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Status of essential elements in autism spectrum disorder: systematic review and meta-analysis

DOI 10.1515/revneuro-2017-0015
Received February 19, 2017; accepted April 16, 2017

Abstract: Autism spectrum disorder (ASD) is a lifelong neurodevelopmental disorder that imposes heavy financial burden on governments and families of affected children. It is considered a multifactorial condition, where trace elements are among environmental factors that may contribute to ASD. Meanwhile, the between-study variance is high. The present systematic review was designed to investigate the difference in trace element measures between patients with ASD and control subjects. Meta-analyses showed that the hair concentrations of chromium ($p=0.024$), cobalt ($p=0.012$), iodine ($p=0.000$), iron ($p=0.017$), and magnesium ($p=0.007$) in ASD patients were significantly lower than those of control subjects, while there were higher magnesium levels in the hair of ASD patients compared to that of controls ($p=0.010$). Patients with ASD had higher blood levels of copper ($p=0.000$) and lower levels of zinc compared to controls ($p=0.021$). Further urinary iodine levels in patients with ASD were decreased in comparison with controls ($p=0.026$). Sensitivity analyses showed that ASD patients in non-Asian but not in Asian countries had lower hair concentrations of chromium compared to controls. Also, such analyses indicated that ASD patients in Asian countries had lower hair zinc concentrations, whereas ASD patients in non-Asian countries had higher hair zinc concentrations in comparison with control subjects. This study found significant differences in the content of trace elements between patients with ASD compared to controls. The findings help highlighting the role of trace elements as environmental factors in the etiology of ASD.

Keywords: Asia; autism; chromium; copper; environmental factors; iodine; iron; trace elements; zinc.

Introduction

Autism spectrum disorder (ASD) refers to a group of heterogeneous neurodevelopmental disorders commonly associated with repetitive behaviors and impairments in social communication behaviors (Bishop et al., 2016). Overall, more than 50 million people suffer from ASD (Baxter et al., 2015), a lifelong disorder that imposes heavy financial burden on governments and families of affected children (Lavelle et al., 2014). Its pathogenesis is assumed to be multifactorial, including genetic variants, immune abnormalities, zinc deficiency, abnormal melatonin synthesis, maternal diabetes, stress, toxins, and parental age.

Human requires a meager supply of essential elements, e.g. zinc, cobalt, copper, selenium, iron, manganese, and iodine, for certain aspects of functioning, importantly brain function. They play a role in regulating the body's systems, importantly the immune and antioxidant system. It is thus understandable that altered profile of trace elements has been observed among different medical conditions and diseases, including neurological and psychiatric disorders (Swardfager et al., 2013; Da Silva et al., 2014; Saghazadeh et al., 2015).

A number of biomarkers (Ming et al., 2005; Ray et al., 2011; Saghazadeh and Rezaei, 2017) for diagnosis and monitoring progression of ASD have been suggested during the last four decades. As yet, none has entered into clinical use. Little is known about molecular mechanism of action through which trace elements might contribute to ASD. However, there have been suggested that dysfunction in excitatory and inhibitory synapses is the culprit behind ASD signs and symptoms and that trace elements mediate their role in ASD by influencing synaptic function (Coghlan et al., 2012). Particularly, zinc is required for scaffolding of ProSAP/Shank proteins related to excitatory synapses

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(Grabrucker et al., 2014). It is, thus, expected that altered zinc profile has been associated with different brain diseases and disorders. Higher zinc values might contribute to epileptogenesis (Saghazadeh et al., 2015), whereas lower zinc levels have been implicated in depression (Swardfager et al., 2013) and ASD (Grabrucker et al., 2014). Interestingly, copper overload is thought to cause zinc deficiency and, therefore, synaptic dysfunction (Baecker et al., 2014). Moreover, disturbed activity of the gamma-aminobutyric acid (GABA) neurotransmitter that mediates a crucial inhibitory synaptic signaling has been linked to ASD (Chao et al., 2010). Magnesium is a regulatory cation that modulates GABA signaling, and thus, altered magnesium profile might contribute to ASD. Iron is an essential element that takes part in important brain functions including gene expression and myelination (Benarroch, 2009). Evidence points to impaired iron hemostasis in neurodegenerative diseases (Benarroch, 2009). Also anemia is associated with depression and anxiety, and more interestingly, iron deficiency status has been shown to have negative effects on social and emotional behaviors in a dose-dependent manner (Lozoff et al., 2008).

Altogether, trace elements are among environmental factors that may contribute to ASD. Many studies have been performed to determine the profile of trace elements in people with ASD. However, due to inconsistencies across studies, it is still hard to explain the association between ASD and trace elements. The present systematic review and meta-analysis study was designed to address the issue by quantitative data synthesis of reports.

Materials and methods

The present systematic review and meta-analysis study was prepared according to the preferred reporting items for systematic reviews and meta-analyses (PRISMA) statement (Moher et al., 2009). The PRISMA statement is a 27-item checklist, which has a rational design for improving the quality of reporting systematic review and meta-analysis studies. Before the study begins, the authors (A.S. and N.R.) developed a study protocol, which is available on request.

Eligibility criteria

Original articles were included if they met the following criteria: (1) studies that measured levels of trace elements in the whole blood, plasma, serum, red cells, hair, and urine specimens in patients with ASD and in control subjects without ASD and (2) articles that provided sufficient data, including the total number of subjects in both patients and controls and mean and standard deviation (SD) of the trace element levels. We also included studies providing enough data (for example, median, the first quartile, and the third quartile, or median and range, or median and standard error) to calculate mean and SD.

Information sources and search strategy

We identified relevant studies by searching the main electronic medical databases, i.e. PubMed and Scopus, through October 2016. We used the following key terms in the title, abstract, or keywords: (1) species ('human' OR 'subject' OR 'patient' OR 'people' OR 'person' OR 'case' OR 'control' OR 'individual' OR 'population' OR 'Child' OR 'kid' OR 'adolescent' OR 'Adult'), (2) participants ('Autism' OR 'autistic' OR 'Asperger' OR 'pervasive developmental disorder' OR 'pervasive developmental delay'), and (3) exposure ('trace element' OR 'trace elements' OR 'trace metal' OR 'trace metals' OR 'essential element' OR 'essential elements' OR 'essential metal' OR 'essential metals' OR 'trace dietary element' OR 'trace dietary elements' OR 'zinc' OR 'iron' OR 'copper' OR 'molybdenum' OR 'iodine' OR 'chromium' OR 'selenium' OR 'cobalt' OR 'boron' OR 'magnesium'). To include as many relevant articles as possible in the present study, backward search was performed through which the reference lists of retrieved results were screened.

Study selection

The present systematic review and meta-analysis was depicted to identify all studies that evaluated concentrations of trace elements in plasma, serum, whole blood, red cells, hair, or urine among patients with ASD and in control subjects without ASD. We applied no language restrictions and time limit in the search strategy and study selection. As recommended by the PRISMA guidelines and graphically illustrated in Figure 1, the study selection is a procedure composed of four main steps: identification, screening, eligibility, and inclusion. The 'identification' step aimed at acquisition of all the relevant papers is a process including forward and backward searches and then removal of duplicate records. The 'screening' step is to screen results based on title/abstract. The apparently relevant papers are examined by the authors for 'eligibility'. The final step is to include articles that met eligible criteria in systematic review and in meta-analysis if

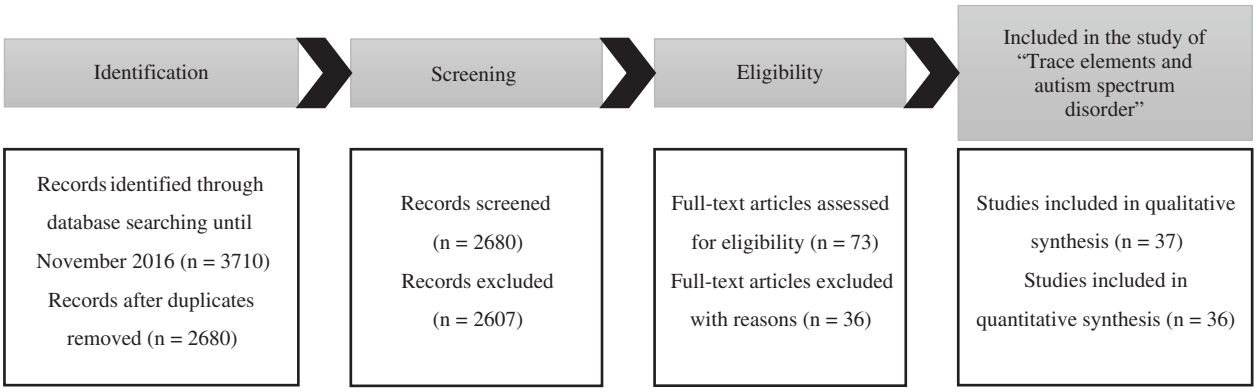


Figure 1: Search strategy.

applicable. In the present study, the study selection was done by four authors (A.S., N.A., K.H., and F.S.), and meta-analysis was performed when there were three or more comparisons regarding the title. More clearly, we did not carry out quantitative synthesis when there were less than three comparisons regarding the title.

Data collection process and data items

We extracted the following data from each included publication: first-named author, year of publication, location of study, the assay that was used for element measurement, type of specimen (serum, plasma, whole blood, red cells, hair, or urine) taken from subjects, number of subjects in the patient and control groups, demographic characteristics (e.g. age and gender) of patients and controls, mean ± SD of the trace element levels in both the patient and the control groups, and the measurement scale.

The extracted data were entered into an excel spread sheet, which is available on request. The plan was to contact the authors for additional data, if the paper does not provide inadequate data. The process of data extraction was done by four authors (A.S., N.A., K.H., and F.S.). A.S. performed the final step of data curation, which was a process composed of data merging, transforming, and conversion.

Quality assessment

We appraised the quality of included studies using the Newcastle-Ottawa Scale (NOS) designed for observational studies (Wells et al., 2000). The NOS is composed for the assessment of three main aspects of observational studies: sample selection, comparability of cases and controls, and exposure. Using this scale, possible scores range from 0 to 9. Studies with scores of 7–9 stars have the lowest risk of bias and represent the highest quality, whereas studies with scores <4 stars have the highest risk of bias and the lowest quality. Studies with scores of 4–6 stars have the moderate risk of bias and quality.

Summary measures and synthesis of results

The standardized mean difference (SMD) and weighted mean difference (WMD) were interchangeably used for measurement of effect. As described elsewhere (Saghazadeh et al., 2014, 2017), the SMD was applied if studies used different measurement scales or assays. Otherwise, we used the WMD for measurement of effect. As well, fixed effects and random effects were interchangeably used as the analysis model. Heterogeneity was determined using Q statistic tests and the I^2 index. According to the Cochrane guidelines, I^2 less than 40% would mean that the inconsistency across studies is not important. In this case, we planned to use the fixed effects model. If I^2 estimates fluctuated more than 40%, we intended to use the random effects procedure as the analysis model. Publication bias was assessed using the degree of funnel plot asymmetry and Egger's test. We performed all of the statistical analyses using STATA version 14.1 (StataCorp. 2015. Stata Statistical Software: Release 14, College Station, TX: StataCorp LP). A p value less than 0.05 was considered statistically significant.

