ab.	203467
8	randomised.ab.	38423
9	6 or 3 or 7 or 2 or 8 or 1 or 4 or 5	734511
10	(animals not (human and animals)).sh.	4482479
11	9 not 10	642665
12	(cohort* or ''case control*'' or cross-sectional* or ''cross sectional'' or case-control* or prospective or ''systematic review*'').mp.	768885
13	exp meta-analysis/ or expmulticenter study/ or follow-up studies/ or prospective studies/ or intervention studies/ or epidemiologic studies/ or case-control studies/ or exp cohort studies/ or longitudinal studies/ or cross-sectional studies/	1013635
14	13 or 12	1203767
15	14 not 10	1154385
16	11 or 15	1599094
17	((zinc or zn or zinc sulphate or zinc gluconate or zinc acetate or methionine or zinc isotope*) adj3 (intake* or diet* or supplement* or deplet* or status or serum or plasma or leukocyte or concentration* or expos* or fortif* or urine or hair)).ti,ab.	16681
18	Nutritional Support/ or Dietary Supplements/ or nutritional requirements/ or Breast feeding/ or exp infant food/ or bottle feeding/ or infant formula/	63098
19	exp Nutritional Status/ or exp Deficiency Diseases/ or supplementation/ or diet supplementation/ or dietary intake/ or exp diet restriction/ or exp mineral intake/ or Diet/ or Food, Fortified/ or nutrition assessment/ or Nutritive Value/	176014
20	(intake* or diet* or supplement* or deplet* or status or serum or plasma or leukocyte or concentration* or expos* or fortif* or urine or hair).ti,ab.	3166092
21	18 or 19 or 20	3263114
22	zinc/	41027
23	22 and 21	20745
24	23 or 17	26943
25	24 and 16	2410

ANEXO III: Búsqueda electrónica COCHRANE

No.	*Search term*	*Results*
#1	(zinc or zn or methionine or ''zinc sulphate'' or ''zinc acetate'' or ''zinc gluconate'' or ''zinc isotope*''), from 2009 to 2010	197
#2	(MeSH descriptor Zinc explode all trees), from 2009 to 2010	15
#3	(#1 OR #2), from 2009 to 2010	197
#4	(deplet* or status* or expo* or plasma or serum or leukocyte or concentration* or fortif* or urine or hair), from 2009 to 2010	11139
#5	(supplement* or diet* or ''diet* restriction*'' or ''mineral intake*'' or intake* or ''infant nutrition'' or ''artificial milk'' or ''breast milk'' or ''bottle feeding'' or ''breast feeding'' or ''lactating''), from 2009 to 2010	3272
#6	(#4 OR #5), from 2009 to 2010	11847
#7	(#3 AND #6), from 2009 to 2010	170

Anexo IV:

In / Out Form

Anexo IV:

In / Out Form

Intervention Studies

Micronutrient: FOLATE / IRON / B12 / ZINC / IODINE

Note 1: Minimum study duration: Iron: wks; Folate: wks; B12: 2 wks; Zinc: 2 wks, Iodine: 2 wks

Note 2: Studies should only be included if answers to all applicable main questions are 'YES'

Main question	Sub-question	Inclusion / Exclusion Criteria	YES	NO	N/A
1		**Study Population:** Does this study use a human population? *If YES, which population group(s)?*			
	1a	Infants: 0-1years			
	1b	Children & adolescents: 1-18years			
	1c	Adults & elderly: males & females 18years +			
	1d	Pregnancy & lactation: pregnant or lactating women			
2		**Study Design:** Is this a randomised controlled trial (RCT)? ***Exclude:*** non-randomised controlled trials, group randomised controlled trial (community trial), uncontrolled trials.			
3		**Type of intervention:** Does the study investigate the effect of micronutrient intake from supplements ***or*** fortified food(s) ***or*** natural dietary sources, compared with placebo ***or*** untreated comparison group? ***Exclude***: Combined interventions e.g. >1 micronutrient ***or*** micronutrient + lifestyle intervention which do not study the effect of the micronutrient separately			
4		**Intervention duration:** Does the study last for the minimum specified duration? (refer to Note 1)			
5		**Study outcomes:** Does the study report the relationship between at least two of the following types of outcome? **(1)**			

		health outcomes; **(2)** status measurements; **(3)** intake measurements *If YES, which relationship(s)?*			
	5a	Status - Health			
	5b	Intake - Health			
	5c	Intake - Status			
	5d	Intake - Status - Health			
6		**Status measures**: If relevant, are the reported status measures included in ***Table RA2: Guidance Status Biomarkers for use in Systematic Reviews***? http://www.eurreca.org/folders/6034 ***Exclude:*** Measures not included in Guidance document			
7		**Health outcomes**: If relevant, are the reported health outcomes included in ***Deliverable RA2.x – 1 / 2. 7-3?*** http://www.eurreca.org/folders/6041 ***Exclude:*** Outcomes not included in above deliverable			
8		**Intake measures:** Does the study report intake using at least one of the following methods (8a-c) **OR** report supplement intake (unless intentionally excluded)? ***Exclude:*** Other assessment methods *If YES, which method(s)?*			
	8a	Validated FFQ / Dietary history			
	8b	24h recall / food records / diary measures for at least 3 days			
	8c	24h recall / food records / diary measure <3 days with adjustment for intra-individual variability			
9		**Baseline information:** Does the study report baseline data for all outcome measures reported**?**			

Studies should only be included if answers to all applicable main questions are 'YES'

Anexo V:

Evaluación de la validez interna de los estudios aleatorizados según protocolo Eurreca

Anexo V: Assessment of internal validity in RCTs

At the end of data extraction, for each study we have a set of indicators of internal validity. The internal validity focuses on the quality of the study and tells us something about the risk of bias. We know:

- Methodology (RCT, cohort, case control)
- Something about various indicators of internal validity (which vary from methodology to methodology)

Randomised controlled trials (RCTs)

For each review question we need to print out the table of internal validity characteristics for all of the RCTs, for all of the cohorts and for all of the cross sectional studies. We should be able to print this table directly from the data extraction database (Adrienne will help you to do this for each study methodology). A sample table for an RCT data set is printed below (your basic output table may need some neatening up to make it look good and read well). For each table the columns with black and purple headings will already be completed, the columns with blue headings will need to be completed independently in duplicate by 2 reviewers and then checked. In your methodology you will need to state how any disagreements were decided.

RCT validity

Study	Method of sequence generation	Adequate sequence generation? (1)	Allocation concealment adequate? (2)	Blinding description and type	Blinding adequate? (3)	Number at start, dropouts & dropout reasons for each arm	Dropouts adequate and outcome data complete? (4)	Funder	Funder adequate? (5)	Compliance check & results	Dose check & results	Dietary intake data reported & results	Outcome (Status or health) comparability & reproducibility	Similarity of most & least exposed groups at baseline?	Lack of other potential threats to validity? (6)	Overall risk of bias (7)
X1	(text)	Yes	Yes	(text)	Yes	(numeric & text)	Yes	(text)	Yes	(text)	(text)	(text)	(text)	(text)	Yes	RCT low risk
X2	(text)	Yes	Yes	(text)	No	(numeric & text)	Yes	(text)	Unclear	(text)	(text)	(text)	(text)	(text)	Yes	RCT high risk
X3	(text)	Yes	Yes	(text)	Unclear	(numeric & text)	Yes	(text)	Yes	(text)	(text)	(text)	(text)	(text)	Yes	RCT moderate risk
X4	(text)	Yes	Unclear	(text)	Yes	(numeric & text)	No	(text)	Unclear	(text)	(text)	(text)	(text)	(text)	Yes	RCT high risk (etc)

The criteria for judging the coloured headings (those in purple, and those in blue) are below, and are adapted from the Cochrane Handbook (General reading is 'Chapter 8: Assessing risk of bias in included studies' in the Cochrane Handbook, available freely on line)[1].

[1] Higgins JPT, Green S (editors). *Cochrane Handbook for Systematic Reviews of Interventions* Version 5.0.0 [updated February 2008]. The Cochrane Collaboration, 2008. Available from www.cochrane-handbook.org.

SEQUENCE GENERATION (1) complete during data extraction **Was the allocation sequence adequately generated? [Short form: *Adequate sequence generation*?]**	
Criteria for a judgement of 'YES' (i.e. low risk of bias).	The investigators describe a random component in the sequence generation process such as: • Referring to a random number table; • Using a computer random number generator; • Coin tossing; • Shuffling cards or envelopes; • Throwing dice; • Drawing of lots; • Minimization*. *Minimization may be implemented without a random element, and this is considered to be equivalent to being random.
Criteria for the judgement of 'NO' (i.e. high risk of bias).	The investigators describe a non-random component in the sequence generation process. Usually, the description would involve some systematic, non-random approach, for example: • Sequence generated by odd or even date of birth; • Sequence generated by some rule based on date (or day) of admission; • Sequence generated by some rule based on hospital or clinic record number. Other non-random approaches happen much less frequently than the systematic approaches mentioned above and tend to be obvious. They usually involve judgement or some method of non-random categorization of participants, for example: • Allocation by judgement of the clinician; • Allocation by preference of the participant; • Allocation based on the results of a laboratory test or a series of tests; • Allocation by availability of the intervention.
Criteria for the judgement of 'UNCLEAR' (uncertain risk of bias).	Insufficient information about the sequence generation process to permit judgement of 'Yes' or 'No'.

ALLOCATION CONCEALMENT (2) complete during data extraction

Was allocation adequately concealed? [Short form: *Allocation concealment*?]

Criteria for a judgement of 'YES' (i.e. low risk of bias).	Participants and investigators enrolling participants could not foresee assignment because one of the following, or an equivalent method, was used to conceal allocation: • Central allocation (including telephone, web-based, and pharmacy-controlled, randomization); • Sequentially numbered drug containers of identical appearance; • Sequentially numbered, opaque, sealed envelopes.
Criteria for the judgement of 'NO' (i.e. high risk of bias).	Participants or investigators enrolling participants could possibly foresee assignments and thus introduce selection bias, such as allocation based on: • Using an open random allocation schedule (e.g. a list of random numbers); • Assignment envelopes were used without appropriate safeguards (e.g. if envelopes were unsealed, not opaque or not sequentially numbered); • Alternation or rotation; • Date of birth; • Case record number; • Any other explicitly unconcealed procedure.
Criteria for the judgement of 'UNCLEAR' (uncertain risk of bias).	Insufficient information to permit judgement of 'Yes' or 'No'. This is usually the case if the method of concealment is not described or not described in sufficient detail to allow a definite judgement – for example if the use of assignment envelopes is described, but it remains unclear whether envelopes were sequentially numbered, opaque and sealed.

BLINDING OF PARTICIPANTS, PERSONNEL AND OUTCOME ASSESSORS (3) complete in table

Was knowledge of the allocated interventions adequately prevented during the study? [Short form: *Blinding*?]

Criteria for a judgement of 'YES' (i.e. low risk of bias).	Any one of the following: • Double blind • No blinding, but the review authors judge that the outcome and the outcome measurement are not likely to be influenced by lack of blinding; • Blinding of participants and key study personnel ensured, and unlikely that the blinding could have been broken; • Either participants or some key study personnel were not blinded, but outcome assessment was blinded and the non-blinding of others unlikely to introduce bias.
Criteria for the judgement of 'NO' (i.e. high risk of bias).	Any one of the following: • Open • Single blind • No blinding or incomplete blinding, and the outcome or outcome measurement is likely to be influenced by lack of blinding; • Blinding of key study participants and personnel attempted, but likely that the blinding could have been broken; • Either participants or some key study personnel were not blinded, and the non-blinding of others likely to introduce bias.
Criteria for the judgement of 'UNCLEAR' (uncertain risk of bias).	Any one of the following: • Unclear • Insufficient information to permit judgement of 'Yes' or 'No'; • The study did not address this outcome.

Dropouts adequate and OUTCOME DATA COMPLETE (4) complete in table **(this is about some participants dropping out or their data not being used for some reason)** **Were incomplete outcome data adequately addressed? [Short form: *Dropouts adequate and outcome data complete*?]**	
Criteria for a judgement of 'YES' (i.e. low risk of bias).	Any one of the following: • No missing outcome data; • Reasons for missing outcome data unlikely to be related to true outcome (for survival data, censoring unlikely to be introducing bias); • Missing outcome data balanced in numbers across intervention groups, with similar reasons for missing data across groups; • For dichotomous outcome data, the proportion of missing outcomes compared with observed event risk not enough to have a clinically relevant impact on the intervention effect estimate; • For continuous outcome data, plausible effect size (difference in means or standardized difference in means) among missing outcomes not enough to have a clinically relevant impact on observed effect size; • Missing data have been imputed using appropriate methods.
Criteria for the judgement of 'NO' (i.e. high risk of bias).	Any one of the following: • Reason for missing outcome data likely to be related to true outcome, with either imbalance in numbers or reasons for missing data across intervention groups; • For dichotomous outcome data, the proportion of missing outcomes compared with observed event risk enough to induce clinically relevant bias in intervention effect estimate; • For continuous outcome data, plausible effect size (difference in means or standardized difference in means) among missing outcomes enough to induce clinically relevant bias in observed effect size; • 'As-treated' analysis done with substantial departure of the intervention received from that assigned at randomization; • Potentially inappropriate application of simple imputation.
Criteria for the judgement of 'UNCLEAR' (uncertain risk of bias).	Any one of the following: • Insufficient reporting of attrition/exclusions to permit judgement of 'Yes' or 'No' (e.g. number randomized not stated, no reasons for missing data provided); • The study did not address this outcome.

Funding adequacy (5) complete in table (this is about potential bias that may be introduced by financial interests) **Was funding unlikely to bias the reporting or publication of the study? [Short form: *Funder adequate*?]**	
Criteria for a judgement of 'YES' (i.e. low risk of bias).	Any one of the following: • Non-profit or statement that no external funding was used to support the study
Criteria for the judgement of 'NO' (i.e. high risk of bias).	Any one of the following: • Profit (even where stated in the text that the funder had no role in the analysis or presentation etc of the data) • Both profit & non-profit • Where any author is employed by a company with a clear financial interest in the study results.
Criteria for the judgement of 'UNCLEAR' (uncertain risk of bias).	Any one of the following: • Unclear • Not stated (no funding source stated)

Lack of other POTENTIAL THREATS TO VALIDITY complete in table (6)

Was the study apparently free of other problems that could put it at a risk of bias? [Short form: *lack of other potential threats to validity*?]

Criteria for a judgement of 'YES' (i.e. low risk of bias).	The study appears to be free of other sources of bias.
Criteria for the judgement of 'NO' (i.e. high risk of bias).	There is at least one important risk of bias. For example, the study: • Had a potential source of bias related to the specific study design used; or • Stopped early due to some data-dependent process (including a formal-stopping rule); or • Had extreme baseline imbalance; or • Has been claimed to have been fraudulent; or • Had problems with compliance check results, or dose check results or status/health outcome comparability and reproducibility • Important outcomes have been assessed but not reported in enough detail to be used in meta-analysis • Had some other problem.
Criteria for the judgement of 'UNCLEAR' (uncertain risk of bias).	There may be a risk of bias, but there is either: • Insufficient information to assess whether an important risk of bias exists; or • Insufficient rationale or evidence that an identified problem will introduce bias.

Overall low risk of bias for RCTs complete in table (7) **This is an overall summary of the various issues for this study**	
Criteria for a judgement of 'RCT, low risk of bias'	• Where sequence generation (1), allocation concealment (2), blinding (3) and funding (5) are all adequate, and there are no serious risks of bias in the other areas (4, 6): the risk of bias is low
Criteria for a judgement of 'RCT, moderate risk of bias'	There is at most one important risk of bias, but other areas are all adequate. For example: • Any one of sequence generation (1), allocation concealment (2), blinding (3) or funding (5) are inadequate OR unclear, or there is a serious risk of bias from one of the other areas (4, 6), AND the remaining areas are all adequate: there is a moderate risk of bias
Criteria for the judgement of 'RCT, high risk of bias'	• There is more than one important risk of bias. This will be any study that does not fit into the 2 categories above.

Anexo VI:

Media y DS de los estudios individuales incluidos en los metaanálisis

ANEXO VI a: Niveles en suero /plasma

ANEXO VI b: Crecimiento: Peso y Longitud

ANEXO VI c: Crecimiento: Circunferencia media de brazo

ANEXO VI d: Crecimiento: Perímetro Cefálico

ANEXO VI e: Crecimiento: WAZ, LAZ y WLZ z-score

ANEXO VI f: Neurodesarrollo

ANEXO VI a: Niveles de Zn en suero /plasma (Status)

Autor	Año	País	Semanas de intervención	Dosis (mg/d)	Intervención			Control		
					n	Media	DS	n	Media	DS
Bates	1993	Gambia	2	20	30	15,2	1,8	28	15,2	1,6
			8	20	46	17,8	1,3	44	16,3	1,3
Chang	2010	Bangladesh	24	2.5	85	10,3	1,1	89	9,9	1,1
Lind	2003	Indonesia	24	10	134	11,58	1,41	143	9,06	1,27
Makonnen	2003	Lesotho	4	10	142	6,66	1,53	121	6,76	1,4
			8	10	141	8,52	2,77	119	7,28	1,4
			12	10	138	10,13	2,93	116	7,84	1,72
Sazawal	1996-2004	India	16	10	223	13,4	5,48	224	9,76	2,1
Lactantes 6-11 me			16	10	78	14,14	5,11	78	9,72	2,83
Lactantes >11 me			16	10	69	12,4	5,05	73	9,96	2,65
Niñas			16	10	115	13,7	5,91	106	9,73	3,69
Niños			16	10	108	13,05	4,97	118	9,78	2,2
Osendarp	2002	Bangladesh	20	5	138	13,3	3,8	133	10,7	2,9
Umeta	2000	Ethiopia								
Lactantes con retraso crecim.			24	8,5	25	15,8	3,7	25	11	1,9
Lactantes sin retraso crecim.			24	8,5	25	17,9	5	25	14,5	2,1
Walravens	1989	USA	24	5,7	16	64	14	25	68	11
Wasantwisut	2006	Thailandia	24	10	58	16,7	5,2	66	9,8	1,9

ANEXO VI b: Crecimiento: Peso y Longitud (Weight and Length)

Autor	Año	País	Semanas de intervención	Dosis (mg/d)	Peso (kg)						Longitud (cm)					
					Intervención			Control			Intervención			Control		
					n	Media	DS	n	Media	DS	n	Media	DS	n	Media	DS
Bates (MEDIA + EE)	1993	Gambia	60	20	53	11,07	0,19 (DS:1,38)	50	11,63	0,19 (DS:1,34)	51	85,8	0,6 (DS:4,43)	46	88	0,6 (DS:4,2)
Berger	2006	Vietnam	24	10	191	8,19	0,92	195	8,05	0,8	191	71,2	2,53	195	71	2,41
Dijkhuizen	2001	Indonesia	24	7	98	8	0,9	90	7,8	1	98	69,4	2,3	90	69	2,7
Fischer Walker	2009	Bangladesh	24	2,8	141	8,08	1,07	140	8,16	1	141	70,9	2,8	140	71	2,6
Gardner	2005	Jamaica	24	10	55	10,03	1,02	59	10,02	1,2	55	83,6	4,4	59	84	5,4
Heinig	2006	USA	16	5	33	7,01	0,85	37	6,96	0,76	33	64,2	2,4	37	63,9	2,4
			40	5	33	9,08	1,03	37	9,11	1,06	33	73	2,3	37	72,9	2,8
Ninh	1996	Vietnam	20	10	73	8,9	1,1	73	8,5	1,1	73	76,2	5,1	73	75	5,5
Osendarp	2002	Bangladesh	20	5	138	2,85	0,73	133	2,79	0,7	138	11,5	1,9	133	11	2,1
Lactantes con niveles séricos de Zn bajos <9,18 µ mol/L			20	5	21	3,15	0,77	16	2,66	0,8	21	12,5	2	16	12	2,2
Lactantes con niveles séricos Zn normales >9,18 µ mol/L			20	5	117	2,79	0,71	115	2,81	0,7	117	11,3	1,8	115	11	2,1
Rivera	1998	Guatemala	28	10	45	8,25	0,95	44	8,12	1,1	45	70	3	44	69	3,7
Walravens (MEDIA + EE)	1989	USA														
Subgrupo Niños			24	5,7	13	9,83	0,24 (DS:0,87)	13	9,54	0,2 (DS:0,72)	13	82,3	1,04 (DS:3,75)	13	80	1,23 (DS:4,43)
Subgrupo Niñas			24	5,7	12	9,34	0,21 (DS:0,73)	12	9,07	0,2 (DS:0,67)	12	79,5	1,24 (DS:4,3)	12	79	1,2 (DS:4,16)
Wasantwisut	2006	Thailandia	24	10	151	8	0,9	153	8,1	1	151	70,6	2,5	153	71	2,4

ANEXO VI c: Crecimiento: Circunferencia media de brazo (MUAC)

Autor	Año	País	Semanas de intervención	Dosis (mg/d)	Intervención			Control		
					n	Media	DS	n	Media	DS
Arsenault	2008	Perú								
Suplemento líquido			24	3	36	0,1	0,9	44	0,01	0,7
Papilla fortificada			24	3	38	0,1	0,7	44	0,01	0,7
Bates (MEDIA+ EE)	1993	Gambia	60	20	53	0,8	0,1 (DS: 0,73)	49	0,7	0,14 (DS:0,98)
Heinig	2006	USA	24	5	33	1,2	1,4	37	1,3	2
Muller	2003	Burkina Faso	24	1,78	332	-0,3	1	329	-0,4	1,1
Osendarp	2002	Bangladesh	20	5	138	2,5	1,1	133	2,6	1
Lactantes con niveles séricos de zinc bajos <9,18 µ mol/L			20	5	21	2,9	1,2	16	2,5	1,1
Lactantes con niveles séricos zinc normales >9,18 µ mol/L			20	5	117	2,5	1	115	2,6	1
Rivera	1998	Guatemala	28	10	45	0,2	0,8	44	0,0	1
Umeta (MEDIA + EE)	2000	Ethiopia								
Lactantes sin retraso crecim.			24	8,57	47	0,1	1,2 (DS:8,22)	47	0	1,1 (DS:7,54)
Lactantes con retraso crecim.			24	8,57	45	0,4	1,3 (DS:8,72)	45	0,3	1,4 (DS:9,89)

ANEXO VI d: Crecimiento: Perímetro Cefálico (Head Circunference)

Autor	Año	País	Semanas de intervención	Dosis (mg/d)	Intervención			Control		
					n	Media	DS	n	Media	DS
Heinig	2006	USA	24	5	33	4,1	0,5	37	4,1	0,4
Meeks Gardner	1998	Jamaica	12	5	31	0,7	0,6	24	0,6	0,6
Osendarp	2002	Bangladesh	20	5	138	5,1	1,1	133	5,2	1,1
Lactantes con niveles séricos de zinc bajos <9,18 µ mol/L			20	5	21	5,9	1	16	5,1	1,2
Lactantes con niveles séricos zinc normales >9,18 µ mol/L			20	5	117	5	1,1	115	5,2	1,1
Rivera	1998	Guatemala	28	10	45	2	2	44	1.9	0.8

ANEXO VI e: Crecimiento: WAZ, LAZ and WLZ z-score

Autor	Año	País	Semanas de Intervención	Dosis (mg/d)	WAZ z-score						LAZ or HAZ z-score						WLZ or WHZ z-score					
					Intervención			Control			Intervención			Control			Intervención			Control		
					n	Media	DS	n	Media	DS	n	Media	DS	n	Media	DS	n	Median	DS	n	Media	DS
Berger	2006	Vietnam	24	10	191	-1,54	0.85	195	-1,68	0,75	191	-1,3	0,83	195	-1,42	0,82	191	-0,71	0,7	195	-0,85	0,69
Dijkhuizen	2001	Indonesia	24	7	98	-1,26	0,85	90	-1,37	0,91	98	-1,29	0,79	90	-1,3	0,88	98	-0,36	0,8	90	-0,44	0,82
Gardner	2005	Jamaica	24	10	55	-2,04	0,56	59	-2	0,58	55	-1,2	0,71	59	-1,08	0,8	55	-1,5	0,51	59	-1,7	0,53
Hamadani	2001	Bangladesh	28	5	103	-1,5	0,9	109	-1,5	1	103	-1,7	1	109	-1,7	1	103	-0,1	0,8	109	-0,1	1
			52	5	97	-2,4	0,9	101	-2,5	0,9	97	-2,3	1	101	-2,4	1	97	-1,2	0,6	101	-1,3	0,8
Lind	2004	Indonesia	24	10	163	-1,46	1,08	164	-1,72	1	163	-0,77	0,92	164	-0,81	0,86	163	-0,7	1,06	164	-1,01	1,16
Ninh	1996	Vietnam	20	10	73	-2,46	0,62	73	-2,76	0,53	73	-2,66	0,76	73	-2,95	0,9	73	-1,18	0,71	73	-1,27	0,6
Olney	2006	Zanzibar	24	10	44	-0,22	0,46	58	-0,15	0,44	44	-0,43	0,26	58	-0,35	0,26						
Rivera	1998	Guatemala	28	10	45	-2,11	0,82	44	-2,07	0,9	45	-2,94	0,84	44	-3	1,16	45	-0,28	0,76	44	-0,09	0,8
Sur	2003	India	1	3,57	50	-1,18	0,79	50	-1,12	0,08												
			2	3,57	50	-1,08	0,84	50	-1,01	0,83												
			3	3,57	50	-0,97	0,8	50	-0,92	0,66												
			4	3,57	50	-0,89	0,79	50	-1,03	0,79												
			5	3,57	50	-0,93	0,78	50	-1,12	0,86												
			6	3,57	50	-1,23	0,99	50	-1,3	0,84												
			7	3,57	50	-1,4	0,96	50	-1,49	0,99												
			8	3,57	50	-1,42	0,96	50	-1,67	0,98												
			9	3,57	50	-1,55	0,92	50	-1,75	0,97												
			10	3,57	50	-1,72	0,89	50	-1,92	0,99												
			11	3,57	50	-1,68	1,02	50	-2,01	1,03												
			12	3,57	50	-1,45	0,95	50	-2,17	1,09												
Wasantwisut	2006	Thailandia	24	10	151	-1,3	0,9	153	-1,3	0,9	151	-1	0,9	153	-0,99	0,86	151	-0,7	0,8	153	-0,7	0,7

ANEXO VI f: Neurodesarrollo (Neurodevelopment)

Autor	Año	País	Semanas de intervención	Dosis (mg/d)	MDI INTERVENCION			MDI CONTROL			PDI INTERVENCION			PDI CONTROL		
					n	Media	DS	n	Media	DS	n	Media	DS	n	Media	DS
Ashworth	1998	Brasil	24	1	56	89,6	6,9	53	91,5	5,4	56	91,3	8,7	53	93,6	6,6
			48	1	48	106,7	11,1	44	109,1	12,2	48	101,1	11	44	100,4	11,3
			24	5	54	90,1	7,4	53	91,5	5,4	54	93,7	8,4	53	93,6	6,6
			48	5	46	106,9	12,1	44	109,1	12,2	46	100	11,6	44	100,4	11,3
Castillo Duran	2001	Chile	24	5	57	90,1	5,6	55	90,8	7,8	57	87,4	8,5	55	87,7	8,9
			48	5	57	90,9	10,5	55	88,9	9,1	57	84,5	11,5	55	87,6	9,9
Hamadani	2001	Bangladesh	28	5	103	98,4	7	109	98,4	6,5	103	101,8	13,3	109	102,1	14
			52	5	97	103,1	11	101	106,4	9,3	97	88	18,9	101	90,6	18,9
Jimenez	2007	Cuba	4	10	87	59	5,6	76	57	4,8	87	47	4,8	76	52	4,9
			13	10	87	84	7,2	76	88	7,6	87	49	4,6	76	55	5,2
			26	10	87	101	8,9	76	105	9	87	87	5,3	76	83	5,7
			39	10	87	107	8,3	76	110	9,1	87	96	6,1	76	88	5,9
			52	10	87	108	9,2	76	113	8,9	87	105	6,6	76	94	6,2
Lind	2004	Indonesia	48	10	161	101	9,3	162	99	10	161	105	10,6	162	103	10,8

Anexo VII

Publicaciones

Effect of zinc intake on serum/plasma zinc status in infants: A meta-analysis.

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(Segunda revisión)

Effect of zinc intake on serum/plasma zinc status in infants: A meta-analysis.

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Abstract

A systematic review and meta-analysis of available RCTs was conducted to evaluate the effect of zinc intake on serum/plasma in infants. Out of 5500 studies identified through electronic searches and reference lists, 9 RCTs were selected after applying the exclusion/inclusion criteria. The influence of zinc intake on plasma/serum Zn concentration was considered in the overall meta-analysis. Other variables were also taken into account as possible effect modifiers: doses of zinc intake, intervention duration, nutritional status and risk of bias. From each selected study, final measures of serum/plasma Zn were assessed. RESULTS: The pooled β of status was 0.09 (95%CI 0.06 to 0.12). However, a substantial heterogeneity was present in the analyses (I^2=95%; p=0.00001). When we performed a meta-regression, the effect of Zn intake on Zn status changed depending on the duration of the intervention, the dose of supplementation and the nutritional situation (p ANCOVA= 0.005; <0.001 and <0.001 respectively). After stratifying the sample according to the effect modifiers the results by duration of intervention showed a positive effect when Zn intake was provided during medium and long period of time (from 4 to 20 and >20 weeks). A positive effect was seen also when doses ranged from 8.1 to 12 mg/day. In all cases, the pooled β showed high evidence of heterogeneity. CONCLUSION: Zinc supplementation increases the status in infants, although high evidence of heterogeneity was found. Further standardized research is urgently needed to reach evidence-based conclusions to clarify the role of zinc supplementation upon infant status, particularly in Europe.

Keywords: EURRECA, zinc intake, status, infants

Introduction

Zinc (Zn) is an essential nutrient, present in all body tissues and fluids. The biologic role of Zn is now recognized in structure and function of proteins, including more than 300 enzymes, transcription factors, hormonal receptor sites, and biologic membranes. Zn has numerous central roles in DNA and RNA metabolism (MacDonald 2000), and it is involved in signal transduction, gene expression, and apoptosis. Zn enzymes are involved in nucleic acid metabolism and cellular proliferation, differentiation, and growth (Chesters 1978).

Plasma Zn accounts for only about 0.1 per cent of the total body content. Zn has a rapid turnover, and its level appears to be under close homeostatic control. There is no 'store' for Zn in the conventional sense (Milne et al. 1983). Zn is present in the body almost exclusively as Zn2+ bound to cellular proteins (Makonnen et al. 2003 II).

Assessment of the Zn nutriture of individuals is complicated by the fact that no generally accepted, sensitive and specific biomarker of Zn status exists (King 1990). Although it is true that serum (or plasma) Zn concentrations decrease within several weeks of the introduction of a diet containing a severely restricted amount of zinc (Baer et al. 1985), serum Zn concentrations are generally maintained within the normal range with small or moderate reductions in Zn intake. Moreover, factors unrelated to the level of Zn nutriture, such as recent meals, time of day, infection, tissue catabolism, and pregnancy, can also affect serum zinc concentrations (King 1990; Hambidge & Krebs 1995). Thus, the serum Zn concentration may not always be a reliable indicator of an individual's true Zn status (Brown et al. 2002). Nevertheless a recent systematic review concluded that plasma (or serum) zinc concentration was responsive to both zinc supplementation and depletion and it remains the most widely used biomarker for zinc (Lowe et al. 2009).

Infants have a relatively high requirement of zinc per unit body weight during a sensitive period of rapid growth and development (Hermoso et al. 2010). Recommendations for zinc intake during infancy vary widely across Europe, ranging from 1 mg/day up to 5 mg/day (Hermoso et al. 2010). The EURRECA project attempts to consolidate the basis for the definition of micronutrient requirements across Europe, taking into account relationships among intake, status and health outcomes, in order to harmonise these recommendations (Ashwell et al. 2008). This paper presents a systematic review of the data from all available randomized controlled trials (RCTs) meeting EURRECA's quality standard (Matthys et al. 2011), which investigated zinc intake and biomarkers

of zinc status in infants, and combines these studies in meta-analyses to model zinc concentrations in serum or plasma as a function of zinc intake.

Materials and Methods

Search strategy

This research was conducted within the framework of the European Micronutrient Recommendations Aligned (EURRECA) Network of Excellence that aims to identify the micronutrient requirements for optimal health in European populations (www.eurreca.org). Databases searched were OVID MEDLINE, OVID EMBASE and Cochrane Library CENTRAL databases from inception to February 2010. The procedure for the identification, selection of articles and data extraction is illustrated in Figure 1.

A search strategy was established to identify the most relevant studies in the electronic databases, using text terms with appropriate truncation and relevant indexing terms.The electronic search used the following keyword: “randomized controlled trial”, “double-blind procedure”, “human”, “zinc or zn or zinc sulphate or zinc gluconate or zinc acetate or methionine or zinc isotope”, “intake or diet or supplement or deplete or status or serum or plasma or leukocyte or concentration or fortify”, “Nutritional Support or Dietary Supplements or nutritional requirements or Breast feeding or infant food or bottle feeding or infant formula”, “Nutritional Status or Deficiency Diseases or supplementation or diet supplementation or dietary intake or diet restriction or mineral intake or Diet or Food, Fortified or nutrition assessment or Nutritive Value”. Languages included were restricted to those spoken in the EURRECA Network (English, Dutch, French, German, Hungarian, Italian, Norwegian, Polish, Spanish, Greek, and Serbian.). Reference lists of retrieved articles and published literature reviews were also checked for relevant studies.

Selection of articles

Titles of articles identified from the searches were entered into an EndNote library. Papers were considered eligible for inclusion if they were RCTs, conducted in human infants (aged 0-12 months), and studied the effect of supplements, fortified foods or micronutrient intake from natural food sources, and assessed zinc concentrations in serum / plasma.

Zinc intake was assessed from breast milk, infant formula and food sources (e.g. complementary foods), fortified foods (e.g. fortified formula or cereal) and supplements.

Exclusion criteria applied were: studies conducted in animals; combined interventions e.g. >1 micronutrient or micronutrient + lifestyle intervention which did not study the effect of the micronutrient separately; non primary studies (e.g. letters & narrative literature reviews); duplicate publications; studies where the zinc intake – status relationship was not reported or biomarkers of zinc other than serum / plasma zinc were used.

Briefly, titles and abstracts of the 10% of the library were screened in duplicate for eligibility by two reviewers. Only when both reviewers agreed that titles and abstracts met the inclusion criteria were the articles included. When a title and abstract could not be included with certainty, the full text of the article was obtained and then further evaluated. The remaining 90% was distributed among the reviewers in even parts.

Following the initial screening process, full-text articles were obtained. Further inclusion and exclusion criteria were then applied. Papers were only included in the meta-analysis if they were: randomised controlled trials; had an intervention duration of at least 2 weeks; and reported baseline data for all outcome measures. Non-randomised controlled trials, uncontrolled trials or trials with insufficient or unclear data reported were excluded.

Data was extracted from each study and organized in a Microsoft Access database file (Microsoft Corp, Redmond, WA).

Data synthesis

Of the select studies, two RCTs were companion papers (Makonnen et al. 2003 I; Sazawal et al. 2004).

When Zn status was measured at different time points within the same population, we used the measures as different estimations (Bates et al. 1993**;** Makonnen et al. 2003 I/II).

One study report data from the total of infants included, and between males and females separately and according to age: <11 month and > 11 month (Sazawal et al. 2004/1996). Then this study was treated as five estimations within the meta-analysis.

One study report data from two groups of infants (stunted and non stunted) and also these were treated as two different estimations within the meta analysis (Umeta et al. 2000).

If dietary intake of Zn (in addition to the intervention) was not reported in the RCTs we imputed a value of 1.3 mg/day, the mean dietary intake level of the RCTs that did report dietary zinc intake. As mean baseline serum/plasma zinc concentrations were infrequently reported in the RCTs, most of the RCTs assumed no differences in baseline serum/plasma Zn concentrations (n= 8). Only one study, Bates et al. 1993, failed to mention anything regarding baseline serum /plasma Zn concentrations.

Exposure and outcome and other covariates assessment:

The influence of Zn intake on plasma/serum Zn concentrations was considered in the overall meta-analysis. Other variables were also taken into account as possible effect modifiers. We considered doses of zinc intake (1 to 4 mg, from 4.1 to 8 mg, from 8.1 to 12 mg and >12.1 mg), intervention duration (1 to 3 weeks, 4 to 20 weeks, and > 20 weeks), nutritional situation (healthy; nutritionally at risk, and poor nutritional status) and risk of bias (low, moderate or high).
Nutritionally at risk was defined as infants who lived in low income families with a low socioeconomic situation and poor nutritional status was defined as infants with protein energy malnutrition (PEM) but without congenital abnormalities or cerebral palsy or heart disease or infants with low birth weight during their first year.

Risk of bias was developed in order to assess the quality of the studies included. The following indicators of internal validity specific to the RCT methodology were collected during data extraction: 1) method of sequence generation and allocation, 2) blinding, 3) potential funding bias, 4) number of participants at start, 5) dropouts and dropout reasons, 6) dose check, 7) dietary intake data reported, 8) outcome comparability and reproducibility, and 9) similarity of most and least exposed groups at baseline. Based on these indicators, two reviewers assessed the overall risk of bias. Disagreements were resolved by discussion. The criteria for judging these indicators were adapted from the Cochrane Handbook (Higgins & Green 2009).

Statistical analysis

Mean and standard deviation (SD) or standard errors (SE) of the outcome (plasma / serum Zn) were assessed. From the mean and SD of each study Beta Values (β) and their SE were calculated because the statistical model that we used to estimate the relation between zinc intake (x-variable) and serum/plasma zinc (y- variable) is based on the assumption that this intake-status curve is a logarithmic function and that both intake and status follow a log-normal distribution (the natural logarithm of intake and status have a normal distribution). Thus, the expected value if the status score is expressed as:

$\mu y = \beta * \mu x + \text{intercept}$

where μy represents the mean of the natural logarithm of the y–variable (= status score), β represents the regression coefficient, and μx represents the mean of the natural logarithm of the x-variable (= Zn intake).

The method used to systematically review differences was a formal meta–analysis (Greenland 1998).

Procedures of formal meta-analysis have been applied to combine the results from previously reported studies (Dickersin 2002).

A random-effects model was considered to be more appropriate than a fixed-effects model. We used the DerSimonian and Laird's (DerSimonian & Laird 1986) to pool the estimates of betas across studies. Under this model, the pooled effect was the beta in the status parameter (serum / plasma), for an increment of 1 unit in Zn intake. A pooled beta estimate was calculated as a weighted average of the beta reported in each study.

The formula we used to estimate the weighted effect size was (Hedges 1982):

$\beta\ pooled = \sum \beta i\ wi\ / \sum wi$

where $\beta\ pooled$ is the pooled estimate of the beta in status parameters; the weight *(wi)* of each study was computed as*:*

$wi = 1 / Vi + \varsigma^2$

where V is the variance of each study and ς^2 is the inter study variance.

Besides this, we calculated a 95% confidence interval for the pooled estimated of effect size:

95% CI= $\beta\ pooled \pm$ (1.96 x SE pooled)

where SE is the standard error of the pooled estimate (Greenland 1998).

A test of heterogeneity was calculated, estimating Q statistics, which follows a chi-square distribution with degrees of freedom n-1, n being the number of studies included in the analysis. The I^2 Index measures the extent of the heterogeneity. A low P value for this statistic (lower than 0.05) indicates the presence of heterogeneity, which somewhat compromises the validity of the pooled estimates (Takkouche et al. 1999).

Because significant heterogeneity was clearly evident in the pooled beta estimates for all studies combined in each outcome, we evaluated potential sources of heterogeneity by linear meta-regressions (Greenland 1998).

We fitted a meta-regression using the duration of the intervention, the doses of zinc intake, the risk of bias and the nutritional situation as independent variables. The betas of the different status parameters according to Zn intake were used as the dependent variable.

Statistical differences in multivariate adjusted mean beta values between each possible heterogeneity sources were determined by ANCOVA.

Additionally we carried out additional meta-analyses by subgroups considering only that groups which provided significant values in the meta-regression.

Microsoft Excel Version (7.0), SPSS 10.0 for Windows and Review Manager 5.1, were used to conduct the statistical analyses.

Results

Five thousand five hundred articles were identified in the initial search strategy. After applying the exclusion / inclusion criteria, 344 articles from the search appeared to be potentially relevant. After applying the additional eligibility criteria and grouping the studies by outcome, 9 randomized controlled trials (17 estimations) were selected for the zinc intake – zinc status meta-analysis (Makonnen et al. 2003 II; Bates et al. 1993; Chang et al 2010; Lind et al. 2003; Makonnen et al. 2003 I; Osendarp_et al. 2002; Sazawal et al. 2004; Sazawal et al. 1996; Umeta et al. 2000; Walravens et al. 1989; Wasantwisut et al. 2006). (Figure 1)

Descriptive characteristics of the studies included in the meta-analysis are presented in Table 1.

Of the 9 studies included, only 4 comply strictly with the age infants (0 to 12 months) (Lind et al. 2003; Osendarp et al. 2002; Umeta et al. 2000; Wasantwisut et al. 2006). The other five studies include this age among their children, but do not detail how many are actually from 0 to 12 month (Bates et al. 1993; Chang et al 2010; Makonnen et al. 2003 I/II; Sazawal et al. 2004/ 1996; Walravens et al. 1989). None of the age extends beyond 27 month, except for one, Makonnen et al. 2003 I/II, which include children up to 5 years. However, this clarifies that almost 45% are within the range of 12 to 23 month, which is calculated that about 55% rest will be less or greater than that range.

Five studies were from Asia, one from North America and three from Asia. The duration of the interventions ranged from 2 to 24 weeks. Doses of zinc intake ranged from 2.5 to 20 mg per day. The age range of the studies included was from 3 weeks to 60 months. The nutritional situation of infants also varied between studies: five studies were conducted on healthy infants (Bates et al. 1993; Lind et al. 2003; Osendarp et al. 2002; Umeta et al. 2000; Wasantwisut et al. 2006), three studies were conducted on infants who were nutritionally at risk (Chang et al 2010; Sazawal et al. 2004/ 1996; Walravens et al. 1989), and one study was conducted on infants with poor nutritional status (Makonnen et al. 2003 I/II). The risk of bias also varied between studies: two studies had a high risk of bias, three had a moderate risk and four had a low risk of bias.

Differences between plasma/serum zinc status measured according to the intervention group in each particular study and in the pooled analysis are showed in Figure 2. The pooled β was 0.09 (95%CI 0.06, 0.12). However, a substantial heterogeneity was present in the analyses (I^2 for status = 95%).

In order to investigate which variables may be potential effect modifiers, we performed a meta-regression (Table 2). The effect of Zn intake on Zn status changed depending on the duration of the

intervention, the dose of supplementation and the nutritional situation (p ANCOVA= 0.005; <0.001 and <0.001) respectively.

After stratifying the sample according to the effect modifiers identified in the meta-regression (Table 3) the results by duration of intervention showed a positive effect when Zn intake was provided during medium (4 to 20 weeks)(β = 0.09; CI 95% 0.06 to 0.13) and long period of time (>20 weeks)(β = 0.11; CI 95% 0.05 to 0.17). However these pooled β still showed high evidence of statistically significant heterogeneity (I^2= 94 and 93 %) respectively.

When doses of Zn ranged from 4.1 to 8 mg/day, there was no significant effect of Zn intake on the serum/plasma Zn; whereas a positive effect was seen when doses ranged from 8.1 to 12 mg/day (β = 0.12; CI 95% 0.08 to 0.15). For doses higher than 12 mg/day we reported no effect. However high evidence of heterogeneity was observed (I^2= from 86 to 93 %).

When studies were categorised by nutritional situation, those studies based on healthy infants and on infant at nutritional risk, reported a positive association between Zn intake and serum/plasma zinc status (β= 0.11; CI 95% 0.06 to 0.16 and β= 0.10; CI 95% 0.05 to 0.14) respectively. However, no association was found when the nutritional situation was poor (β= 0.05; CI 95% -0.02 to 0.12). Once again, the pooled β still showed high evidence of heterogeneity (I^2= from 85 to 97 %).

Due to the high heterogeneity found in all the analyses, we decided to avoid calculating dose-response relationship between Zn intake and Zn status.

Discussion

Our results indicate that zinc supplementation increases the status in infants, although high evidence of heterogeneity was found. Moreover, after carrying out several subgroup analyses, the pooled β for each sub analysis still showed high evidence of heterogeneity.

Therefore, interpretation of these results should be carefully considered for a number of reasons. First, the number of studies that were eligible for inclusion in this meta-analysis was small, which limited the statistical power of the analyses to examine the relation between status responses to zinc supplementation. Thus, the small effect size we found may be explained by of the limited amount of available information. Also, it is well acknowledged that when many statistical comparisons are carried out, one or more might reach significance due to chance alone (Bland & Altman 1995).

It is also important to consider the scientific quality of included studies. Although meta-analyses are increasingly used to consolidate results from multiple studies of the same topic and to develop evidence-based policies for clinical practice and public health programmes, the reliability of reached conclusions depend on the methodological quality of the original studies, the appropriateness of the study inclusion criteria, and the thoroughness of the review and synthesis of

information (Brown et al. 2002). While strict systematic review protocols were followed adhering to EURRECA's quality standards (Matthys et al 2011), an assessment of the risk of bias of included studies revealed that the majority (n=5) had a high- moderate risk of bias.

Positive effects of zinc supplementation on mean serum zinc concentrations have also been reported in a previous meta-analysis conducted in children and pregnant women (Brown et al. 2002; Hess et al. 2007). In both meta-analyses, there was a significantly positive effect of zinc supplementation over the mean serum zinc concentrations of the studied population. However, to our knowledge, meta-analytical methods have not yet been used to model zinc status as a function of zinc intake levels. Understanding the relationship between dietary intake and micronutrient status is essential for deriving dietary recommendations.

Population mean concentration of serum zinc is a useful indicator of the successful delivery and absorption of zinc supplements in infants. Both serum and plasma zinc concentrations are the most widely used biochemical indicators of zinc status but their levels are not necessarily identical. For instance, several biochemical studies designed to compare plasma and serum zinc concentrations observed higher levels of zinc in serum than in plasma (Kasperek et al. 1981; English & Hambidge 1988). These differences may have occurred because serum samples were separated from blood cells after a longer period of time than plasma samples, so more zinc went out from the cells into serum than into plasma. By controlling both, the amount of blood collected and the time of cell separation, no differences were found in the zinc concentrations of serum and plasma (English & Hambidge 1988). For the sake of simplicity, this paper referred to "serum / plasma zinc" without making any distinction between them.

Some confounders should be considered in evaluating the effect of zinc intake on infant zinc status. Those confounders include low birth weight, breastfeeding, protein energy malnutrition, poverty, and social deprivation. The pre-existing zinc status of the study subjects, the content and bioavailability of zinc in the local diets, and the incidence of common infections that can affect individual's zinc status are others important confounders to take into account. Moreover, methodology aspects of these studies, such as variations in the dose, chemical form, method of administration of zinc and duration of supplementation, may have influenced their results (Brown et al. 2002). However, with the exception of Bates et al, all the RCTs included in the meta-analysis assumed no baseline differences in serum/plasma Zn. Age of the studies considering in this meta-analysis was other important point. We believe that there was no reason to exclude any study that did not adhere exclusively to the group of 0 to 12 months of age. For this reasons, we took into account all the studies which included this age group in the study, even if they were not analysed according to their age group (Bates et al. 1993; Chang et al. 2010; Makonnen et al. 2003 I/II; Sazawal et al. 2004/1996; Walravens et al. 1989) and assumed the consequences of this possible

bias. Another confounding factor that might explain the inconsistency in our findings is that serum zinc concentrations vary according to the time of day, proximity of previously consumed meals, and occurrence of recent physical activity or other forms of stress, fluctuating by as much as 20% during a 24-hour period (Hambidge et al. 1989). The diurnal variation in circulating zinc concentration is largely a result of metabolic changes after meal consumption, although some variation may occur as a result of normal circadian variation in metabolism (Guillard et al. 1979; Wallock et al. 1993). Meal consumption results in a decrease in serum zinc concentrations, which add up following repeated meals (Wallock et al. 1993; Goode 1991), whereas overnight and daytime fasting result in increased circulating zinc concentrations (Wallock et al. 1993). Of the studies included in our meta-analyses, only those conducted by Osendrap et al. 2002, Umeta et al. 2000, Walravens et al. 1989 and Wasantwisut et al. 2006 reported the time of the day were the blood samples were collected. The samples were collected at morning hours.

Moreover, infection and inflammation can decrease serum zinc values, with the magnitude of change depending on the severity and stage of infection (Brown 1998). In community- based surveys, the reductions in serum zinc concentration due to infection average ~10% to 12% compared with healthy reference groups (Thurnham et al. 2005). Several other factors, such as low serum albumin, elevated white blood cell counts, use of hormones, can also affect serum zinc levels and must be considered in the interpretation of laboratory results (IZiNCG 2004). In our meta-analysis, all studies account for presence of disease during the length of intervention and whether or not zinc levels were affected by that.

Infants suffering from protein-energy malnutrition have low concentrations of zinc in plasma, muscle and liver (Hansen & Lehman 1969; Cheek et al. 1970). Because zinc is needed for tissue synthesis during nutritional rehabilitation, the amount required may exceed dietary supply (Castillo-Duran et al. 1987; Gibson et al. 1998). Makonnen et al 2003 I/II were the only authors in our meta-analysis which included infants with protein – energy malnutrition. In this study, improvement in zinc status became evident only after 60 days. It takes over one month for serum levels to increase significantly. This could explain the limited effect zinc supplementation had on serum zinc levels after 30 days. Inclusion of studies conducted in malnourished children might have contributed to the lack of significance in the present meta-analysis.

In conclusion, a positive significant association was found between zinc intake and status in infants. The magnitude of effect we found was in all cases, rather small. Based on this limited group of studies and their heterogeneity, we found insufficient information to current date to suggest that supplementation of zinc has a positive effect on infants' status or to recommend mean serum zinc concentration of a given population as a useful predictor of response to zinc supplementation.

Further standardized research is urgently needed to reach evidence-based conclusions to clarify the role of zinc supplementation upon infant status, particularly in Europe and other affluent societies.

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The authors´ responsibilities were as follows: MN: analysis of the data and writing the manuscript, ALS & MN: review the papers, MWM: contribution to selection of papers and data extraction, ASV: support in data-analysis, DFL, PHS, JDA, NL, VMH and LSM provision of significant advice. All authors directly participated in the planning, execution or analysis of the study and reviewed the manuscript.

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Figure 1: Flow diagram for the systematic review.

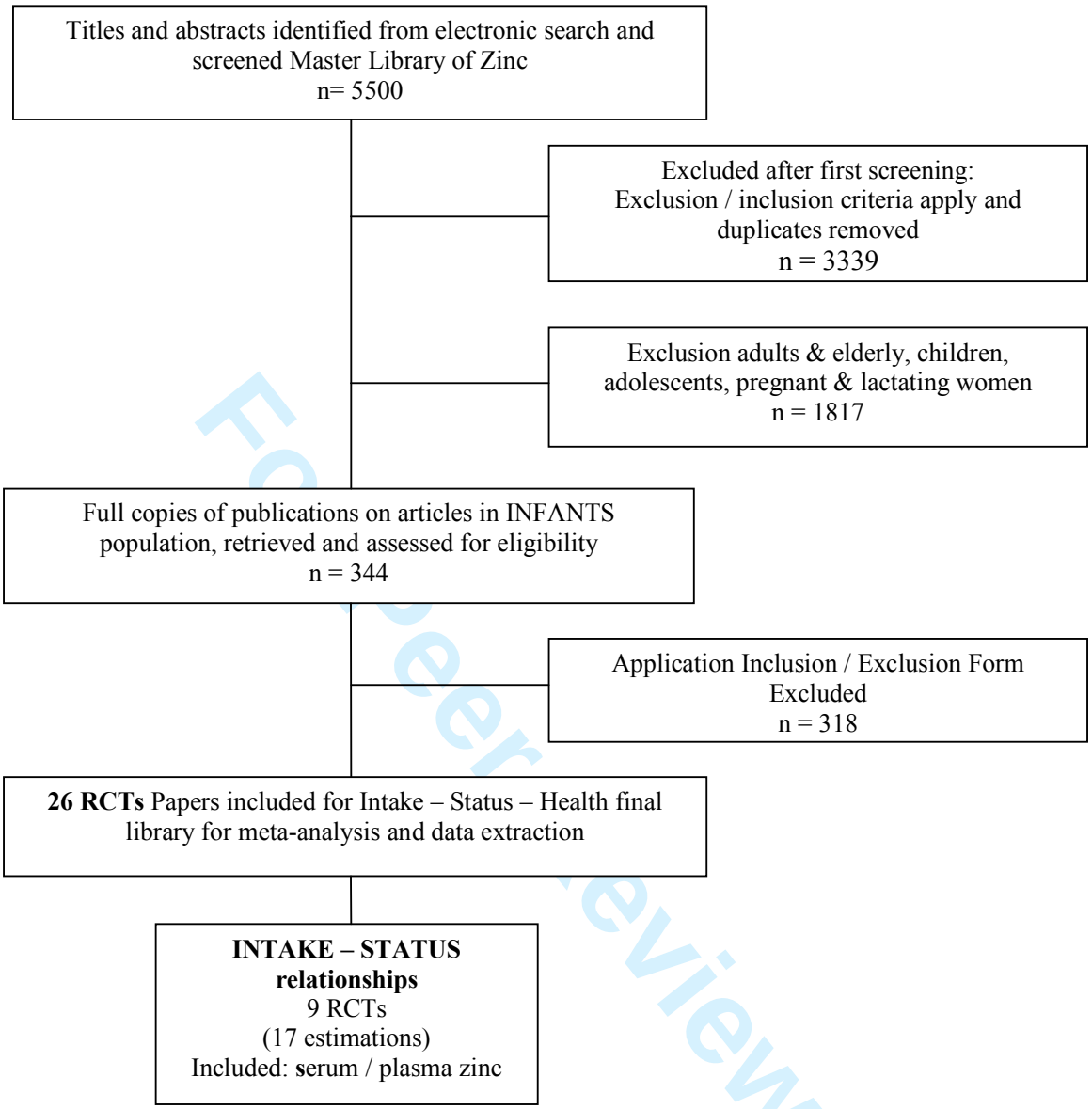

Figure 2: Status

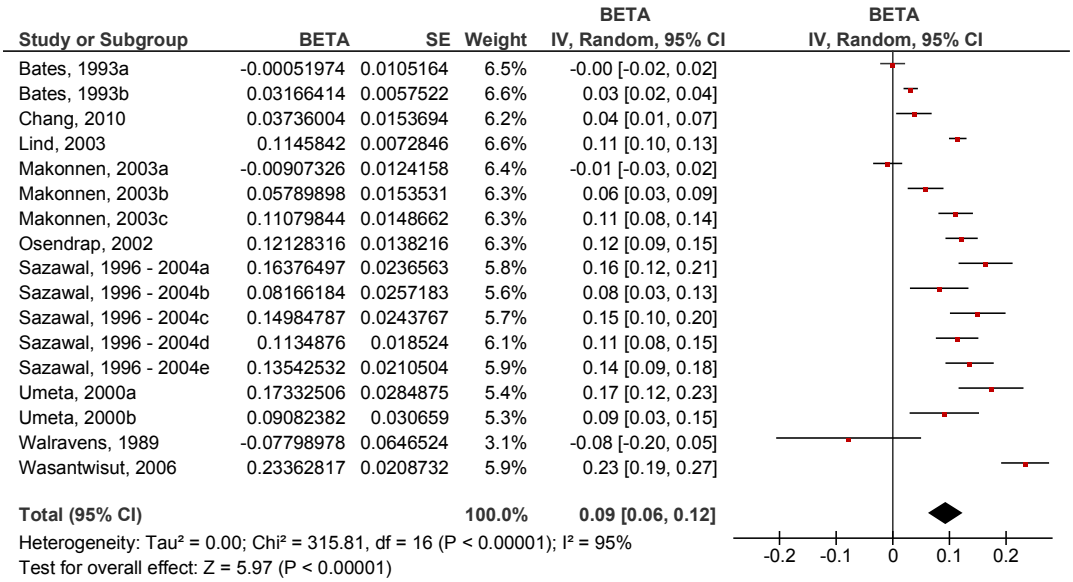

Table 1: Characteristics of the 9 (17 estimations) Status studies included in the meta-analysis

Author	Study year	Country	Sample Age range or Mean (SD)	Number of Infants (n)		Doses of Zinc	Weeks of the intervention	Outcome (measure)	Nutritional situation	Risk of bias [2]
				Zn [1]	C[1]					
Bates (a) (19) (b)	1993	Gambia	5.7 to 27 months	30 46	28 44	20 mg	2 w 8 w	Status (plasma)	Healthy	High risk
Chang (20)	2010	Bangladesh	6 to 18 months	85	89	2,5 mg	24 w	Status (serum)	Nutritionally at risk	Low risk
Lind (21)	2003	Indonesia	6.1 (0.5) months	134	143	10 mg	24 w	Status (serum)	Healthy	Low risk
(a) Makonnen (b) (22,4[3]) (c)	2003	Lesotho	6 to 60 months	142 141 138	121 119 116	10 mg	4 w 8 w 12 w	Status (serum)	Poor nutritional status	Moderate risk
Osendarp (23)	2002	Bangladesh	3 to 5 weeks	138	133	5 mg	20 w	Status (serum)	Healthy	Moderate risk
Sazawal (a) (24,25[3]) (b) (c) (d) (e)	1996-2004[3]	India	6 to 35 months 6 to 11 months > 11 months Females Males	223 78 69 115 108	224 78 73 106 118	10 mg	16 w	Status (plasma)	Nutritionally at risk	Moderate risk
(a) Umeta (26) (b)	2000	Ethiopia	Zinc stunted 9.5 (2.0) mo Placebo stunted 9.7 (2.0) mo Zinc non stunted 9.3 (2.1) mo Placebo non stunted 9.2 (2.0) mo	25 25	25 25	8,57 mg	24 w	Status (serum)	Healthy	High risk
Walravens (27)	1989	USA	8 to 27 months	16	25	5,7 mg	24 w	Status (plasma)	Nutritionally at risk	Low risk
Wasantwisut (28)	2006	Thailand	4 to 6 months	58	66	10 mg	24 w	Status (serum)	Healthy	Low risk

(a - e): Estimations

[1]Zn: Zinc group / [1]C: Control group

[2] Low risk of bias meant that the study was randomized, the randomization method was at least partially described, reasons for and numbers of dropouts were stated (or there were no dropouts), and the method used to assess compliance and some assessment of compliance were reported. All others studies were considered as moderate when they meet any of the above criteria or high risk of bias when they meet any of the criteria. (Higgins 2009, Cochrane Handbook)

[3]Compagnion paper

Table 2: Meta-regression. Multivariate adjusted mean beta for Status (95% confidence interval) by different characteristics of the studies included in the meta-analysis

	n	Mean Beta's	CI (95%)	P Ancova*
Status				
By duration of the intervention				
1 to 3 weeks	1	0.0390	-0.0556 to 0.1337	
4 to 20 weeks	10	0.0712	0.0318 to 0.1107	
> 20 weeks	6	-0.0535	-0.1137 to 0.0068	
				0.005
By Dose				
1 to 4 mg	1	0.1011	0.0074 to 0.1948	
4,1 to 8 mg	2	-0.0143	-0.0763 to 0.0477	
8,1 to 12 mg	12	0.1070	0.0663 to 0.1478	
> 12 mg	2	-0.1181	-0.1921 to -0.0440	
				<0.001
By Nutritional situation				
Healthy	7	0.1304	0.0819 to 0.1789	
Nutritionally at risk	7	-0.0004	-0.0408 to 0.0400	
Poor nutritional situation	3	-0.0732	-0.1312 to -0.0152	
				<0.001
By Risk of Bias				
Low	4	0.0470	-0.0104 to 0.1043	
Moderate	9	0.0049	-0.0381 to 0.0480	
High	4	0.0049	-0.0381 to 0.0480	
				0.241

* Adjusted for the rest of variables in the table

Table 3: Pooled beta (95% confidence intervals) in Status according to the intervention group. Subgroup analyses.

	Pooled estimates (β)	Chi² (df, P)	I²
Status			
All Studies (n=17)	0.09 (0.06 to 0.12)	315.81 (16, < 0.00001)	95%
By duration of the intervention			
1 to 3 weeks (n=1)	0 (-0.02 to 0.02)		
4 to 20 weeks (n=10)	0.09 (0.06 to 0.13)	141.21 (9, < 0.00001)	94%
> 20 weeks (n=6)	0.11 (0.05 to 0.17)	71.40 (5, < 0.00001)	93%
By dose			
1 to 4 mg (n=1)	0.04 (0.01 to 0.07)		
4,1 to 8 mg (n=2)	0.04 (-0.17 to 0.25)	9.85 (1, 0.002)	90%
8,1 to 12 mg (n=12)	0.12 (0.08 to 0.15)	151.38 (11, < 0.00001)	93%
> 12 mg (n=2)	0.02 (-0.01 to 0.05)	7.21 (1, 0.007)	86%
By Nutritional Situation			
Healthy (n=7)	0.11 (0.06 to 0.16)	215.29 (6, < 0.00001)	97%
Nutritionally at risk (n=7)	0.10 (0.05 to 0.14)	39.48 (6, < 0.00001)	85%
Poor nutritional status (n=3)	0.05 (-0.02 to 0.12)	39.26 (2, < 0.00001)	95%

*I² Index measures the extent of the heterogeneity

Effect of zinc intake on growth in infants: A meta-analysis.

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Effect of zinc intake on growth in infants: A meta-analysis.

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Abstract

A systematic review and meta-analysis of RCTs was conducted to evaluate the effect of zinc intake on growth in infants. Out of 5500 studies identified through electronic searches, 19 RCTs were selected after applying the exclusion/inclusion criteria. The influence of zinc intake on growth was considered in the overall meta-analysis. Other variables were also considered as possible effect modifiers: doses of intake, intervention duration, nutritional status and risk of bias. From each study, final measures of Weight, Length, MUAC (Mid upper arm circumference), Head circumference, WAZ (Weight for age z-score), LAZ (Length for age z-score) and WLZ (Weight for Length z-score) were assessed. Pooled β and 95% CI were calculated. RESULTS: Zn intake was not associated to Weight, Length, MUAC, Head Circumference and LAZ in the pooled analyses. However, had a positive and statistically effect on WAZ and WLZ. The dose response relationship indicated that a doubling of Zn intake increased WAZ and WLZ by approximately 4%. Substantial heterogeneity was present only in Length analyses. Zn intake was positively associated with length values at short time and at medium doses of Zn. Nevertheless, the effect magnitude was small. Zinc intake increases growth parameters of infants. Nonetheless, interpretation of these results should be carefully considered.

Keywords: EURRECA, zinc intake, growth, infants

Introduction

Zinc (Zn) is an essential nutrient, present in all body tissues and fluids. The biologic role of Zn is now recognized in structure and function of proteins, including more than 300 enzymes, transcription factors, hormonal receptor sites, and biologic membranes. Zn has numerous central roles in DNA and RNA metabolism (MacDonald 2000), and it is involved in signal transduction, gene expression, and apoptosis. Zn enzymes are involved in nucleic acid metabolism and cellular proliferation, differentiation and growth (Chesters 1978). Zinc is a critical micronutrient for normal growth, haematopoiesis, immune function and neurologic development during infancy. Infants have a relatively high requirement of zinc per unit body weight during a sensitive period of rapid growth and development (Hermoso et al. 2010).

Physiological functional consequences (e.g. growth retardation) of mild zinc deficiency are often apparent before the zinc concentrations in plasma and/or tissues are significantly reduced.(Gibson et al. 1989; Ruz et al. 1991) Inadequate zinc intake is likely to be an important contributing factor of growth failure in children that are malnourished, because diets low in protein tend to be low in zinc.(Golden & Golden 1981) Growth faltering starts at 6 mo of age in less-developed countries with rapid progression (The World Bank 2006) and coincides with a critical time in the dietary supply of Zn, labelled as a ‘‘problem’’ nutrient in complementary feeding by WHO. (Dewey & Brown 2003)

Human Zn deficiency was described since the early 1960s. But it was not until 1990, when the Zn became to be a micronutrient of major interest until the current date, due the important function for immune system integrity (Shankar & Prasad 1998), the know losses of Zn in diarrheal fluids (Ruz & Solomons 1990), and pilot data on the association between Zn deficiency and diarrhea (Hambidge 1992). Zn supplementation RCT´s focused on growth velocity in young children. A comprehensive meta-analysis of results of 33 studies provided convincing evidence of a significant increase in linear growth (Brown et al. 1998; IZNCG 2004).

A considerable number of intervention trials have been completed in multiple countries to assess the effect of supplemental Zn on children's growth. However, these studies have yielded inconsistent results, possibly because of differences in 1) the pre-existing Zn status of the study subjects, 2) the content and bioavailability of Zn in the local diets, and 3) the incidence of common infections that can affect growth independently of an individual's Zn status. Moreover, methodology aspects of these studies, such as variations in the dose, the sample sizes, the method of administration or the duration of supplementation, may have influenced their results (Brown et al. 2002).

Recommendations for zinc intake during infancy vary widely across Europe, ranging from 1 mg/day up to 5 mg/day (Hermoso et al. 2010). The EURRECA project attempts to consolidate the basis for the definition of micronutrient requirements across Europe, taking into account relationships among intake, status and health outcomes, in order to harmonise these recommendations (Ashwell et al. 2008). This paper presents a systematic review of the data from all available randomized controlled trials (RCTs) meeting EURRECA's quality standard (Matthys et al. 2011), which investigated zinc intake and growth parameters in infants, and combines these studies in meta-analyses to model of growth as a function of zinc intake.

Materials and Methods

Search strategy

This research was conducted within the framework of the European Micronutrient Recommendations Aligned (EURRECA) Network of Excellence that aims to identify the micronutrient requirements for optimal health in European populations (www.eurreca.org). Databases searched were OVID MEDLINE, OVID EMBASE and Cochrane Library CENTRAL databases from inception to February 2010. The procedure for the identification, selection of articles and data extraction is illustrated in Figure 1.

A search strategy was established to identify the most relevant studies in the electronic databases, using text terms with appropriate truncation and relevant indexing terms. The electronic search used the following keyword: "randomized controlled trial", "double-blind procedure", "human", "zinc or zn or zinc sulphate or zinc gluconate or zinc acetate or methionine or zinc isotope", "intake or diet or supplement or deplete or status or serum or plasma or leukocyte or concentration or fortify", "Nutritional Support or Dietary Supplements or nutritional requirements or Breast feeding or infant food or bottle feeding or infant formula", "Nutritional Status or Deficiency Diseases or supplementation or diet supplementation or dietary intake or diet restriction or mineral intake or Diet or Food, Fortified or nutrition assessment or Nutritive Value". Languages included were restricted to those spoken in the EURRECA Network (English, Dutch, French, German, Hungarian, Italian, Norwegian, Polish, Spanish, Greek, and Serbian.). Reference lists of retrieved articles and published literature reviews were also checked for relevant studies.

Selection of articles

Titles of articles identified from the searches were entered into an EndNote library. Papers were considered eligible for inclusion if they were RCTs, conducted in human infants (aged 0-12 months), and studied the effect of supplements, fortified foods or micronutrient intake from natural food sources, and assessed zinc concentrations in serum / plasma.

Zinc intake was assessed from breast milk, infant formula and food sources (e.g. complementary foods), fortified foods (e.g. fortified formula or cereal) and supplements.

Exclusion criteria applied were: studies conducted in animals; combined interventions e.g. >1 micronutrient or micronutrient + lifestyle intervention which did not study the effect of the micronutrient separately; non primary studies (e.g. letters & narrative literature reviews); duplicate publications; studies where the zinc intake – growth relationship was not reported or health outcomes other than growth assessed.

Briefly, titles and abstracts of the 10% of the library were screened in duplicate for eligibility by two reviewers. Only when both reviewers agreed that titles and abstracts met the inclusion criteria were the articles included. When a title and abstract could not be included with certainty, the full text of the article was obtained and then further evaluated. The remaining 90% was distributed among the reviewers in even parts.

Following the initial screening process, full-text articles were obtained. Further inclusion and exclusion criteria were then applied. Papers were only included in the meta-analysis if they were: randomised controlled trials; had an intervention duration of at least 2 weeks; and reported baseline data for all outcome measures. Non-randomised controlled trials, uncontrolled trials or trials with insufficient or unclear data reported were excluded.

Data was extracted from each study and organized in a Microsoft Access database file (Microsoft Corp, Redmond, WA).

Data synthesis

When growth was measured at different time points within the same population, we used the measures as different analyses or estimations (Hamadani et al. 2001; Heinig et al. 2006; Sur et al 2003).

Moreover, one study reported data separately for boys and girls (Walravens et al. 1989).

One study report data from two groups of infants (stunted and non stunted) and also these were treated as two different estimations within the meta analysis (Umeta et al. 2000). Different estimations were also considered for the following studies: the study of Osendrap et al (2002) that analyzed three groups of infants (all, infants with low serum Zn < 9.18 μmol/L, and infants with normal serum Zn > 9.18 μmol/L) and the study of Arsenault et al (2008) that assessed two groups (zinc intake in a liquid supplement and in a fortified porridge).

If dietary intake of Zn (in addition to the intervention) was not reported in the RCTs we imputed a value of 1.3 mg/day, the mean dietary intake level of the RCTs that did report dietary zinc intake.

As mean baseline growth parameters were infrequently reported in the RCTs, most of the RCTs assumed no differences in baseline measures (n= 16). Arsenault and Rivera et al (2008; 1998) performed an adjustment for initial differences. Only the study of Bates et al (Bates et al. 1993) failed to mention anything regarding this matter.

Exposure and outcome and other covariates assessment:

The influence of Zn intake on growth parameters was considered in the overall meta-analysis. From each select growth study, final measures were assessed: Weight, Length, MUAC (Mid upper arm circumference), Head circumference, WAZ (Weight for age z-score), LAZ (Length for age z-score) and WLZ (Weight for Length z-score) in all the included studies.

Other variables were also taken into account as possible effect modifiers. We considered doses of zinc intake (1 to 4 mg, from 4.1 to 8 mg, from 8.1 to 12 mg and >12.1 mg), intervention duration (1 to 3 weeks, 4 to 20 weeks, and > 20 weeks), nutritional situation (poor nutritional status, nutritionally at risk and healthy) and risk of bias (low, moderate or high).

Poor nutritional status was defined as infants with low birth weight during their first year, undernourished or current growth retardation evidenced by a WAZ and LAZ scores below -2; nutritionally at risk was defined as infants who lived in low income families with a low socioeconomic situation.

Risk of bias was developed in order to assess the quality of the studies included. The following indicators of internal validity specific to the RCT methodology were collected during data extraction: 1) method of sequence generation and allocation, 2) blinding, 3) potential funding bias, 4) number of participants at start, 5) dropouts and dropout reasons, 6) dose check, 7) dietary intake data reported, 8) outcome comparability and reproducibility, and 9) similarity of most and least exposed groups at baseline. Based on these indicators, two reviewers assessed the overall risk of bias. Disagreements were resolved by discussion. The criteria for judging these indicators were adapted from the Cochrane Handbook (Higgins JPT & Green S 2009).