Results

Study selection

As schematically summarized in Figure 1, the initial search resulted in 3710 records (Figure 1). A total of 2680

discrete manuscripts were identified for review after removing duplicate publications (n = 1030). Of these, 2607 publications were excluded based on the title/abstract. We reviewed in detail the remaining 73 publications. Based on detail review, we excluded 36 additional publications due to the following reasons. Sixteen articles were excluded owing to lacking a normal control group (Shannon and Graef, 1996; Adams et al., 2009; Faber et al., 2009; Bilgiç et al., 2010; Clark et al., 2010; Song et al., 2010; Yasuda et al., 2011; Blaucok-Busch et al., 2012a,b; Geier et al., 2012; Herguner et al., 2012; Almogren et al., 2013; Yasuda and Tsutsui, 2013; Yasuda et al., 2013; Russo, 2015; Kim et al., 2016). Ten articles were excluded owing to the fact that they did not report adequate data or provided data in terms of deficiency or anemia (Campbell et al., 1980; Soden et al., 2007; Russo, 2010; Rahbar et al., 2012; Rahbar et al., 2013, 2014a,b, 2015; Sidrak et al., 2014; Lane et al., 2015). There was a duplicate record (Desoto and Hitlan, 2007). Some abstracts or titles were likely to be related, but the full texts were not available to obtain sufficient data for analysis or to ensure they were relevant (Cohen et al., 1976; Sohler et al., 1977; Fido et al., 2002; Lubkowska and Sobieraj, 2009; Albizzati et al., 2012). One study was excluded because the patient group enrolled patients other than ASD (Dikme et al., 2013). One study was excluded because the patient group had other medical complaints (sleep disturbances) (Youssef et al., 2013). One study excluded because the type of specimen (brain tissue) was not of interest to the present study (Pamphlett and Kum Jew, 2016). One study was excluded because it measured elements other than 19 elements of interest (Liu et al., 2016). As previously explained in the Materials and methods section, meta-analysis was performed if there were three or more comparisons with regard to the certain question. Of the 37 eligible studies included in the present systematic review (Jackson and Garrod, 1978; Shearer et al., 1982; Massaro et al., 1983; Wecker et al., 1985; Yorbik et al., 2004; Al-Ayadhi, 2005; Torsdottir et al., 2005; Adams et al., 2006; Mousain-Bosc et al., 2006; Strambi et al., 2006; Adams et al., 2007; Jory and Mcginnis, 2008; Jung et al., 2008; Yorbik et al., 2010; Adams et al., 2011; Blaurock-Busch et al., 2011; Elsheshtawy et al., 2011; Lakshmi Priya and Geetha, 2011; Obrenovich et al., 2011; Russo, 2011; Russo and Devito, 2011; Vergani et al., 2011; De Palma et al., 2012; Parellada et al., 2012; Semprún-Hernández et al., 2012; Adams et al., 2013; Al-Farsi et al., 2013; Hamza et al., 2013; Bener et al., 2014; Li et al., 2014; Macedoni-Luksic et al., 2015; Blazewicz et al., 2016; Craciun et al., 2016; Kondolot et al., 2016; Skalny et al., 2016a,b,c) (Table 1), 36 studies were included in meta-analyses (Jackson and Garrod, 1978; Shearer et al., 1982; Massaro et al., 1983; Wecker et al., 1985; Yorbik et al., 2004; Al-Ayadhi, 2005; Torsdottir et al., 2005; Adams et al., 2006; Mousain-Bosc et al., 2006; Strambi et al., 2006; Jory and Mcginnis, 2008; Jung et al., 2008; Yorbik et al., 2010; Adams et al., 2011; Blaurock-Busch et al., 2011; Elsheshtawy et al., 2011; Lakshmi Priya and Geetha, 2011; Obrenovich et al., 2011; Russo, 2011; Russo and Devito, 2011; Vergani et al., 2011; De Palma et al., 2012; Parellada et al., 2012; Semprún-Hernández et al., 2012; Adams et al., 2013; Al-Farsi et al., 2013; Hamza et al., 2013; Bener et al., 2014; Li et al., 2014; Macedoni-Luksic et al., 2015; Blazewicz et al., 2016; Craciun et al., 2016; Kondolot et al., 2016; Skalny et al., 2016a,b,c) (Table 2).

Meta-analysis results

Boron

There were five studies that measured boron concentrations in the hair of patients with ASD and control subjects (Adams et al., 2006; Jung et al., 2008; Blaurock-Busch et al., 2011; Al-Farsi et al., 2013; Skalny et al., 2017). Meta-analysis of six comparisons showed no significant difference ($p = 0.147$) in hair boron concentrations between ASD patients (n = 205) and controls (n = 188) (Table 2). After excluding an outlier record (Al-Farsi et al., 2013) from analysis, significant heterogeneity was no longer present ($p = 0.290$, $I^2 = 19.6\%$) but effect size remained insignificant ($p = 0.667$). Only one study (Adams et al., 2011) was retrieved that investigated erythrocyte boron concentrations, and therefore, we could not perform meta-analysis (Table 1).

Chromium

There were studies (Wecker et al., 1985; Al-Ayadhi, 2005; Adams et al., 2006; Jung et al., 2008; Yorbik et al., 2010; Blaurock-Busch et al., 2011; Vergani et al., 2011; De Palma et al., 2012; Al-Farsi et al., 2013; Skalny et al., 2016a,b) that assessed chromium concentrations in hair, erythrocyte, blood, and urine of patients with ASD and those of the control subjects. According to the number of comparisons available, we could perform meta-analysis of data related to blood and hair chromium levels. A meta-analysis of three comparisons demonstrated no difference ($p = 0.827$) in blood chromium levels between patients with ASD (n = 76) and control subjects (n = 80). The first

Table 1: Publications related to concentrations of trace elements in patients with autism compared to control subjects.

Element (symbol)	Specimen	Number of relevant publications	Element (symbol)	Specimen	Number of relevant publications
Copper (Cu)	Erythrocyte	2 (Jory and Mcginnis, 2008; Adams et al., 2011)	Magnesium (Mg)	Blood	2 (Massaro et al., 1983; Adams et al., 2011)
	Hair	14 (Shearer et al., 1982; Massaro et al., 1983; Wecker et al., 1985; Al-Ayadhi, 2005; Adams et al., 2006; Jung et al., 2008; Blaurock-Busch et al., 2011; Elsheshtawy et al., 2011; Lakshmi Priya and Geetha, 2011; Obrenovich et al., 2011; De Palma et al., 2012; Al-Farsi et al., 2013; Skalny et al., 2016a,c)		Erythrocytes	4 (Mousain-Bosc et al., 2006; Strambi et al., 2006; Jory and Mcginnis, 2008; Adams et al., 2011)
	Nail	1 (Lakshmi Priya and Geetha, 2011)		Hair	9 (Shearer et al., 1982; Massaro et al., 1983; Wecker et al., 1985; Al-Ayadhi, 2005; Adams et al., 2006; Jung et al., 2008; Blaurock-Busch et al., 2011; Lakshmi Priya and Geetha, 2011; Al-Farsi et al., 2013)
	Plasma	6 (Jackson and Garrod, 1978; Torsdottir et al., 2005; Russo, 2011; Russo and Devito, 2011; Vergani et al., 2011; Parellada et al., 2012)		Nail	1 (Lakshmi Priya and Geetha, 2011)
	Serum	4 (Semprún-Hernández et al., 2012; Li et al., 2014; Macedoni-Luksic et al., 2015; Skalny et al., 2016b)		Plasma	1 (Strambi et al., 2006)
	Urine	1 (Blaurock-Busch et al., 2011)		Serum	4 (Mousain-Bosc et al., 2006; Adams et al., 2011; Semprún-Hernández et al., 2012; Bener et al., 2014)
	Whole blood	3 (Massaro et al., 1983; Adams et al., 2011; Craciun et al., 2016)		Urine	1 (Blaurock-Busch et al., 2011)
Iodine (I)	Hair	4 (Adams et al., 2006; Blaurock-Busch et al., 2011; Skalny et al., 2016a,c)	Zinc (Zn)	Blood	3 (Massaro et al., 1983; Adams et al., 2011; Craciun et al., 2016)
	Serum	1 (Skalny et al., 2016b)		Erythrocyte	3 (Yorbik et al., 2004; Jory and Mcginnis, 2008; Adams et al., 2011)
	Urine	4 (Adams et al., 2011; Blaurock-Busch et al., 2011; Hamza et al., 2013; Blazewicz et al., 2016)		Hair	14 (Shearer et al., 1982; Massaro et al., 1983; Wecker et al., 1985; Yorbik et al., 2004; Al-Ayadhi, 2005; Adams et al., 2006; Jung et al., 2008; Blaurock-Busch et al., 2011; Elsheshtawy et al., 2011; Lakshmi Priya and Geetha, 2011; De Palma et al., 2012; Al-Farsi et al., 2013; Skalny et al., 2016a,c)
Chromium (Cr)	Erythrocyte	2 (Jory and Mcginnis, 2008; Adams et al., 2011)		Nail	1 (Lakshmi Priya and Geetha, 2011)
	Hair	8 (Wecker et al., 1985; Al-Ayadhi, 2005; Adams et al., 2006; Jung et al., 2008; Blaurock-Busch et al., 2011; De Palma et al., 2012; Al-Farsi et al., 2013; Skalny et al., 2016a)		Plasma	5 (Jackson and Garrod, 1978; Yorbik et al., 2004; Russo, 2011; Russo and Devito, 2011; Vergani et al., 2011)
	Plasma	1 (Vergani et al., 2011)		Serum	4 (Semprún-Hernández et al., 2012; Li et al., 2014; Macedoni-Luksic et al., 2015; Skalny et al., 2016b)
	Serum	1 (Skalny et al., 2016b)		Teeth	(Adams et al., 2007)

Table 1 (continued)

Element (symbol)	Specimen	Number of relevant publications	Element (symbol)	Specimen	Number of relevant publications
	Urine	2 (Yorbik et al., 2010; Blaurock-Busch et al., 2011)		Urine	(Blaurock-Busch et al., 2011)
Cobalt (Co)	Erythrocyte	1 (Jory and Mcginnis, 2008)	Iron (Fe)	Erythrocyte	1 (Adams et al., 2011)
	Hair	9 (Wecker et al., 1985; Al-Ayadhi, 2005; Adams et al., 2006; Jung et al., 2008; Blaurock-Busch et al., 2011; De Palma et al., 2012; Al-Farsi et al., 2013; Skalny et al., 2016a,c)		Hair	10 (Wecker et al., 1985; Al-Ayadhi, 2005; Adams et al., 2006; Jung et al., 2008; Blaurock-Busch et al., 2011; Obrenovich et al., 2011; De Palma et al., 2012; Al-Farsi et al., 2013; Skalny et al., 2016a,c)
	Plasma	1 (Vergani et al., 2011)		Plasma	2 (Vergani et al., 2011; Parellada et al., 2012)
	Serum	1 (Skalny et al., 2016b)		Serum	1 (Adams et al., 2011; Skalny et al., 2016b)
	Urine	1 (Blaurock-Busch et al., 2011)		Urine	1 (Blaurock-Busch et al., 2011)
	Whole blood	1 (Adams et al., 2013)	Selenium (Se)	Erythrocyte	3 (Jory and Mcginnis, 2008; Adams et al., 2011; Kondolot et al., 2016)
Molybdenum (Mo)	Erythrocyte	2 (Jory and Mcginnis, 2008; Adams et al., 2011)		Hair	8 (Al-Ayadhi, 2005; Adams et al., 2006; Jung et al., 2008; Blaurock-Busch et al., 2011; Lakshmi Priya and Geetha, 2011; De Palma et al., 2012; Skalny et al., 2016a,c)
	Hair	6 (Al-Ayadhi, 2005; Adams et al., 2006; Jung et al., 2008; Blaurock-Busch et al., 2011; De Palma et al., 2012; Al-Farsi et al., 2013)		Nail	1 (Lakshmi Priya and Geetha, 2011)
	Urine	1 (Blaurock-Busch et al., 2011)		Serum	1 (Skalny et al., 2016b)
	Whole blood	1 (Adams et al., 2011)			
Boron (B)	Hair	5 (Jung et al., 2008; Adams et al., 2006; Blaurock-Busch et al., 2011; Al-Farsi et al., 2013; Skalny et al., 2016a)		Whole blood	1 (Adams et al., 2011)
	Erythrocyte	1 (Adams et al., 2011)			

Table 2: Summary of meta-analyses of the mean difference in toxic heavy metal levels between patients with autism and control subjects.