Statistical analysis

Mean and standard deviation (SD) or standard errors (SE) of the outcome (Weight, Length, MUAC, Head circumference, WAZ, LAZ and WLZ) were assessed. From the mean and SD of each study Beta Values (β) and their SE were calculated because the statistical model that we used to estimate the relation between zinc intake (x-variable) and growth (y- variable) is based on the assumption that this intake-growth linear relationship is a logarithmic function and that both intake and growth follow a log-normal distribution (the natural logarithm of intake and growth have a normal distribution). Thus, the expected value if the growth score is expressed as:

$\mu y = \beta * \mu x + \text{intercept}$

where μy represents the mean of the natural logarithm of the y–variable (= growth score), β represents the regression coefficient, and μx represents the mean of the natural logarithm of the x-variable (= Zn intake).

This shape of this linear relationship on the $\log_e$–$\log_e$-scale corresponds to a monotonic concave function on the original scale for $\beta < 1$. This shape is assumed to be realistic for the biological relationship between zinc intake and growth parameters. As the true dose-response curve is unknown, this approximation provides a practical methodology to estimate the dose-response relationship.

The method used to systematically review differences was a formal meta–analysis (Greenland 1998).

Procedures of formal meta-analysis have been applied to combine the results from previously reported studies (Dickersin 2002).

A random-effects model was considered to be more appropriate than a fixed-effects model. We used the DerSimonian and Laird's (DerSimonian & Laird 1986) to pool the estimates of betas across studies. Under this model, the pooled effect was the beta in the growth parameters (Weight, Length, MUAC, Head circumference, WAZ, LAZ and WLZ), for an increment of 1 unit in Zn

intake. A pooled beta estimate was calculated as a weighted average of the beta reported in each study.

The formula we used to estimate the weighted effect size was (Hedges 1982):

$\beta\ pooled = \sum \beta i\ wi\ / \sum wi$

where $\beta\ pooled$ is the pooled estimate of the beta in growth parameters; the weight *(wi)* of each study was computed as:

$wi = 1 / Vi + \varsigma^2$

where V is the variance of each study and ς^2 is the inter study variance.

Besides this, we calculated a 95% confidence interval for the pooled estimated of effect size:

95% CI= $\beta\ pooled \pm$ (1.96 x SE pooled)

where SE is the standard error of the pooled estimate (Greenland 1998).

A test of heterogeneity was calculated, estimating Q statistics, which follows a chi-square distribution with degrees of freedom n-1, n being the number of studies included in the analysis. The I^2 Index measures the extent of the heterogeneity. A low P value for this statistic (lower than 0.05) indicates the presence of heterogeneity, which somewhat compromises the validity of the pooled estimates (Takkouche et al. 1999).

Because significant heterogeneity was clearly evident in the pooled beta estimates for Length studies, we evaluated potential sources of heterogeneity by linear meta-regressions (Greenland 1998).

We fitted a meta-regression using the duration of the intervention, the doses of zinc intake, the risk of bias and the nutritional situation as independent variables. The betas obtained in each study for the Length parameter according to Zn intake were used as the dependent variable.

Statistical differences in multivariate adjusted mean beta values between each possible heterogeneity sources were determined by ANCOVA.

Additionally we carried out additional meta-analyses by subgroups considering only that groups which provided significant values in the meta-regression.

Sensitivity analyses were also conducted. We excluded the studies considered outliers and recalculated the pool estimate of the beta in each growth parameter.

Microsoft Excel Version (7.0), SPSS 10.0 for Windows and Review Manager 5.1, were used to conduct the statistical analyses.

Results

Five thousand five hundred articles were identified in the initial search strategy. After applying the exclusion / inclusion criteria 344 articles from the search appeared to be potentially relevant. After applying the additional eligibility criteria and grouping the studies by outcome, 19 randomized controlled trials (38 estimations) were selected for the growth meta-analysis (Arsenault et al. 2008; Bates et al. 1993; Berger et al. 2006; Dijkhuizen et al. 2001; Fischer Walker et al. 2009; Gardner et al. 2005; Hamadani et al. 2001; Heinig et al. 2006; Lind et al. 2004; Meeks Gardner et al. 1998; Müller et al. 2003; Ninh et al. 1996; Olney et al. 2006; Osendarp et al. 2002; Rivera et al. 1998; Sur et al. 2003; Umeta et al. 2000; Walravens et al. 1989; Wasantwisut et al. 2006). (Figure 1)

Descriptive characteristics of the studies included in the meta-analysis are presented in Table 1.

Of the 19 studies included, only 12 comply strictly with the age infants (0 to 12 months) (Arsenault et al. 2008; Berger et al. 2006; Dijkhuizen et al. 2001; Fischer Walker et al. 2009; Heinig et al. 2006; Lind et al. 2004; Olney et al. 2006; Osendarp et al. 2002; Rivera et al. 1998; Sur et al. 2003; Umeta et al. 2000; Wasantwisut et al. 2006). The other 7 studies include this age among their children, but do not detail how many are actually from 0 to 12 month (Bates et al. 1993; Gardner et al. 2005; Hamadani et al. 2001; Meeks Gardner et al. 1998; Müller et al. 2003; Ninh et al. 1996; Walravens et al. 1989). None of the age extends beyond 27 month, except for Gardner and Ninh et al (2005; 1996), which includes children up to 30 and 36 months respectively.

Four studies were from Latin America and the Caribbean, 2 from North America, 9 from Asia and 4 from Africa. The duration of the interventions ranged from 1 to 60 weeks. Some studies assessed growth parameters at several time points within the study (Hamadani et al. 2001; Heinig et al. 2006; Sur et al. 2003). Doses of zinc intake ranged from 1.78 to 20 mg per day. The nutritional situation of infants varied also between studies: six studies were conducted within healthy infants (Bates et al. 1993; Heinig et al. 2006; Lind et al. 2004; Osendarp et al. 2002; Umeta et al. 2000; Wasantwisut et al. 2006), six studies with infants who were nutritionally at risk (Arsenault et al. 2008; Berger et al. 2006; Fischer Walker et al. 2009; Müller et al. 2003; Rivera et al. 1998; Walravens et al. 1989), and seven studies were carried out within infants with poor nutritional status (Dijkhuizen et al. 2001; Gardner et al. 2005; Hamadani et al. 2001; Meeks Gardner et al. 1998; Ninh et al. 1996; Olney et al. 2006; Sur et al. 2003). The risk of bias varied also between studies: six studies had a high risk of bias, seven had a moderate risk and six showed a low risk of bias.

Differences in Growth outcomes (Weight, Length, MUAC, Head Circumference, WAZ, LAZ and WLZ) according to Zn intake in each particular study and in the pooled analyses are showed in Figures 2 to 8. Zn intake was not associated to Weight, Length, MUAC, Head Circumference and LAZ in the pooled analyses. However, Zn intake had a positive and statistically effect on WAZ (β = 0.06; 95 % CI 0.02 to 0.10) and WLZ (β = 0.05; 95 % CI 0.01 to 0.08).

Since we applied a base-*e* logarithmic transformation on the zinc intake and growth parameters before calculation of the study-specific β´s, the overall β represents the difference in the $\log_e$transformed predicted value of WAZ and WLZ for each one-unit difference in the $\log_e$transformed value in zinc intake. Therefore, an overall β of 0.06 means that for every doubling in zinc intake, the difference in WAZ is 2^{β} ($2^{0.06}$ = 1.04). For an overall β of 0.05, the difference in WLZ is 1.035. That means that a person with a double intake of Zn has aproximately 4% higuer WAZ and WLZ than a person with the half intake. (Figure 9, 10)

Excepting for Length (I^2 = 45 %, p= 0.03), heterogeneity was not present in any analysis. In order to find out those variables which may be potential effect modifiers on Length, we performed a meta-

regression (Table 2). The effect of Zn supplementation on Length changed depending on the duration of the intervention and the dose (p ANCOVA= 0.008 and 0.023) respectively.

Table 3 shows the results of Length analyses after stratifying the studies according to the effect modifiers identified in the meta-regression. After stratifying by duration of the intervention and by dose, the heterogeneity disappeared.

At short time (4 to 20 weeks), Zn intake was positively associated with length values (β=0.01; CI 95% 0 to 0.02). However, no effect was found when supplementation lasted for more than 20 weeks (β = -0.001; CI 95% -0.003 to 0.002).

At medium doses of Zn (4.1 to 8 mg/day), Zn intake was positively associated with length values (β= 0.003; CI 95% 0 to 0.01). Nevertheless, the effect magnitude was small. However, no effect was found at low or high doses of Zn (1 to 4 or >12 mg/day) (β= 0; CI 95% -0.01 to 0.004 and β= 0.01; CI 95% -0.02 to 0) respectively.

The results of the sensitivity analyses are shown in Table 4.

The study of Osendrap et al (b) (2002), Walravens et al (a) and Walravens et al (b) (1989) were considered as outliers in the analysis of weight because the limits of beta were very wide (from CI 95% 0.01 to 0.23; CI 95% -0.04 to 0.10 and CI 95% -0.04 to 0.10) respectively. When we excluded these studies, the null association previously seen remained.

In WAZ studies the study of Sur et al (d, e, f, g, h, I, j, k, l) (2003) were considered as outliers for the same reasons. When we excluded these studies, we observed an attenuation of the positive effect of Zn supplementation on WAZ (β = 0.03; CI 95% 0 to 0.07).

The study of Osendrap et al (b) (2002) was considered as an outlier in the analysis of Length. When we excluded this study, the null association previously seen persisted and also the heterogeneity (I^2= 47%; p= 0.03).

Discussion

Our results indicate that zinc supplementation increases some growth parameters in infants. Zn intake had a positive and statistically effect on WAZ (β = 0.06; 95 % CI 0.02 to 0.10) and WLZ (β = 0.05; 95% CI 0.01 to 0.08). We only found significant heterogeneity while analysing length (I^2 = 45 %, p = 0.03). After stratifying by several factors, heterogeneity disappeared.

To our knowledge, this meta-analysis is the only in providing an estimate of the dose-response relationship of zinc intake and growth parameters in infants aged 1–12 months. An infant with a Zn intake of 10 mg/day has a WAZ and WLZ that is 4 % higher than an infant who has a zinc intake of 5 mg/day. However, interpretation of these results should be carefully considered for different reasons. It is a well known fact that when many comparisons are done, one or more might reach significance due to pure chance (Bland & Altman 1995). Although meta-analysis are increasingly used to consolidate results from multiple studies of the same topic and to develop evidence-based policies for clinical practice and public health programmes, the reliability of reached conclusions depends on the methodological quality of the original studies (such as the control of confounders), the appropriateness of the inclusion criteria, and the thoroughness of the review and synthesis of information (Brown et al. 2002). It is unlikely that confounding factors might have affected our results since all the studies included in our meta-analyses are RCT. However, if some baseline differences were observed because any failure in the randomize process, several authors performed an adjustment of initial differences (Arsenault et al and Rivera et al) (Arsenault et al. 2008; Rivera et al. 1998). Other authors assumed no initial differences. The only exception was Bates et al (1993) that did not mention anything regarding this matter.

A limitation of this study was the small amount of studies included to measure growth parameters. Although 19 intervention trials of the effect of zinc supplementation on infant's growth were included, for the association between zinc intake and head circumference the meta-analysis only included 4 studies which led to a reduction of the statistical power to detect significant differences.

Age of the studies considering in this meta-analysis was other important point. We believe that there was no reason to exclude any study that did not adhere exclusively to the group of 0 to 12 months of age. For this reasons, we took into account all the studies which included this age group in the study, even if they were not analysed according to their age group (Bates et al. 1993; Gardner et al. 2005; Hamadani et al. 2001; Meeks Gardner et al. 1998; Müller et al. 2003; Ninh et al. 1996; Walravens et al. 1989) and assumed the consequences of this possible bias.

The small magnitude of effect that we observed might be due to some effect modifiers that should be considered whenever the effect of zinc deficiency on growth is being evaluated. Those include factors in close relation to infancy such as prematurity, low birth-weight, breastfeeding, protein energy malnutrition, infectious morbidity, poverty, and social deprivation, the pre-existing zinc status of the study subjects; and others directly related to zinc such as content and bioavailability of zinc in local diets. Moreover, methodological aspects of these studies, such as variations in the dose, chemical form, method of administration of zinc and duration of supplementation, may have influenced our results (Brown et al. 2002). Some of these aspects were analysed in the sub-groups analyses of the meta-analysis. Duration of the intervention in some studies was other possible explication to the small magnitude of the effect founded. Time of the intervention appears to be too short to obtain a positive impact on growth (Heinig et al. 2006; Lind et al. 2004; Meeks Gardner et al. 1998; Sur et al. 2003). However, this becomes more relevant when studies are conducted in LBW infants because the low weight together with the immaturity associated with premature infants requires adjustment of gestational age with chronological age for proper assessment of catch-up growth (Rugolo 2005). This is the case of the Meeks Gardner and Sur´s studies (Meeks Gardner 1998; Sur et al. 2003) which were conducted in infants with a poor nutritional status. Nonetheless, on healthy infants Lind et al (2004) reported improvements in growth in a 3 month period. Opposite results were obtained by Bates et al and Heinig et al (1993; 2006) which failed to observe a positive effect in longer periods of time. Also Rivera et al and Umeta et al (1998; 2000) found that MUAC did not change due to the extension of the supplementation period which was

apparently too short to find any measurable effect. Thus, clinical trials are required to analyze the long-term effects on growth of zinc supplementation before reaching significant conclusions.

Other consideration to take into account is that the data from our meta-analysis was obtained generally from countries in a developing stage and included data from LBW and malnourished infants which might have resulted in the poor effect we found. Several studies have been carried out worldwide and many of these showed a positive effect of zinc supplementation on growth among groups of children who were nutritionally disadvantaged in some way, including stunted children (Walravens et al. 1989; Wasantwisut et al. 2006), and in particular among malnourished children (Arsenault et al. 2008; Ninh et al. 1996; Rivera et al. 1998). On the other hand, there was no growth response to supplementation in healthy Gambian nor healthy USA infants (Bates et al. 1993; Heinig et al. 2006). A meta-analysis of 25 studies (Brown et al. 1998) of zinc supplements on growth of children in developing countries found smaller but significant effects on growth (an effect size of +0.22 for height and +0.26 for weight increments). However, an updated version of that meta-analysis (Brown et al. 2002) based on 33 randomized controlled trials, showed a highly significant aggregate effect size of 0.350 (95% CI: 0.189, 0.511) for height, 0.309 (95% CI: 0.178, 0.439) for weight, and ≈0 for WLZ increments. Thus zinc supplementation on child growth has been studied extensively in developing countries, but relatively little information is available from industrialized ones (Brown et al. 2002). Therefore, it is unclear whether children in industrialized countries would benefit from increased zinc intakes.

In conclusion, our meta-analyses provided us with an estimate of the dose-response relation between zinc intake and some growth's parameters (WAZ and WLZ) in infant population. These data can be used as complementary evidence for underpinning zinc reference values, although restrictions on extrapolation of our results to other populations should be acknowledged mainly to developed populations.

For the others growth's parameters included in the meta-analyses, no effect was found. Further standardized research is urgently needed to reach evidence-based conclusions to clarify the role of zinc supplementation upon infant growth mainly in Western populations.

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The authors´ responsibilities were as follows: MN: analysis of the data and writing the manuscript, JDA: contribution to selection of papers and data extraction, ASV: support in data-analysis, DFL, PHS, JDA, LPQ, CR and LSM provision of significant advice. All authors directly participated in the planning, execution or analysis of the study and reviewed the manuscript.

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Figure 1: Flow diagram for the systematic review.

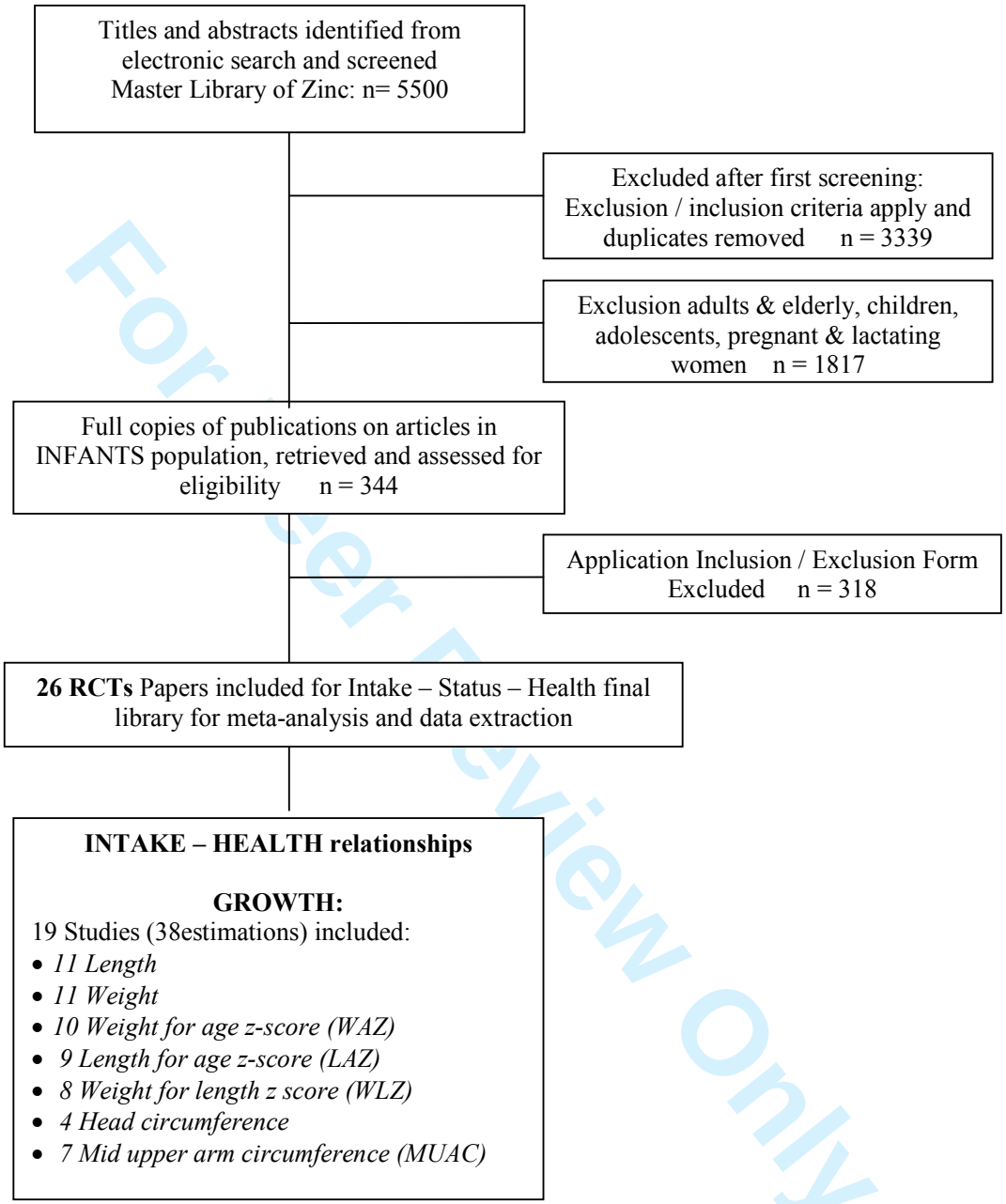

Figure 2: Growth – Weight –

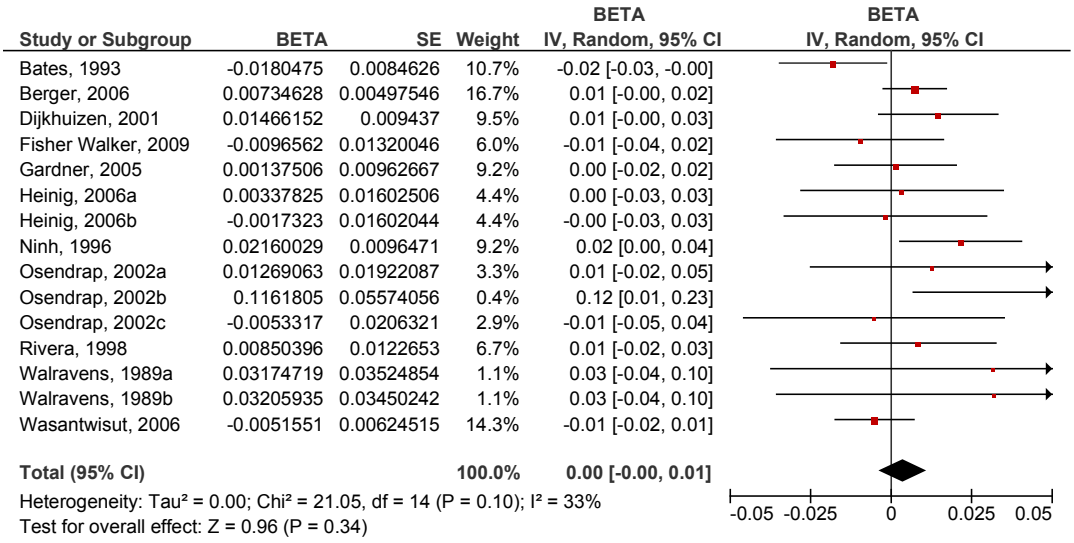

Figure 3: Growth – Length –

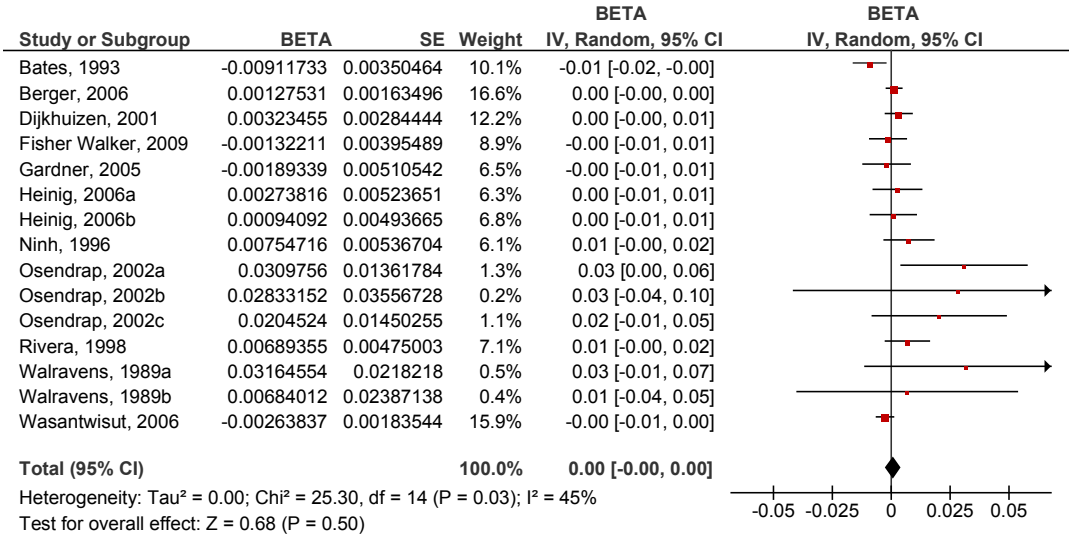

Figure 4: Growth – Head Circunference –

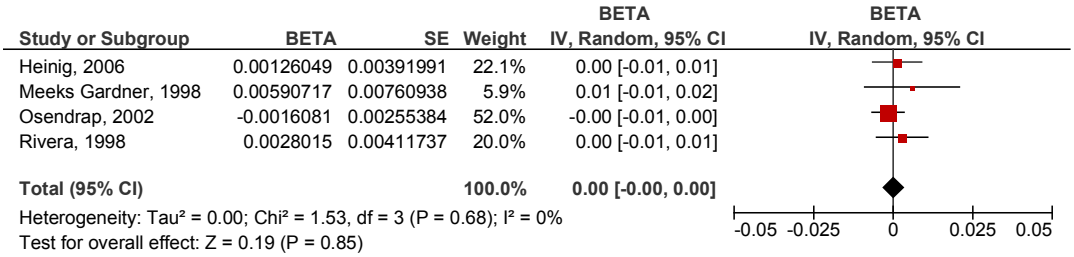

Figure 5: Growth –MUAC (Mid upper arm circumference)

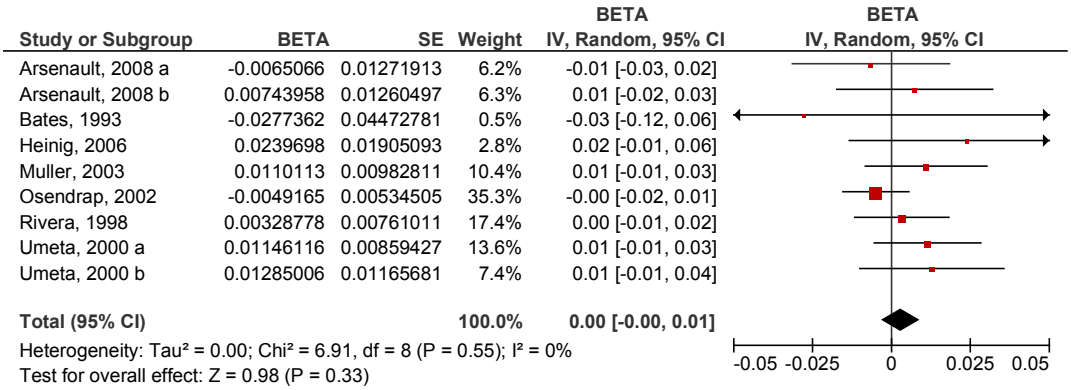

Figure 6: Growth – WAZ (Weight for age Z – score)

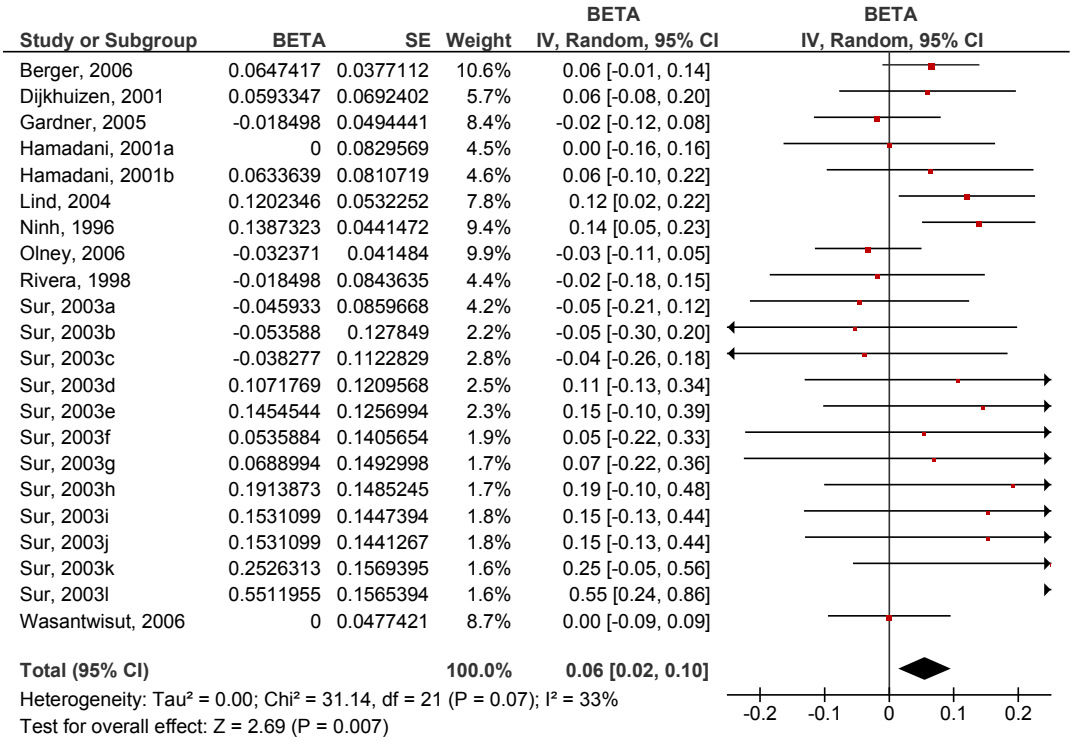

Figure 7: Growth – LAZ (Length for age Z- score)

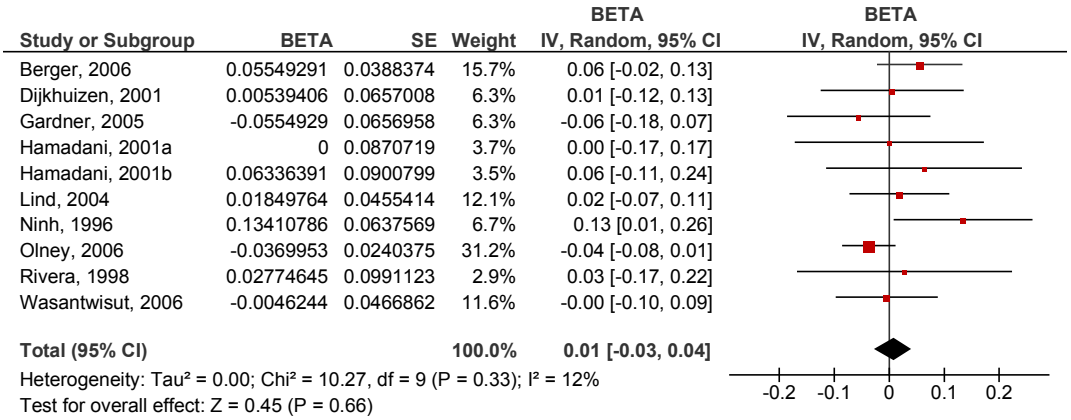

Figure 8: Growth – WLZ (Weight for length Z- score)

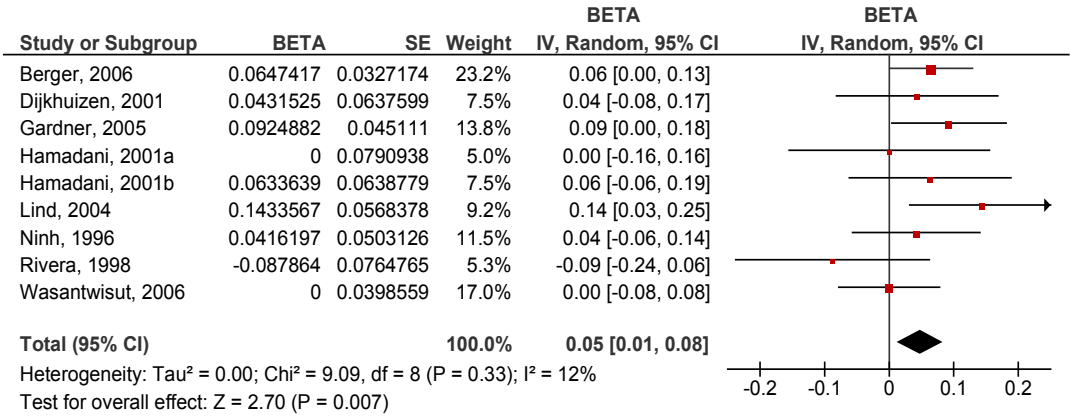

Figure 9: WAZ (Kg/age z-score) as a function of dietary zinc intake (mg/day), estimated by random effects meta-analyses of RCTs of infants.

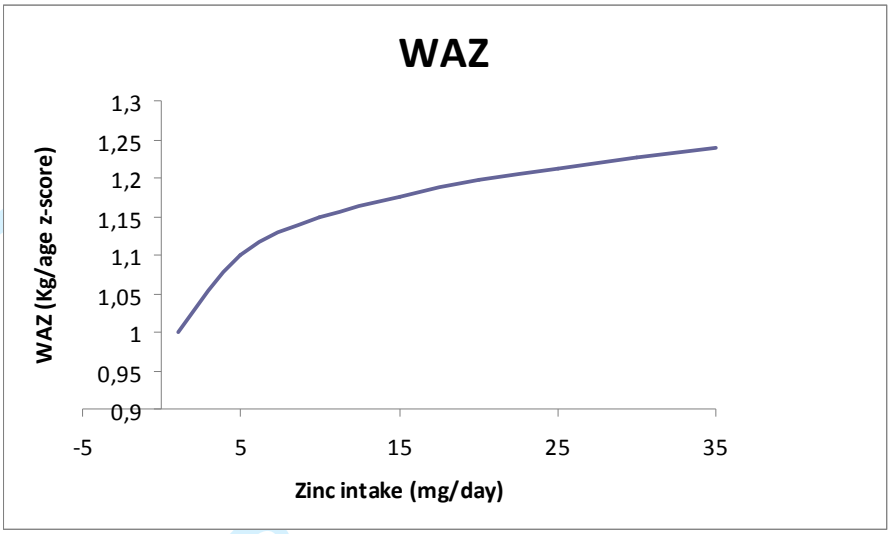

Figure 10: WLZ (Kg/Length z-score) as a function of dietary zinc intake (mg/day), estimated by random effects meta-analyses of RCTs of infants.