Element Specimen	No. of comparisons	No of patients/ controls	Heterogeneity χ^2	Inconsistency	Effect measure (95% CI)	Overall effect (p value)	Egger's test
Boron Hair (overall)	6	205/188	$\chi^2=80.18$ $p=0.000$	$I^2=93.8\%$	SMD = 0.640 (−0.255 to 1.505)	$Z=1.45$ $p=0.147$	$T=3.92$ $p=0.017$
Boron Hair (without outlier record)	5	178/161	$\chi^2=4.97$ $p=0.290$	$I^2=19.6\%$	SMD = −0.047 (−0.262 to 0.167)	$Z=0.43$ $p=0.667$	$T=0.37$ $p=0.736$
Cobalt Blood	3	130/124	$\chi^2=2.35$ $p=0.309$	$I^2=14.8\%$	SMD = −0.217 (−0.465 to 0.030)	$Z=1.72$ $p=0.086$	$T=-1.33$ $p=0.410$
Cobalt Hair (overall)	11	367/384	$\chi^2=114.75$ $p=0.000$	$I^2=92.2\%$	SMD = 0.202 (−0.383 to 0.786)	$Z=0.68$ $p=0.499$	$T=3.08$ $p=0.015$
Cobalt Hair (without outlier record)	10	340/357	$\chi^2=17.56$ $p=0.025$	$I^2=54.4\%$	SMD = −0.310 (−0.551 to 0.069)	$Z=2.52$ $p=0.012$	$T=0.77$ $p=0.469$
Chromium Blood	3	76/80	$\chi^2=24.18$ $p=0.000$	$I^2=91.7\%$	SMD = −0.128 (−1.269 to 1.014)	$Z=0.22$ $p=0.827$	$T=-7.97$ $p=0.079$
Chromium Hair (overall)	9	334/351	$\chi^2=137.56$ $p=0.000$	$I^2=94.2\%$	SMD = 0.200 (−0.491 to 0.890)	$Z=0.57$ $p=0.571$	$T=3.28$ $p=0.013$
Chromium Hair (without outlier record)	8	307/324	$\chi^2=34.58$ $p=0.000$	$I^2=79.8\%$	SMD = −0.421 (−0.787 to −0.056)	$Z=2.26$ $p=0.024$	$T=0.65$ $p=0.537$
Chromium Hair (Asian studies)	3	118/127	$\chi^2=19.73$ $p=0.000$	$I^2=89.9\%$	SMD = −0.395 (−1.274 to 0.484)	$Z=0.88$ $p=0.378$	$T=6.43$ $p=0.098$
Chromium Hair (European and American studies)	5	189/197	$\chi^2=11.43$ $p=0.022$	$I^2=65.0\%$	SMD = −0.400 (−0.752 to −0.049)	$Z=2.23$ $p=0.025$	$T=-2.04$ $p=0.134$
Copper Blood (overall)	14	604/380	$\chi^2=21.96$ $p=0.056$	$I^2=40.8\%$	WMD = 9.118 (5.491–12.745)	$Z=4.93$ $p=0.000$	$T=1.03$ $p=0.322$
Copper Whole blood	3	71/88	$\chi^2=0.16$ $p=0.924$	$I^2=0.0\%$	WMD = 5.093 (0.816–9.370)	$Z=2.33$ $p=0.020$	$T=0.05$ $p=0.966$
Copper Plasma	6	348/144	$\chi^2=9.59$ $p=0.088$	$I^2=47.8\%$	WMD = 13.301 (5.349–21.254)	$Z=3.28$ $p=0.001$	$T=-0.14$ $p=0.896$
Copper Serum	5	185/148	$\chi^2=6.57$ $p=0.161$	$I^2=39.1\%$	SMD = 8.731 (5.081–12.381)	$Z=4.69$ $p=0.000$	$T=0.36$ $p=0.740$
Copper Hair	16	505/533	$\chi^2=497.91$ $p=0.000$	$I^2=97\%$	SMD = − 0.980 (−1.815 to −0.145)	$Z=2.30$ $p=0.021$	$T=-2.51$ $p=0.025$
Copper Hair	14	413/426	$\chi^2=164.51$ $p=0.000$	$I^2=92.1\%$	SMD = −0.031 (−0.550 to 0.488)	$Z=0.12$ $p=0.907$	$T=-0.59$ $p=0.566$
Iron Blood	5	131/158	$\chi^2=59.66$ $p=0.000$	$I^2=93.3\%$	SMD = 0.256 (−0.712 to 1.225)	$Z=0.52$ $p=0.604$	$T=0.84$ $p=0.462$
Iron Hair	12	393/423	$\chi^2=1290.13$ $p=0.000$	$I^2=99.1\%$	WMD = 2.778 (−2.230 to 7.787)	$Z=1.09$ $p=0.277$	$T=0.71$ $p=0.492$
Iron Hair	11	366/396	$\chi^2=43.4$ $p=0.000$	$I^2=77\%$	WMD = −1.410 (−2.571 to 0.248)	$Z=2.38$ $p=0.017$	$T=-1.44$ $p=0.183$
Iodine Urine	4	135/159	$\chi^2=100.48$ $p=0.000$	$I^2=97.0\%$	SMD = −1.972 (−3.705 to −0.240)	$Z=2.23$ $p=0.026$	$T=-2.86$ $p=0.103$
Iodine Hair	6	183/172	$\chi^2=11.23$ $p=0.047$	$I^2=55.5\%$	SMD = −0.432 (−0.645 to −0.219)	$Z=3.98$ $p=0.000$	$T=-0.05$ $p=0.965$
Magnesium Blood	7	409/428	$\chi^2=19.69$ $p=0.003$	$I^2=69.5\%$	SMD = −0.228 (−0.548 to 0.093)	$Z=1.39$ $p=0.164$	$T=1.32$ $p=0.244$
Magnesium Serum	4	337/354	$\chi^2=4.66$ $p=0.198$	$I^2=35.6\%$	WMD = −0.105 (−0.140 to −0.070)	$Z=5.88$ $p=0.000$	$T=1.86$ $p=0.204$
Magnesium Erythrocyte	4	121/109	$\chi^2=31.30$ $p=0.000$	$I^2=90.4\%$	SMD = −0.581 (−1.497 to 0.335)	$Z=1.24$ $p=0.214$	$T=-0.70$ $p=0.554$
Magnesium Hair (overall)	9	296/294	$\chi^2=138.49$ $p=0.000$	$I^2=94.2\%$	SMD = −0.005 (−0.770 – 0.759)	$Z=0.01$ $p=0.989$	$T=1.06$ $p=0.323$

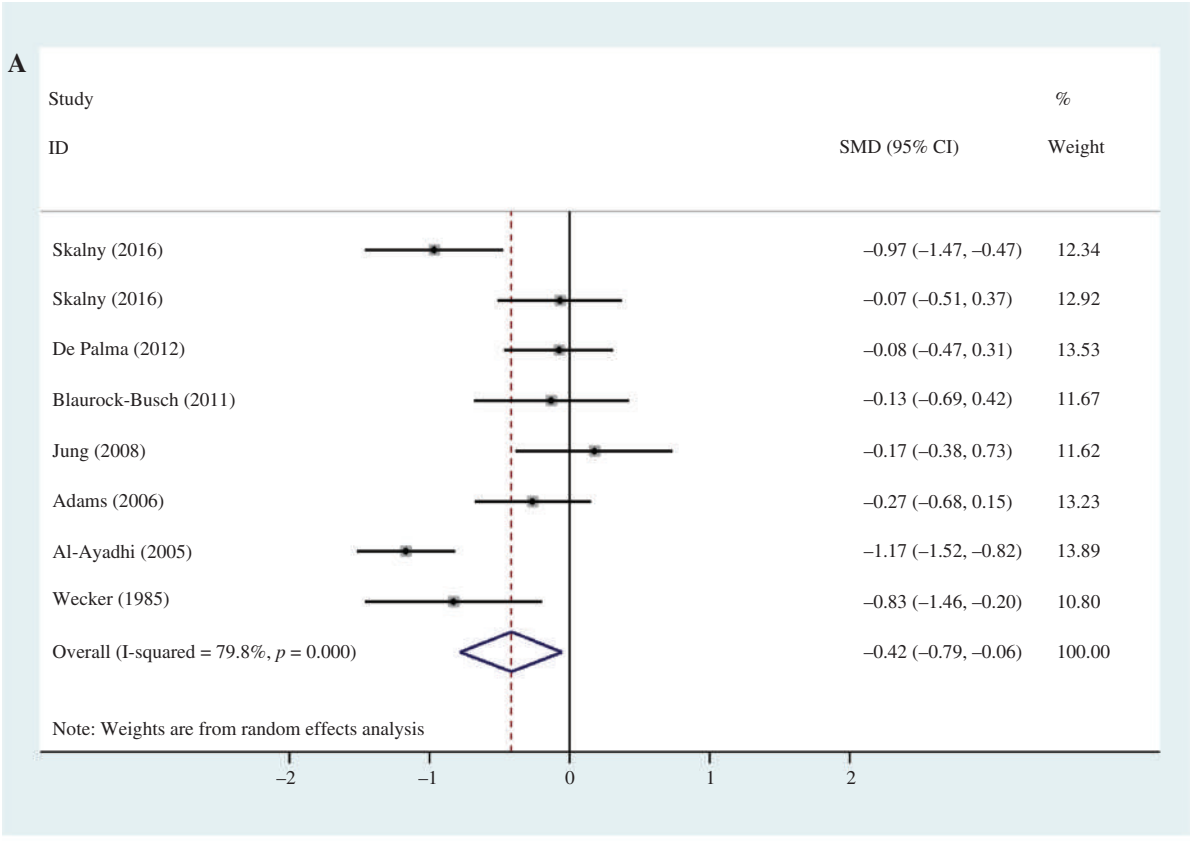
Table 2 (continued)

Element Specimen	No. of comparisons	No of patients/ controls	Heterogeneity χ^2	Inconsistency	Effect measure (95% CI)	Overall effect (p value)	Egger's test
Magnesium Hair (without outlier record)	8	269/267	$\chi^2=41.21$ $p=0.000$	$I^2=83.0\%$	SMD = −0.612 (−1.060 to −0.164)	$Z=2.68$ $p=0.007$	$T=-1.01$ $p=0.353$
Molybdenum Hair	5	240/255	$\chi^2=85.34$ $p.000$	$I^2=95.3\%$	SMD = 1.293 (0.303 to 2.283)	$Z=2.56$ $p=0.010$	$T=5.73$ $p=0.011$
Selenium Erythrocyte	3	88/107	$\chi^2=16.80$ $p=0.000$	$I^2=88.1\%$	SMD = − 0.027 (−0.934 to 0.880)	$Z=0.06$ $p=0.953$	$T=-2.70$ $p=0.226$
Selenium Hair	10	365/385	$\chi^2=186.81$ $p=0.000$	$I^2=95.2\%$	SMD = 0.614 (−0.114 to 1.342)	$Z=1.65$ $p=0.098$	$T=0.50$ $p=0.629$
Zinc Blood, serum, or plasma (overall)	13	586/406	$\chi^2=137.49$ $p=0.000$	$I^2=91.3\%$	SMD = − 0.347 (−0.833 to 0.138)	$Z=1.40$ $p=0.160$	$T=0.43$ $p=0.676$
Zinc Blood, serum, or plasma (without outlier record)	11	513/333	$\chi^2=39.74$ $p=0.000$	$I^2=74.8\%$	SMD = −0.361 (−0.668 to −0.055)	$Z=2.31$ $p=0.021$	$T=0.20$ $p=0.844$
Zinc Plasma (overall)	6	328/171	$\chi^2=971$ $p.000$	$I^2=95\%$	SMD = −0.280 (−1.262 to 0.702)	$Z=0.56$ $p=0.576$	$T=0.39$ $p=0.718$
Zinc Plasma (without outlier record)	4	255/98	$\chi^2=1.54$ $p=0.673$	$I^2=0.0\%$	SMD = −0.266 (−0.527 to −0.006)	$Z=2.01$ $p=0.045$	$T=-1.62$ $p=0.246$
Zinc Serum	4	187/147	$\chi^2=29.47$ $p=0.000$	$I^2=89.8\%$	SMD = −0.538 (−1.270 to 0.195)	$Z=1.44$ $p=0.151$	$T=-0.16$ $p=0.887$
Zinc Blood	3	71/88	$\chi^2=5.98$ $p=0.050$	$I^2=66.6\%$	SMD = −0.222 (−0.789 to 0.346)	$Z=0.77$ $p=0.444$	$T=0.35$ $p=0.783$
Zinc Erythrocyte	3	85/100	$\chi^2=3.23$ $p=0.199$	$I^2=38.1\%$	SMD = −0.194 (−0.492 to 0.104)	$Z=1.27$ $p=0.203$	$T=-0.40$ $p=0.760$
Zinc Hair (overall)	16	520/591	$\chi^2=223.66$ $p=0.000$	$I^2=93.3\%$	SMD = −0.316 (−0.816 to 0.184)	$Z=1.24$ $p=0.216$	$T=-0.62$ $p=0.545$
Zinc Hair (without outlier record)	15	493/564	$\chi^2=178.93$ $p=0.000$	$I^2=92.2\%$	SMD = −0.462 (−0.935 to 0.011)	$Z=1.92$ $p=0.055$	$T=-1.18$ $p=0.259$
Zinc Hair (Asian studies)	6	236/306	$\chi^2=108.57$ $p=0.000$	$I^2=95.4\%$	SMD = −1.493 (−2.430 to −0.557)	$Z=3.12$ $p=0.002$	$T=-3.60$ $p=0.023$
Zinc Hair (non-Asian studies)	9	257/258	$\chi^2=10.93$ $p=0.206$	$I^2=26.8\%$	WMD = 10.384 (0.044 to 20.724)	$Z=1.97$ $p=0.049$	$T=0.87$ $p=0.415$

meta-analysis of nine comparisons showed that patients with ASD (n = 334) and control subjects (n = 351) did not differ in hair chromium levels ($p = 0.571$). As shown in Table 2, there existed publication bias, but after removal of one outlier study (Al-Farsi et al., 2013), significant publication bias was no longer present and that an effect size of −0.421 represented significantly ($p = 0.024$) lower chromium levels in the hair of patients with ASD (n = 307) than in that of control subjects (n = 324) (Figure 2). Sensitivity analyses revealed that this significant impact does exist among non-Asian (European and American) (Figure 2) but not among Asian subjects (Figure 2). However, the result should be treated with caution because of a small number of comparisons (n = 3) included in the meta-analysis of Asian studies.