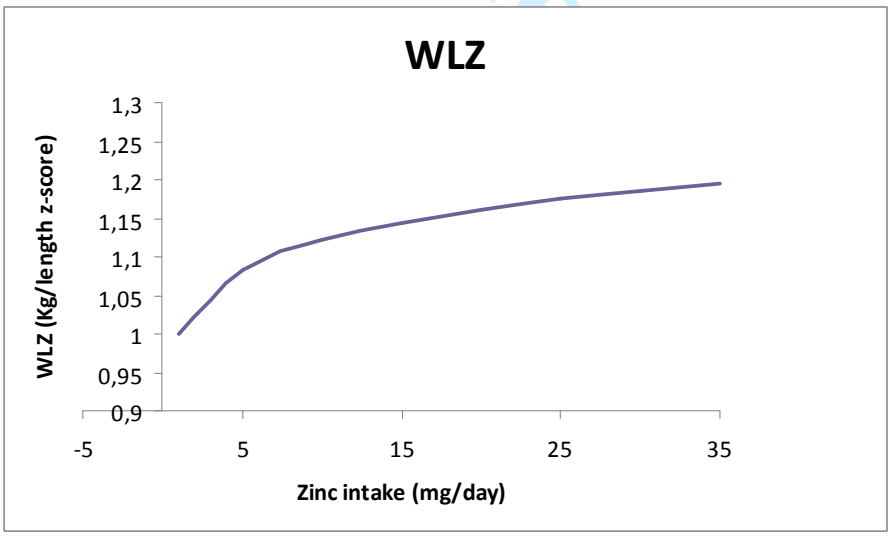

Table 1: Characteristics of the 19 (38 estimations) Growth studies included in the meta-analysis

Author	Study year	Country	Sample (age range)	Number of Infants (n)		Doses of Zinc	Weeks of the intervention	Outcome	Nutritional situation	Risk of bias[2]
				Zn[1]	C[1]					
Arsenault (a)	2008	Peru	6 to 8 months	44	36	3 mg in a liquid supplement	24 w	Growth: MUAC	Nutritionally at risk	High risk
(b)				44	38	3 mg in a fortified porridge				
Bates	1993	Gambia	5.7 to 27 months	50	53	20 mg	60 w	Growth: Weight - Length MUAC	Healthy	High risk
Berger	2006	Vietnam	4 to 7 months	195	191	10 mg	24 w	Growth: Weight - Length WAZ – LAZ - WLZ	Nutritionally at risk	Moderate risk
Dijkhuizen	2001	Indonesia	Mean 4.2 months	90	98	7 mg	24 w	Growth: Weight- Length WAZ – LAZ – WLZ	Poor nutritional status	Moderate risk
Fischer Walker	2009	Bangladesh	6.3 ± 0.3 months	140	141	2,8 mg	24 w	Growth: Weight - Length	Nutritionally at risk	Moderate risk
Gardner	2005	Jamaica	9 to 30 months	59	55	10 mg	24 w	Growth: Weight - Length WAZ – LAZ - WLZ	Poor nutritional status	Moderate risk
Hamadani (a)	2001	Bangladesh	1 to 13 months	109	103	5 mg	28 w	Growth: WAZ – LAZ WLZ	Poor nutritional status	High risk
(b)				101	97		52 w			
Heinig (a)	2006	USA	3 to 10 months	37	33	5 mg	16 w	Growth: Weight - Length MUAC	Healthy	Low risk
(b)				37	33		40 w	Head Circumference		
For Head Circunf.				37	33		24 w			
Lind	2004	Indonesia	6 to 12 months	164	163	10 mg	8 w	Growth: WAZ - LAZ- WLZ	Healthy	Low risk
Meeks Gardner	1998	Jamaica	6 to 24 months	24	31	5 mg	12 w	Growth: Head Circumference	Poor nutritional status	Moderate risk

Muller	2003	Burkina Faso	Zn group: 18.7 ± 7.0 mo Placebo group: 17.6 ± 6.5 mo	329	332	1,78 mg	24 w	Growth: MUAC	Nutritionally at risk	Low risk
Ninh	1996	Vietnam	4 to 36 months	73	73	10 mg	20 w	Growth: Weight - Length WAZ – LAZ - WLZ	Poor nutritional status	Low risk
Olney	2006	Zanzibar	5 to 12 months	58	44	10 mg	24 w	Growth: WAZ – LAZ	Poor nutritional status	High risk
Osendarp (a) (b) low serum zn < 9.18 µmol/L (c) normal serum zn > 9.18 µmol/L	2002	Bangladesh	3 to 5 weeks	133 16 115	138 21 117	5 mg	20 w	Growth: Weight - Length MUAC Head Circumference	Healthy	Moderate risk
Rivera	1998	Guatemala	6 to 9 months	44	45	10 mg	28 w	Growth: Weight - Length WAZ – LAZ - WLZ- MUAC Head Circumference	Nutritionally at risk	High risk
Sur (a) (b) (c) (d) (e) (f) (g) (h) (i) (j) (k) (l)	2003	India	0 to 12 months	50 50 50 50 50 50 50 50 50 50 50 50	50 50 50 50 50 50 50 50 50 50 50 50	3,57 mg 3,57 mg 3,57 mg 3,57 mg 3,57 mg 3,57 mg 3,57 mg 3,57 mg 3,57 mg 3,57 mg 3,57 mg 3,57 mg	1 w 2 w 3 w 4 w 5 w 6 w 7 w 8 w 9 w 10 w 11 w 12 w	Growth : WAZ	Poor nutritional status	Moderate risk
Umeta (a) (b)	2000	Ethiopia	Zinc stunted: 9.5 ± 2.0 mo Placebo stunted: 9.7 ± 2.0 mo Zinc non stunted: 9.3 ± 2.1 mo Placebo non stunted: 9.2 ± 2.0 mo	47 45	47 45	8,57 mg	24 w	Growth: MUAC	Healthy (stunted – non stunted)	High risk
Walravens (a) Boys (b) Girls	1989	USA	8 to 27 months	13 12	13 12	5,7 mg 5,7 mg	24 w	Growth: Weight - Length	Nutritionally at risk	Low risk
Wasantwisut	2006	Thailand	4 to 6 months	153	151	10 mg	24w	Growth: Weight - Length WAZ – LAZ - WLZ	Healthy	Low risk

Zn[1]: Zinc Group; C[1]: Control group
[2] Low risk of bias meant that the study was randomized, the randomization method was at least partially described, reasons for and numbers of dropouts were stated (or there were no dropouts), and the method used to assess compliance and some assessment of compliance were reported. All others studies were considered as moderate when they meet any of the above criteria or high risk of bias when they meet any of the criteria. (Higgins 2009, Cochrane Handbook)
(a - l): Sub-studies
MUAC: Mid upper arm circumference
WAZ: Weight for age z – score
LAZ: Length for age z- score
WLZ: Weight for length z-score

Table 2: Meta-regression. Multivariate adjusted mean beta for Growth (Length) (95% confidence interval) by different characteristics of the studies included in the meta-analysis

	n	Mean Beta's	CI (95%)	P Ancova*
Growth: Length				
By time				
4 to 20 weeks	5	0.0113	0.008 to 0.0219	
> 20 weeks	10	-0.0026	-0.0089 to 0.0037	
				0.008
By Dose				
1 to 4 mg	1	-0.0058	-0.0245 to 0.0130	
4,1 to 8 mg	8	0.0162	0.0078 to 0.0245	
8,1 to 12 mg	5	0.0057	-0.0016 to 0.0130	
> 12 mg	1	0.0014	-0.0200 to 0.0229	
				0.023
By Nutritional situation				
Healthy	7	0.0010	-0.0066 to 0.0086	
Nutritionally at risk	5	0.0128	0.0025 to 0.0230	
Poor nutritional situation	3	-0.0006	-0.0122 to 0.0110	
				0.083
By Risk of Bias				
Low	6	0.0016	-0.0084 to 0.0116	
Moderate	7	0.0074	-0.0013 to 0.0161	
High	2	0.0042	-0.0105 to 0.0189	
				0.409

* Adjusted for the rest of variables in the table

Table 3: Pooled beta (95% confidence intervals) for Growth according to the intervention group. Subgroup analyses.

	Pooled estimates (β)	Chi² (df, P)	I² *
Growth: Length			
All Studies (n=15)	0.001 (-0.002 to 0.004)	25.30 (14, 0.03)	45%
By time			
4 to 20 weeks (n=5)	0.01 (0 to 0.02)	4.93 (4, 0.29)	19%
> 20 weeks	-0.001 (-0.003 to 0.002)	15.18 (9, 0.09)	41%
By dose			
1 to 4 mg (n=1)	0 (-0.01 to 0.01)		
4,1 to 8 mg (n=8)	0.003 (0 to 0.01)	7.81 (7, 0.35)	10%
8,1 to 12 mg (n=5)	0 (-0.002 to 0.004)	6.85 (4, 0.14)	42%
> 12 mg (n=1)	0.01 (-0.02 to 0)		

*I² Index measures the extent of the heterogeneity

Table 4: Pooled beta (95% confidence intervals) for Growth according to the intervention group. Sensitivity Analyses

	Pooled estimates (β)	Chi² (dif, P)	I²
Growth: Weight			
All studies (n=15)	0.004 (-0.004 to 0.01)	21.05 (14, 0.10)	33%
All Studies excluding (n=3)	0 (-0.005 to 0.01)	15.53 (11, 0.16)	29%
Osendrap et al. 2002 b	0.12 (0.01 to 0.23)		
Walravens et al. 1989 a	0.03 (-0.04 to 0.10)		
Walravens et al. 1989 b	0.03 (-0.04 to 0.10)		
Growth: MUAC			
All studies (n=9)	0.003 (-0.003 to 0.01)	6.91 (8, 0.55)	0%
All Studies excluding (n=1)	0 (-0.003 to 0.01)	6.43 (7, 0.49)	0%
Bates et al.1993	-0.03 (-0.12 to 0.06)		
Growth: WAZ			
All studies (n=22)	0.06 (0.02 to 0.10)	31.14 (21, 0.07)	33%
All Studies excluding (n=9)	0.03 (0 to 0.07)	15.67 (12, 0.21)	23%
Sur et al. 2003 d	0.11 (-0.13 to 0.34)		
Sur et al. 2003 e	0.15 (-0.10 to 0.39)		
Sur et al. 2003 f	0.05 (-0.22 to 0.33)		
Sur et al. 2003 g	0.07 (-0.22 to 0.36)		
Sur et al. 2003 h	0.19 (-0.10 to 0.48)		
Sur et al. 2003 i	0.15 (-0.13 to 0.44)		
Sur et al. 2003 j	0.15 (-0.13 to 0.44)		
Sur et al. 2003 k	0.25 (-0.05 to 0.56)		
Sur et al. 2003 l	0.55 (0.24 to 0.86)		
Growth: Length			
All studies (n=15)	0.001 (-0.002 to 0.004)	25.30 (14, 0.03)	45%
All Studies excluding (n=1)	0 (-0.002 to 0.004)	24.67 (13, 0.03)	47%
Osendrap et al. 2002 b	0.03 (-0.04 to 0.10)		

I^2 Index measures the extent of the heterogeneity

Effect of zinc intake on neurodevelopment in infants: A meta-analysis

Enviado a: Maternal & Child Nutrition: Julio 2012

(Segunda revisión)

Effect of zinc intake on neurodevelopment in infants: A meta-analysis.

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Abstract

A systematic review and meta-analysis of available RCTs was conducted to evaluate the effect of zinc intake on neurodevelopment in infants. After applying the exclusion / inclusion criteria, 5 RCTs were selected. The influence of zinc intake on neurodevelopment was considered in the overall meta-analysis. Other variables were also taken into account as possible effect modifiers: doses of zinc intake, intervention duration, nutritional status and risk of bias. Indices of neurodevelopment assessed in the included studies were MDI (Mental Development Index) and PDI (Psychomotor Development Index). The method used to systematically review differences was a formal meta–analysis. Additionally we carried out a sensitivity analysis.

RESULTS: The pooled β was -0.01 (95%CI -0.02, 0) for MDI and 0 (95%CI -0.02, 0.02) for PDI, with a substantial heterogeneity in both analyses. When we performed a meta-regression, the effect of Zn supplementation on MDI changed depending on the dose (p ANCOVA= 0.002). Regarding PDI, there was a differential effect of Zn intake depending on duration of the intervention, dose, nutritional status and risk of bias.

Zn supplementation showed a negative, weak and significant effect on PDI score in those studies with a length of 4 to 20 weeks (β= -0.05; CI 95% -0.06 to -0.04).

In conclusion, a negative but not statistically significant association was found between zinc intake and neurodevelopment. The magnitude of effect was small in all cases. Further standardized research is urgently needed to clarify the role of zinc supplementation upon infant neurodevelopment, particularly in Europe.

Keywords: EURRECA, zinc intake, neurodevelopment, infants

Introduction

Our perception of zinc (Zn) has progressed from that of a rather obscure essential trace mineral of doubtful significance for human health to that of a micronutrient of exceptional biologic and public health importance, in a remarkably short time. This is most evident in relation to both prenatal and postnatal development (Hambidge KM & Krebs NF 2007).

Zn is an essential nutrient, present in all body tissues and fluids. The biologic role of Zn is now recognized in structure and function of proteins, including more than 300 enzymes, transcription factors, hormonal receptor sites, and biologic membranes. Zn has numerous central roles in DNA and RNA metabolism (MacDonald RS 2000), and it is involved in signal transduction, gene expression, and apoptosis. Zn enzymes are involved in nucleic acid metabolism and cellular proliferation, differentiation and growth (Hambidge KM & Krebs NF 2007).

Zn is also present in the brain and contributes to its structure and function. Limited evidence from animal and human studies suggests that its deficiency may lead to delays in cognitive development. Severe Zn deficiency in animals has been associated with structural malformations of the brain, such as anencephaly, microcephaly and hydrocephaly; with behavioural problems, such as reduced activity and deficits in short-term memory and spatial learning. In humans, severe Zn deficiency can cause abnormal cerebellar function and impaired behavioural and emotional responses (Black MM 1998).

Although the mechanisms linking Zn deficiency with cognitive development remain unclear, it appears that Zn deficiency may lead to deficits infant's neuropsychological functioning, activity, or motor development, and thus interfere with cognitive performance (Black MM 1998).

A considerable number of intervention trials have been completed in multiple countries to assess the effect of supplemental Zn on infant's neurodevelopment. However, these studies have yielded inconsistent results, possibly because of differences in the pre-existing Zn status of the study subjects, in the content and bioavailability of Zn in the local diets, and in the incidence of common infections that can affect growth independently of an individual's Zn status. Moreover, methodological aspects of these studies, such as variations in the dose, in the method of administration or in the duration of supplementation, may have influenced their results (Brown KH et al. 2002). Finally, in some cases, the sample sizes may have been inadequate to detect potentially important differences in growth with statistical confidence.

To our knowledge meta-analytic methods have not yet been used to evaluate the influence of zinc intake on infant neurodevelopment. This paper presents a systematic review of the data from all available randomized controlled trials (RCTs) meeting EURRECA's quality standard (Matthys C et al. 2011) that assessed the effect of zinc supplementation on infant neurodevelopment.

Materials and Methods

Search strategy

This research was conducted within the framework of the European Micronutrient Recommendations Aligned (EURRECA) Network of Excellence that aims to identify the micronutrient requirements for optimal health in European populations (www.eurreca.org). Databases searched were OVID MEDLINE, OVID EMBASE and Cochrane Library CENTRAL databases from inception to February 2010. The procedure for the identification, selection of articles and data extraction is illustrated in Figure 1.

A search strategy was established to identify the most relevant studies in the electronic databases, using text terms with appropriate truncation and relevant indexing terms.The electronic search used the following keywords: "randomized controlled trial", "double-blind procedure", "human", "zinc or zn or zinc sulphate or zinc gluconate or zinc acetate or methionine or zinc isotope", "intake or diet or supplement or deplete or status or serum or plasma or leukocyte or concentration or fortify", "Nutritional Support or Dietary Supplements or nutritional requirements or Breast feeding or infant food or bottle feeding or infant formula", "Nutritional Status or Deficiency Diseases or supplementation or diet supplementation or dietary intake or diet restriction or mineral intake or Diet or Food, Fortified or nutrition assessment or Nutritive Value". Languages included were restricted to those spoken in the EURRECA Network (English, Dutch, French, German, Hungarian, Italian, Norwegian, Polish, Spanish, Greek, and Serbian.). Reference lists of retrieved articles and published literature reviews were also checked for relevant studies.

Selection of articles

Titles of articles identified from the searches were entered into an EndNote library. Papers were considered eligible for inclusion if they were RCTs, conducted in human infants (aged 0-12 months), and studied the effect of supplements, fortified foods or micronutrient intake from natural food sources on neurodevelopment.

Zinc intake was assessed from breast milk, infant formula and food sources (e.g. complementary foods), fortified foods (e.g. fortified formula or cereal) and supplements.

Exclusion criteria applied were: studies conducted in animals; combined interventions e.g. >1 micronutrient or micronutrient + lifestyle intervention which did not study the effect of the micronutrient separately; non primary studies (e.g. letters & narrative literature reviews); studies where the zinc intake – cognitive development/functioning association was not reported; or health outcomes other than neurodevelopment assessed.

Briefly, titles and abstracts of the 10% of the library were screened in duplicate for eligibility by two reviewers. Only when both reviewers agreed that titles and abstracts met the inclusion criteria were the articles included. When a title and abstract could not be included with certainty, the full text of the article was obtained and then further evaluated. The remaining 90% was distributed among the reviewers in even parts.

Following the initial screening process, full-text articles were obtained. Further inclusion and exclusion criteria were then applied. Papers were only included in the meta-analysis if they were: randomised controlled trials, had an intervention duration of at least 2 weeks, and reported baseline data for all outcome measures. Non-randomised controlled trials, uncontrolled trials or trials with insufficient or unclear data reported were excluded.

Data was extracted from each study and organized in a Microsoft Access database file (Microsoft Corp, Redmond, WA).

Data synthesis

When neurodevelopment was measured at different time points within the same population, we used all measured as different analysis or estimations (Ashworth A et al. 1998; Castillo-Duran C et al. 2001; Hamadani JD et al. 2001; Jiménez R et al. 2007).

In one study (Ashworth A et al. 1998) doses of Zn was different in two groups, and in each group, the duration of the intervention also change.

If dietary intake of Zn (in addition to the intervention) was not reported in the RCTs we imputed a value of 1.3 mg/day, the mean dietary intake level of the RCTs that did report dietary zinc intake. As mean baseline MDI or PDI were infrequently reported in the RCTs, most of the RCTs assumed no differences in baseline measures (Castillo-Duran C et al. 2001; Hamadani JD et al. 2001; Lind T et al. 2004). Ashworth et al performed an adjustment for initial differences. Only one study of Jimenez et al failed to mention anything regarding this matter.

Exposure and outcome and other covariates assessment:

The influence of Zn intake on infant neurodevelopment measures was considered in a pooled meta-analysis. Other variables were also taken into account as possible effect modifiers. We considered doses of zinc intake (1 to 4 mg, from 4.1 to 8 mg, from 8.1 to 12 mg and >12.1 mg), intervention duration (1 to 3 weeks, 4 to 20 weeks, and > 20 weeks), nutritional situation (healthy; nutritionally at risk, and poor nutritional status) and risk of bias (low, moderate or high).

Nnutritionally at risk was defined as infants who lived in low income families with a low socioeconomic situation, and poor nutritional status was defined as infants with protein energy malnutrition (PEM), but without congenital abnormalities or cerebral palsy or heart disease, or infants with low birth weight during their first year.

Risk of bias was developed in order to assess the quality of the studies included. The following indicators of internal validity specific to the RCT methodology were collected during data extraction: 1) method of sequence generation and allocation, 2) blinding, 3) potential funding bias, 4) number of participants at start, 5) dropouts and dropout reasons, 6) dose check, 7) dietary intake data reported, 8) outcome comparability and reproducibility, and 9) similarity of most and least exposed groups at baseline. Based on these indicators, two reviewers assessed the overall risk of bias. Disagreements were resolved by discussion. The criteria for judging these indicators were adapted from the Cochrane Handbook (Higgins JPT & Green S 2009).

Indices of neurodevelopment assessed in the included studies were MDI (Mental Development Index) and PDI (Psychomotor Development Index). Both indices were measured by Bayley Scales of Infant Development II in all studies.

Statistical analysis

Mean and standard deviation (SD) or standard errors (SE) of the outcome (MDI and PDI Index) were assessed. From the mean and SD of each study Beta Values (β) and their SE were calculated because the statistical model that we used to estimate the relation between zinc intake (x-variable) and neurodevelopment (y- variable) is based on the assumption that this intake – neurodevelopment curve is a logarithmic function and that both intake and neurodevelopment follow a log-normal distribution (the natural logarithm of intake and neurodevelopment have a normal distribution). Thus, the expected value if the neurodevelopment score is expressed as:

$\mu y = \beta * \mu x + intercept$

where μy represents the mean of the natural logarithm of the y–variable (= neurodevelopment score), β represents the regression coefficient, and μx represents the mean of the natural logarithm of the x-variable (= Zinc intake).

The method used to systematically review differences was a formal meta–analysis (Greenland S 1998). Procedures of formal meta-analysis have been applied to combine the results from previously reported studies (Dickersin K 2002).

A random-effects model was considered to be more appropriate than a fixed-effects model. We used the DerSimonian and Laird's (DerSimonian R. & Laird N 1986) to pool the estimates of betas across studies. Under this model, the pooled effect was the beta in the Neurodevelopment parameters (MDI – PDI), for an increment of 1 unit in Zn intake. A pooled beta estimate was calculated as a weighted average of the beta reported in each study.

The formula we used to estimate the weighted effect size was (Hedges LV 1982):

$\beta\ pooled = \sum \beta i\ wi\ / \sum wi$

where $\beta\ pooled$ is the pooled estimate of the beta in neurodevelopment parameters; the weight *(wi)* of each study was computed as: $wi = 1 / Vi + \tau^2$

where V is the variance of each study and τ^2 is the inter study variance.

Besides this, we calculated a 95% confidence interval for the pooled estimated of effect size:

95% CI= $\beta\ pooled \pm$ (1.96 x SE pooled)

where SE is the standard error of the pooled estimate (Greenland S 1998).

A test of heterogeneity was calculated, estimating Q statistics, which follows a chi-square distribution with degrees of freedom n-1, n being the number of studies included in the analysis. The I^2 Index measures the extent of the heterogeneity. A low P value for this statistic (lower than 0.05) indicates the presence of heterogeneity, which somewhat compromises the validity of the pooled estimates (Takkouche B et al 1999).

Because significant heterogeneity was clearly evident in the pooled beta estimates for all studies combined in each outcome, we evaluated potential sources of heterogeneity by linear meta-regressions (Greenland S 1998).

We fitted a meta-regression using the duration of the intervention, the doses of zinc intake, the nutritional situation and the risk of bias as independent variables. The betas of the different neurodevelopment parameters according to Zn intake were used as the dependent variable.

Statistical differences in multivariate adjusted mean beta values between each possible heterogeneity sources were determined by ANCOVA.

Additionally we carried out additional meta-analyses by subgroups considering only that groups which provided significant values in the meta-regression.

Sensitivity analyses were also conducted. We excluded the studies considered outliers and recalculated the pool estimate of the beta in each neurodevelopment parameter.

Microsoft Excel Version (7.0), SPSS 10.0 for Windows and Review Manager 5.1, were used to conduct the statistical analyses.

Results

Five thousand five hundred articles were identified in the initial search strategy. After applying the exclusion / inclusion criteria, 344 articles from the search appeared to be of potentially relevance. After applying the additional eligibility criteria and grouping the studies by outcome, 5 randomized controlled trials (9 estimations) were selected for the neurodevelopment meta-analysis (Ashworth A et al. 1998; Castillo-Duran C et al. 2001; Hamadani JD et al. 2001; Jiménez R et al. 2007; Lind T et al. 2004). (Figure 1)

Descriptive characteristics of the studies included in the meta-analysis are presented in Table 1.

Three studies were from Latin America and the Caribbean and two from Asia. The duration of the interventions ranged from 4 to 52 weeks. Doses of zinc varied from 1 to 10 mg per day. Age of infants also was different between 1 to 12 months. However, one study (Hamadani JD et al. 2001) with 13 months olds infants was included. The nutritional situation was also different between surveys: two studies were conducted on healthy infants (Castillo-Duran C et al. 2001; Lind T et al. 2004) and three were on infants with poor nutritional status (Ashworth A et al. 1998; Hamadani JD et al. 2001; Jiménez R et al. 2007). There were not studies including infants nutritionally at risk. Risk of bias varied between the studies: two studies had a high risk of bias (Ashworth A et al. 1998; Hamadani JD et al. 2001), one had a moderate risk (Castillo-Duran C et al. 2001) and two had low risk of bias (Jiménez R et al. 2007; Lind T et al. 2004).

Differences between neurodevelopment (MDI and PDI) outcomes according to the Zn intake in each particular study and in the pooled analysis are showed in Figures 2 and 3. The pooled β was -0.01 (95%CI -0.02, 0) for MDI and 0 (95%CI -0.02, 0.02) for PDI. However, a substantial heterogeneity was present in both analyses (I^2 for MDI = 72% and I^2 for PDI = 96%).

In order to explore which variables may be potential effect modifiers, we performed a meta-regression (Table 2). The effect of Zn supplementation on MDI changed depending on the dose (p ANCOVA= 0.002). Regarding PDI, there was a differential effect of Zn intake depending on duration of the intervention, dose, nutritional status and risk of bias (p ANCOVA = <0.001, <0.001, <0.001, <0.002) respectively.

Table 3 shows the results after stratifying the sample according to the effect modifiers identified in the meta-regression. After stratifying by dose, the pooled β for MDI still showed evidence of heterogeneity (I^2= 87%) for high dose of zinc (8.1 to 12 mg/day), but this heterogeneity disappeared within some strata such as low and medium doses of zinc (1 to 4 mg/day and 4.1 to 8 mg/day). However, there was no significant effect on Zn supplementation at these dosages.

In the case of the pooled β for PDI, the heterogeneity disappeared when we grouped studies with an intervention period of 4 to 20 weeks, with low and medium doses of zinc (1 to 4 mg/day and 4.1 to 8 mg/day) and in studies with moderate or high risk of bias. Heterogeneity was maintained for the following studies: for >20 weeks of intervention (I^2 = 88%); for high dose of zinc (8.1 to 12 mg/day) (I^2 = 98%), for healthy and poor nutritional status (I^2 = 54 and 96%) and for low bias risk (I^2 = 98%).

Zn supplementation showed a negative, weak and significant effect on PDI score in those studies with a length of 4 to 20 weeks (β= -0.05; CI 95% -0.06 to -0.04).

The results of the sensitivity analyses are shown in Table 4. We did not find a significant association between Zn intake and MDI and PDI.

The study by Ashworth et al (b) was considered as an outlier in the analysis of MDI because the limits of beta were very wide (from -0.11 to 0.04). When we excluded this study, the null association previously seen persisted as did the degree of heterogeneity. The same was true when the Ashworth et al studies (a) and (b) were removed from the PDI meta-analysis.

Due to the high heterogeneity found in most of the analyses, we decided not perform dose-response relationship assessment.

Discussion

Our pooled meta-analysis indicated that zinc intake does not appear to influence mental or psychomotor neurodevelopment of infants. Only a negative effect on infant neurodevelopment (PDI) was found when the time of Zn supplementation ranged between 4 to 20 weeks. Nonetheless, interpretation of these results should be carefully considered for a number of reasons. First of all, the magnitude of effect was rather small. Also, it is a well known fact that when many comparisons are done, one or more might reach significance due to pure chance (Bland JM & Altman DG 1995). Furthermore, significant heterogeneity existed in the methods across pooled studies, and in general, after stratifying on several factors, heterogeneity persisted through various analyses and finally the few number of articles included in each sub-analyses.

It is also important to consider the scientific quality of included studies. Although meta-analysis techniques are being increasingly used to consolidate results and to develop evidence-based policies for clinical practice and public health programmes, the reliability of the conclusions derived from meta-analyses depend on the methodological quality of the original studies, the appropriateness of the study inclusion criteria, and the thoroughness of the review and synthesis of information (Brown KH et al.2002). While strict systematic review protocols were followed adhering to EURRECA's

quality standards (Matthys C et al. 2011), an assessment of the risk of bias of included studies revealed that the majority (n=3) had a high- moderate risk of bias.

For the interpretation of our results, one should bear in mind that sample size was considerably small. Information on the mean zinc intake and neurodevelopment was available only from 5 studies, which limited the statistical power of the analyses to examine the relation between neurodevelopment responses to zinc supplementation. Thus, the lack of significance in the current analysis may be due to the limited amount of available information, and more studies will be needed to resolve this issue.

It is also important to keep in mind that all of the included studies in this meta-analysis, used Bayley Scales for the measurement of neurodevelopment. This is the most commonly used instrument to test psychomotor development. Although the Bayley Scales of Infant Development were standardized in the United States, they have been used in many others countries, mostly in research focused on nutrition and development. It is possible that slight differences in administration of the test occur in different countries. Additionally, infants in different cultures are exposed to very different environments, which would be expected to affect their development. Therefore, it is difficult to interpret the differences infant's scores across cultures (Black MM & Matula K 2000). Therefore, although all the included studies used the same instrument for measuring neurodevelopment, this might be a source of heterogeneity by itself and another factor contributing to the lack of effect of zinc supplementation upon neurodevelopment.

Some confounders should be considered in evaluating the effect of zinc intake on neurodevelopment in infancy. Those confounders include low birth weight, breastfeeding, protein energy malnutrition, infectious morbidity, poverty, and social deprivation. For instance, in our meta-analysis, Ashworth et al performed an adjustment of initial differences, whilst others (Castillo Duran et al 2001, Hamadani et al 2001, Lind et al 2004) assumed no initial differences and or failed to mention anything regarding this matter (Jimenez et al 2007). However, all the studies included in our meta-analysis are RCT. So, if we assume the randomization has been correct, none of these factors should bias the results.

In some studies the difference in neurodevelopment scores for Zn intake was rather small (Hamadani et al 2001). However the infants included in their study were undernourished and required more nutrients other than zinc. The same observation could be made regarding Ashworth et al and Jimenez et al conducted in low birth weight (LBW) infants. However, the first group of authors concluded that the duration of their intervention was insufficient and in the second study they found some benefit of zinc on PDI but not in MDI. It is very important to consider the duration of the intervention in studies conducted in LBW infants because the low weight and the immaturity associated with premature infants requires adjustment of gestational age with chronological age for

proper assessment of catch-up growth (Rugolo LM 2005). The study by Jimenez et al, also conducted in LBW infants showed an increment in development after following zinc supplementation. This increment was more evident in motor than in mental development, especially from 3 to 6 months of age. However, these authors do not assure that time of supplementation could be responsible for the reported effect. New studies are required to analyze the long-term effects on psychomotor development of zinc deficiency.

Other factors to consider are the influence of social context, the caregiving environment and the developmental stimulation, all of which play very important role in the evolution of the cognitive development in infants (Black MM 1998). It is clear that all these factors play a relevant role for neuropsychological functioning, activity and motor development and all of the studies included in our meta-analysis account for these variables, except Jimenez et al.

In infants of 6 to 8 months of age, special considerations need to be acknowledged since they are particularly vulnerable to suffer from zinc deficiency while they are in the feeding transition period because gradual introduction of solids is still taking place. This problem is particularly relevant to underdeveloped countries but there is growing evidence that suggests that this is present also in adequately nourished populations (Skinner JD et al 1997). More studies that examine the response to zinc supplementation or fortification in populations that are zinc deficient in the absence of poverty clearly need to be conducted to clarify the relationship between zinc and neurodevelopment. Zinc deficiency poses a serious public health problem that compromises the adequate development of millions of children worldwide in both developing and developed countries (Sandstead HH 1996).

In conclusion, a negative but not statistically significant association was found between zinc intake and neurodevelopment in infants. The magnitude of effect was small in all cases. Based on this limited group of studies and their heterogeneity, we found insufficient information to suggest that zinc supplementation has a positive effect on neurodevelopment of infants. Further standardized research is urgently needed to clarify the role of zinc supplementation upon infant neurodevelopment mainly in Western populations.

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The authors´ responsibilities were as follows: MN: analysis of the data and writing the manuscript, ALS & MN: review the papers, MWM: contribution to selection of papers and data extraction, ASV: support in data-analysis, DFL, PHS, JDA, NL, VMH and LSM provision of significant advice. All authors directly participated in the planning, execution or analysis of the study and reviewed the manuscript.

CONFLICT OF INTEREST: Authors declare no conflicts of interest.

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Figure 1: Flow diagram for the systematic review.

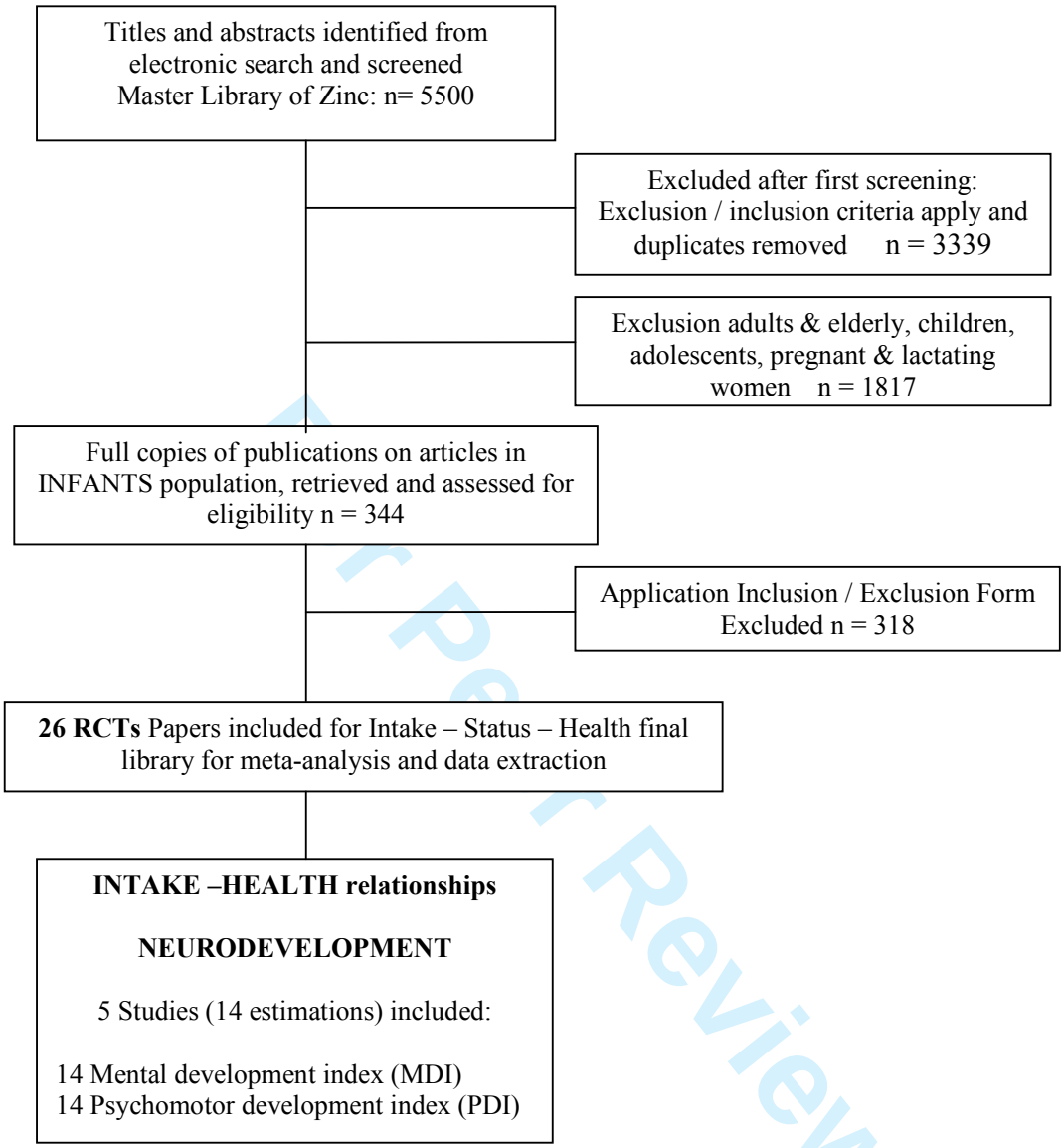

Figure 2: Neurodevelopment: MDI

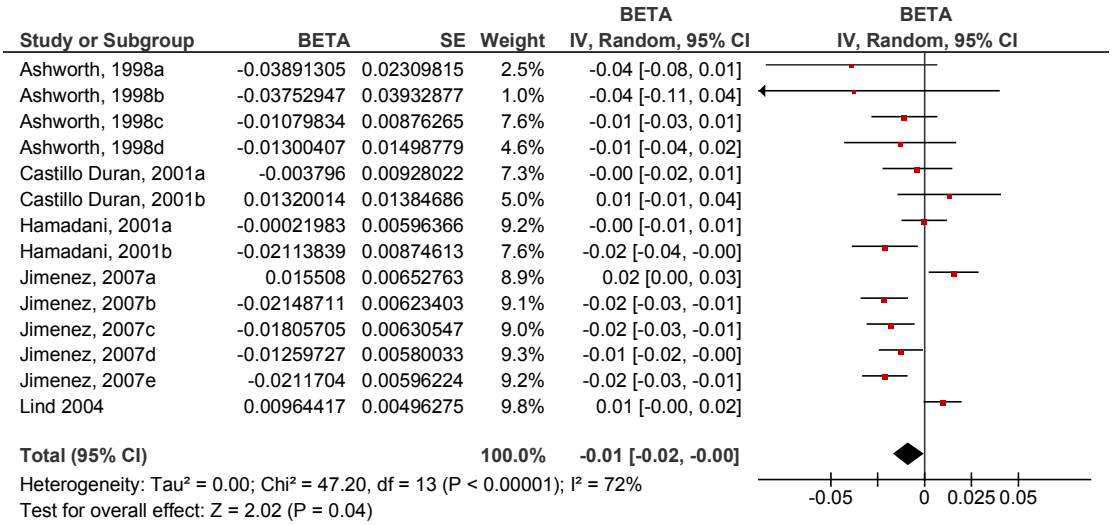

Figure 3: Neurodevelopment: PDI

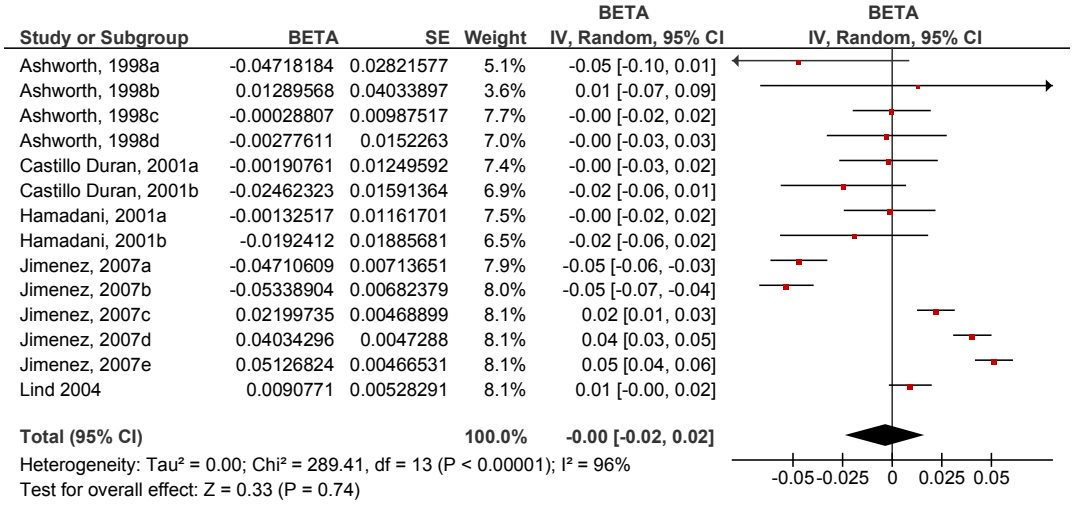

Table 1: Characteristics of the 5 (14 estimations) Neurodevelopment studies included in the meta-analysis

Author	Study year	Country	Sample (age range or Mean)	Number of Infants (n)		Doses of Zinc	Weeks of the intervention	Outcome	Nutritional situation	Risk of bias[2]
				Zn[1]	C[1]					
Ashworth (a) (6) (b)	1998	Brazil	6 to 12 months	53 44	56 48	1 mg	24 w 48 w	MDI-PDI	Poor nutritional status	High risk
(c) (d)				53 44	54 46	5 mg	24 w 48 w			
Castillo Duran (a) (7) (b)	2001	Chile	Mean 6 months	45 45	57 57	5 mg	24 w 48 w	MDI-PDI	Healthy	Moderate risk
Hamadani (a) (8) (b)	2001	Bangladesh	1 to 13 months	109 101	103 97	5 mg	28 w 52 w	MDI-PDI	Poor nutritional status	High risk
(a) Jiménez (b) (9) (c) (d) (e)	2007	Cuba	1 to 12 months	76 76 76 76 76	87 87 87 87 87	10 mg	4 w 13 w 26 w 39 w 52 w	MDI-PDI	Poor nutritional status	Low risk
Lind (10)	2004	Indonesia	6 to 12 months	162	161	10 mg	8 w	MDI-PDI	Healthy	Low risk

[1]Zn: Intervention group / C: Control group

[2] Low risk of bias meant that the study was randomized, the randomization method was at least partially described, reasons for and numbers of dropouts were stated (or there were no dropouts), and the method used to assess compliance and some assessment of compliance were reported. All others studies were considered as moderate when they meet any of the above criteria or high risk of bias when they meet any of the criteria. (Higgins 2009, Cochrane Handbook)

(a - e): Sub-studies

MDI: Mental Development Index

PDI: Psychomotor Development Index

Table 2: Meta-regression. Multivariate adjusted mean beta for Neurodevelopment (MDI; PDI) (95% confidence interval) by different characteristics of the studies included in the meta-analysis

	n	Mean Beta's	CI (95%)	P Ancova *
MDI				
By duration of the intervention				
4 to 20 weeks	3	-0.0005	-0.0143 to 0.0132	
> 20 weeks	11	-0.0148	-0.0224 to -0.0073	
				0.076
By Dose				
1 to 4 mg	2	-0.0236	-0.0386 to -0.0087	
4,1 to 8 mg	6	0.0033	-0.0067 to 0.0133	
8,1 to 12 mg	6	-0.0027	-0.0113 to 0.0060	
				0.002
By Nutritional situation				
Healthy	3	-0.0014	-0.0145 to 0.0118	
Poor nutricional situation	11	-0.0140	-0.0272 to -0.0008	
				0.242
By Risk of Bias				
Low	6	-0.0088	-0.0189 to 0.0013	
Moderate	2	-0.0054	-0.0261 to 0.0152	
High	6	-0.0088	-0.0189 to 0.0013	
				0.800
PDI				
By duration of the intervention				
4 to 20 weeks	3	-0.0757	-0.0977 to -0.0538	
> 20 weeks	11	0.0124	0.0004 to 0.0244	
				<0.001
By Dose				
1 to 4 mg	2	-0.0538	-0.0776 to -0.0299	
4,1 to 8 mg	6	-0.0425	-0.0584 to -0.0266	
8,1 to 12 mg	6	0.0012	-0.0125 to 0.0150	
				<0.001
By Nutritional situation				
Healthy	3	-0.0020	-0.0230 to 0.0189	
Poor nutricional situation	11	-0.0613	-0.0823 to -0.0404	
				<0.001
By Risk of Bias				
Low	6	-0.0095	-0.0256 to 0.0067	
Moderate	2	-0.0761	-0.1090 to -0.0433	
High	6	-0.0095	-0.0256 to 0.0067	
				0.002

* Adjusted for the rest of variables in the table

Table 3: Pooled beta (95% confidence intervals) in Neurodevelopment according to the intervention group. Subgroup analyses.