Cobalt

Thirteen studies made cobalt levels of erythrocyte (Jory and Mcginnis, 2008), hair (Wecker et al., 1985; Al-Ayadhi, 2005; Adams et al., 2006; Jung et al., 2008; Blaurock-Busch et al., 2011; De Palma et al., 2012; Al-Farsi et al., 2013; Skalny et al., 2016a,c), plasma (Vergani



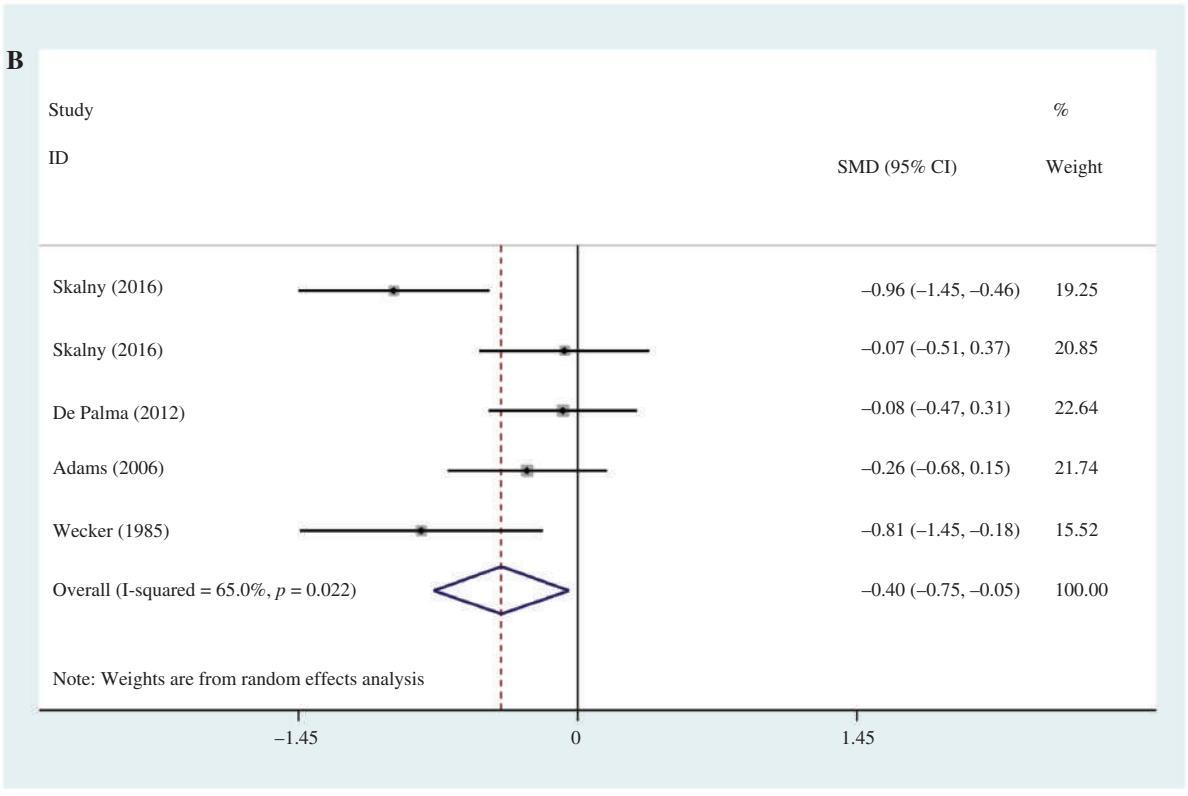


Figure 2: Meta-analyses of hair chromium levels.
(A) Overall meta-analysis of hair chromium levels. (B) Subgroup meta-analysis of hair chromium levels: non-Asian countries. (C) Subgroup meta-analysis of hair chromium levels: Asian countries.

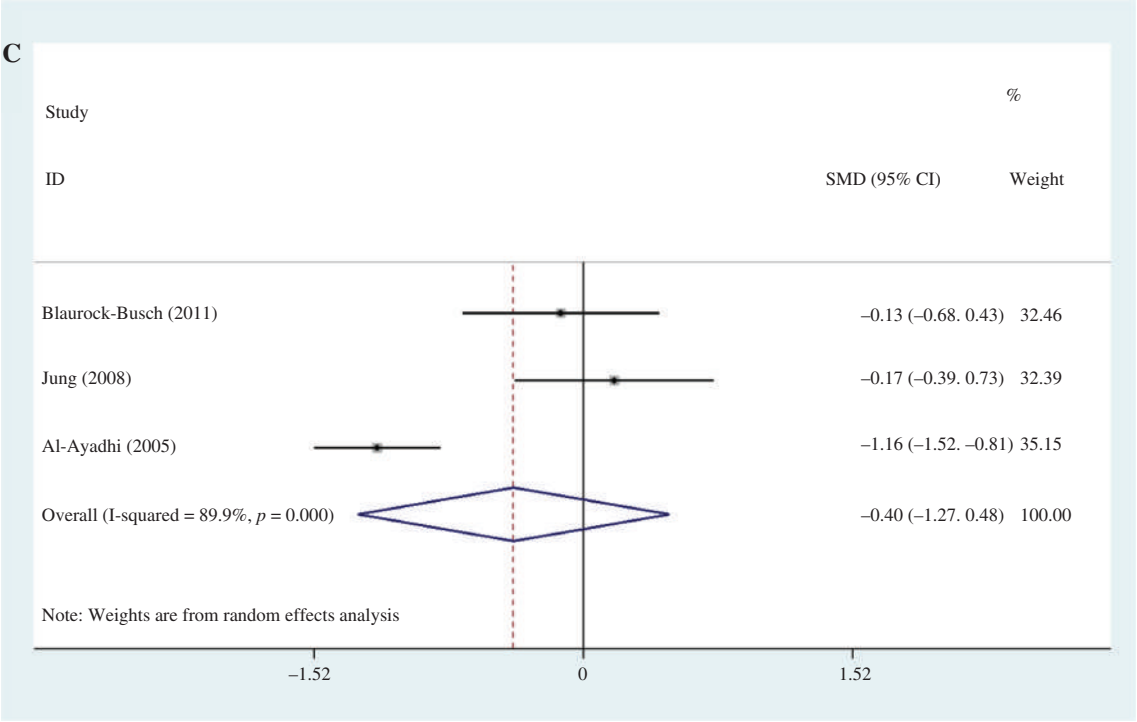


Figure 2 (continued)

et al., 2011), serum (Skalny et al., 2016b), urine (Blaurock-Busch et al., 2011), and whole blood (Adams et al., 2013) for patients with ASD and control subjects. We were unable to perform meta-analyses of comparisons regarding erythrocyte, plasma, serum, urine, and whole blood cobalt levels because of inadequate comparisons available. Regarding hair cobalt levels, an initial analysis of 11 comparisons including 367 patients with ASD and 384 control subjects revealed insignificant effect size (SMD = 0.202, $p = 0.499$). Due to the presence of publication bias ($t = 3.08$, $p = 0.015$), we excluded an outlier record (Al-Farsi et al., 2013) and conducted second meta-analysis of ten comparisons. An overall effect size of −0.310 showed that patients with ASD (n = 340) have significantly lower hair cobalt levels compared with controls (n = 357) (Figure 3) and publication bias was no longer present ($t = 0.77$, $p = 0.469$).

Copper

As noted in Table 1, it was the most well-documented trace element in ASD; there were 27 studies (Jackson and Garrod, 1978; Shearer et al., 1982; Massaro et al., 1983; Wecker et al., 1985; Al-Ayadhi, 2005; Torsdottir et al., 2005; Adams et al., 2006; Jory and Mcginnis, 2008; Jung et al., 2008; Adams et al., 2011; Blaurock-Busch et al., 2011; Elsheshtawy et al., 2011; Lakshmi Priya and Geetha, 2011; Obrenovich et al., 2011; Russo, 2011; Russo and Devito, 2011; Vergani et al., 2011; De Palma et al., 2012; Parellada et al., 2012; Semprún-Hernández et al., 2012; Al-Farsi et al., 2013; Li et al., 2014; Macedoni-Luksic et al., 2015; Craciun et al., 2016; Skalny et al., 2016a,b,c) that provided copper concentrations in erythrocyte, hair, nail, plasma, serum, urine, and whole blood for patients with ASD and controls. An overall effect size of 9.118 showed that copper levels in the blood in the ASD group (n = 604) are significantly ($p = 0.000$) increased compared with the control group (n = 380) (Figure 4). On the basis of blood specimen type (e.g. plasma, serum, and whole blood), several subgroup meta-analyses were performed, which all indicated significant effect sizes in favor of increased copper levels in patients with ASD compared with controls (Figure 4). As shown in Table 2, there were significantly lower copper levels in the hair of patients with ASD compared with controls. However, the effect size did not remain significant when two outlier records were removed from the analysis.

Iodine

Eight studies assessed iodine measures in the hair, urine, and serum for patients with ASD and control subjects

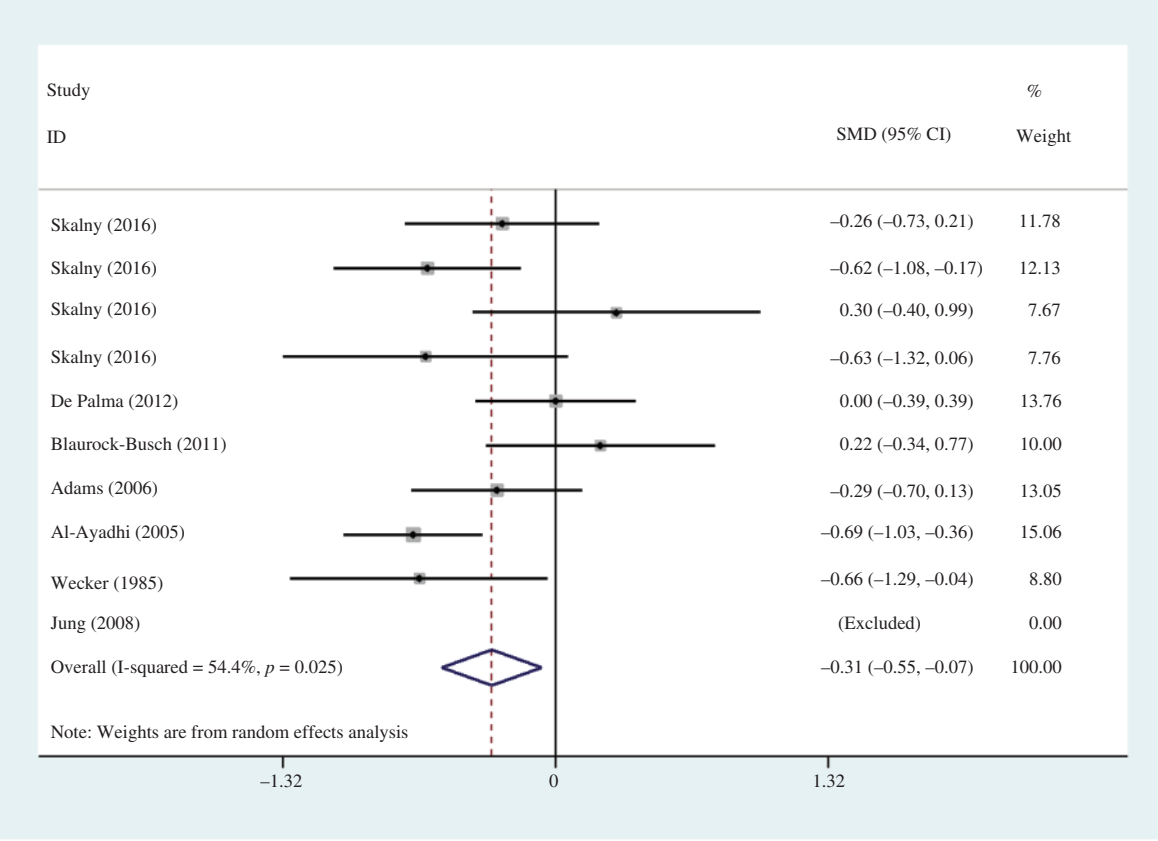


Figure 3: Meta-analysis of hair cobalt levels.

(Adams et al., 2006; 2011; Blaurock-Busch et al., 2011; Hamza et al., 2013; Blazewicz et al., 2016; Skalny et al., 2016a,b,c). A meta-analysis of four comparisons showed significantly ($p = 0.026$) lower urinary iodine concentrations in patients with ASD (n = 135) than in control subjects (n = 159) with a summary effect size of –1.972 (Figure 5). Also an overall effect size of –1.410 indicated that iodine concentrations of hair are decreased ($Z = 4.53$, $p = 0.000$) in patients with ASD (n = 183) compared with controls (n = 172) (Figure 5).

Iron

There were studies (Wecker et al., 1985; Al-Ayadhi, 2005; Adams et al., 2006; Jung et al., 2008; Adams et al., 2011; Blaurock-Busch et al., 2011; Obrenovich et al., 2011; Vergani et al., 2011; De Palma et al., 2012; Parellada et al., 2012; Al-Farsi et al., 2013; Skalny et al., 2016a,b,c) that assessed iron levels of erythrocyte, hair, blood, and urine for ASD and control individuals. Meta-analysis of five comparisons showed no difference ($p = 0.604$) in blood iron levels between the ASD and control group. There was no difference ($p = 0.277$) in hair iron levels between ASD and control subjects as an initial meta-analysis of 12 comparisons showed. We identified one study (Al-Farsi et al., 2013) as outlier considering forest plots and heterogeneity test results. Therefore, second meta-analysis of eleven comparisons was performed, which indicated significant effect size of –1.410 ($Z = 2.38$, $p = 0.017$) in favor of lower iron levels in the hair of patients with ASD (n = 366) compared with controls (n = 366) (Figure 6).

Magnesium

The measurement of magnesium levels in blood, erythrocyte, hair, nail, plasma, serum, and urine for patients with ASD and control subjects was made by 15 studies (Shearer et al., 1982; Massaro et al., 1983; Wecker et al., 1985; Al-Ayadhi, 2005; Adams et al., 2006; Mousain-Bosc et al., 2006; Strambi et al., 2006; Jory and Mcginnis, 2008; Jung et al., 2008; Adams et al., 2011; Blaurock-Busch et al., 2011; Lakshmi Priya and Geetha, 2011; Semprún-Hernández et al., 2012; Al-Farsi et al., 2013; Bener et al.,

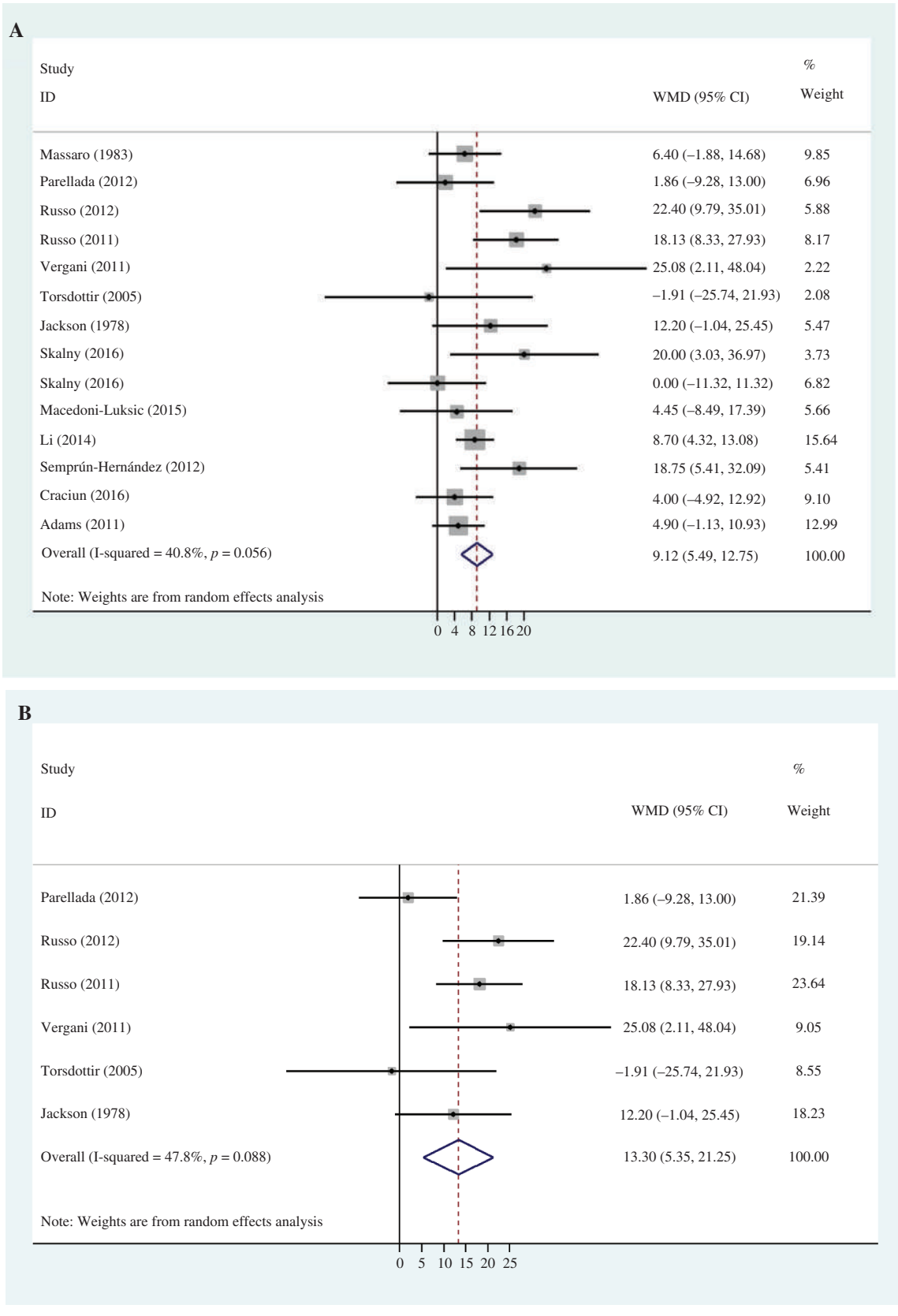


Figure 4: Meta-analyses of copper levels.
(A) Meta-analysis of blood copper levels. (B) Meta-analysis of plasma copper levels. (C) Meta-analysis of serum copper levels. (D) Meta-analysis of whole blood copper levels.

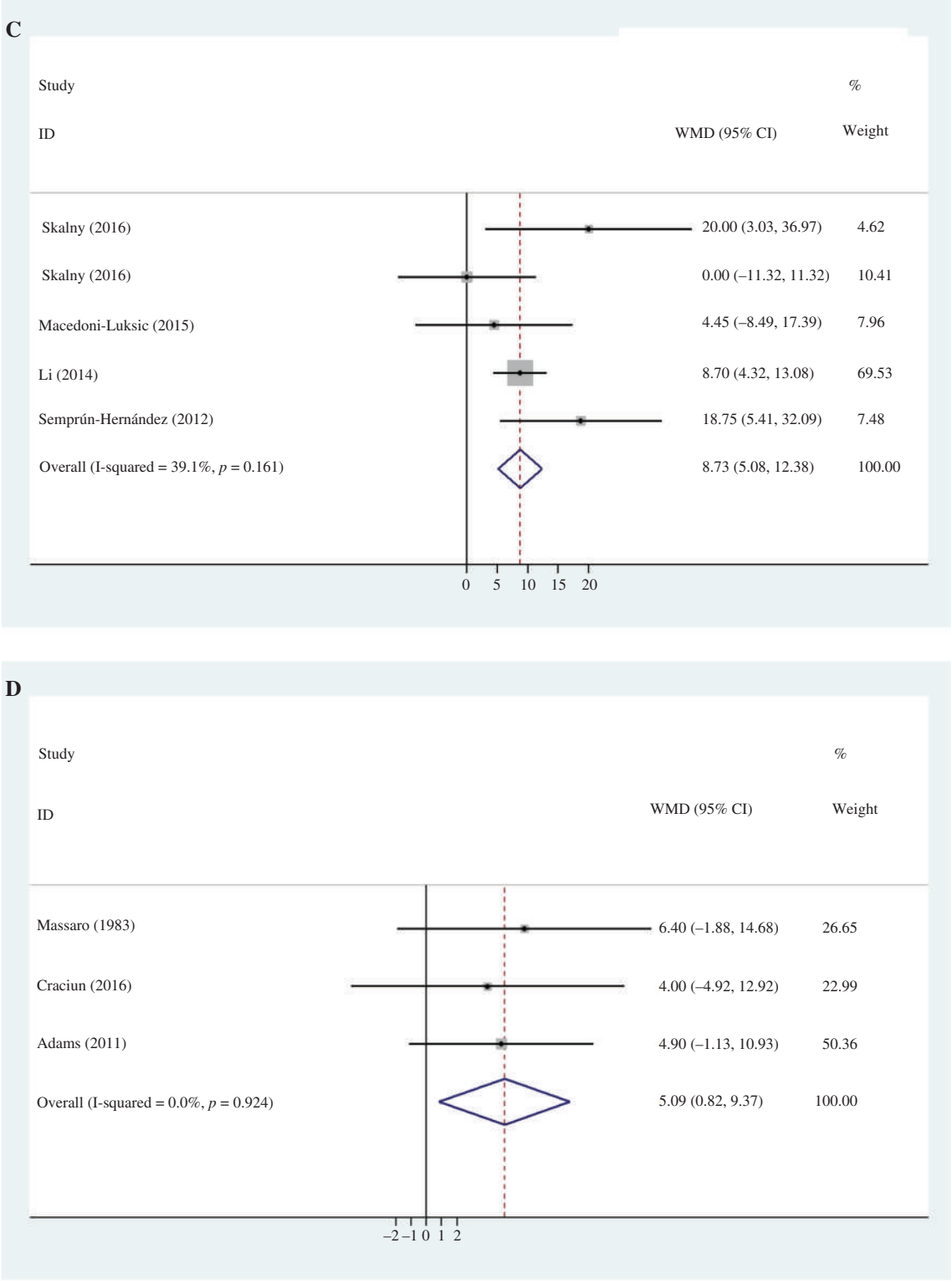


Figure 4 (continued)

2014). According to the number of comparisons (Table 1), we could perform meta-analysis regarding magnesium levels in blood, serum, erythrocyte, and hair. As shown in Table 2, meta-analysis for blood and erythrocyte magnesium levels estimated insignificant effect sizes. Regarding serum magnesium levels, an overall effect size of

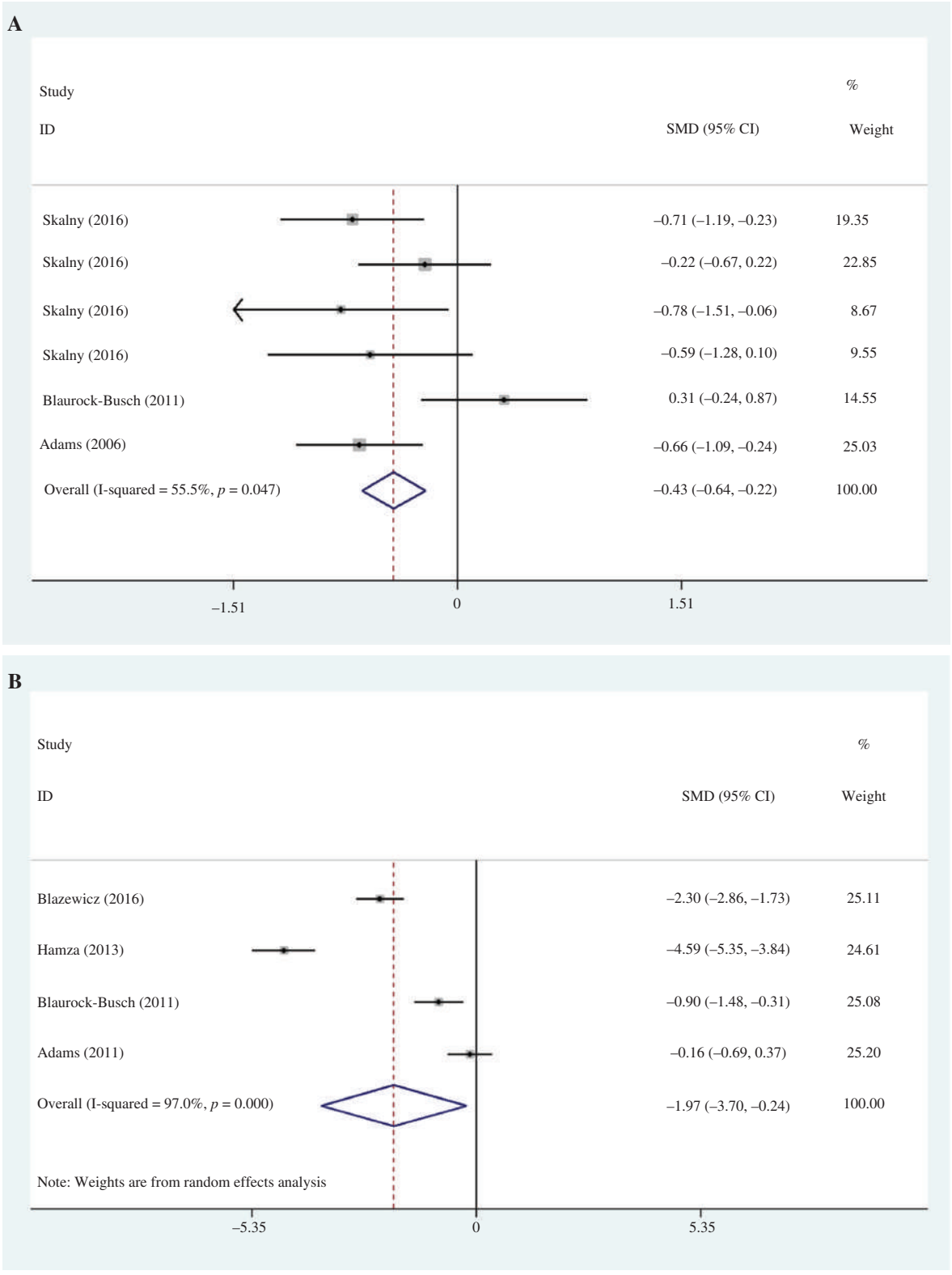


Figure 5: Meta-analyses of iodine concentrations.
(A) Meta-analysis of urinary iodine concentrations. (B) Meta-analysis of hair iodine concentrations.