	Pooled estimates (β)	Chi² (df, P)	I² *
MDI			
All Studies (n=14)	-0.01 (-0.02 to 0)	47.20 (13, < 0,00001)	72%
By dose			
1 to 4 mg (n=2)	-0.04 (-0.08 to 0)	0 (1, 0.98)	0%
4,1 to 8 mg (n=6)	-0.01 (-0.01 to 0)	6.43 (5, 0.27)	22%
8,1 to 12 mg (n=6)	-0.01 (-0.02 to 0.01)	38.19 (5, <0.00001)	87%
PDI			
All Studies (n=14)	0 (-0,02 to 0,02)	289.41 (13, < 0,00001)	96%
By duration of the intervention			
4 to 20 weeks (n=2)	-0.05 (-0.06 to -0.04)	0.40 (1, 0,52)	0%
> 20 weeks (n=12)	0.01 (-0.01 to 0.02)	93.24 (11, < 0,00001)	88%
By dose			
1 to 4 mg (n=2)	-0.02 (-0.08 to 0.03)	1.49 (1, 0.22)	33%
4,1 to 8 mg (n=6)	-0.01 (-0.02 to 0.01)	2.50 (5, 0.78)	0%
8,1 to 12 mg (n=6)	0 (-0.03 to 0.04)	268.60 (5, < 0,00001)	98%
By Nutritional situation			
Healthy (n=3)	0 (-0.02 to 0.02)	4.36 (2, 0.11)	54%
Poor nutritional situation (n=11)	0 (-0.03 to 0.02)	281.40 (10, < 0,00001)	96%
By Risk of Bias			
Low (n=6)	0 (-0.03 to 0.04)	268.60 (5, < 0,00001)	98%
Moderate (n=2)	-0.01 (-0.03 to 0.01)	1.26 (1, 0,26)	21%
High (n=6)	0 (-0.02 to 0.01)	3.35 (5, 0,65)	0%

*I² Index measures the extent of the heterogeneity

Table 4: Pooled beta (95% confidence intervals) in Neurodevelopment according to the intervention group. Sensitivity Analyses

	Pooled estimates (β)	Chi² (dif, P)	I²
MDI			
All studies (n=14)	-0.01 (-0.02 to 0)	47.20 (13, <0.00001)	72%
All Studies excluding (n=1)	-0.01 (-0.02 to 0)	46.60 (12, <0.00001)	74%
Ashworth et al. 1998 b	-0.04 (-0.11 to 0.04)		
PDI			
All studies (n=14)	0 (-0.02 to 0.02)	289.41 (13, <0.00001)	96%
All Studies excluding (n=2)	0 (-0.02 to 0.02)	284.88 (11, <0.00001)	96%
Ashworth et al. 1998 a	-0.05 (-0.10 to 0.01)		
Ashworth et al. 1998 b	0.01 (-0.07 to 0.09)		

I² Index measures the extent of the heterogeneity

The Relationship between Zinc Intake and Serum/Plasma Zinc Concentration in Children: A Systematic Review and Dose-Response Meta-Analysis

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Review

The Relationship between Zinc Intake and Serum/Plasma Zinc Concentration in Children: A Systematic Review and Dose-Response Meta-Analysis

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Abstract: Recommendations for zinc intake during childhood vary widely across Europe. The EURRECA project attempts to consolidate the basis for the definition of micronutrient requirements, taking into account relationships among intake, status and health outcomes, in order to harmonise these recommendations. Data on zinc intake and biomarkers of zinc status reported in randomised controlled trials (RCTs) can provide estimates of dose-response relationships which may be used for underpinning zinc reference values. This systematic review included all RCTs of apparently healthy children aged 1–17 years published by February 2010 which provided data on zinc intake and biomarkers of zinc status.

An intake-status regression coefficient ($\hat{\beta}$) was calculated for each individual study and calculated the overall pooled $\hat{\beta}$ and SE ($\hat{\beta}$) using random effects meta-analysis on a double log scale. The pooled dose-response relationship between zinc intake and zinc status indicated that a doubling of the zinc intake increased the serum/plasma zinc status by 9%. This evidence can be utilised, together with currently used balance studies and repletion/depletion studies, when setting zinc recommendations as a basis for nutrition policies.

Keywords: zinc; children; serum zinc; systematic review; dose-response; dietary recommendations; EURRECA

1. Introduction

Suboptimal dietary zinc intake is increasingly recognised as an important public health issue. Although the lack of generally accepted biomarkers of zinc status has impeded estimation of the global prevalence of zinc deficiency, based on information regarding the amount of zinc present in national food supplies, it has been estimated that the risk of low dietary intake of absorbable zinc and consequent zinc deficiency affects between one-third and one-half of the world's population [1] and rates of deficiency may approach 73% in some regions [2]. Although severe zinc deficiency is uncommon in European populations, marginal deficiency is likely to be much more prevalent [3], with associations to immune system dysfunction and restricted physical development [4]. Children are particularly vulnerable to suboptimal zinc status during periods of rapid growth that create increased zinc needs that may not be met [5,6]. It is estimated that over 450,000 deaths per year (4.4% of all mortalities) among children between six months and five years of age are attributable to zinc deficiency [7]. Zinc deficiency also has an impact of child morbidity, impairing growth and contributing to childhood stunting [8,9].

Physiological requirements for zinc peak at the time of the pubertal growth spurt, the onset of which varies according to gender. In girls the onset of the growth spurt (OGS) occurs at 10.1 years and peak height velocity (PHV) occurs at 12.0 years. In boys OGS and PHV occur at 11.8 and 14.2 years, respectively [10]. Even after the growth spurt has ceased, adolescents may require additional zinc to replete tissue zinc pools depleted during puberty [11]. Marginal zinc status during the pubertal growth spurt has been associated with slower skeletal growth, maturation, and reduced bone mineralisation [12–14]. As nearly a third of total skeletal mineral is accumulated in the 3–4 year period immediately after the onset of puberty [15] suboptimal zinc intake may have long-term consequences on bone health.

Although a sensitive and specific biomarker has yet to be identified, a recent systematic review concluded that plasma (or serum) zinc concentration was responsive to both zinc supplementation and depletion and is the most widely used biomarker for zinc [16]. However, meta-analytic methods have not yet been used to model zinc status as a function of zinc intake levels. Understanding the relationship between dietary intake and micronutrient status is essential for deriving dietary recommendations.

The recommendations for zinc intake during childhood vary widely across Europe and comparisons are difficult due to differences in categorisation. For example between 4 and 6 age categories are used

to describe micronutrient recommendations in childhood with different age cut-off points being used by different European countries [17,18]. Recommendations for zinc intake differ between males and females at the age of 15 years in most countries, but also differ at the age of 10 years in some countries. Zinc intake values range from 2.9 to 10.0 mg/day in children aged 5 years, 5.7 to 15.5 mg/day (boys) and 4.6 to 15.0 mg/day (girls) in children aged 10–15 years [17]. The EURRECA project attempts to consolidate the basis for the definition of micronutrient requirements across Europe, taking into account relationships among intake, status and health outcomes, in order to harmonise these recommendations [19].

This paper presents a systematic review of the data from all available randomised controlled trials (RCTs) meeting EURRECA's quality standard [20], which investigated zinc intake and biomarkers of zinc status, and combines these studies in meta-analyses to model zinc concentrations in serum or plasma as a function of zinc intake.

2. Methods

2.1. Search Strategy

This research was conducted within the framework of the European Micronutrient Recommendations Aligned (EURRECA) Network of Excellence that aims to identify the micronutrient requirements for optimal health in European populations (EURRECA [21]). This review was part of a wider review process to identify studies assessing the effect of zinc intake on different outcomes (biomarkers of zinc status and health outcomes). The wider searches were performed of literature published up to and including February 2010 using MEDLINE, Embase, and Cochrane using search terms for "study designs in humans" and "zinc" and "intake OR status". Both indexing and text terms were used and languages included were restricted to those spoken in the EURRECA Network (English, Dutch, French, German, Hungarian, Italian, Norwegian, Polish, Spanish, Greek, and Serbian). The full Ovid MEDLINE search strategy can be found in Table 1. Reference lists of retrieved articles and published literature reviews were also checked for relevant studies. Authors were contacted to request missing data or clarify methods or results. The search process is illustrated in Figure 1.

Table 1. Search strategy: MEDLINE February 2010 [22].

No.	Search Term	Results
1	randomised controlled trial.pt.	280,821
2	controlled clinical trial.pt.	79,998
3	randomised.ab.	196,604
4	placebo.ab.	117,891
5	clinical trials as topic.sh.	146,242
6	randomly.ab.	145,491
7	trial.ab.	203,467
8	randomised.ab.	38,423
9	6 or 3 or 7 or 2 or 8 or 1 or 4 or 5	734,511
10	(animals not (human and animals)).sh.	4,482,479
11	9 not 10	642,665
12	(cohort* or "case control*" or cross-sectional* or "cross sectional" or case-control* or prospective or "systematic review*").mp.	768,885

Table 1. *Cont.*

13	exp meta-analysis/ or expmulticenter study/ or follow-up studies/ or prospective studies/ or intervention studies/ or epidemiologic studies/ or case-control studies/ or exp cohort studies/ or longitudinal studies/ or cross-sectional studies/	1,013,635
14	13 or 12	1,203,767
15	14 not 10	1,154,385
16	11 or 15	1,599,094
17	((zinc or Zn or zinc sulphate or zinc gluconate or zinc acetate or methionine or zinc isotope*) adj3 (intake* or diet* or supplement* or deplet* or status or serum or plasma or leukocyte or concentration* or expos* or fortif* or urine or hair)).ti,ab.	16,681
18	Nutritional Support/ or Dietary Supplements/ or nutritional requirements/ or Breast feeding/ or exp infant food/ or bottle feeding/ or infant formula/	63,098
19	exp Nutritional Status/ or exp Deficiency Diseases/ or supplementation/ or diet supplementation/ or dietary intake/ or exp diet restriction/ or exp mineral intake/ or Diet/ or Food, Fortified/ or nutrition assessment/ or Nutritive Value/	176,014
20	(intake* or diet* or supplement* or deplet* or status or serum or plasma or leukocyte or concentration* or expos* or fortif* or urine or hair).ti,ab.	3,166,092
21	18 or 19 or 20	3,263,114
22	zinc/	41,027
23	22 and 21	20,745
24	23 or 17	26,943
25	24 and 16	2410

2.2. Criteria for the Consideration of Studies for This Review

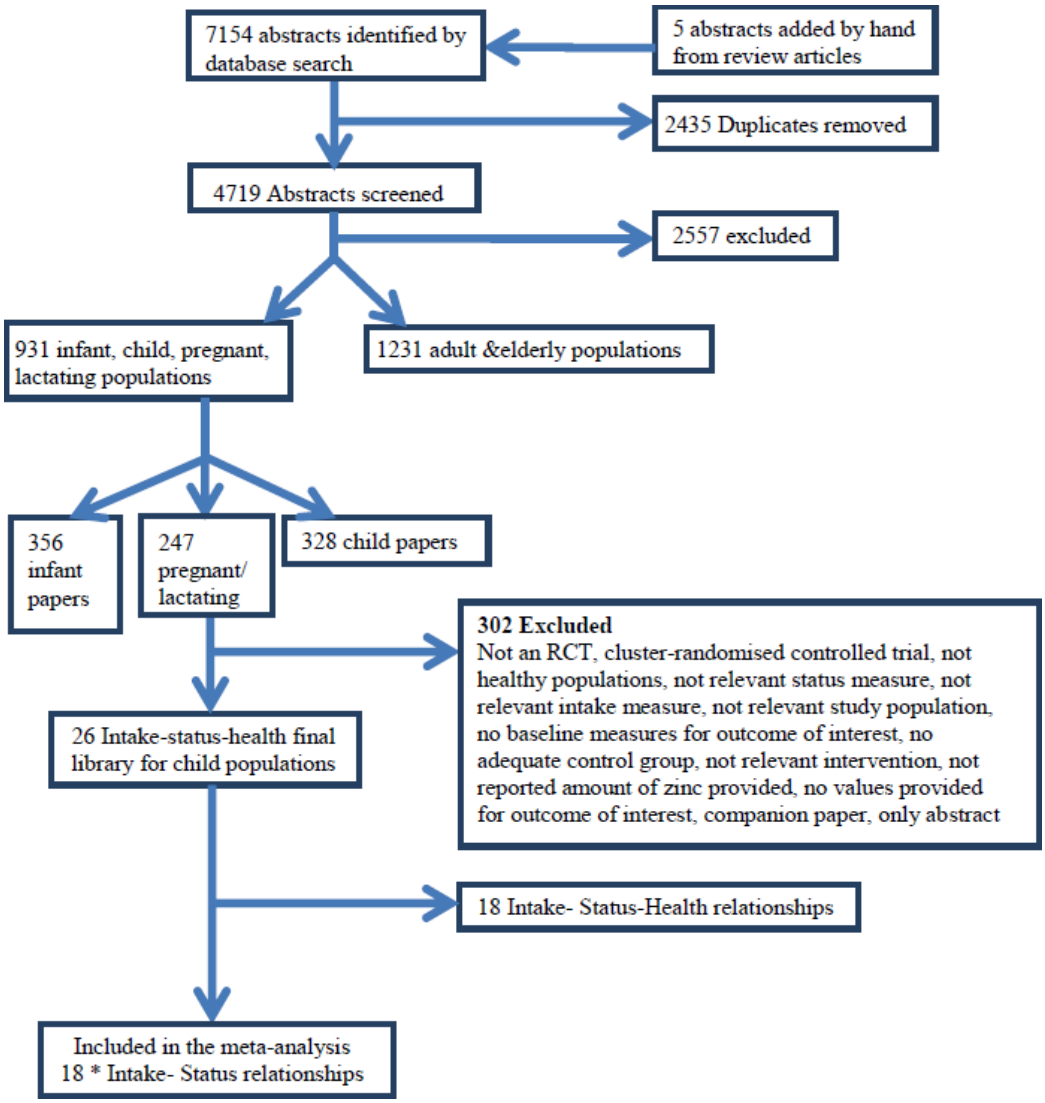
Included studies were RCTs in apparently healthy child (human) populations aged from 1 to 17 years that supplied zinc supplementation either as capsules or part of a fortified meal. If supplemental zinc was provided as a component of a fortified meal, studies were only considered acceptable if zinc was the only constituent that was different between treatment groups. Only studies that measured zinc status as serum or plasma zinc were included; and those that reported sufficient data or had sufficient data obtainable from the authors to estimate $\hat{\beta}$ and SE ($\hat{\beta}$) for the assumed linear relation on the $\log_e$–$\log_e$ scale. Studies were excluded if they were a group RCT (community trial), or were commentaries, reviews, or duplicate publications from the same study. Studies were excluded if children were hospitalised, had severe protein-energy malnutrition or a chronic disease or if supplemental zinc was provided for less than 6 weeks.

2.3. Selection of Articles

Of 4719 identified articles in the wider search on zinc intake, status and priority health outcomes in all populations, 2557 were excluded based upon screening of the title and abstract. Two independent reviewers screened 10% of the abstracts in duplicate and any discrepancies were discussed before screening the remaining references. Following subdivision into appropriate population groups the full texts of the 328 manuscripts were assessed to determine inclusion and exclusion by two independent reviewers and disagreements rectified through discussion. 302 studies were excluded because they did not meet the inclusion criteria. Of the remaining 26 studies, 8 studies were excluded as they had not

investigated the relation between zinc intake and zinc related biomarkers, but related either intake or status directly to a health endpoint. For the purpose of this paper, 18 RCTs remained. Table 2 presents the characteristics of the included studies.

Figure 1. Study selection process for systematic review (* some papers reported more than one relationship).



2.4. Data Extraction

For each of the identified manuscripts, data was extracted independently by two reviewers into a standardised database. Extracted data included population characteristics, dose of elemental zinc in intervention and placebo supplements, duration of the study, dietary intake of zinc, and mean concentration of zinc in plasma or serum at the end of the intervention period. Serum/plasma zinc concentrations were converted to μmol/L when applicable.

Table 2. Summary of included trials reporting the effect of dietary zinc intake on serum/plasma zinc status in children.

First Author, Year, Country	Participants	Treatment Groups (*n*)	Mean Zn Intake (mg/day)	Mean (SD) Plasma/Serum Zn (µmol/L)	Duration	Zinc Status Biomarker [Analytical Method]	Main Results
Mahloudji, 1975, Iran [23]	Males & females aged 6–12 years	Fe only (12); Fe + 20 mg/day Zn (13)	5.65; 25.65	8.95 (1.80) 8.50 (1.93)	8 months	Plasma Zn [AAS]	No significant difference between plasma Zn of the supplemented and placebo groups
Hambidge, 1979, USA [24]	Males & females aged 33–90 months	Male placebo (15); Male Zn FM 2.57 mg/day (20); Female placebo (14); Female Zn FM 2.57 mg/day (11)	6.3; 9.27; 6.3; 9.27	11.06 (2.23) 11.85 (2.23) 10.61 (1.81) 11.96 (1.81)	9 months	Plasma Zn [AES]	Plasma Zn significantly higher in Zn supplemented compared to placebo (girls and combined sexes only $p < 0.05$)
Walravens, 1983, USA [25]	Males & females aged 2–6 years	Placebo (16); 10 mg/day Zn (16)	4.6; 15.9	11.32 (2.14) 10.86 (2.14)	12 months	Plasma Zn [AES]	No significant difference between plasma Zn of the supplemented and placebo groups
Gibson, 1989, Canada [26]	Males aged 59–95 months	Placebo (21); 10 mg Zn/day (18)	6.4; 16.7	15.8 (3.5) 17.9 (3.4)	6 months	Serum Zn [AAS]	No significant correlation between serum Zn and dietary Zn levels
Cavan, 1993, Guatemala [27]	Males & females, mean age 81.5 (±7.0) months [1]	Placebo (74); 10 mg Zn/day (71)	5.65; 15.65	14.9 (2.1) 16.2 (2.9)	25 weeks	Plasma Zn [AAS]	Plasma Zn significantly higher in Zn supplemented compared to placebo ($p < 0.01$)
Friis, 1997, Zimbabwe [28]	Males and females aged 11–17 years	Placebo (121); 30–50 mg/day Zn (122)	5.65; 45.65 [2]	10.89 (2.5) 11.71 (2.4)	12 months	Serum Zn [AAS]	The decline in zinc concentration was significantly lower in the Zn supplemented group compared to the placebo group ($p < 0.02$)
Rosado, 1997, Mexico [29]	Males & females aged 18–36 months	Placebo (55); 20 mg Zn/day (54)	5.65; 25.65	14.4 (4.45) 16.8 (5.88)	12 months	Plasma Zn [AAS]	Plasma Zn increased significantly in the Zn supplemented group over the 12 months period ($p < 0.01$)
Ruz, 1997, Chile [30]	Males & females aged 27–50 months	Placebo (33); 10 mg/day Zn (36)	6.4; 17.1	17.7 (1.9) 17.6 (2.2)	6 months	Plasma Zn [AAS]	No significant difference between plasma Zn of the supplemented and placebo groups
Sandstead, 1998, China [31] (3 regions)	Males & females aged 6–9 years	*Chonqing* MN, no Zn (35); 20 mg/day Zn + MN (35); *Quindgdao* MN, no Zn (36); 20mg/day Zn + MN (36); *Shanghai* MN, no Zn (37); 20 mg/day Zn + MN (37)	5.65; 25.65; 5.65; 25.65; 5.65; 25.65	19.83 (4.12) 23.6 (4.12) 20.42 (4.08) 22.97 (4.08) 17.9 (2.75) 17.97 (2.75)	10 weeks	Plasma Zn [AAS]	Plasma Zn significantly higher in Zn supplemented compared to placebo ($p < 0.05$) in Chonqing and Quindgdao groups.

Table 2. *Cont.*

Clark, 1999, UK [32]	Peripubertal females, mean age 12.2 (±0.3) years	Placebo (19); 15 mg Zn/day (23)	6.6; 21.6	12.6 (1.0) 16.7 (4.9)	6 weeks	Serum Zn [no method given]	Serum Zn significantly higher in Zn supplemented compared to placebo ($p < 0.001$)
Smith, 1999, Belize [33]	Males & females aged 22–66 months	Placebo (10); 70 mg Zn/day (12)	5.65; 75.65	11.7 (0.68) 13.5 (0.68)	6 months	Serum Zn [AAS]	Serum Zn significantly higher in Zn supplemented compared to placebo ($p < 0.001$)
Munoz, 2000, Mexico [34]	Males & females aged 18–36 months	Placebo (54); 20 mg/day Zn (47)	5.65; 25.65	14.3 (4.7) 16.8 (5.6)	6 months	Plasma Zn [AAS]	Serum Zn significantly higher in Zn supplemented compared to placebo ($p < 0.0001$)
Lopez de Romana, 2005, Peru [35]	Males & females aged 3–4 years	Fe FM (12); Fe + 3 mg/day Zn FM (10); Fe + 9 mg/day Zn FM (12);	4.71; 8.72; 15.7	11.87 (1.88) 11.65 (1.25) 12.60 (1.51)	70 days	Plasma Zn [ICP-MS]	No significant differences in plasma Zn were found between treatments
Silva, 2006, Brazil [36]	Males & females aged 12–59 months [3]	Placebo (30); 10 mg/day Zn (28)	5.65; 15.65	8.0 (0.58) 13.4 (0.25)	4 months	Serum Zn [AAS]	Serum Zn significantly higher in Zn supplemented compared to placebo ($p < 0.05$)
Sandstead, 2008, USA (Mexican Americans) [37]	Males & females aged 6–7 years	MN, no Zn (25); 20 mg/day Zn + MN (25)	5.65; 25.65	15.4 (1.5) 15.6 (1.2)	10 weeks	Plasma Zn [AAS]	Mean plasma Zn increased significantly in both groups compared to baseline ($p < 0.05$)
Wuehler, 2008, Ecuador [38]	Males & females aged 12–30 months	Placebo (56); 3 mg Zn/day (50); 7 mg Zn/day (52); 10 mg Zn/day (54)	5.65; 8.65; 12.65; 15.65	10.6 (1.6) 12.3 (1.6) 13.3 (1.7) 14.0 (1.7) [4]	6 months	Plasma Zn [ICP-MS]	The mean change in plasma zinc concentrations from baseline increased progressively with higher doses of supplemental Zn ($p < 0.001$)
de Oliveira, 2009, Brazil [39]	Pubescent males, mean age 13 (±0.4) years	Placebo (26); 22 mg Zn/day (21)	5.65; 27.65	16.9 (2.1) 18.7 (3.5)	12 weeks	Plasma Zn [ICP-MS]	Plasma Zn significantly higher in Zn supplemented compared to placebo ($p < 0.05$)
Uckarde, 2009, Turkey [40]	Males & females aged 8–9 years	Placebo (109); 15 mg/day Zn (109)	5.65; 20.65	19.19 (1.80) 19.50 (2.41)	10 weeks	Serum Zn [CS]	Both supplemented and placebo groups had significantly higher serum Zn at follow up ($p < 0.05$)

AAS, atomic absorption spectroscopy; AES, atomic emission spectroscopy; ICP-MS, inductively coupled plasma mass spectrometry; CS, caloric spectrophotometry; MN, micronutrients; FM, fortified meal; [1] all participants also received MN supplements; [2] children weighing <29.5 kg were given 30 mg Zn/day and those >29.5 kg were given 50 mg Zn/day, this figure is an average of the two doses; [3] all participants also received Fe fortified milk; [4] geometric means.

2.5. Data Synthesis

One study that included two zinc-treated groups and two control groups (males and females) was treated as two independent estimates in the analysis [24] and one study that included three zinc-treated groups and three control groups (from different regions of China) [31] and was treated as three independent estimates in the analysis. Where studies provided outcome data for two or more zinc-treated groups they were included as separate estimates in the meta-analysis [35,38]. In one study zinc status was measured at 6 months and 12 months in the same population [26] and only the measure at 6 months was used in the analysis (where *n* was the largest). If dietary intake of zinc (in addition to the intervention) was not reported we imputed a value of 5.65 mg/day, the mean dietary intake level of the RCTs (n = 7) that did report dietary zinc intake. As mean baseline serum/plasma zinc concentrations were infrequently reported, the serum/plasma zinc concentrations in the control group of the RCTs were used as a proxy of the baseline serum/plasma zinc concentrations for our analyses.

2.6. Pre-Specified Potential Factors Modifying the Association

A pooled meta-analysis was performed combining the evidence from all the available RCTs. In addition, we investigated whether age, dose of zinc, duration of the supplementation, and type of supplement (zinc only *vs.* zinc with other micronutrients) were variables that modified the association.

2.7. Statistical Analyses

As we wanted to estimate the dose-response relation between zinc intake and serum/plasma zinc, the data presented in the studies had to be transformed to a common statistic, namely a regression coefficient ($\hat{\beta}$) and the standard error (SE ($\hat{\beta}$)) of this regression coefficient. The transformations used to derive this common single-study estimate from the available summary statistics per study have been described elsewhere [41]. In short, we estimated an intake-status regression coefficient ($\hat{\beta}$) for each individual study, based on the assumption of a linear relation on the $\log_e$–$\log_e$-scale (natural logarithm of intake *vs.* natural logarithm of status). This shape of this linear relationship on the $\log_e$–$\log_e$-scale corresponds to a monotonic concave function on the original scale for $\beta < 1$. This shape is assumed to be realistic for the biological relationship between zinc intake and plasma/serum zinc concentrations. As the true dose-response curve is unknown, this approximation provides a practical methodology to estimate the dose-response relationship. We calculated the overall pooled $\hat{\beta}$ and SE ($\hat{\beta}$) using random effects meta-analysis, which estimates the between-study variance using the method of DerSimonian and Laird and used this estimate to modify the weights used to calculate the summary estimate. Residual heterogeneity between studies was evaluated using the I^2 statistic. Pre-specified potential factors that could modify the association were explored using stratified random effects meta-analyses. The statistical transformations to obtain $\hat{\beta}$'s and SE ($\hat{\beta}$)'s were performed using GenStat version 13-SP2 (VSN International Ltd. [42]) and the meta-analysis was performed using STATA version 11.0 (College Station, TX, USA), with statistical significance defined as $p < 0.05$.

2.8. Assessment of Risk of Bias in Included Studies

In order to assess the quality of the included studies and the risk of bias, indicators of internal validity were collected during data extraction (Table 2). Based on the indicators two independent reviewers assessed the overall risk of bias and disagreements resolved by discussion. The criteria for judging these indicators were adapted from the Cochrane Handbook for Systematic Reviews [43].

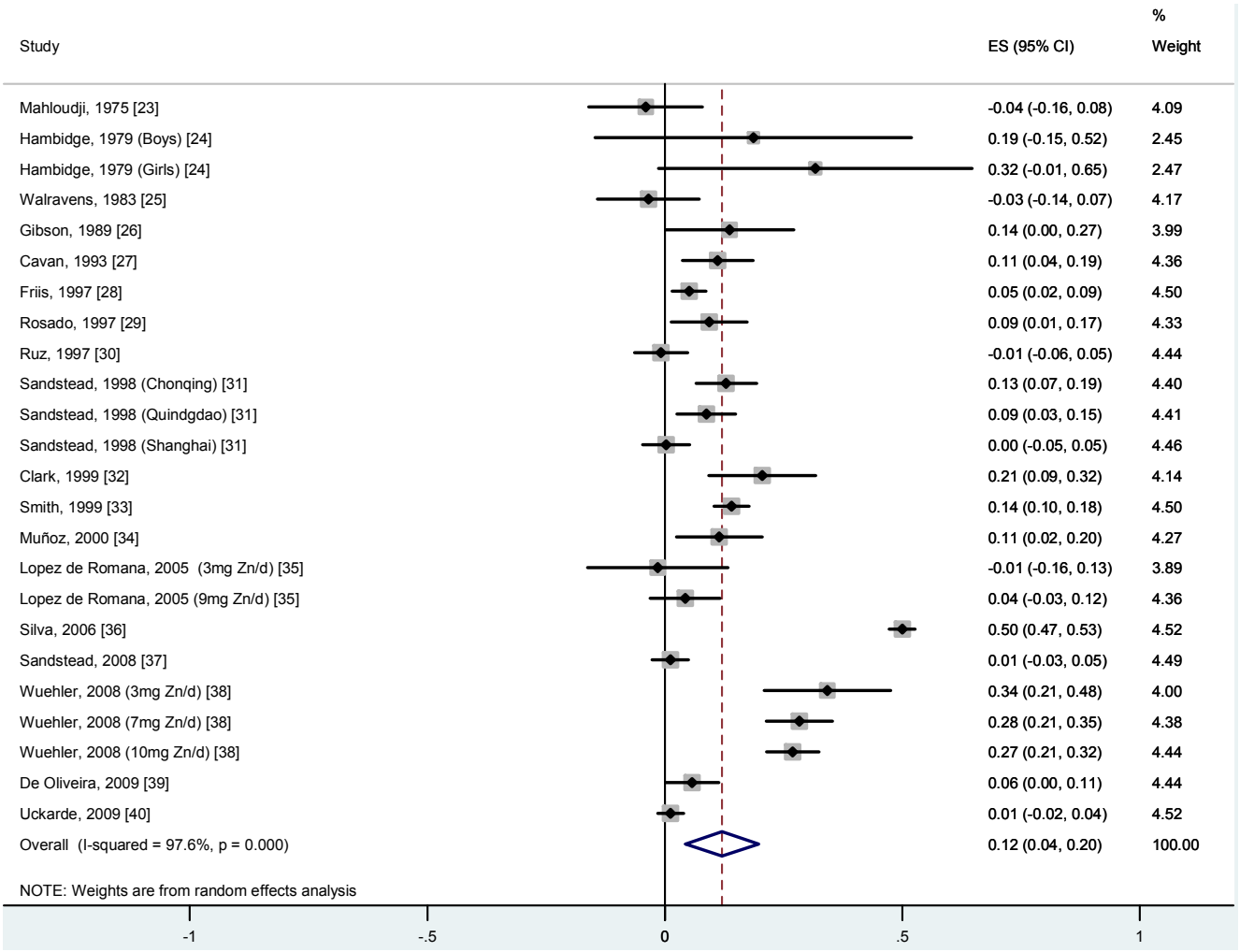
3. Results

Twenty-four estimates of zinc intake and serum/plasma zinc status in 18 RCTs with children were eligible for meta-analysis. All studies were RCTs published between 1975 and 2009 which reported zinc intake and plasma/serum zinc concentrations. The 24 estimates included 1722 participants in total with sample sizes ranging from 10 to 122. The median duration of the trials was 24 weeks (range 6–52 weeks). In 11 studies zinc was supplemented alone at doses ranging from 3 to 70 mg/day and in 7 studies participants also received other micronutrients. Zinc was provided with iron supplements [23] or iron fortified milk [36], as part of a fortified meal [24,35], and with other micronutrients [27,31,37]. The zinc dose ranged from 10 to 20 mg/day when combined with other vitamin/minerals and 2.57 to 9 mg/day when provided in fortified meals. Most studies (n = 7) provided the zinc supplements in the form of zinc sulphate, but others used zinc citrate [32], zinc gluconate [33], zinc carbonate [23], zinc methionine [29,34], amino acid chelate [27], and elemental zinc in a syrup [28,40]. Studies were conducted in Latin America (n = 9), North America (n = 4), Asia (n = 3), Africa (n = 1) and Europe (n = 1). Habitual zinc intakes ranged from 4.6 to 7.1 mg/day (where data was provided) and ages of children ranged from 2 to 17 years. Most studies included, but did not differentiate between, males and females, but one study provided separate male and female data [24], one included only females [32], and one only males [39].

The majority of studies (n = 13) reported that zinc supplementation significantly increased zinc plasma/serum status or significantly reduced the decline in zinc serum values compared to placebo. Of these, two studies also reported increased plasma/serum zinc concentrations in the placebo group. Five studies failed to find a significant relationship between zinc supplementation and zinc status [23,25,26,30,35], four of which provided zinc supplements or fortified meals with a zinc concentration of 10 mg/day or less.

Our meta-analysis of available studies suggested that zinc supplementation was associated with increased serum/plasma zinc concentrations. Combining the 18 RCTs in one meta-analysis yielded an overall pooled β-coefficient of 0.12 (95% CI 0.04, 0.20; $p < 0.005$; I^2 97.6%) (Figure 2). Since we applied a base-e logarithmic transformation on the zinc intake and serum/plasma zinc concentration before calculation of the study-specific $\hat{\beta}$'s, the overall $\hat{\beta}$ represents the difference in the $\log_e$ transformed predicted value of serum/plasma zinc status for each one-unit difference in the $\log_e$ transformed value in zinc intake. Therefore, an overall $\hat{\beta}$ of 0.12 means that for every doubling in zinc intake, the difference in zinc serum or plasma concentration is $2^{\hat{\beta}}$ ($2^{0.12} = 1.09$), which is 9%. This means that a person with a zinc intake of 14 mg/day has a zinc serum/plasma concentration that is 9% higher than a person who has a zinc intake of 7 mg/day.

Figure 2. Random effects meta-analyses of RCTs evaluating the effect of dietary zinc on serum/plasma zinc in children. Beta's represent the regression coefficients for the linear association between $\log_e$ transformed zinc intake and $\log_e$ transformed serum/plasma zinc status (lines represent the confidence intervals of each study).



As the physiological requirements for zinc peak at the time of the pubertal growth spurt, which generally occurs in girls between 10 and 15 years and in boys between 12 and 15 years, a separate subgroup analysis compared zinc intake and status according to age. As the onset of puberty was rarely assessed in papers, arbitrary age groups of <10 year, and ≥10 year were used. One study for which mean serum/plasma zinc values were given for children whose ages spanned both age groups were excluded from this analysis [23]. A meta-analysis of 18 studies performed in children under 10 years yielded an overall β of 0.13 (95% CI 0.04, 0.22; I^2 69.7%), compared to a meta-analysis of 3 studies performed in children over 10 years which yielded a β of 0.08 (95% CI 0.02, 0.15; I^2 97.9%), although care should be taken with interpreting this finding as the latter analysis is based on limited available data.

There is statistical evidence for substantial between-study heterogeneity in the overall meta-analysis (I^2 97.6%, $p < 0.0001$). To evaluate potential sources of heterogeneity, the variables duration, age, dose of zinc, and zinc status of the placebo groups (as a proxy for baseline zinc status) were added simultaneously to a meta-regression model as continuous variables. The analysis revealed that only zinc status of the placebo group was a statistically significant determinant of the overall β. The model

explained 26.5% of between-study variance but the residual variation due to heterogeneity remained high (91.7%). It is important to note that serum/plasma zinc levels of the placebo groups were used as a proxy of baseline serum/plasma zinc as the mean baseline serum/plasma zinc levels were infrequently reported in the papers, however it does suggest that the β vary according to zinc status.

Table 3 summarises the internal validity of the included studies, assessed as described in the methods section. The risk of bias was high in all but three where the risk was low/moderate [25,37,38]. Papers were given a high risk of bias rating due to insufficient information provided on sequence generation and/or allocation, drop-outs and funding bodies.

Table 3. Assessment of validity of included RCTs reporting zinc intake and serum/plasma zinc in children (adapted from the Cochrane Handbook [43]).

Author, Year	Adequate Sequence Generation	Allocation Concealment Adequate	Blinding Adequate	Dropouts Adequate and Outcome Data Complete	Funder Adequate	Lack of other Potential Threats to Validity	Overall Risk of Bias
Mahloudji, 1975 [23]	Unclear	Yes	Unclear	Unclear	Yes	Unclear	High
Hambidge, 1979 [24]	Unclear	Unclear	Yes	Unclear	No	Unclear	High
Walravens, 1983 [25]	Unclear	Yes	Yes	Yes	Yes	Yes	Moderate
Gibson, 1989 [26]	Unclear	Yes	Yes	Yes	No	Yes	High
Cavan, 1993 [27]	Unclear	Yes	Yes	Unclear	Yes	No	High
Friis, 1997 [28]	Unclear	Yes	Yes	Yes	Yes	Yes	High
Rosado, 1997 [29]	Unclear	Yes	Yes	Unclear	Yes	Yes	High
Ruz, 1997 [30]	Unclear	Yes	Yes	Unclear	Yes	Yes	High
Sandstead, 1998 [31]	Unclear	Unclear	Yes	Unclear	No	No	High
Clark, 1999 [32]	Yes	Yes	Yes	Unclear	No	Unclear	High
Smith, 1999 [33]	Unclear	Unclear	Unclear	Yes	Yes	Yes	High
Muñoz, 2000 [34]	Unclear	No	Yes	Yes	Nor	Yes	High
Lopez de Romana, 2005 [35]	Unclear	Unclear	Unclear	Yes	Yes	Yes	High
Silva, 2006 [36]	Unclear	Unclear	No	Yes	No	Yes	High
Sandstead, 2008 [37]	Yes	Yes	Yes	Unclear	No	Yes	Moderate
Wuehler, 2008 [38]	Yes	Unclear	Yes	Yes	Yes	Yes	Low
de Oliveira, 2009 [39]	Unclear	Unclear	No	Unclear	No	Yes	High
Uckarde, 2009 [40]	Unclear	Yes	Yes	Yes	No	Yes	High

4. Discussion

This study is unique in providing an estimate of the dose-response relationship of zinc intake and serum/plasma zinc concentrations in children aged 1–17 years. Similar to findings published in an earlier systematic review [44], this meta-analysis of 24 estimates in 18 RCTs found a significant effect of zinc intake and serum/plasma zinc concentrations in children. In addition we have provided an estimate of the dose-response relationship between zinc intake and serum/plasma concentrations. An overall $\hat{\beta}$ of 0.12 means that for every doubling in zinc intake, the difference in zinc serum or plasma concentration is 9%. In other words, a child with a zinc intake of 14 mg/day has a zinc serum/plasma concentration that is 9% higher than a person who has a zinc intake of 7 mg/day. It is important to note however that, due to homeostatic regulatory mechanisms, the amount of dietary zinc absorbed decreases as intake increases, and plasma zinc concentration is homeostatically controlled within a narrow physiological range, therefore this dose response relationship can only be applied to the range of intakes used to derive this relationship. The studies included in this meta-analysis were different in a number of aspects, such as using various designs, follow-up times, zinc doses, and populations.

Therefore, it is no surprise that, when combining these studies in a meta-analysis, a large heterogeneity is observed between the studies (I^2 = 97.6% p = 0.0001). This between-study heterogeneity may be caused by methodological factors, such as biological factors, e.g., differences in study population characteristics (age, socio-economic status), differences in doses of provided zinc (amount, one or more doses per day, study duration). We have considered the dose of zinc provided, study duration, age, and supplement type and these factors did not significantly explain the between-study heterogeneity. An individual participant data meta-analysis may have provided a more conclusive explanation of the between-study heterogeneity in this meta-analysis. However, this type of analysis would involve the input of raw individual participant data provided by the original study investigators for re-analysis and combination in a pooled analysis and as such would be a major undertaking in terms of time, costs, and collaboration. Moreover, an inability to include individual participant data from all relevant studies could introduce selection bias. The meta-analytic approach used in this paper is not an attempt to accurately describe the biological relation between actual zinc intake and zinc concentrations in blood under strict experimental conditions and on an individual level, but rather to simulate a dose-response relationship between zinc intake and status that is useful for surveillance studies with a public health point of view and, as such, deliberately incorporates the differences between dietary assessment methods, laboratory assessment methods and participant characteristics to ensure a broad external validity. Thus, the heterogeneity reflects the lack of standardisation of methods and the true heterogeneity between study populations and necessarily enters as uncertainty into the application of such data for public health purposes [45].

The relationship observed between serum/plasma zinc concentration and zinc intake may have been weakened by the limitation of this particular biomarker for zinc status. It is well established that plasma zinc concentration can fall in response to factors unrelated to zinc status or dietary zinc intake, such as infection, inflammation, exercise, stress or trauma. Conversely, tissue catabolism during starvation can release zinc into the circulation, causing a transient increase in circulating zinc levels. Postprandial plasma zinc concentrations have been reported to fall up to 19% [46]. Twelve studies used fasting blood samples in their analyses (usually overnight). Other factors related to the adequacy of serum/plasma sampling, such as storage and separation of samples, was often inadequately reported. Whilst all studies included in the analysis were undertaken in individuals without chronic disease or severe protein-energy malnutrition, other factors such as stress, infection and inflammation may also have gone unreported. For example, only three studies screened for parasitic infection [28,30,36]. Clearly such confounders have a strong influence on the interpretation of plasma zinc concentrations. However, as more sensitive indices of zinc status have yet to be identified, plasma serum zinc remains by far the most commonly used biomarker of zinc status [16].

It is important to note that the majority of studies in our meta-analysis (n = 13 of 18) were conducted in countries where participants are likely to have dietary patterns with low-moderate zinc bioavailability with higher fibre and phytate content which may have weakened the overall β. Although suboptimal zinc status may be caused by inadequate dietary intake of zinc in some cases, inhibitors of zinc absorption are likely the most common causative factor [47], and recent evidence in adults suggests that the inhibitory effect of dietary factors such as phytate on zinc absorption is likely to be much greater than previously recognised [48], although whether this is the case for children is less certain [49]. Indeed, a proxy measure for initial nutritional status of participants (zinc

concentration in placebo groups) was found to be a significant effect modifier of β. However as very few studies reported baseline zinc or gave details of the concentration of indigestible zinc binding ligands in participants' diets we were unable to investigate this important effect further.

Zinc was given in combination with other micronutrients including iron in several studies. As iron and zinc are known to compete for absorption [50] it is possible that iron supplements may impair child zinc status [47]. It is possible therefore that additional supplementation of iron may have reduced the effect of zinc supplementation on zinc plasma levels. A review on the interaction between zinc and iron in supplementation trials reported that, in the 4 RCTs reviewed, addition of iron to zinc supplementation did not affect plasma zinc status in children [51]. As three of the included trials were in infants aged 4–6 months and as the iron-regulatory mechanisms of infants may differ before and after 9 months of age [52], further studies in older age groups are needed to understand more fully the interaction effects of micronutrient supplementation.

To conduct our meta-analysis some assumptions related to the availability of the required data or related to statistical issues had to be made. The meta-analysis required transformations of the intake and biomarker data to a common scale, as the studies included in our meta-analyses had different ways of reporting the relation between zinc and biomarkers of zinc status in blood. We standardised the different ways of reporting by transformation of both the intake and biomarker data to double $\log_e$-scale, which allowed us to derive a standardised estimate from each study of the regression coefficient and its standard error as a basis for comparing these heterogeneously reported results. We also assumed a linear relationship on the double $\log_e$-scale. This rigorous but flexible transformation allowed us to pool β's and report these as a dose-response relationship between zinc intake and serum/plasma zinc concentrations. As compared to a conventional meta-analysis of mean differences between high and low exposed subjects, a linear relationship on the double $\log_e$-scale with a slope lower than 1 allows us to model biomarker levels as a non-linear but monotonic concave function of dose, which is considered a more likely shape in biology. The meta-analyses were conducted within the context of the EURRECA project as a means to provide additional evidence for underpinning reference values for zinc intake of populations [53]. Whether the dose-response relationship, as provided in this paper, could be used as either qualitative or quantitative evidence to substantiate the daily zinc intake dose necessary to achieve normal or optimal levels of biomarkers for zinc status, remains a matter of discussion regarding the cut-off levels for biomarkers of zinc and the predictive value of serum/plasma zinc concentration for relevant functional health outcomes such as growth, immune function, cognitive function and psychomotor development.

Due to the wide heterogeneity that exists in the published literature on the relation between zinc intake and zinc status, such data cannot be combined in a conventional meta-analysis. Our paper not only provides a useful summary of this data in a systematic review, but also demonstrates a new meta-analytic approach to summarise all this data while appreciating the heterogeneity of it. The mathematical basis of this novel approach has recently been published [41] and is beyond the scope of the paper under review here, but in summary, we modelled a dose-response relation as a monotonic concave function between zinc intake and biomarkers. This is an innovative way to use all the data available, albeit heterogeneous data, to model the dose-response relationship; information which is essential when appraising micronutrient recommendations. When more research on zinc intake and

status becomes available, this meta-analytical approach can be improved to strengthen the evidence on which we base our zinc recommendations.

5. Conclusion

In conclusion, based on 24 estimates among 1722 participants, the results indicate that a doubling of zinc increases plasma/serum levels by 9%. Although it is recognised that serum/plasma zinc is a somewhat flawed biomarker of zinc status, in the absence of suitable alternatives it remains by far the most commonly used method and as such this review provides a valuable source of information for those seeking to assess zinc nutriture. As serum/plasma zinc levels are considered intermediates in the causal path to health benefits and because the heterogeneity between study results reflects real uncertainty in the evidence base, these issues must be taken into account when our dose-response data are used as complementary evidence for underpinning zinc reference values.

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The relationship between zinc intake and serum/plasma zinc concentration in adults. A systematic review and dose-response meta-analysis by the EURRECA Network

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The relationship between zinc intake and serum/plasma zinc concentration in adults. A systematic review and dose-response meta-analysis by the EURRECA Network

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Short title: Zinc intake and plasma zinc concentration

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Key words

EURRECA, zinc, dose-response, systematic review, meta-analysis.

Abstract

Dietary zinc recommendations vary widely across Europe due to the heterogeneity of approaches used by expert panels. Under the EURRECA consortium a protocol was designed to systematically review and undertake meta-analyses of research data to create a database that includes "best practice" guidelines which can be used as a resource by future panels when setting micronutrient recommendations. As part of this process, the objective of the present study was to undertake a systematic review and meta-analysis of previously published data describing the relationship between zinc intake and status in adults. Searches were performed of literature published up to February 2010 using MEDLINE, Embase, and Cochrane Library. Data extracted included population characteristics, dose of zinc, duration of study, dietary intake of zinc, and mean concentration of zinc in plasma or serum at the end of the intervention period. An intake-status regression coefficient ($\hat{\beta}$) was estimated for each individual study, and pooled meta-analysis undertaken. The overall pooled $\hat{\beta}$ for zinc supplementation on serum/plasma zinc concentrations from RCTs and observational studies was 0.08 (95% CI 0.05, 0.11; $p<0.0001$; I^2 84.5%). An overall $\hat{\beta}$ of 0.08 means that for every doubling in zinc intake, the difference in zinc serum or plasma concentration is $2^{\hat{\beta}}$ ($2^{0.08}$ = 1.06), which is 6%. Whether the dose-response relationship, as provided in this paper, could be used as either qualitative or quantitative evidence to substantiate the daily zinc intake dose necessary to achieve normal or optimal levels of biomarkers for zinc status, remains a matter of discussion.

Introduction

Dietary zinc recommendations vary widely across Europe due to the heterogeneity of approaches used by expert panels [1]. There is a need for a harmonised approach that is transparent and based on the best quality data and methods available. Traditionally, the factorial approach is used in the determination of zinc requirements. This method seeks to estimate the zinc intake required to meet physiological requirements for growth, metabolism and tissue repair while replacing obligatory losses. An alternative approach is to examine the dose-response relationship between intake and biomarkers of status and also between intake and health outcomes. This information could then be integrated using a mathematical model to provide an insight into the level of zinc intake required for optimal health based on a range of parameters and indices of health that are known to be dependent upon dietary zinc intake [2]. To this end, the members of the European Micronutrient Recommendations Aligned (EURRECA) Network of Excellence have undertaken a series of systematic reviews of zinc-intake-status relationships, according to rigorous protocols defined by consortium members and external experts [2] . This paper presents the results of the systematic review and meta-analysis of the dose response relationship between dietary zinc intake and zinc status using novel methodology developed by members the EURRECA consortium.

The assessment of zinc status is notoriously problematic for zinc, as a sensitive, specific biomarker for zinc has not yet been identified [3]. A systematic review and meta-analysis of biomarkers of zinc status was undertaken in 2009 [4]. For many putative biomarkers (such as the zinc concentrations found in the cellular components of whole blood) there were insufficient data to arrive at a definitive conclusion regarding their efficacy as a biomarker of zinc status, however plasma (or serum) zinc concentration was responsive to both zinc supplementation and zinc depletion and is the most widely reported biomarker for zinc. Hair and urine zinc concentrations were also considered to be potentially useful biomarkers in response to zinc supplementation.

The purpose of this study was to systematically and quantitatively assess the dose response relationships relevant to deriving zinc recommendations based on intervention studies, cohort (nested case control) studies and cross-sectional studies. The specific questions to be addressed were; what is the effect of intake on indicators of exposure or body stores (i.e. biomarkers)? What factors affect this relationship?

The data used in this meta-analysis were extracted from published studies (RCTs, prospective cohort studies, nested case-control studies and cross-sectional), performed in healthy adult and elderly populations, reporting the relationship between zinc status (plasma or serum zinc, hair or urine zinc concentration) and intake from supplements, fortified diets or natural food diets.

Methods

Search strategy

This research was conducted within the framework of the European Micronutrient Recommendations Aligned (EURRECA) Network of Excellence that aims to identify the micronutrient requirements for optimal health in European populations (www.eurreca.org). This research was part of a wider review process to identify studies assessing the effect of zinc intake on different outcomes (biomarkers of zinc status and health outcomes). The wider searches were performed of literature published up to and including February 2010 using Ovid MEDLINE, Embase (Ovid), and the Cochrane Library (CENTRAL) using search terms for ('study designs in humans') AND (zinc) AND (intake OR status). Both indexing and text terms were used and languages included were restricted to those spoken in the EURRECA Network (English, Dutch, French, German, Hungarian, Italian, Norwegian, Polish, Spanish, Greek, and Serbian.). The full Ovid MEDLINE search strategy can be found in Table 1. Reference lists of retrieved articles and published literature reviews were also checked for relevant studies. Authors were contacted to request missing data or clarify methods or results. The search process is illustrated in Figure 1.

Criteria for the consideration of studies for this review

Included studies were RCTs, prospective cohort studies, nested case-control studies and cross-sectional studies in healthy human populations that supplied zinc supplementation (RCTs) or measured dietary zinc intake with either a validated food frequency questionnaire, a dietary history method, a 24-hour recall method for at least 3 days, or a food record/diary for at least 3 days (observational studies). Studies had to be conducted in apparently healthy adult and elderly (human) populations aged ≥18 years and supplied zinc supplementation either as capsules or part of a fortified meal. If supplemental zinc was provided as a component of a fortified meal, studies were only considered acceptable if zinc was the only

constituent that was different between treatment groups. Biomarkers of zinc status included plasma/serum, urine and hair zinc concentrations. Only studies that reported sufficient data or had sufficient data obtainable from the authors to estimate $\hat{\beta}$ and SE($\hat{\beta}$) for the assumed linear relation on the $\log_e$-$\log_e$ scale were included. Studies were excluded if they were a group RCT (community trial), or were commentaries, reviews, or duplicate publications from the same study. Studies were excluded if adults were hospitalised, had a chronic disease or if supplemental zinc was provided for less than 2 weeks.

Selection of articles

Of 4719 identified articles in the wider search on zinc intake, status and priority health outcomes in all populations, 2557 were excluded based upon screening of the title and abstract. Two independent reviewers screened 10% of the abstracts in duplicate and any discrepancies were discussed before screening the remaining references. Following subdivision into appropriate population groups the full texts of the 1231 manuscripts were assessed to determine inclusion and exclusion by two independent reviewers and disagreements rectified through discussion. 1147 studies were excluded because they did not meet the inclusion criteria. Of the remaining 84 studies, 54 studies were excluded as they related either zinc intake or status directly to a health endpoint, but they had not investigated the relationship between zinc intake and zinc related to biomarkers. A further 17 studies were excluded from the meta-analysis because study participants were not healthy, insufficient data was reported, data was duplicated, or the dosage and duration was unclear. For the purpose of this meta-analysis, 10 RCTs and 3 observational studies remained. The characteristics of the included studies are presented in Table 2 and Table 3 respectively.

Data extraction

For each of the identified manuscripts, data was extracted independently by two reviewers into a standardized database. Extracted data included population characteristics, dose of zinc in intervention and placebo supplements, duration of the study, dietary intake of zinc, and mean concentration of zinc in plasma or serum at the end of the intervention period. Serum/plasma zinc concentrations were converted to µmol/L when applicable.

Data synthesis

Two RCTs that reported data for two zinc-treated groups and two control groups were treated as two independent estimates in the analysis [5; 6]. Where RCTs provided outcome data for two or more zinc-treated group, they were included as separate estimates in the meta-analysis [7; 8; 9; 10; 11]. Where zinc status was measured at different time points within the same population only the final measure was used in the analysis [12; 13]. One observational study reported data from males and females and these were treated as two estimates in the meta-anaysis [14]. If dietary intake of zinc (in addition to the intervention) was not reported in the RCTs, a value of 9.7 mg/day was imputed, which was the mean dietary intake level of the RCTs that did report dietary zinc intake. As mean baseline serum/plasma zinc concentrations were infrequently reported in the RCTs, the serum/plasma zinc concentrations in the control group were used as a proxy of the baseline serum/plasma zinc concentrations for our analyses.

Statistical analyses

A stratified random effects meta-analysis was conducted using STATA version 11 (College Station, TX), with one subgroup combining the evidence from RCTs and the other subgroup combining the evidence from observational studies. As serum/plasma zinc levels have been reported to decline with age [15], a separate stratified random effects meta-analysis compared zinc intake and status according to age in RCTs (< 55 years and ≥ 55 years). In addition, stratified meta-analyses were also conducted on dose of zinc (<35 mg/day and ≥ 35 mg/day) and trial duration (in weeks). It was not possible to perform a stratified meta-analysis for gender, because most studies included both men and women and data were not available at the individual level.

The transformations used to derive coherent single-study estimates from the available summary statistics per study have been described elsewhere [16] . In short, an intake-status regression coefficient (β) for each individual study was estimated from the mean serum/plasma zinc concentrations, based on the assumption of a linear relation on the $\log_e$-$\log_e$-scale (natural logarithm of intake versus natural logarithm of status). Algebraically deriving an estimate from each study of the regression coefficient ($\hat{\beta}$) and its standard error (SE($\hat{\beta}$)) enabled a comparison of the results from studies with heterogeneously reported associations and effects. The overall pooled $\hat{\beta}$ and SE($\hat{\beta}$) was calculated using random effects meta-analysis, which estimates the between-study variance using the method of DerSimonian and Laird [17]. This was then used to modify the weights used to calculate the summary

estimate. Residual heterogeneity between studies was evaluated using the I^2 statistic. To evaluate potential sources of heterogeneity, the variables study duration, age, gender and zinc dose were added simultaneously to a meta-regression model as continuous variables. The statistical transformations to obtain $\hat{\beta}$'s and SE($\hat{\beta}$)'s were performed using GenStat version 13-SP2 (VSN International Ltd. Hemel Hemptead, UK) and the meta-analysis was performed using STATA version 11.0, with statistical significance defined as $P<0.05$.

Assessment of risk of bias in included studies

In order to assess the quality of the included studies and the risk of bias, indicators of internal validity were collected during data extraction (Table 3). Based on the indicators two independent reviewers assessed the overall risk of bias and disagreements resolved by discussion. The criteria for judging these indicators were adapted from the Cochrane Handbook for Systematic Reviews [18].

Results

Twenty estimates of zinc intake and serum/plasma zinc status in 10 RCTs and four estimates in 3 observational studies were eligible for meta-analysis. All studies were published between 1979 and 2010. Although plasma/serum, urine and hair zinc concentrations were included as markers of status in the systematic review protocol, only plasma/serum zinc concentration was reported universally and sufficiently frequently to be used in the meta-analysis. Most studies included, but did not differentiate between, males and females, but three studies included only females [19; 9; 20], two included only males [13; 8] and one provided both male and female data [14]. Studies were conducted in Europe (n=7), North America (n=3), South Asia (n=1), East Asia (n=1) and Australasia (n=1) and ages of participants ranged from 18 to 106 years.

All but one RCTs used a parallel design. Boukaïba and colleagues employed a cross-over RCT design [6]. The RCTs included 1285 participants in total with sample sizes ranging from 5-201. The median duration of the trials was 25 weeks (range 2-52 weeks). In 9 studies zinc was supplemented alone at doses ranging from 15-135.3 mg/day and in 1 study zinc was provided within a multi-micronutrient supplement [12]. Most studies (n=7) provided the zinc supplements in the form of zinc gluconate, but others used zinc sulphate [21], zinc acetate [7], or zinc carnosine [11]. Habitual zinc intakes ranged from 5.4-10.8 mg/day (where data was provided).

The observational studies included 1184 participants in total with sample sizes ranging from 170-500. Zinc intake was measured using a combination of FFQ and 24 hour recall, or 24 hour recall alone and values ranged from 8.6-12.2 mg/day. The meta-analysis of available studies suggested that zinc supplementation was associated with increased serum/plasma zinc concentrations. The estimated effect for zinc supplementation on serum/plasma zinc concentrations from RCTs and observational studies was 0.08 (95% CI 0.05, 0.11; $p<0.0001$; I^2 84.5%) (Fig 2). When data sets were grouped according to study design, only the RCTs showed a significant effect size (0.09 95% CI 0.07, 0.120; $p<0.0001$; I^2 79.1%).

Since a base-*e* logarithmic transformation was applied to the zinc intake and serum/plasma zinc concentration before calculation of the study-specific $\hat{\beta}$'s, the overall $\hat{\beta}$ represents the difference in the $\log_e$transformed predicted value of serum/plasma zinc status for each one-unit difference in the $\log_e$transformed value in zinc intake. Therefore, an overall $\hat{\beta}$ of 0.08 means that for every doubling in zinc intake, the difference in zinc serum or plasma concentration is $2^{\hat{\beta}}$ ($2^{0.08} = 1.06$), which is 6%. This means that a person with a zinc intake of 14 mg/day has a zinc serum/plasma concentration that is 6% higher than a person who has a zinc intake of 7 mg/day (Fig 3).

As plasma/serum zinc concentrations have been reported to decline with age [15], a separate subgroup analysis compared zinc intake and status according to age in RCTs (< 55 years and ≥ 55 years). Two studies for which mean serum/plasma zinc values were given for adults whose ages spanned both age groups were excluded from this analysis [12; 11]. A stronger effect size was found in adults aged under 55 years (0.14 95% CI 0.04, 0.24; $p<0.005$; I^2 92.1%) compared to adults aged 55 years and over (0.09 95% CI 0.07, 0.11; $p<0.0001$; I^2 32.8%), although care should be taken with interpreting this finding as the younger age group analysis is based on only three estimates in two studies. Stratifying the analysis for dose of zinc (<35 mg/day and ≥ 35 mg/day) revealed a stronger effect size for a zinc dose ≥ 35mg/d (0.14 95% CI 0.08, 0.21; $p<0.0001$; I^2 85.2%) compared to <35mg/d (0.09 95% CI 0.07, 0.10; $p<0.005$; I^2 27.6%). Similar effect sizes were demonstrated for study duration (0-12 weeks 0.13 CI 0.05, 0.20 I^2 92.4% and > 12 weeks 0.10 CI 0.07, 0.12 I^2 75.8%).

To evaluate potential sources of heterogeneity, the variables duration, age, gender and dose were added simultaneously to a meta-regression model as continuous variables. The analysis revealed that only zinc dose was a statistically significant determinant of the overall

beta. The model explained 50% of between-study variance and the residual variation due to heterogeneity was reduced to 48.2%.

Table 4 summarises the internal validity of the included studies, assessed as described in the methods section. The risk of bias was high in 5 out of the 10 papers [21; 6; 22; 23; 11]. Papers were given a high risk of bias rating due to insufficient information provided on sequence generation and/or allocation, drop-outs and funding bodies.

Discussion

The current study is unique in providing an estimate of the dose-response relationship of zinc intake and serum/plasma zinc concentrations in adults. A meta-analysis of 20 estimates in 10 RCTs and 4 estimates in 3 observational studies found that zinc supplementation produced a statistically significant increase in serum/plasma zinc concentrations and provided an estimate of the dose-response relationship between zinc intake and serum/plasma concentrations. An overall $\hat{\beta}$ of 0.08 means that for every doubling in zinc intake, the difference in zinc serum or plasma concentration is 6%. In other words, an adult with a zinc intake of 14 mg/day has a zinc serum/plasma concentration that is 6% higher than a person who has a zinc intake of 7 mg/day. This association was slightly stronger when considering only the RCTs, as no observational studies found a significant association between zinc intake and plasma zinc concentrations. The intake-status regression coefficient for the observational studies is likely to be attenuated by random and intake-related errors in assessing dietary zinc intake [24], whereas in RCTs zinc intake can be considered as fixed at each level of dosage and random errors arise only through assessment of biomarkers.

The studies included in this meta-analysis were different in a number of aspects, such as using various designs, follow-up times, zinc doses, and populations. Therefore, it is no surprise that, when combining these studies in a meta-analysis, a large heterogeneity is observed between the studies (I^2 = 84.5% p=0.0001). This between-study heterogeneity may be caused by methodological factors, such as differences in study population characteristics (age, socio-economic status) or differences in doses of provided zinc (amount, one or more doses per day, study duration). When considering some key variables (study duration, zinc dose, age, and gender) in a meta-regression model, only dose explained some between-study heterogeneity. An individual participant data meta-analysis may have provided a more conclusive explanation of the between-study heterogeneity in this meta-analysis. However,

this type of analysis would involve the input of raw individual participant data provided by the original study investigators for re-analysis and combination in a pooled analysis and as such would be a major undertaking in terms of time, costs, and collaboration. Moreover, an inability to include individual participant data from all relevant studies could introduce selection bias. The meta-analytic approach used in this paper is not an attempt to accurately describe the biological relation between actual zinc intake and zinc concentrations in blood under strict experimental conditions and on an individual level, but rather to simulate a dose-response relationship between zinc intake and status that is useful for surveillance studies with a public health point of view and, as such, deliberately incorporates the differences between dietary assessment methods, laboratory assessment methods and participant characteristics to ensure a broad external validity. Thus, the heterogeneity reflects the lack of standardization of methods and the true heterogeneity between study populations and necessarily enters as uncertainty into the application of such data for public health purposes [25].

To conduct this meta-analysis some assumptions related to the availability of the required data or related to statistical issues had to be made. First, when two or more intervention groups were compared to the same control group (5 RCTs), independence of estimates was assumed. As a consequence bias may have been introduced, by either increasing the estimates of the intervention effect (if the control group values were in fact lower), or decreasing the estimates of the intervention effect (if the control group values were higher). Second, the meta-analysis required transformations of the intake and biomarker data to a common scale, as the studies included in this meta-analyses had different ways of reporting the relation between zinc and serum/plasma zinc concentration . The different ways of reporting by transformation of both the intake and biomarker data were standardized to double $\log_e$-scale, which allowed the derivation of a standardized estimate from each study of the regression coefficient and its standard error as a basis for comparing these heterogeneously reported results. A linear relationship on the double $\log_e$-scale was also assumed. This transformation allowed the pooling of beta values and enable these to be reported as a dose-response relationship between zinc intake and serum/plasma zinc concentrations [16].

The meta-analyses were conducted within the context of the EURRECA project as a means to provide additional evidence for underpinning reference values for zinc intake of populations. This dose-response relationship methodology may be used as either qualitative or quantitative evidence to substantiate the daily zinc intake dose necessary to achieve normal

or optimal levels of biomarkers for zinc status. The dose-response relationship between zinc intake and plasma zinc concentration is of course subject to the debate around the usefulness of plasma/serum zinc concentration as a biomarker of zinc status, and the it's predictive value for relevant functional health outcomes, such as markers of immune function.

The relationship observed between serum/plasma zinc concentration and zinc intake may have been weakened by the limitation of this particular biomarker for zinc status. It is well established that plasma zinc concentration can fall in response to factors unrelated to zinc status or dietary zinc intake, such as infection, inflammation, exercise, stress or trauma [26]. Conversely, tissue catabolism during starvation can release zinc into the circulation, causing a transient increase in circulating zinc levels. Six studies used non-fasted blood samples in their analyses [5; 7; 27; 20; 11; 14]. As postprandial plasma zinc concentrations have been reported to fall up to 19% [28], the inclusion of these studies may have weakened the observed relationship between zinc intake and status. Whilst all studies included in the analysis were undertaken in individuals without chronic disease or severe protein-energy malnutrition, other factors such as stress, infection and inflammation may also have gone unreported. In addition, serum zinc concentration has been reported to decrease with age [15]. Clearly such confounders have a strong influence on the interpretation of plasma zinc concentrations. However, as more sensitive indices of zinc status have yet to be identified, plasma serum zinc remains by far the most commonly used biomarker of zinc status [4].

In conclusion, the current study presents the application of a novel technique to analyse data from 10 RCT's and 3 observational studies reporting the relationship between zinc intake and serum/plasma zinc concentration. This meta-analysis has provided an estimate of the dose-response relationship between zinc intake and serum/plasma zinc concentration in adults and elderly populations. Based on 24 estimates among 2469 participants, the results indicate that a doubling of zinc intake increases plasma/serum levels by 6%. There is a high level of heterogeneity in the data obtained from the studies included in this meta-analysis. Analysis of the factors that may contribute to this, namely study duration, zinc dose, age, and gender, indicated that zinc dose was able to explain 50% of this heterogeneity. This novel method of analyzing intake/biomarker relationships may be useful for the setting of future dietary zinc recommendations.

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The original conception of the systematic review was undertaken by the EURRECA Network and coordinated by partners based at Wageningen University (WU), the Netherlands and the University of East Anglia (UEA), United Kingdom. Susan Fairweather-Tait (UEA), Lisette de Groot (WU), Pieter van' t Veer (WU), Kate Ashton (UEA), Amélie Casgrain (UEA), Adriënne Cavelaars (WU), Rachel Collings (UEA), Rosalie Dhonukshe-Rutten (WU), Esmée Doets (WU), Linda Harvey (UEA) and Lee Hooper (UEA) designed and developed the review protocol and search strategy.

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454 **Figure Legends**

455 **Figure 1** Study selection process for systematic review

456 **Figure 2** Random effects meta-analyses of RCTs and observational studies evaluating the
457 pooled effect of dietary zinc on serum/plasma zinc in adults. Beta values (♦) represent the
458 regression coefficients for the linear association between $\log_e$ transformed zinc intake and
459 $\log_e$ transformed serum/plasma zinc status.