–0.105 indicated that patients with ASD (n = 337) have significantly ($Z = 5.88$, $p = 0.000$) lower serum magnesium levels compared with normal controls (n = 354) (Figure 7). There were nine studies that assessed magnesium measures in the hair of patients with ASD and control subjects. An initial analysis of these studies showed no

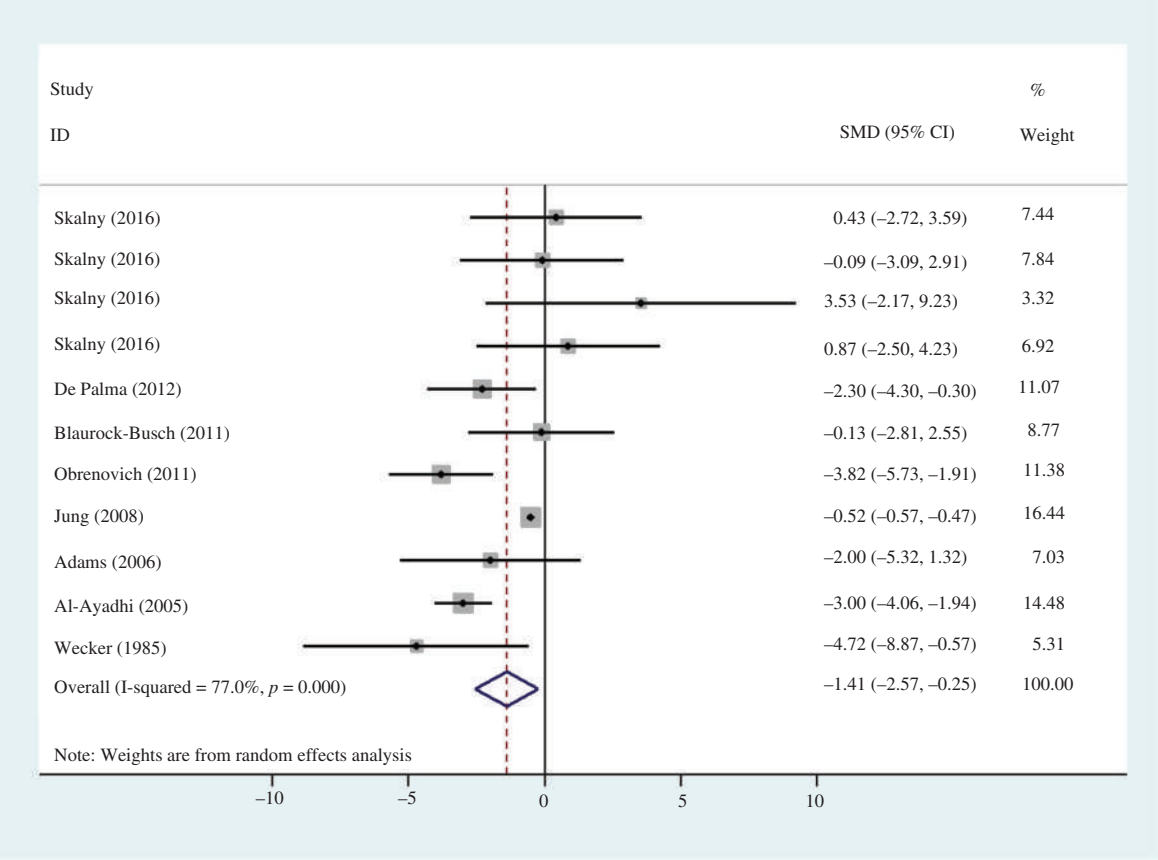


Figure 6: Meta-analysis of hair iron concentrations.

difference ($p = 0.989$) in hair magnesium levels between patients and controls. After removal of an outlier study (Al-Farsi et al., 2013) from analysis, a significant ($Z = 2.68$, $p = 0.007$) effect size of −0.612 indicated that the measurement of magnesium in the hair of patients with ASD (n = 269) is decreased compared with that of controls (n = 267) (Figure 7).

Molybdenum

Concentrations of molybdenum in erythrocytes, hair, urine, and whole blood for patients with ASD and control subjects were extracted from eight studies (Al-Ayadhi, 2005; Adams et al., 2006; Jory and Mcginnis, 2008; Jung et al., 2008; Adams et al., 2011; Blaurock-Busch et al., 2011; De Palma et al., 2012; Al-Farsi et al., 2013). The effect size of 1.293 was significant ($Z = 2.56$, $p = 0.010$) in favor of higher hair molybdenum levels in patients with ASD (n = 240) compared with control subjects (n = 255) (Figure 8). The number of comparisons that measured erythrocyte, urine, and blood molybdenum levels was not enough to perform meta-analysis.

Selenium

There were 12 studies (Al-Ayadhi, 2005; Adams et al., 2006; Jory and Mcginnis, 2008; Jung et al., 2008; Adams et al., 2011; Blaurock-Busch et al., 2011; Lakshmi Priya and Geetha, 2011; De Palma et al., 2012; Kondolot et al., 2016; Skalny et al., 2016a,b,c) that determined selenium concentrations in erythrocyte, hair, nail, serum, and whole blood of patients with ASD and of control subjects. According to the number of comparisons (Table 1), we could perform meta-analyses to determine the mean difference in erythrocyte and hair selenium concentrations between patients with ASD and control subjects. As shown in Table 2, patients did not differ significantly from controls, neither in the hair selenium concentrations ($p = 0.098$) nor in the erythrocyte selenium concentrations ($p = 0.953$).

Zinc

Twenty-six studies (Jackson and Garrod, 1978; Shearer et al., 1982; Massaro et al., 1983; Wecker et al., 1985;

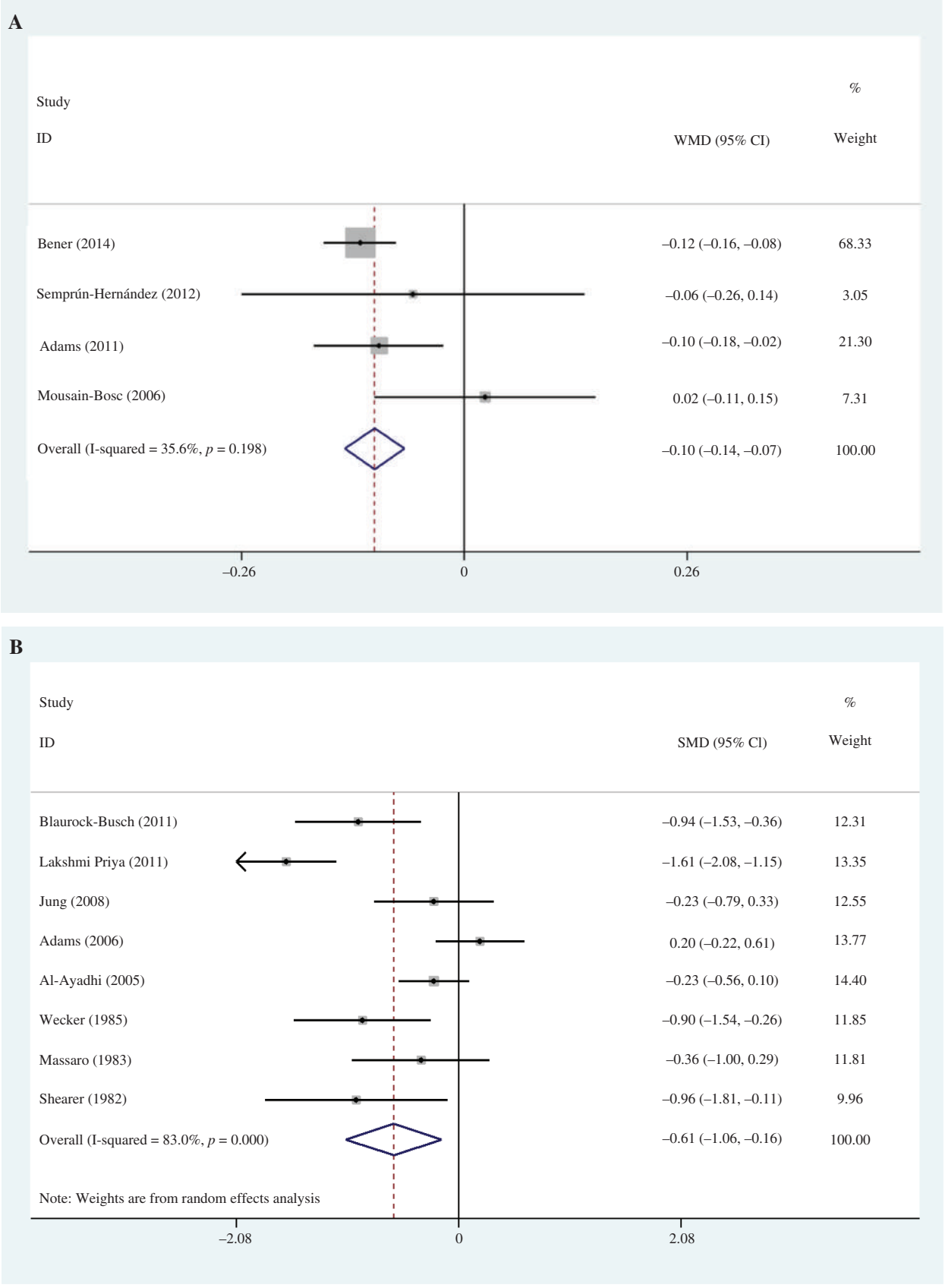


Figure 7: Meta-analysis of magnesium concentrations.
(A) Meta-analysis of serum magnesium concentrations. (B) Meta-analysis of hair magnesium concentrations.

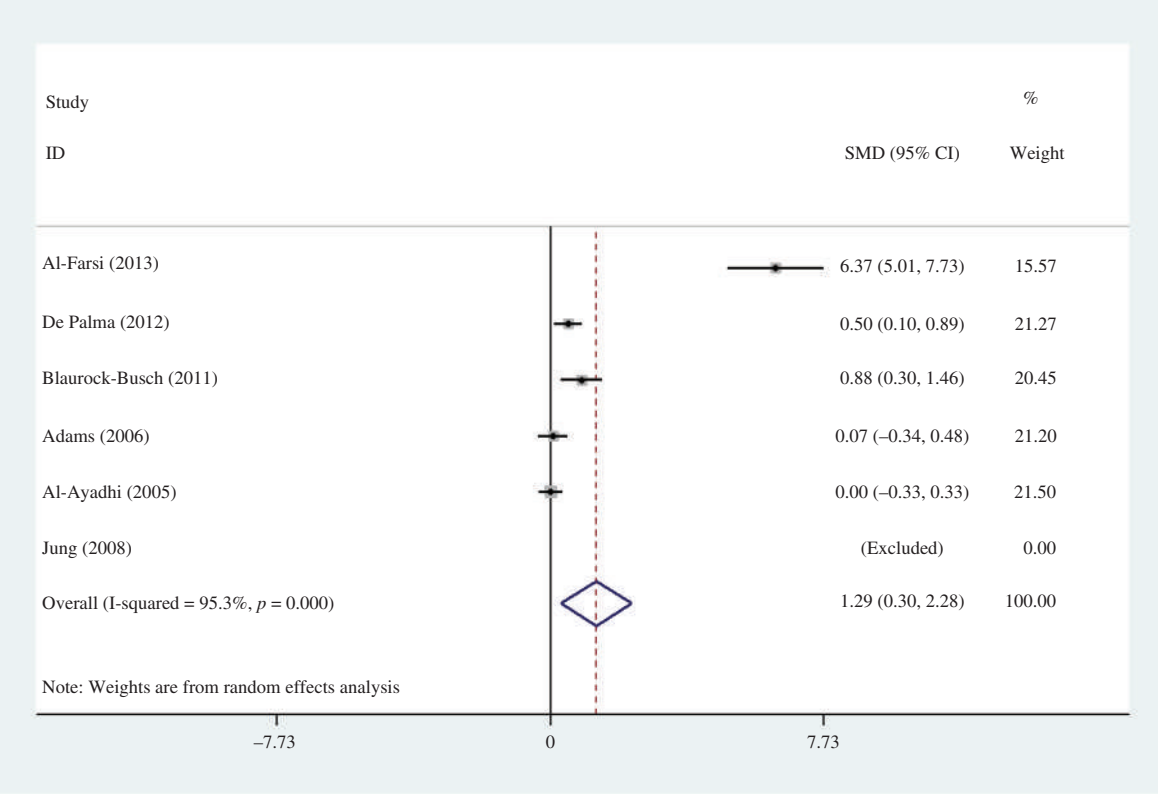


Figure 8: Meta-analysis of hair molybdenum concentrations.

Yorbik et al., 2004; Al-Ayadhi, 2005; Adams et al., 2006; 2007; 2011; Jory and Mcginnis, 2008; Jung et al., 2008; Blaurock-Busch et al., 2011; Elsheshtawy et al., 2011; Lakshmi Priya and Geetha, 2011; Russo, 2011; Russo and Devito, 2011; Vergani et al., 2011; De Palma et al., 2012; Semprún-Hernández et al., 2012; Al-Farsi et al., 2013; Li et al., 2014; Macedoni-Luksic et al., 2015; Craciun et al., 2016; Skalny et al., 2016a,b,c) assessed zinc status in the blood, erythrocyte, hair, nail, plasma, serum, teeth, and urine of patients with ASD and of control subjects. We could perform several meta-analyses to investigate whether zinc profile in patients with ASD is altered compared with controls. Overall, there were 13 studies that determined zinc levels in blood specimens (e.g. serum, plasma, and blood) for patients with ASD and for control individuals. The initial effect size was not significant ($p = 0.160$). However, when two outlier records (Yorbik et al., 2010; Vergani et al., 2011) were excluded from analysis, a significant ($Z = 2.31$, $p = 0.021$) effect size of −0.361 indicated that patients with ASD (n = 513) have lower blood zinc levels than controls (n = 333) (Figure 9). On the basis of blood specimen type (e.g. plasma, serum, and blood), several subgroup meta-analyses were performed (Table 2). There were no significant differences in serum ($p = 0.151$) and blood ($p = 0.444$) zinc levels between patients and controls. Also, an initial analysis of studies that measured zinc levels in plasma showed no significant ($p = 0.576$) difference between patients and controls. After two outlier records (Yorbik et al., 2010; Vergani et al., 2011) were excluded from analysis, the effect size of −0.266 ($Z = 2.01$, $p = 0.045$) indicated that the measurement of zinc in plasma is decreased in patients with ASD (n = 255) compared with control subjects (n = 98) (Figure 9). A meta-analysis of three studies revealed no difference ($p = 0.203$) in erythrocyte zinc levels between patients with ASD and control subjects. There were 16 comparisons concerning zinc concentrations in hair of patients with ASD (n = 520) and in that of controls (n = 591). The effect size estimated by analysis of these studies was not significant ($p = 0.216$). However, there was a trend toward conventional significance ($Z = 1.92$, $p = 0.055$) when an outlier record (Al-Farsi et al., 2013) was excluded from analysis (Figure 9). As noted in Table 2, sensitivity analyses indicated that Asian patients with ASD (n = 236) have lower hair levels of zinc (SMD = − 1.493, $p = 0.002$) compared with their Asian counterparts (n = 306) (Figure 9), whereas non-Asian patients with ASD (n = 257) have higher hair levels of zinc (WMD = 10.384, $p = 0.049$) compared with their non-Asian counterparts (n = 258) (Figure 9).

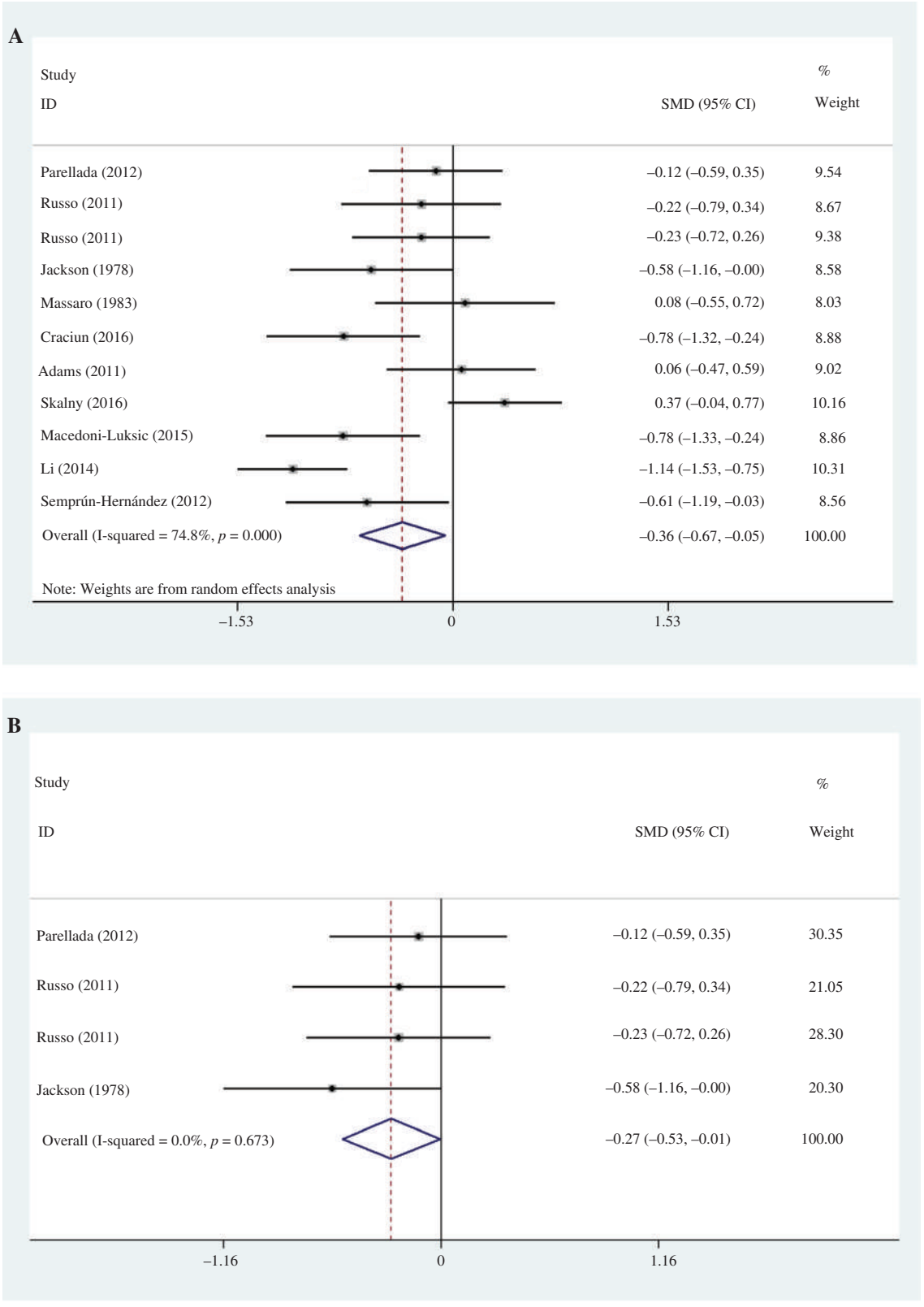
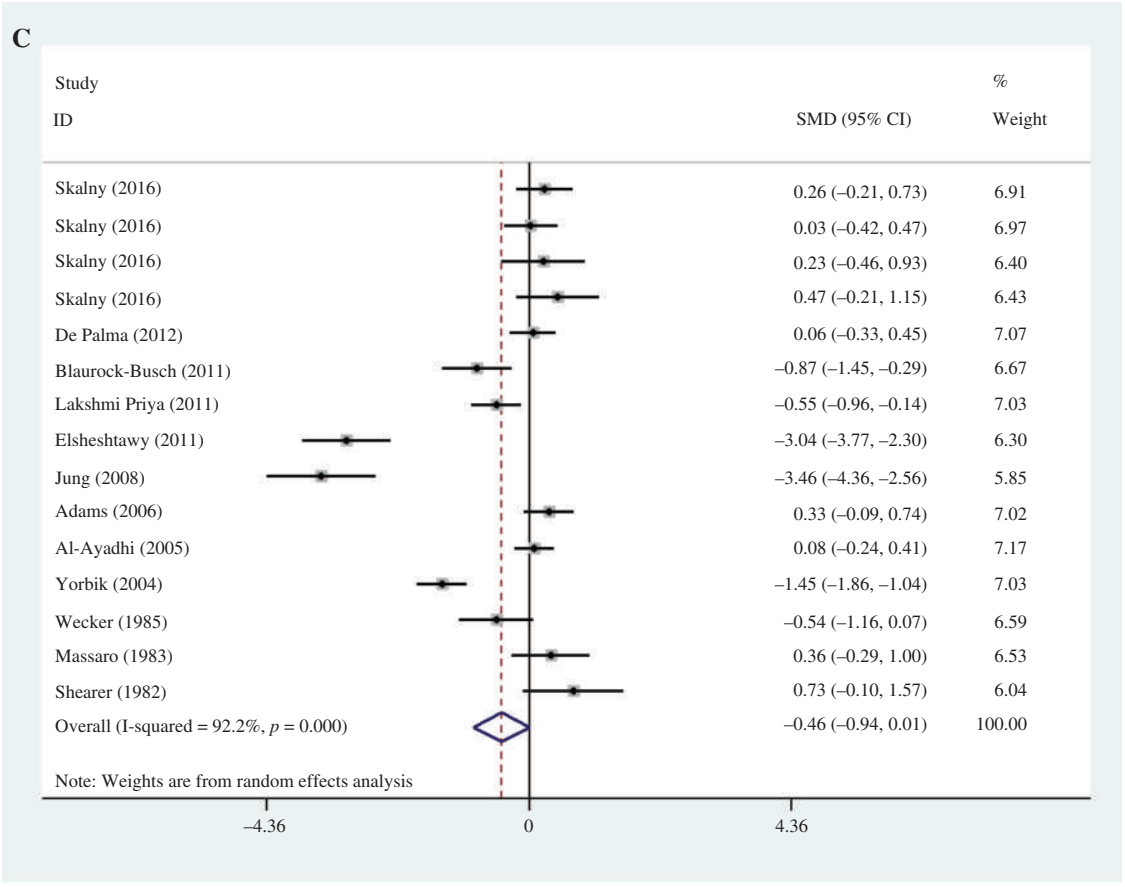
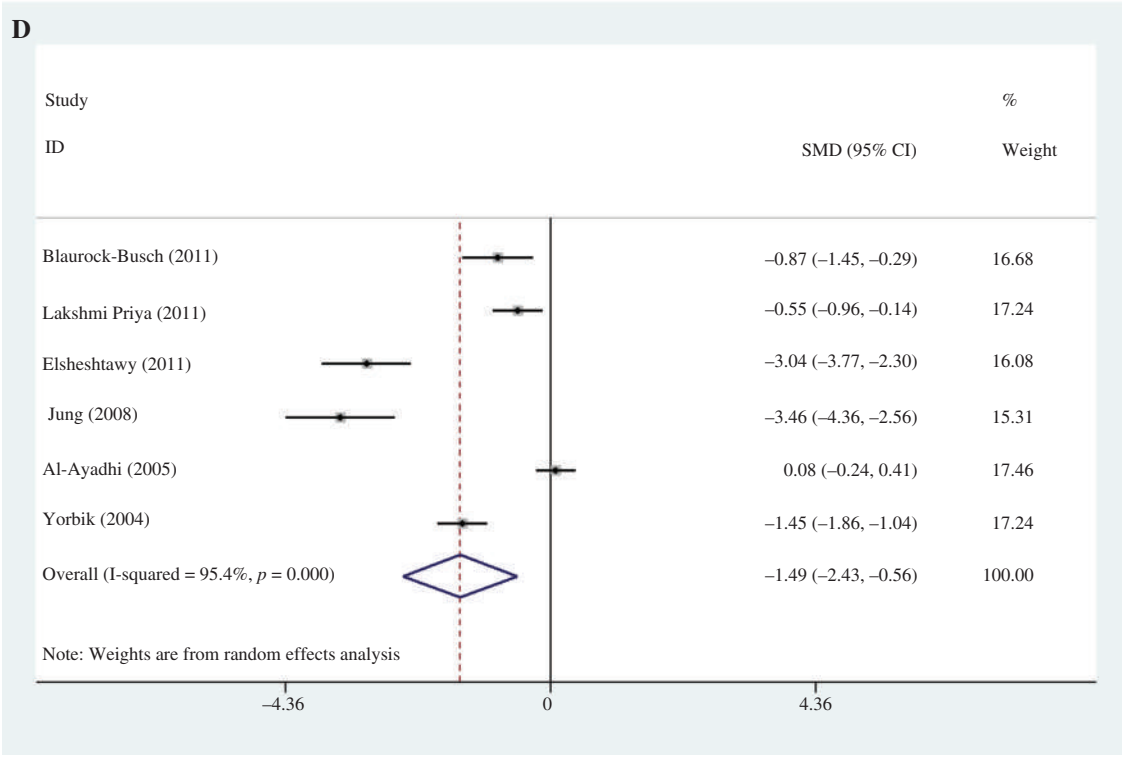


Figure 9: Meta-analysis of zinc concentrations.
(A) Meta-analysis of blood zinc concentrations. (B) Meta-analysis of plasma zinc concentrations. (C) Overall meta-analysis of hair zinc concentrations. (D) Subgroup meta-analysis of hair zinc concentrations: Asian countries. (E) Subgroup meta-analysis of hair zinc concentrations: non-Asian countries.