460 **Figure 3** Serum/plasma zinc concentration (μmol/L) as a function of dietary zinc intake
461 (mg/day), estimated by random-effects meta-analyses of RCTs of adults

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Table 1. Ovid MEDLINE search strategy.

No.	*Search term*	*Results*
1	randomized controlled trial.pt.	280821
2	controlled clinical trial.pt.	79998
3	randomized.ab.	196604
4	placebo.ab.	117891
5	clinical trials as topic.sh.	146242
6	randomly.ab.	145491
7	trial.ab.	203467
8	randomised.ab.	38423
9	6 or 3 or 7 or 2 or 8 or 1 or 4 or 5	734511
10	(animals not (human and animals)).sh.	4482479
11	9 not 10	642665
12	(cohort* or "case control*" or cross-sectional* or "cross sectional" or case-control* or prospective or "systematic review*").mp.	768885
13	exp meta-analysis/ or expmulticenter study/ or follow-up studies/ or prospective studies/ or intervention studies/ or epidemiologic studies/ or case-control studies/ or exp cohort studies/ or longitudinal studies/ or cross-sectional studies/	1013635
14	13 or 12	1203767
15	14 not 10	1154385
16	11 or 15	1599094
17	((zinc or zn or zinc sulphate or zinc gluconate or zinc acetate or methionine or zinc isotope*) adj3 (intake* or diet* or supplement* or deplet* or status or serum or plasma or leukocyte or concentration* or expos* or fortif* or urine or hair)).ti,ab.	16681
18	Nutritional Support/ or Dietary Supplements/ or nutritional requirements/ or Breast feeding/ or exp infant food/ or bottle feeding/ or infant formula/	63098
19	exp Nutritional Status/ or exp Deficiency Diseases/ or supplementation/ or diet supplementation/ or dietary intake/ or exp diet restriction/ or exp mineral intake/ or Diet/ or Food, Fortified/ or nutrition assessment/ or Nutritive Value/	176014
20	(intake* or diet* or supplement* or deplet* or status or serum or plasma or leukocyte or concentration* or expos* or fortif* or urine or hair).ti,ab.	3166092
21	18 or 19 or 20	3263114
22	zinc/	41027
23	22 and 21	20745
24	23 or 17	26943
25	24 and 16	2410

Table 2: Randomised controlled trials (n=10) reporting the effect of dietary zinc intake on serum/plasma zinc status in adults.

First author, year, country	Gender, age	Treatment groups	Micronutrient type	Duration	Status marker reported [analytic method]
Abdulla, 1979 Sweden (5)	Mean age 25 y. SD, age range, gender not reported	*Study 1* Placebo (n=5) 135.3mg/d Zn (n=7) *Study 2* Placebo (n=8) 45mg/d Zn (n=7)	Zinc sulphate	12 wk	Plasma Zn [AAS]
Bodgen, 1988 USA (6)	Males and females aged 60- 89 y	Placebo (n=36) 15 mg/d Zn (n=36) 100 mg/d Zn (n=31)	Zinc acetate	3 mo	Plasma Zn [AAS]
Boukaïba, 1993	Males and females aged 73-106 y	*BMI ≤ 21* Placebo (n=21)	Zinc gluconate	8 wk	Serum Zn [AAS]

France (4)		20mg/d Zn (n=21) *BMI ≥ 24* Placebo (n=23) 20mg/d Zn (n=23)			
Preziosi, 1998 France (12)	Males and females aged 35-60 y	Placebo (n=200) Multi-micronutrient supplement (20mg/d Zn) (n=201)	Zinc gluconate	3 & 6 mo	Serum Zn [AAS]
Sullivan, 1998 USA (11)	Males aged 19- 35 y	Placebo (n=13) 50mg/d Zn (n=13)	Zinc gluconate	15 d	Plasma Zn [AAS]
Feillet-Coudray, 2005 France (8)	Males aged 58-68 y	Placebo (n=16) 15 mg/d Zn (n=16) 30 mg/d Zn (n=16)	Zinc gluconate	6 mo	Plasma Zn [ICP-MS]
Feillet-Coudray, 2006 France (7)	Females aged 55-70 y	Placebo (n=16) 15 mg/d Zn (n=16) 30 mg/d Zn (n=15)	Zinc gluconate	6 mo	Serum Zn [ICP-MS]

Hininger-Favier, 2007 France, UK, Italy (9)	Males and females aged 55-85 y	*Age 55-70y* Placebo (n=63) 15mg/d Zn (n=60) 30mg/d Zn (n=65) *Age >70 y* Placebo (n=67) 15 mg/d Zn (n=66) 30 mg/d Zn (n=66)	Zinc gluconate	6 mo	Serum Zn [AAS]
Prasad, 2007 USA (21)	Males and females aged 55-87 y	Placebo (n=25) 45 mg/d Zn (n=24)	Zinc gluconate	12 mo	Plasma Zn [AAS]
Sakagami, 2009 Japan (10)	Males and females aged 21-77 y	Placebo (n=28) 17 mg/d Zn (n=27) 34 mg/d Zn (n=26) 68 mg/d Zn (n=28)	Zinc carnosine	12 wk	Serum Zn [AAS]

AAS atomic absorption spectroscopy; ICP-MS inductively coupled plasma mass spectrometry

Table 3: Observational studies (n=3) reporting the association between dietary zinc intake and serum/plasma zinc status in adults.

First author, year, country	**N**	**Mean (SD) zinc intake (mg/day)**	**Mean (SD) plasma/serum zinc (µmol/L)**	**Zinc intake (source)**	**Zinc intake (assessment)**	**Zinc status biomarker [analytical method]**
Gibson 2001 (New Zealand) (17)	330 females aged 18-40 y	10.44 (3.51)	12.00 (1.36)	Diet	FFQ & 24 hr recall	Serum zinc [AAS]
Chandyo, 2009 (Nepal) (16)	500 females aged 13-35 y	8.6 (3.3)	8.5 (2.4)	Diet	FFQ & 24 hr recall (2 days)	Plasma zinc [ICP-AES]
Sánchez 2009 (Spain) (13)	170 males aged	12.24 (7.16)	17.48 (6.68)	Diet	24 hr recall (2	Plasma zinc [AAS]

	25-60 y			days)
	184 females aged 25-60 y	9.07 (4.40)	16.32 (6.21)	

AAS atomic absorption spectroscopy; ICP-MS inductively coupled plasma mass spectrometry

Table 4 Assessment of validity of included RCTs reporting zinc intake and serum/plasma zinc in adults

Study	Adequate sequence generation	Adequate Blinding	Dropouts adequate and outcome date complete	Funder adequate	Compliance check & results	Dose check & results	Dietary intake data reported & results	Status reproducibility reported	Similarity of most & least exposed groups at baseline	Lack of other potential threats to validity	Overall risk of bias
Abdulla 1979	no	no	unclear	no	unclear	unclear	nr	No	yes	no	High
Bodgen 1988	yes	yes	yes	yes	nr	yes	yes	no	yes	yes	Low
Boukaiba 1993	unclear	yes	yes	unclear	yes	nr	yes	nr	yes	yes	High
Preziosi 1998	yes	yes	yes	unclear	yes	yes	nr	yes	yes	yes	High
Sullivan 1998	unclear	unclear	yes	yes	yes	nr	nr	no	yes	yes	High
Feillet-Coudray 2005	unclear	yes	yes	yes	yes	yes	unclear	yes	yes	yes	Low
Feillet-Coudray	unclear	yes	yes	yes	yes	yes	yes	yes	yes	yes	Low

2006											
Hininger-Favier 2007	unclear	yes	unclear	yes	yes	yes	yes	yes	yes	yes	Low
Prasad 2007	yes	yes	yes	yes	yes	yes	nr	yes	yes	yes	Low
Sakagami 2009	unclear	yes	yes	unclear	nr	yes	nr	yes	yes	unclear	High

nr: not reported

Figure 1 Study selection process for systematic review

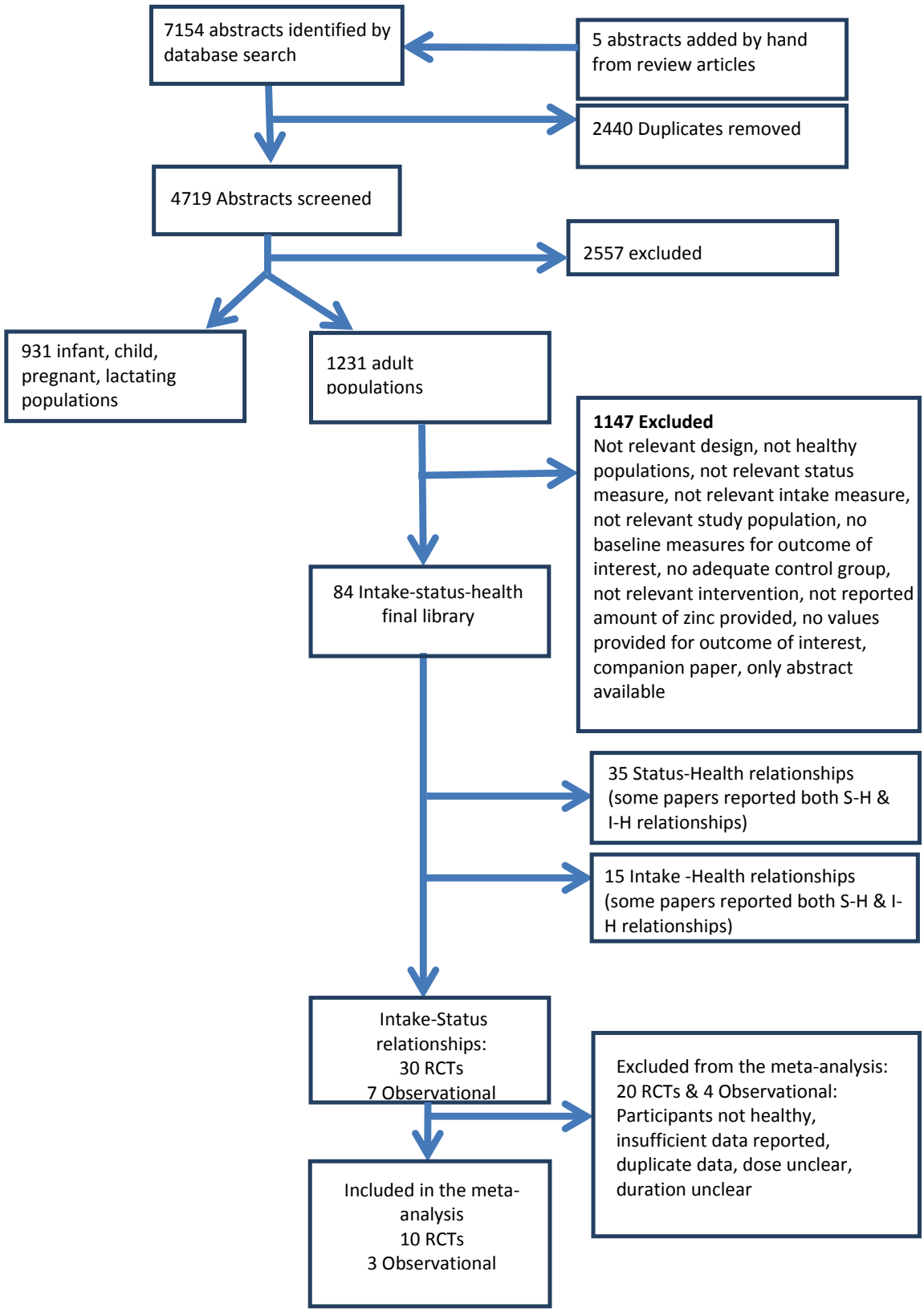
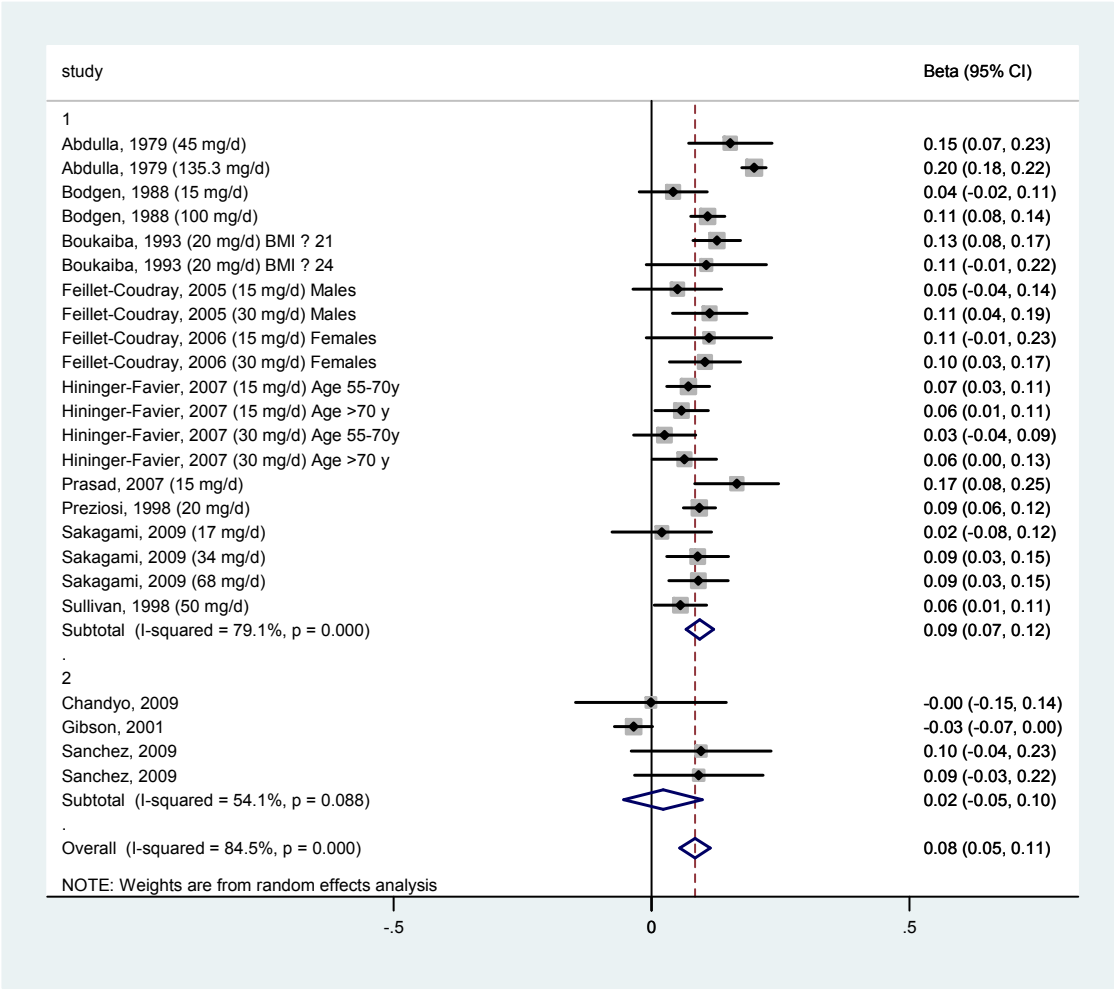
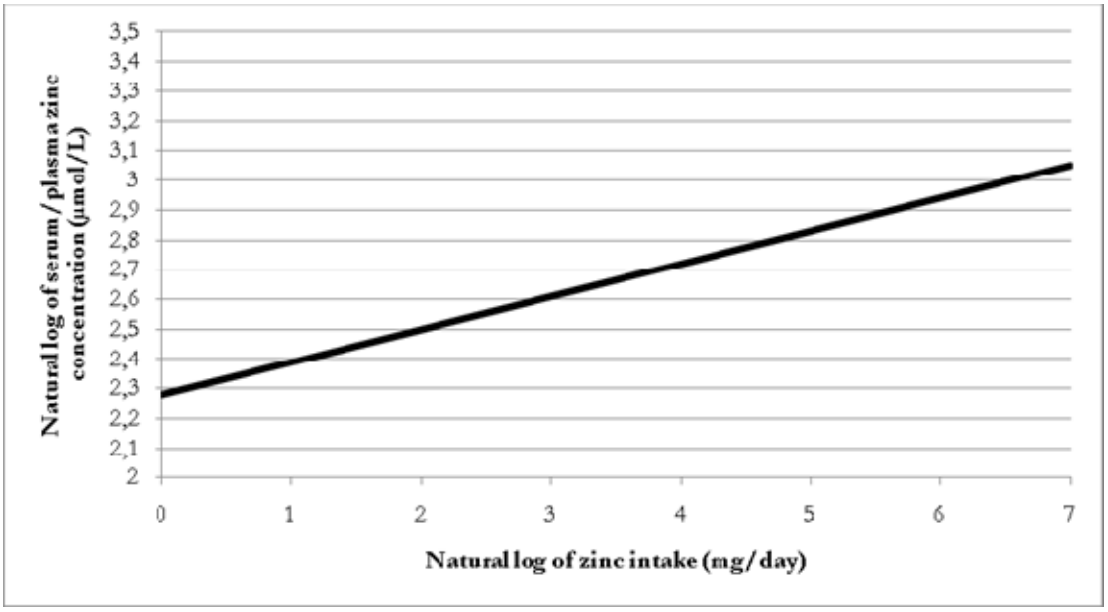


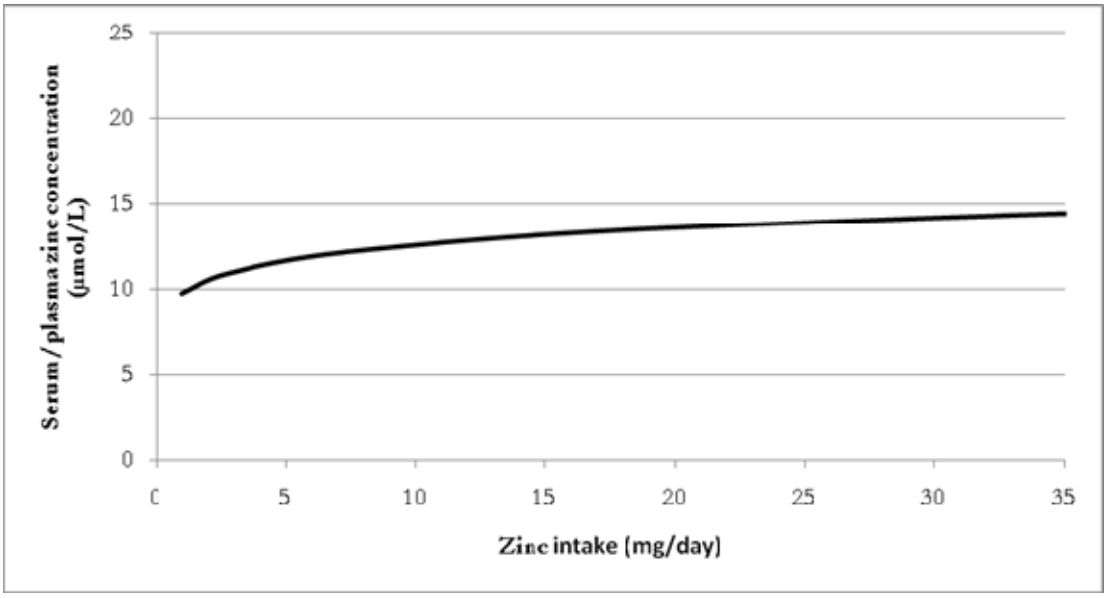
Figure 2 Random effects meta-analyses of RCTs and observational studies evaluating the pooled effect of dietary zinc on serum/plasma zinc in adults. Beta values (♦) represent the regression coefficients for the linear association between $\log_e$ transformed zinc intake and $\log_e$ transformed serum/plasma zinc status.



1: RCTs; 2: Observational studies

Figure 3 Serum/plasma zinc concentration (μmol/L) as a function of dietary zinc intake (mg/day), estimated by random-effects meta-analyses of RCTs of adults





EURRECA – Estimating zinc requirements for deriving dietary reference values

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EURRECA – Estimating zinc requirements for deriving dietary reference values

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Abbreviations

ACE Angiotensin-converting enzyme

ApoE Apolipoprotein E

EAR Estimated Average Requirement

EURRECA European Micronutrient Recommendations Alligned

DNA Deoxyribonucleic acid

DRV Dietary Reference Value

FFQ Food frequency questionnaire

GDS Geriatric depression scale

GST Glutathione S transferase

HbH Haemoglobin H

LBW Low birth weight

MMSE Mini mental state examination

PSS Perceived stress scale

RA Research activity

RCT Randomised controlled trials

RNA Ribonucleic acid

SES Socioeconomic status

SNP Single nucleotide polymorphism

WHO World Health Organisation

Abstract

Zinc was selected as a priority micronutrient for EURRECA, because there is significant heterogeneity in the Dietary Reference Values (DRVs) across Europe. In addition, the prevalence of inadequate zinc intakes was thought to be high among all population groups worldwide, and the public health concern is considerable. In accordance with the EURRECA consortium principles and protocols, a series of literature reviews were undertaken in order to develop best practice guidelines for assessing dietary zinc intake and zinc status. These were incorporated into subsequent literature search strategies and protocols for studies investigating the relationships between zinc intake, status and health, as well as studies relating to the factorial approach (including bioavailability) for setting dietary recommendations. EMBASE (Ovid), Cochrane Library CENTRAL and MEDLINE (Ovid) databases were searched for studies published up to February 2010 and collated into a series of Endnote databases that are available for the use of future DRV panels. Meta-analyses of data extracted from these publications were performed where possible in order to address specific questions relating to factors affecting dietary recommendations. This review has highlighted the need for more high quality studies to address gaps in current knowledge, in particular the continued search for a reliable biomarker of zinc status and the influence of genetic polymorphisms on individual dietary requirements. In addition, there is a need to further develop models of the effect of dietary inhibitors of zinc absorption and their impact on population dietary zinc requirements.

Key words: Zinc, Dietary recommendations, Zinc intake, systematic review, zinc status, zinc bioavailability, zinc requirements.

Introduction

Zinc is well established as an essential micronutrient for human health, having numerous structural and biochemical functions at the cellular and sub-cellular level, including enzyme function, DNA and RNA metabolism, protein synthesis, gene expression, cell growth and differentiation, and cell mediated immunity. Inadequate zinc intake has profound consequences at all points of the human lifecycle from the point of conception through to old age. Zinc was selected as a priority micronutrient for EURRECA, because the prevalence of inadequate zinc intakes was thought to be high among all population groups, and the public health concern is considerable. In addition, new scientific evidence has recently become available that demonstrates a large heterogeneity among current recommendations on zinc intake across Europe (Cavelaars et al., 2010; Doets et al., 2008) (See Activity 1 in (Dhonukshe-Rutten et al., 2013).

The total amount of zinc present in the adult human body ranges from 1.5 to 2.5mg, most of which is intracellular, within skeletal muscle tissue (57%), bone (29%) and other tissues including skin and organs (Jackson, 1989). The zinc located within these tissues has a relatively slow turnover rate and is not readily responsive to changes in dietary zinc intake. Kinetic studies suggest that only a small proportion of total body zinc (approximately 10%) represents the "functional pool" of zinc, which is comprised of zinc, located within the liver and other tissues, that exchanges rapidly with the plasma, and when this functional pool is depleted zinc deficiency ensues (King, 1990). Zinc deficiency in adults can lead to dermatitis, hair loss, diarrhoea, loss of appetite, reproductive failure, hypogeusia, loss of cognitive function, susceptibility to infections and depressed immune function (Shankar and Prasad, 1998), delayed wound healing and depression (Andrews and Gallagher-Allred, 1999). Zinc deficiency has also been associated with three major health diseases prevalent in Europe: Diabetes, cancer and coronary heart disease (Singh et al., 1998). Zinc deficiency may be a

serious public health problem that compromises the development of millions of children (Sandstead and Smith, 1996). The recent Lancet series on maternal and child under nutrition concluded that zinc deficiency is responsible for about 4% of child mortality and disability-adjusted life-years (Black et al., 2008). The consequences and manifestations of severe zinc deficiency in infants and children and adolescents can be retardation of linear growth and development, poor appetite, delayed sexual maturation and hypogonadism, frequent infections (Maret and Sandstead, 2008), alopecia, dermatitis, delayed wound healing, diarrhoea, pneumonia, malaria (Fischer Walker et al., 2009), limitation on the senses of taste and smell, night blindness (Christian et al., 2001; Seiler et al., 2002). Zn deficiency may be associated with deficits in activity, attention, and motor development (Bhatnagar and Taneja, 2001). Results of several studies indicated that supplementation with zinc can significantly reduce the rates of diarrhoea and pneumonia in young children and increase the growth rate of stunted children (Brooks et al., 2005a; Brooks et al., 2005b; Brown et al., 2002). During the acute diarrhoea, zinc supplementation reduces the duration and severity of the disease, so that now the WHO recommends zinc supplementation as an adjunct to rehydration therapy, to replace the excessive losses of zinc during periods of diarrhoea (WHO). The involvement of maternal zinc status in pregnancy outcome is still unclear, animal models have shown that severe maternal zinc deficiency results in impaired implantation, abortions and foetal malformations (Keen et al., 2003). The consideration that zinc deficiency is a teratogenic risk in humans may be supported by the correlation of low plasma zinc concentrations in the first and third trimesters of pregnancy with an increased risk for malformations and low birth weight (LBW), respectively. Zinc deficiency is thought to influence embryonic and foetal development through reduced cell proliferation, or reduced protein synthesis or reductions in rates of tubulin polymerization rather than increased rates of cellular oxidative damage or increased rates of apoptosis and reduced binding of hormones and transcription factors

dependent on zinc-finger regions (Jankowski-Hennig et al., 2000; Mackenzie et al., 2002; WHO).

Current dietary recommendations

Dietary recommendations for zinc intake have been mainly based on balance studies focusing on the prevention of deficiency and use the factorial approach which assumes that the zinc requirement is the lowest intake which replaces obligatory zinc endogenous loss. The method computes the dietary zinc requirement by dividing the endogenous zinc loss by the fractional zinc absorption (King, 1986). In Europe and in other non-European countries, zinc recommendations for infants are generally set either based on zinc concentration of breast milk, using a factorial approach or extrapolating values from those given for adults.

There is significant heterogeneity among current recommendations on zinc intake across Europe and worldwide (Doets et al., 2008). Table 1 illustrates the range of dietary zinc recommendations for various countries worldwide. This heterogeneity is due to a number of factors including the data used to derive the value, and differences in expert opinion between panels convened to review the data.

Current European intakes

As part of the EURRECA programme of work, Roman-Vinas *et al* undertook an analysis of population dietary surveys from across Europe in order to determine the prevalence of inadequate nutrient intake in Europe using the Nordic Nutritional Recommendations as the standard (Roman-Vinas et al., 2010). This study revealed that the failure to meet the EAR (estimated average requirement) of 6.4mg/day or 5.7mg/d for adult males and female respectively was greatest in Ireland with dietary zinc intakes falling below this cut off value

in 11.9% men and 28.8% women. A similar picture emerged for elderly people (aged >64 years), with those living in Ireland having the highest percentage failing to meet the EAR, 13.6% and 13.1% of elderly men and women respectively (Vinas et al., 2011)

A review of available micronutrient intake and status data in Europe (Novakovic R, Submitted 2011) showed that data on intake of zinc were very limited for all life stages, so no cross country comparison could have been made. However, available data for zinc status (based on serum/plasma zinc concentrations) in children, adolescents and adults showed no regional differences when Central and Eastern Europe, Scandinavia, Western and Mediterranean countries were compared. All levels were within the optimal range indicating adequacy in zinc status (Novakovic R, Submitted 2011).

A systematic review of the relationship between micronutrient intake and socioeconomic determinants in Europe revealed that there were almost no differences in zinc intake between different socioeconomic status (SES) groups. On the other hand, status data in adults showed 5% higher serum zinc level in the low SES group. In comparison to reference values (Nordic nutrient recommendations for intake and the WHO for status (de Benoist et al., 2007)), all observed intake and status levels were within the optimal range, with the exception of levels of the low SES group in UK children (Novakovic R, Submitted 2012)

On an individual basis, an inadequate dietary intake of zinc could be the result of a strict vegan diet, a diet which is primarily based on grain products (Solomons and Slavin, 2001) or through a restrictive diet due, for example, to anorexia or alcohol or drug addiction. Certain disease states such as acrodermatitis enteropathica, Celiac disease, Crohn's disease and ulcerative colitis may disrupt the absorption of zinc (Solomons and Slavin, 2001). Other

health states may increase zinc losses primarily through diarrhoea, or increase requirements, such as the post-operative state (Solomons and Slavin, 2001).

The purpose of this review is to provide a summary of the methods used, and the results obtained from the systematic literature searches and subsequent meta-analysis of the data retrieved that were performed by partners in the EURRECA network of excellence. These activities were designed to answer specific questions regarding zinc-intake-status relationships. In addition, a comprehensive review of the factorial approach to setting zinc recommendations was undertaken. The overall aim of these activities was to generate new data and approaches that could assist future panels to derive dietary zinc recommendations using robust and transparent methodology.

Methods

Assessing dietary zinc intake:

One of the initial activities in the EURRECA process was to establish the most robust methodology of assessing zinc intake and status ((Matthys et al., 2010) and activity 3 in (Dhonukshe-Rutten et al., 2013)). The accurate determination of dietary micronutrient intake is notoriously problematic. Following a series of reviews of the methods used to assess micronutrient intake in Europe (Serra-Majem L, 2009) best practice guidelines were developed and adopted by the EURRECA network for all subsequent nutrient review activities. These are described in detail in "RA1.1 Best practice guidelines" in www.eurreca.org. In summary, only studies that used the following methodologies were included in the systematic reviews:

1) validated FFQ/Dietary History

2) validated 24h recall / food records / diary measurements for at least 3 days

3) validated 24h recall / food records / diary measurements < 3 days with adjustment for intra-individual variability

Since interventions commonly involve supplements, these were considered, taking into account the possible differences in bioavailability.

Assessing zinc status:

The assessment of zinc status is also problematic and it is generally accepted that there is currently no specific, reliable biomarker of zinc status. A systematic review and meta-analysis of the literature examining the efficacy of potential biomarkers of zinc status was undertaken (Lowe et al., 2009). This review presented an analysis of data from over 32 potential biomarkers however for many there was insufficient evidence to assess their reliability (Table 2)

Table 2. Biomarkers identified in systematic review (Lowe et al., 2009)

Plasma/serum zinc concentration was the most commonly used marker of zinc status and therefore the biomarker for which there were most data. It was found to respond to both increases and decreases in zinc intake, and was identified as being a useful biomarker however there are considerable reservations due to the effect of multiple confounders, such as infection, inflammatory status and time of last meal. Urine and hair were also considered useful biomarkers.

Health outcomes associated with inadequate zinc intake:

Important health problems related to zinc intake in adults and elderly people were identified by a literature search. These include: compromised immunity, dermatitis, hypogeusia, impaired cognitive functioning (dementia), depression, diabetes (reduced glucose tolerance), ischemic heart disease, carcinogenesis and anorexia. These were discussed and prioritized by the experts in zinc research within the RA2 team (Matthys C, 2011). Prioritisation was based on the strength of evidence of the deficiency and role of zinc on the health outcome, the relevance of the health outcome to the European population groups and the amount of evidence based research literature available based on a pilot literature search. The health outcomes that were identified for each population group are shown in Table 3, and are listed in order of priority.

Table 3. Priority health outcomes associated with inadequate zinc intake for each population group.

The best practice guidelines were then used to design the search protocols for the subsequent systematic reviews of the zinc intake- status- health relationships, the factorial approach for assessing dietary zinc requirements, zinc bioavailability and the influence of polymorphisms on zinc requirements. [Details of the search protocols can be found at: www.eurreca.org]

Results

Factorial approach and bioavailability

A technique commonly used when setting dietary zinc recommendations is the factorial approach which combines zinc required to replace obligatory losses with additional needs for zinc during different stages in the life cycle and makes adjustments for the bioavailability of zinc in the diet (See Table 3 in (Dhonukshe-Rutten et al., 2013). Additional needs for zinc during different stages of life include that required for foetal growth during pregnancy, lactation and growth through infancy to adulthood. Literature searches were therefore designed to answer the following research questions: What are the key factors that affect zinc losses in all population groups? What are the key factors that affect zinc gains in all population groups? What are the additional needs for zinc during pregnancy, lactation and for growth? How well is zinc absorbed from meals and whole diets?

All titles and abstracts were screened for potential relevance and sorted into the population groups; infants, children and adolescents, pregnant and lactating women, adults, and elderly people as defined by the EURRECA consortium (See Activity 1 in (Dhonukshe-Rutten et al., 2013). An EndNote library was created compiling all the papers that met the inclusion criteria. The data regarding zinc losses and gains were extracted and collated using Excel (Microsoft Office Excel 2003). The quality and the risk of bias were assessed as indicators of validity. The studies included in this review were checked for a minimum quality score system developed by the EURRECA consortium which was adapted from The Cochrane Handbook for Systematic Reviews (Higgins and Green, 2008).

Factors affecting zinc losses and gains

From a total of 491 abstracts retrieved from electronic and hand searches, 105 appeared potentially relevant studies and were assessed for inclusion once the full paper had been obtained. Seventy-two papers were finally considered relevant across all population groups (adults and elderly, infants, children and adolescents, and pregnant and lactating women, adults and elderly). Despite the relatively stringent inclusion criteria, the included studies displayed a broad variety of methodological approaches, and were therefore unsuitable for meta-analysis. Therefore the data extracted from the papers were tabulated and summarised narratively (Silvia Bel-Serrat, In Progress). Overall, balance studies have shown that zinc losses and gains are a function of the initial zinc status of an individual, the amount of bioavailable zinc in the diet and are modulated by homeostatic mechanisms. That means that dietary zinc recommendations should be estimated on the basis of the target population diet making difficult the possibility of establishing a value valid for the entire population. In addition, age, physical activity level, malabsorption syndromes, disease status can all affect zinc losses and gains. Moreover, interactions among nutrients should be also taken into account as they may also play an important role by means of affecting zinc utilization. As suggested by Taylor et al. (1991), the interaction between these homeostatic changes and zinc availability from different dietary sources should be better characterized to improve the accuracy of dietary zinc recommendations.

Factors affecting zinc bioavailability

The systematic review identified 120 studies as relevant to the research question of which 87 studies were conducted in adults and elderly, 2 in pregnancy and lactating women, 14 in children and adolescents and 17 in infants. Potential modifiers of zinc bioavailability were identified as illustrated in **Figure 1**. Phytate was the most frequently investigated modifier of

zinc absorption. Twenty four estimates from seventeen studies that investigated the effect of dietary phytate level on zinc absorption were combined in a random effects meta-analysis. A forest plot showing the overall effect size of high versus low dietary phytate intake on zinc absorption is shown in **Figure 2**. The mean difference in fractional zinc absorption between low and high phytate diets was 0.11 (95% CI: 0.07, 0.16) however there was a high degree of between study heterogeneity (I^2 94% , P<0.0001). Further analysis of this data set is underway to examine the factors that contribute to this heterogeneity and the overall effect of phytate:zinc molar ratio on zinc absorption.

The influence of gene polymorphisms zinc metabolism

The primary aim of this activity was to generate a database containing relevant information related to the impact of functional gene polymorphisms on zinc metabolism. Specifically, this involved identifying data assessing the impact of functional polymorphisms (e.g. single nucleotide polymorphisms, or SNPs) on micronutrient status biomarkers and associated health outcomes. The research questions used to develop the search protocol were: How do genetic polymorphisms affect zinc status? Are there any interactions between functional polymorphisms which affect zinc status and various health outcomes? Information was collated from studies of individuals who are either homozygous, heterozygous or wild type for specific polymorphisms. Where data exists for zinc status, functional polymorphisms and linked health outcome, this information was also recorded. Data were collated from all population groups including infants, children, adolescents and adults including the elderly into a database that is available at www.eurreca.org.

Of the 167 papers identified by the systematic search of the literature databases, 12 papers met the inclusion criteria and reported statistically significant results for altered zinc biomarker status in groups of people with differing gene variants. The gene interleukin 6

which regulates the amount of circulating proteins involved in inflammatory responses associated with hyperglycaemia and non-insulin dependent diabetes mellitus and coronary artery disease is thought to be influenced by zinc status (Giacconi et al., 2006; Giacconi et al., 2005). Two papers reported a relationship between SNP's of the Interleukin 6 gene and plasma zinc concentration and health outcomes, including perceived stress scale (PSS), geriatric depression scale (GDS) and mini mental state examination (MMSE) (Mariani et al., 2008; Mocchegiani et al., 2008)..

Another gene angiotensin-converting enzyme (ACE) was also reported. An impaired zinc status can alter enzyme activity, with adverse effects on angiotensin- conversion from I to II affecting vasoconstriction and hypertension and subjects with the DD genotype polymorphism in the ACE gene have been shown to have the highest enzyme activity increasing the risk of hypertension (Tamura et al., 1996). Tamura *et al* found a significant correlation between plasma zinc concentration and ACE activity in pregnant women at 33 weeks of gestation. However as this was the only significant correlation found in this study, it was stated that the significant result may have occurred by chance.

The metallothionine gene has been included in the zinc database. Some polymorphisms of the metallothionein gene have been correlated to chronic inflammation and may affect zinc release (Mocchegiani et al., 2006; Richards et al., 2002). Another gene reported in the zinc database was apolipoprotein E (ApoE). Gonzalez *et al* (1999), reported that serum zinc concentrations in epsilon 4 ApoE carriers were significantly higher in patients with Alzheimer's disease than in healthy control patients.

The TP53 mutation in exon 5 through to 8, found in esophageal squamous cell carcinoma tumours was reported in a paper by Dar *et al* (2008). There is a notion that an imbalance of copper and zinc levels may lead to a higher prevalence of TP53 tumour mutations. Dar *et al*

found that cancer patients with the TP53 tumour mutation had lower plasma zinc levels than those with no mutation.

The glutathione S transferase (GST) gene was reported by 3 papers (Jin et al., 2011; Reszka et al., 2007; Reszka et al., 2005). The detoxifying enzyme GST metabolises tobacco smoke derived compounds; zinc deficiency therefore can increase the risk of mutations occurring and can decrease the activity of the antioxidant GST enzyme increasing the risk of some cancers including lung cancer. Some polymorphisms in the GST gene may be associated with an elevated risk of lung cancer and therefore the effect of zinc status on each variant allele needs to be investigated.

The gene CYP1A1 was also investigated by Jin et al (2011). The CYP1A1 gene has a polymorphism at exon 7 where a new Mspl restriction site is introduced. The CYP1A1 gene is thought to influence metabolic activation and detoxification of some toxins and therefore can increase susceptibility to increasing risk of lung cancer. Jin *et al* reported that the risk of lung cancer decreased with a zinc level >1200ng/ml for both CYP1A1 variants and CYP1A1 carriers suggesting that a higher concentration of serum zinc may protect against lung cancer.

Haemoglobin H disease (HbH) was also included in the database and was thought to influence zinc status. Zinc deficiency is thought to be involved with impaired growth and hypogonadism traits observed in patients with polymorphic diseases such as the thalassemic diseases (Ajayi, 1997; Kajanachumpol et al., 1997) and cystic fibrosis (Van Biervliet et al., 2007).

The final gene reported as having a significant association with blood biomarker zinc status is SLC30A4 gene with a polymorphism on exon 5 915 T-C. The SLC30A4 gene encodes one of the zinc transport proteins and therefore it is thought that a polymorphism in this gene may

affect zinc absorption and foetal development. Akar *et al* (2006) studied this gene and zinc status association and found that three hours after a zinc tolerance test there was a significant difference in plasma zinc level for TT and CC carriers, indicating a functional property of this polymorphism.

Table 5. Results of the systematic search for studies of the effect of gene polymorphisms on zinc metabolism.

Intake- status-health relationships

EURRECA is developing a method for the quantitative integration of evidence for deriving nutrient intake recommendations using bivariate dose-response relationships for intake-health (I-H) as well as intake-status (I-S) and status-health (S-H) relationships. These data will be combined in a new integrated trivariate intake-status-health (I-S-H) dose-response model with data from classical nutrition studies and bioavailability factors (See Activity 6 in (Dhonukshe-Rutten et al., 2013). Search protocols were therefore designed to answer the following questions: What is the effect of zinc intake on functional or clinical outcomes (intake-health) and what factors affect this relationship? What is the effect of zinc intake on indicators of exposure or body stores/biomarkers (intake-status) and what factors affect this relationship? What is the effect of indicators of exposure or body stores (i.e. biomarkers) on functional or clinical outcome (status-health) and what factors affect this relationship?

The result of the systematic search for studies addressing zinc intake status health relationships yielded over 1000 articles that were obtained in full text for eligibility evaluation. Due to the heterogeneity of the methodological approaches and outcome measures used in these studies, it is unlikely that meta-analysis of the data will be possible.

Intake- status relationships

Sufficient high quality RCT studies were identified to enable a meta-analysis of data describing the relationship between plasma zinc intake and plasma zinc concentration in each of the population categories. Units of measurement were converted to a standard form to facilitate comparison across studies. Intake-status regression coefficients ($\hat{\beta}$) were estimated for each individual study as described in detail elsewhere (Souverein et al., 2012). An overall pooled $\hat{\beta}$ and SE($\hat{\beta}$) was calculated using random effects meta-analysis The statistical transformations to obtain $\hat{\beta}$ s and SE($\hat{\beta}$)'s were performed using GenStat version 13-SP2 (VSN International Ltd., http://www.vsni.co.uk/) and the meta-analysis was performed using STATA version 11.0 (College Station, TX), with statistical significance defined as $P<0.05$.

For all population groups, with the exception of lactating women, the intake-status analyses revealed a positive and significant relationship between zinc intake and plasma zinc concentration, however a high degree of heterogeneity between the studies was observed (Table 4).

Table 4: Results of the Meta-analysis of intake-status in all populations groups.

Discussion

The determination of dietary zinc recommendations has relied primarily on the factorial approach, with extrapolation to population groups for which data are limited or missing. A complementary approach involves examining the associations between dietary intake, status and health, to arrive at intakes that result in optimal status levels and are sufficient to prevent disease due to deficiency at one end of the spectrum, or toxic effects due to excess dietary

zinc at the other end of the spectrum. The difficulty of this approach for zinc is the lack of a reliable and sensitive marker of zinc status, and the non-specific nature of the diseases symptoms associated with sub-optimal zinc intake. However, one of the overarching aims guiding the work described in this review was to gather the best quality data using the most robust methodology to provide a database for future panels to use when setting recommendations. This included both a comprehensive review of the data available using both the classical factorial approach and a more novel intake-status-health association approach for zinc.

Regarding the factorial approach, a key factor that has been highlighted in this review and in discussion with experts is the need to consider bioavailability more closely as a modifier of the amount of dietary zinc required to meet requirements (Hambidge, 2010). This review identified a broad range of dietary components that may impact on the amount of dietary zinc that is absorbed and utilised, the majority of which had a deleterious effect on zinc bioavailability (figure 1). Many of these food components require further studies to generate sufficient high quality data to enable conclusive evaluation of their effect at different levels of intake, however the most widely studied modifier of zinc absorption is dietary phytate. This systematic review and meta-analysis confirms that phytate is a potent modifier of zinc absorption and should be taken into consideration when using the factorial approach to setting dietary zinc recommendations for any given population. Mathematical models that combine the effects of varying levels of phytate and zinc intake on true zinc absorption are potentially valuable tools in this process. A trivariate model (zinc intake-absorption-phytate intake), published by Hambidge *et al* in 2010 has helped to explain much of the variability of zinc absorption from human diets (Hambidge et al., 2010). This mathematical model is based on the accepted view that zinc absorption is a carrier-mediated process, phytate inhibits absorption by binding with zinc in the gut to form an insoluble complex, and that dietary zinc

and phytate are the primary dietary factors determining zinc absorption. The model predicts that the quantity of zinc absorbed from 40 mg dietary zinc at zero phytate intake is 6.4 mg Zn/d and that the dietary zinc intake required to meet the requirements for zinc doubles with every 1000 mg phytate consumed in the diet per day (Hambidge et al., 2010).

Another potential modifier of the amount of dietary zinc needed to meet the requirement is genotype (Hambidge, 2010). This has been shown to have profound effects on the bioavailability of some micronutrients such as folate and iron (Casgrain et al., 2010). Most ZnT and Zip families show evidence of polymorphisms, which could produce structurally different proteins and hence, transporter activity and /or specificity for zinc. Such polymorphisms could influence the amount of dietary zinc needed to meet the requirements and alter zinc metabolism (Cousins et al., 2006; Liuzzi and Cousins, 2004). The systematic search for studies on micronutrient metabolism yielded a very small number of relevant studies. This is clearly an important area for future research development.

Investigation of the zinc intake-status relationships in some population groups yielded some potentially useful new data. A dose-response curve was constructed from the extracted data, where the slope was based on the pooled $\hat{\beta}$ from the meta-analysis of the RCTs expressed on a $\log_e$-scale. Reported means and standard deviations of zinc intake and zinc status were extracted from the observational studies and was used to estimate the intercept of this curve (See Activity 6 in (Dhonukshe-Rutten et al., 2013). The dose response curves for the Adult and Elderly, and the Pregnant and Lactating women population groups are shown in **Figure 3** and **Figure 4** respectively. These data can be used as complementary evidence for underpinning zinc reference values, however, the limitations of serum/plasma zinc concentration as a biomarker for zinc status should be acknowledged. Serum/plasma zinc is recognised as being a relatively insensitive index of zinc nutritional status due to efficient

homeostatic regulation which responds to alterations in zinc intake, up-regulating absorption and conserving losses via the gastrointestinal tract and kidneys when intakes fall. In addition, whilst all studies included in the analysis were undertaken in apparently healthy individuals, factors such as stress, infection and inflammation, which are known to affect plasma zinc concentrations, may have gone unreported. Unfortunately, more sensitive indexes of zinc status have yet to be identified and plasma serum zinc remains by far the most commonly used biomarker of zinc status (Lowe et al., 2009). It is anticipated that this approach may be used to model the relationships between zinc intake or status with the health outcomes for zinc, and that these can be combined with the intake status relationships described above to form a trivariate model of intake status and health (See Activity 6 in (Dhonukshe-Rutten et al., 2013). It is unclear at the moment whether or not our systematic searches have yielded sufficient data to enable this but it is likely that further studies are required.

This process has highlighted the need for more high quality studies to address gaps in current knowledge. Some of the key issues that came out of EURRECA workshops through discussion with experts external to the EURRECA Network focussed around zinc bioavailability and the need to model the effect of inhibitors of zinc absorption such as phytate, calcium and iron (Casgrain et al., 2010). Most current knowledge of zinc homeostasis is based on research in healthy adult males. In order to avoid scaling efforts should be made to obtain data on both genders at all ages, including pregnancy and lactation. In particular there is a paucity of data from studies in young children which necessitates the use of scaling to arrive at dietary recommendations, where this is the only option, there needs to be consensus regarding which growth/weight data to use.

In summary, a series of systematic reviews and meta-analyses were conducted in accordance with the protocols and procedures developed by the EURRECA consortium. This process

has gathered together information relating to the setting dietary zinc recommendations which will be available as a valuable resource for future DRV panels. It has also generated new intake-status-health association data that may be used in combination with the classical factorial approach to model dietary zincs necessary to meet physiological requirements. This process has also highlighted the key areas for further research, in particular the urgent need for a reliable biomarker of zinc status, the further development of models of the impact of dietary factors on zinc bioavailability and the influence of genetic polymorphisms on individual dietary requirements.

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Figure 1. Flow diagram showing the results of the systematic review of studies investigating zinc bioavailablity.

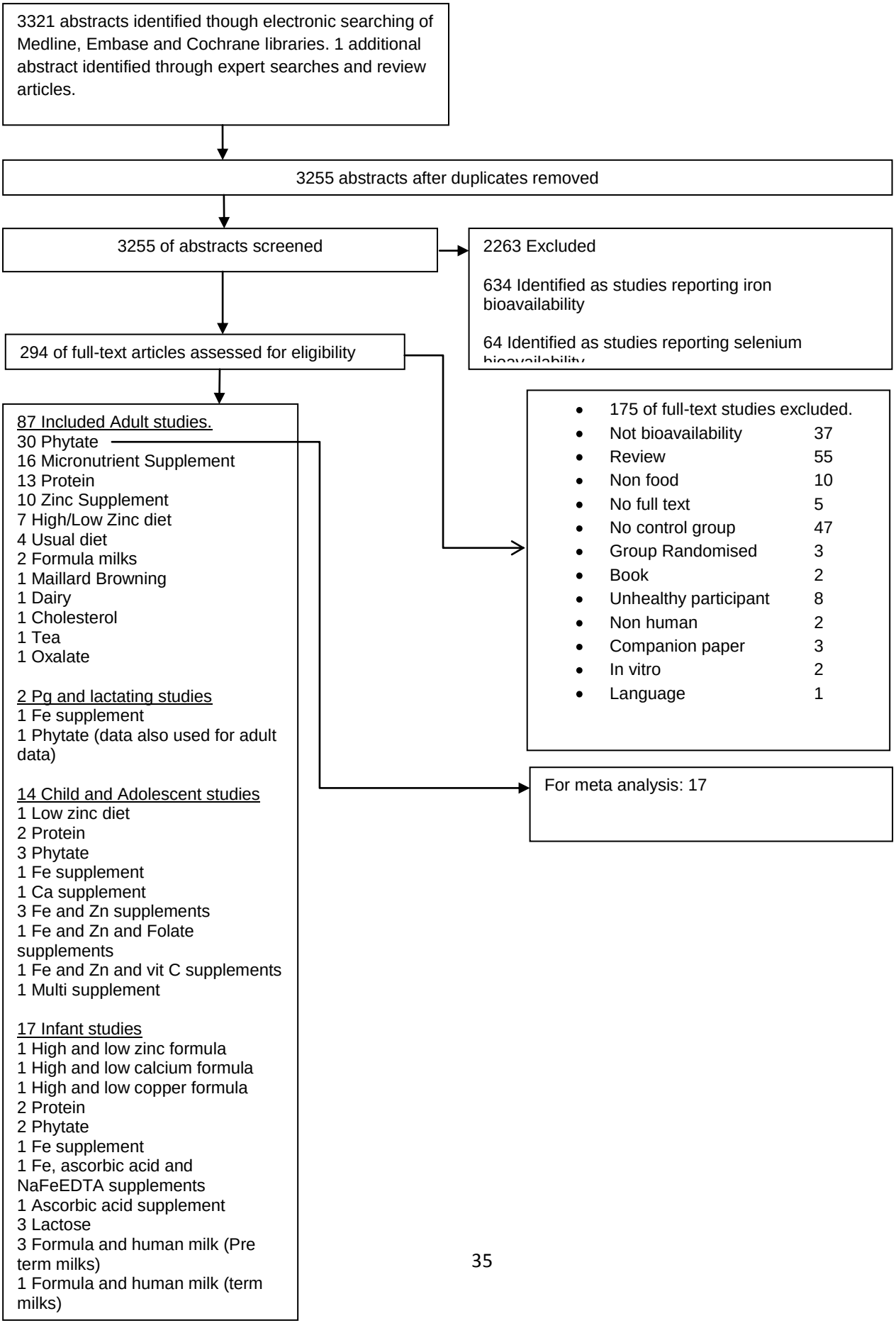


Figure 2 . Forest plot of high and low phytate meals and zinc absorption as % of the diet.

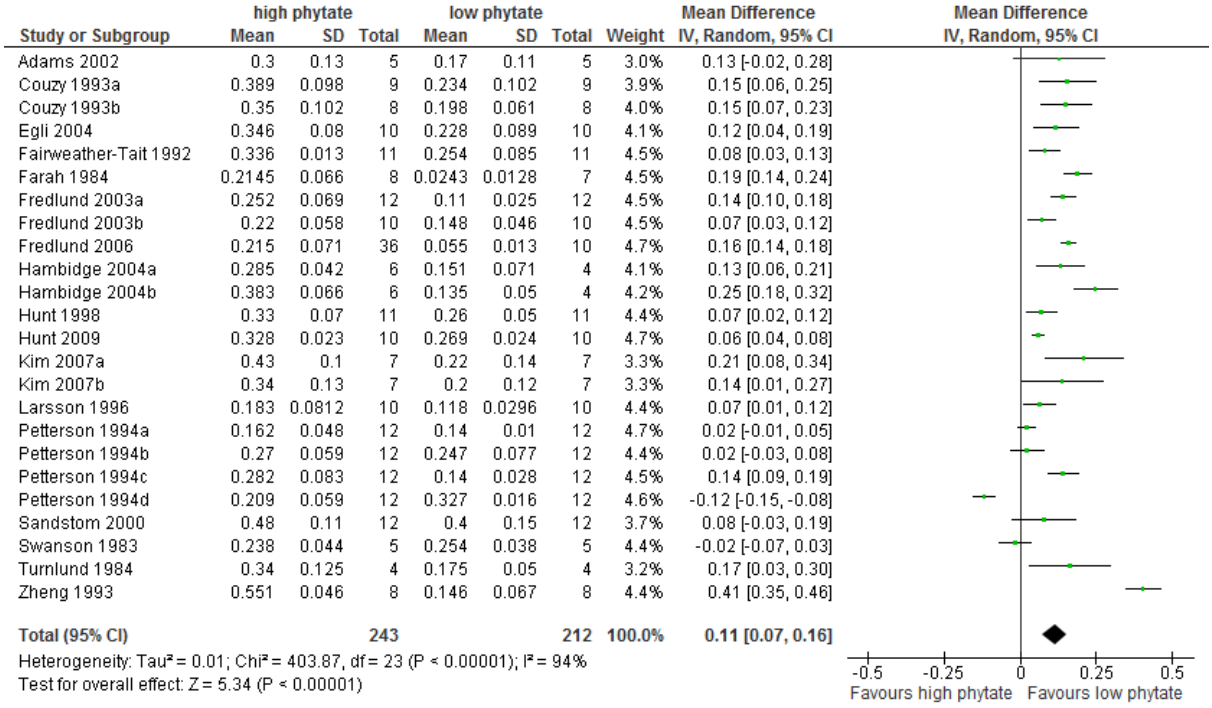
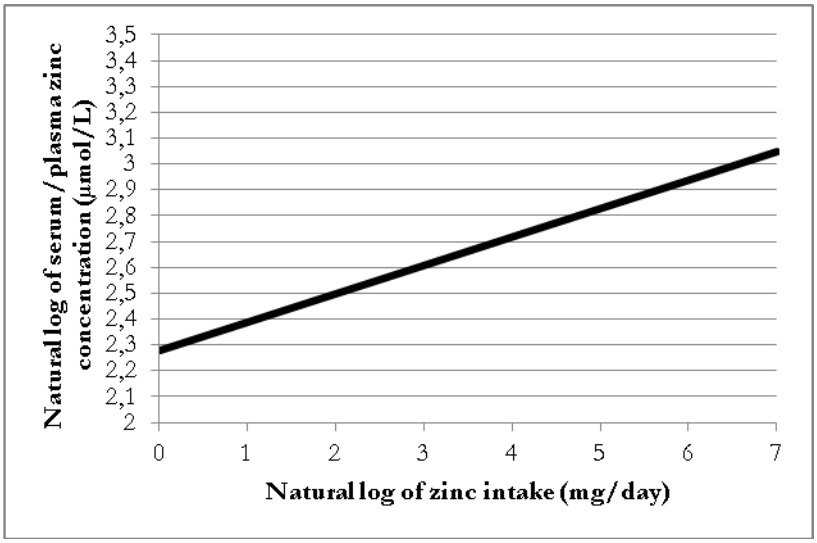


Figure 3. Serum/plasma zinc concentration (µmol/L) as a function of dietary zinc intake (mg/day), estimated by random-effects meta-analyses of RCTs. In figure 3a the data are presented on natural log scale, where Y=0.11*x+2.28.

a



b.

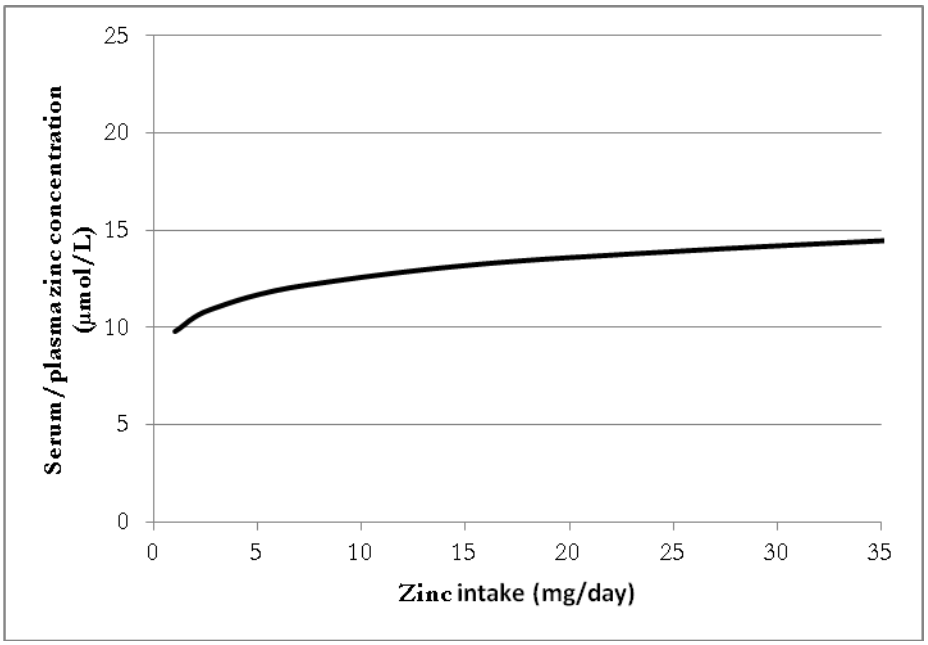
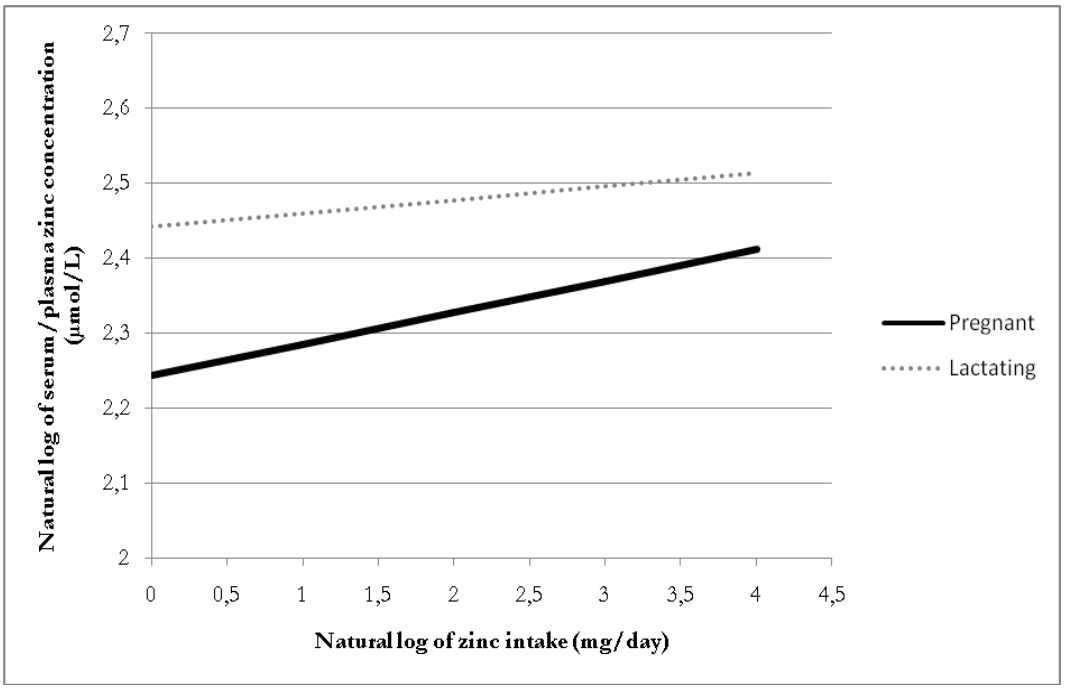


Figure 4 Serum/plasma zinc concentration (µmol/L) as a function of dietary zinc intake (mg/day), estimated by random-effects meta-analyses of RCTs of pregnant (solid line) and lactating (dashed line) women. In figure 4a the data are presented on natural log scale.

4a



4b.

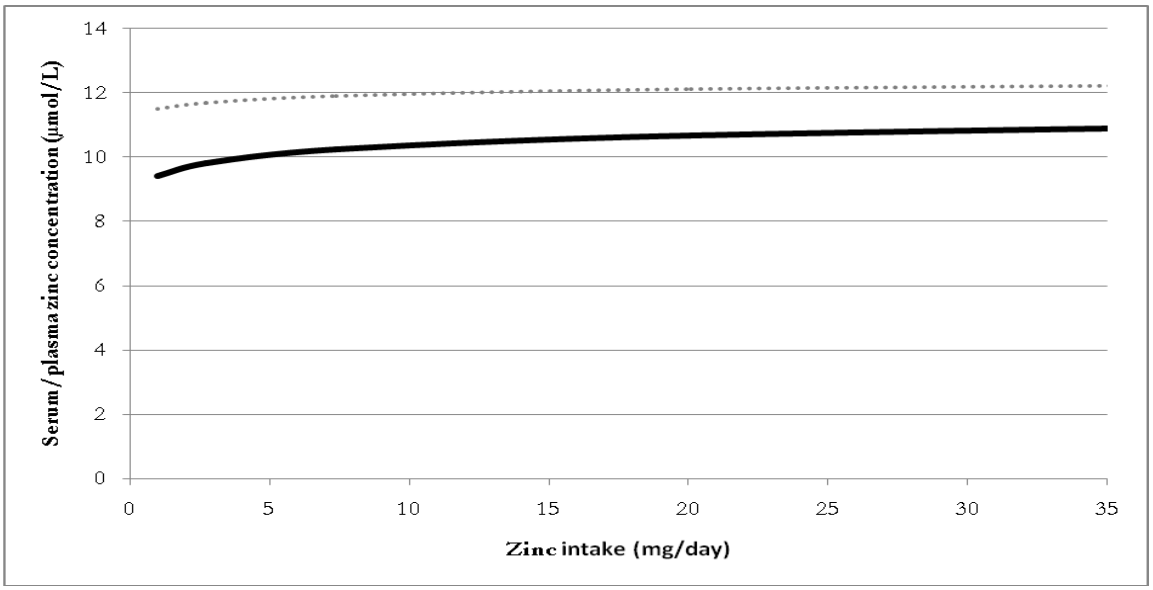


Table 1. - Selected recommended intake levels for zinc (mg)

DATA SOURCE	Gender	Special conditions	POPULATION GROUP						
			Infants	*Children*	*Adolescents*	*Adults*	*Elderly*	*Lactation*	*Pregnancy*
WHO / FAO	*Male*	High bioavailability	*0-6m* 1.1 *7-12m* 0.8	*1-3y* 2.4 *4-6y* 2.9 *7-9y* 3.3	*10-18y* 5.1	*19-65y* 4.2	*>65y* *4.2*	-	-
	Female	Low bioavailability	*0-6m* 6.6 *7-12m* 8.4	*1-3y* 8.3 *4-6y* 9.6 *7-9y* 11.2	*10-18y* 14.4	*19-50y* 9.8	*>65y* 9.8	*0-3m* 19	*< 3m.* 11
Nordic (Norway)	*Male*	-	*<6m* NULL *6-11m* 5	*2-5y* 6 *6-9y* 7 *10-13y* 11	*14-17y* 12	*18-30y* 9 *31-60y* 9	*≥75y* 9	-	-
	Female	-	*<6m* NULL *6-11m* 5	*2-5y* 6 *6-9y* 7 *10-13y* 8	*14-17y* 9	*18-30y* 7 *31-60y* 7	*≥75y* 7	-	-
Australia / NZ	*Male*	-	*0-6m* 2 *7-12m* 3	*1-3y* 3 *4-8y* 4 *9-13y* 6	*14-18y* 13	*19-30y* 14 *31-50y* 14	*>70y* 14	-	-
	Female	-	*0-6m* *2* *7-12m* *3*	*1-3y* 3 *4-8y* 4 *9-13y* 6	*14-18y* 7	*19-30y* 8 *31-50y* 8	*>70y* 8	*14-18y* 11 *19-50y* 12	*14-18y* 10 *19-30y* 11
DACH (Germany)	*Male*	-	*0-3m* 1 *4-11m* *2*	*1-3y* 3 *4-6y* 5 *7-9y* 7 *10-12y* *7*	*13-14y* 9.5 *15-18y* 10	*19-64y* 10	*≥65y* 10	-	-
	Female	-	*0-3m* 1 *4-11m* 2	*1-3y* 3 *4-6y* 5 *7-9y* 7 *10-12y* 7	*13-14y* 7 *15-18y* 7	*19-64y* 7	*≥65y* 7	10	*>4m* 10
EC	*Male*	-	*6-11m* 4	*1-3y* 4 *4-6y* 6 *7-10y* 7	*11-14y* 9 *15-17y* 9	*≥18y* 9.5	*≥18y* 9.5	-	-
	Female	-	*6-11m* 4	*1-3y* 4 *4-6y* 6 *7-10y* 7	*11-14y* 9 *15-17y* 7	*≥18y* 7	*≥18y* 7	12	7
IOM (US/ Canada)	*Male*	-	*0-6m* *2* *7-12m* *3*	*1-3y* 3 *4-8y* 5 *9-13y* 8	*14-18y* 11	*19-70y* 11	*>70y* 11	-	-
	Female	-	*0-6m* *2* *7-12m* *3*	*1-3y* 3 *4-8y* 5 *9-13y* 8	*14-18y* 9	*19-70y* 8	*>70y* 8	*14-18y* 12 *19-30y* 12	*19-30y* 11 *31-50y* 11
United Kingdom	*Male*	-	*0-3m* 4 *4-6m* 4	*1-3y* 5 *4-6y* 6.5 *7-10y*	*11-14y* 9	*19-50y* 9.5	*>50y* 9.5	-	-
	Female	-	*0-3m* 4 *4-6m* 4	*1-3y* 5 *4-6y* 6.5 *7-10y* 7	*14-18y* 9	*19-50y* 7	*>50y* 7	NULL	NULL

Data presented in the Table was obtained from online EURRECA web resource *NutriRecQuest*: http://www.serbianfood.info/eurreca/ (Cavelaars et al., 2010) from original

source documents (Australian Government Department of Health and Aging New Zealand Ministry of Health and National Health and Medical Research Council, 2005; Department of Health, 1991; Institute of Medicine, 2000; Ministry of Health Labour and Welfare Japan, 2005; Nordic Council of Ministers, 2004; Scientific Committee for Food, 1993)

† RDA, Recommended Dietary Allowance (USA, Japan); equivalent to RNI, Reference Nutrient Intake (UK); PRI, Population Reference Intake (EFSA); RNI, Recommended Nutrient Intake (WHO/FAO); RDI, Recommended Dietary Intake (AU/NZ); RI, Recommended Intake (Nordic)

**upper level of safe intake for European Community from EC2006, other EC data from 1993*

#Safe upper limit of selenium intake for UK from Expert Group on Vitamins and Minerals 2003, Safe Upper Levels for Vitamins and Minerals Food Standards Agency (full document available at http://www.food.gov.uk/multimedia/pdfs/vitmin2003.pdf

'DACH' refers to reference intakes for Germany, Austria and Switzerland

'Nordic countries' refers to Denmark, Finland, Norway and Sweden.

'EC' refers to European Community.

Table 2. Biomarkers identified in systematic review (Lowe et al., 2009).

Potentially useful	• Plasma/serum zinc concentration • Hair zinc concentration • Urinary zinc excretion
Not useful	• Erythrocyte zinc concentration • Mononuclear cell zinc concentration • Polymorphonuclear cell zinc concentration • Platelet zinc concentration • Plasma alkaline phosphatise activity

Inconclusive due to lack of data	• Aminolevulinic acid dehydratase • Erythrocyte metallothionein • Monocyte metallothionein cDNA • Salivary zinc • Salivary-sediment zinc • Mixed saliva zinc • Plasma extracellular superoxide dismutase • Lymphocyte zinc • Lymphocyte ecto-5'-nucleotidase • Nail zinc • Plasma angiotensin-converting enzyme • Neutrophil zinc • T lymphocyte metallothionein -2A mRNA • Plasma 5'-nucleotidase • Endogenous zinc excretion • Plasma zinc flux • Exchangeable zinc pool • Carbonic anhydrase • Fecal Zinc • Neutrophil α-D-mannosidase • Neutrophil alkaline phosphatase • Erythrocyte membrane zinc • Erythrocyte membrane alkaline phosphatase • Erythrocyte membrane neutral phosphatase

Table 3. Priority health outcomes associated with inadequate zinc intake for each population group.

Infants	Children & Adolescent	Pregnant & lactating women	Adults/Elderly
Growth	Growth	Foetus	Immune function
Immune response to vaccination	Immune function	Foetal growth	Cognitive function
Neurodevelopment	Cognitive functions and Psychomotor development	Foetal malformation	Dermatitis
	Dermatitis	Mother	Anorexia
		Preeclampsia	Hypogeusia
		Preterm delivery	Ischemic heart disease
			Depression
			Diabetes Mellitus
			Carcinogenesis

Table 4: Results of the Meta-analysis of intake-status in all populations groups.

Population group	Overall Beta	95% CI's	I^2	p
Adults and Elderly	0.09	0.07,0.12	79.1%	P<0.0001
Pregnant women	0.04	0.02, 0.07	55%	p=0.002
Lactating women	0.02	-0.01,0.05	0%	p=0.28
Children and adolescents	0.12	0.04, 0.20	97.6%	p<0.005
Infants	0.09	0.06,0.12	95%	P<0.00001

Table 5. Results of the systematic search for studies of the effect of gene polymorphisms on zinc metabolism.

Author	Year	Reference	Gene with significant interaction with Zn
(Tamura et al.)	1996	Obstet Gynecol 88:497-502	ACE insertion/deletion DD, DI and II
(Mariani et al.)	2008	Experimental Gerontology 43:462-471	MT1a +674 A/C transition and Interleukin 6 IL-6 +174C/G transition
(Gonzalez et al.)	1999	Eur J Clin Investigation 29:637-642	Epsilon 4 apoE
(Giacconi et al.)	2005	Biogerontology 6:407-413	MT2A rs1610216 AA and AG, 246bp, 131 and 115bp
(Dar et al.)	2008	Nutrition and Cancer 60(5):585-591	TP53 mutation at exon 5-8
(Reszka et al.)	2005	Trace Elements and Electrolytes 22(1):23-32	GSTP1, GSTT1 and GSTM1
(Jin et al.)	2011	Cancer Epidemiology 32:182-187	CYP1A1 mspl Aa or aa and GSTM1null
(Reszka et al.)	2007	Genes Nutr 2:287-294	GSTM1 and GSTT1
(Mocchegiani et al.)	2008	Experimental Gerontology 43:433-444	IL-6 +174G/C
(Kajanachumpol et al.)	1997	Southeast Asian J Trop Med Public Health 28(4):877-880	HbH, ß-thal/HbE, ß-thal major
(Ajayi)	1997	Trace Elements and Electrolytes 14(2):69-71	HbAA, HbSS, HbAS and HbAC
(Akar et al.)	2006	Trace Elements and Electrolytes 23(4):266-269	SLC30A4 ZNT4 915T-C at exon 5