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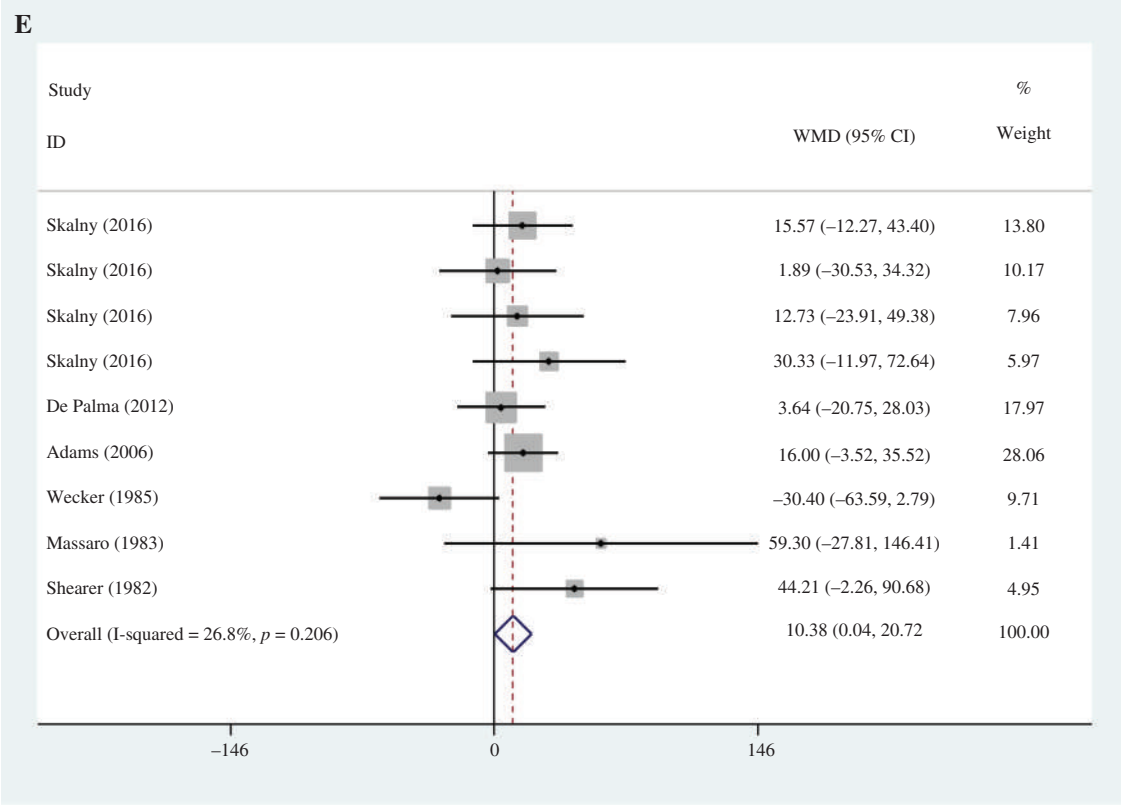

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Discussion

Chromium

It is an industrial byproduct that can cause different degrees of toxicity depending on its oxidation state (Barnhart, 1997). Generally, chromium toxicity is not a common event due to its low absorption and high excretion. However, it is an essential micronutrient needed by the human body for carbohydrate and lipid metabolism. Chromium deficiency, unlike chromium toxicity, seems to be a common condition because most popular diets contain insufficient amounts of this micronutrient (Calton, 2010). Chromium deficiency has been associated with obesity, diabetes, and cardiovascular events (Schroeder, 1966; Schroeder et al., 1970). Interestingly, chromium infusion has been shown to be an effective treatment for neuropathy and glucose intolerance in patients under long-term total parenteral nutrition (Freund et al., 1979).

This study showed lower chromium concentrations in the hair of patients with ASD as compared to that in healthy controls. Studies have revealed that chromium supplementation might result in weight loss in people who are obese or overweight (Pittler et al., 2003; Onakpoya et al., 2013). Interestingly, obesity in patients with ASD is of serious concern (Ho et al., 1997), because obesity and overweight are common among people with ASD and that there is a positive association between obesity and disease severity. Therefore, low chromium levels might explain obesity or overweight in people with ASD, and it is recommended to assess hair chromium levels in people with ASD who are overweight or obese, especially those who live in European or American countries.

Copper

It exists as integral part of vital enzymes, such as ferroxidase I (also known as ceruloplasmin), dopamine β-monooxygenase, and peptidylglycine α-hydroxylating monooxygenase, and therefore, its deficiency may cause serious health problems, such as immune abnormalities (neutropenia), anemia, (Dunlap et al., 1974; Lukasewycz, 1981), impaired glucose tolerance, and cardiac hypertrophy (Fields et al., 1983). Copper deficiency is commonly seen among neonates (Al-Rashid and Spangler, 1971), people under parenteral nutrition (Karpel and Peden, 1972), and patients who have Menkes disease (Williams,

1983). Careful attention must be paid toward copper toxicity as well as copper deficiency. Because of its role in the generation of hydroxyl radicals, it results in oxidative damage if the body is overloaded with copper or exposed to excessive copper (Gaetke and Chow, 2003). Oxidative stress affects structure and function of major cellular components, e.g. membranes, DNA, RNA, and enzymes (Cadenas and Davies, 2000). As a result, oxidative stress has long been known as a mechanism that underlies numerous diseases and related complications, notably neurodegenerative diseases (Coyle and Puttfarcken, 1993), diabetes (Baynes, 1991), and cancers (Valko et al., 2006).

Meta-analysis of 14 comparisons indicated significantly higher blood levels of copper in people with ASD compared to controls without ASD. Subgroup meta-analyses confirmed this result for serum, plasma, and whole blood copper concentrations. The findings have important implications. First, similarly to previous studies that have indicated an altered profile of oxidative stress-related biomarkers in people with ASD (James et al., 2004), the current study supports the hypothesis of oxidative damage in pathogenesis of ASD. Moreover, the fact that copper in people with ASD increases above control levels might point out that excitatory glutamatergic synapses do not function properly, because copper overload is believed to be the causative factor of zinc deficiency at synapses and thereby contribute to ASD progression (Baecker et al., 2014).

Iron

It is a trace metal required by the human body for the regulation of various metabolic processes, particularly for the electron transport chain (Lieu et al., 2001) and oxygen metabolism (Theil, 2003). It is of importance to control body iron hemostasis as there have been documented many health problems arising from the altered profile of iron (Andrews, 1999). Increased loss and inadequate absorption are two main causes of iron deficiency. The blood loss via any route (e.g. gastrointestinal, pulmonary, and genitourinary) results in iron loss. Inadequate iron absorption is linked with inflammatory bowel disease, celiac disease, and bowel resection. On the other side, iron overload and toxicity and consequent stimulated oxidative stress will predispose individuals to organ failure and cancer (Papanikolaou and Pantopoulos, 2005). It is of particular importance that neuronal iron accumulation in specific brain regions is implicated in common neurodegenerative conditions, e.g. Alzheimer's disease and Parkinson's disease (Moos and Morgan, 2004).

A meta-analysis of 11 comparisons showed significantly lower levels of iron in hair of ASD people compared to that of control subjects. Iron deficiency with or without anemia has been correlated with a spectrum of neuropsychiatric problems, favorably developmental delay, cognitive deficits, and behavioral impairment, in children (Deinard et al., 1986; Yager and Hartfield, 2002; Mccann and Ames, 2007). More interestingly, iron deficiency has been associated with emotional disturbances and behavioral abnormalities in a dose-dependent manner (Lozoff et al., 2008). Therefore, iron deficiency might explain behavioral problems common to people with ASD. Assessment of iron status is suggested for people with ASD, particularly for those who have emotional and behavioral problems.

Iodine

The thyroid gland requires this essential trace mineral to function properly. Adults should consume 150 μg of iodine per day, while it is estimated that the average consumption of iodine is about 140 μg per day (Haldimann et al., 2005). Some populations including non-Hispanic black individuals, pregnant women, and neonates are more likely to have iodine deficiency. Although it mainly affects the thyroid gland, iodine deficiency has been associated with a variety of disorders, e.g. goiter, stillbirths, abortions, cretinism, mental retardation, deaf-mutism, spasticity, rigidity, and bradykinesia (Hetzel, 1983; Delange, 2001). It is considered as the most prominent cause of mental retardation as congenital iodine deficiency has been correlated with 10–15-point decrease in intelligence quotient (Delange, 2001). Moreover, developmental iodine deficiency disrupts normal development of the brain (Gong et al., 2010), resulting in impaired cognitive and motor performance (Connolly et al., 1979).

A meta-analysis of four comparisons showed decreased urinary iodine excretion in ASD group compared to control group. As the measurement of iodine in urine can serve as a reliable indicator of iodine status (World Health, 2013), it can be hypothesized that patients with ASD have iodine deficiency in comparison with non-ASD control group. Further, there were lower iodine levels in the hair of ASD patients than in control subjects. Because of a two-fold reason that (i) iodine deficiency is correlated with cognitive and mental impairment, which are displayed by ASD people in different degrees, and (ii) meta-analysis results that reveal people with ASD are likely to have iodine deficiency, the assessment of iodine status is necessary for all patients with ASD.

Magnesium

Because of its light weight, magnesium has greater automobility than other metals used for making alloys (Mordike and Ebert, 2001). Magnesium, in general, appears to act as a cofactor assisting with the formation of protein (Terasaki and Rubin, 1985) and nucleic acid (Cowan, 1998) and also with regulation of energy metabolism (Boska et al., 2002). Therefore, magnesium deficiency by slowing down or stopping these vital chemical reactions may result in a variety of pathologies such as migraine (Mauskop et al., 1993), seizure (Bac et al., 1998), cardiovascular disorders (Turlapaty and Altura, 1980; Altura et al., 1984), parathyroid dysfunction (Anast et al., 1972), insulin resistance (Nadler et al., 1993), preeclampsia, and growth retardation (Altura et al., 1983). Particularly, in neuronal membranes, it is an essential cation responsible for regulating the opening and closing of glutamate-activated channels in a voltage-dependent way (Nowak et al., 1984). Thus, it is expected that magnesium deficiency has been shown to impair fear conditioning and emotional memory (Bardgett et al., 2005) as well as to cause depression and anxiety (Singewald et al., 2004) possibly via dysregulation of hypothalamic-pituitary-adrenal (HPA) axis (Sartori et al., 2012). Both depression (Ghaziuddin et al., 2002) and anxiety (White et al., 2009) are common among people with ASD. This would correspond to a higher level of morbidity in this special population.

This study indicated lower serum magnesium levels in patients with ASD than control subjects. There were lower levels of magnesium in the hair of patients with ASD compared with that of controls as well. Low magnesium levels can lead to impaired GABAergic signaling contributing to ASD pathogenesis. Therefore, estimation of magnesium status is recommended for patients with ASD, especially for who suffer from neurological (seizure) and psychiatric disorders (depression and anxiety).

Zinc

More than 300 enzymes require the presence of zinc for being activated. It is an essential micronutrient that contributes to vital cellular and subcellular processes particularly metabolism, gene expression, and transport processes (Vallee and Auld, 1990). Its role in the brain is probably of utmost importance because neural excitability over the entire brain is thought to be regulated by zinc (Frederickson et al., 2005). However, because of its unique properties (e.g. its presence at both presynaptic and postsynaptic clefts and the existence of various zinc-secreting cells in the human body), zinc signaling is not restricted to specific part of the human body but extends to the main systems of the human body, importantly the CNS, immune system, and gastrointestinal system (Frederickson et al., 2005). As reviewed in detail elsewhere (Adamo and Oteiza, 2010), zinc is involved in different dimensions of neural development, e.g. neurogenesis, neuronal migration, differentiation, and apoptosis. Therefore, zinc deficiency can affect both prenatal and postnatal life, thereby causing serious neurological complications mainly neurodevelopmental problems (Hambidge, 2000; Uriu-Adams and Keen, 2010). It has also shown anti-inflammatory and anti-oxidative effects (Prasad, 2009) to the extent that zinc deficiency is considered a common type of nutritional immunodeficiency (Raker, 1984). Accordingly, its deficiency may make individuals vulnerable to inflammatory and other immune abnormalities, importantly infections (pneumonia) and autoimmune disorders (inflammatory bowel disease) (Hendricks and Walker, 1988; Shankar and Prasad, 1998; Black, 2003). Zinc deficiency is predominantly seen among people who live in developing countries, elderly people, and patients with nutritional Dwarfism (Prasad et al., 1963; Halsted et al., 1972; Prasad et al., 1992).

Meta-analysis of 11 comparisons revealed that patients with ASD have lower blood zinc levels than controls. As well, subgroup analyses showed that Asian patients with ASD have lower hair levels of zinc compared with their Asian counterparts, whereas non-Asian patients with ASD have higher hair levels of zinc compared with their non-Asian counterparts. Recent *in vitro* and *in vivo* studies have shown that zinc deficiency interferes with excitatory synaptic activities whereby behavioral abnormalities common to ASD occur (Grabrucker et al., 2014). Therefore, it is suggested to evaluate zinc status in patients with ASD especially for those who (a) suffer from autoimmune disorders and other immune disorders, (b) have resistant and recurrent infection, (c) have serious neuropsychiatric problems such as growth and mental retardation and hyperactivity and for women of childbearing age who have (a) malnutrition or (b) a child with developmental disorders.

Conclusion

The present study found significant differences in the content of trace elements in patients with ASD compared to controls. More interestingly, sensitivity analyses showed the difference in trace element status between Asian and

non-Asian countries. The findings help in highlighting the role of trace elements as environmental factors in the etiology of ASD. The strengths of our study are as follows: (i) it is a comprehensive meta-analysis study without any restrictions (language and timing of publications) about including reports, (ii) it considered all of the most convenient specimen types (whole blood, serum, plasma, erythrocyte, hair, urine, and nail), and (iii) it estimated the influence of study location (Asia versus Europe/America) on the effect size. Low chromium levels can cause obesity or overweight, common problems that people with ASD face. High copper values may aggravate oxidative damage and synaptic dysfunction, which are the well-known hypotheses on the pathogenesis of ASD. Iron and iodine deficiency can explain emotional and behavioral abnormities associated with ASD. Also, low magnesium and zinc values may contribute to ASD by influencing synaptic function. More *in vivo* and *in vitro* research is needed to unravel the exact molecular mechanism of action of trace elements in the pathogenesis of ASD. Additionally, clinical studies must monitor the influence of diet and trace element supplementation on disease severity and patient performance.

Acknowledgments: This study was supported by a grant from Tehran University of Medical Sciences and Health Services (grant no. 96-01-30-34676).

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