Cellular Phone User's Age or the Duration of Calls Moderate Autonomic Nervous System? A Meta-Analysis

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1 Introduction

In many countries, more than half of the population uses cellular phones, and the use is rapidly expanding. In some parts of the world, cellular phones are the most reliable or the only phones available. The World Health Organization has recognized that "given the large number of mobile phone users (6.9 billions in 2014), it is important to investigate, understand and monitor any potential public health impact." Scientific research has held a skeptic approach over the possible short- and long-term health effects of the cellular phone use (radiofrequency fields). Yet, WHO identifies and promotes research priorities for radiofrequency fields in relation to health in order to fill gaps in current knowledge.

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The Pew Research Center and the Berkman Center for Internet and Society at Harvard University conducted a survey based on 802 teenagers and their parents. They concluded that 78% of adolescents possess cellular phones versus 90% of adult owners. More importantly, in 2015, 23% of US teenagers are reported to be online constantly (Lenhart 2015), while a considerable prevalence of addiction is reported in the UK (Lopez-Fernandez et al. 2014).

The enormous penetration of the body tissues from antenna radiowaves reveals the need for profound research in this scientific field. Thermal and nonthermal effects have been detected and published in the environmental health literature during the last decade. At the same time, there is little knowledge on the impact of the electromagnetic fields exposure on the cardiac autonomic system. The autonomic nervous system (ANS) and the parasympathetic tone in particular is proposed to play a role in the regulation of allostatic systems associated with inflammation, hypothalamus-pituitary-adrenal axis (HPA) function, glucose regulation (Thayer & Sternberg 2006), and even autoimmune disorders, such as systemic lupus erythematodes (SLE) disease activity (Aydemir et al. 2010; Thanou 2014). On the other hand, it is well established that the vagus nerve plays a significant role in health and disease (Darwin 1872/1999; Porges 2007).

Heart rate variability (HRV) has been a useful tool for evaluating vagus nerve outflow. It represents the beat-to-beat variation in the duration of the R-R interval (heart period), reflecting complex interactions between parasympathetic, sympathetic, mechanical, and other factors on the pacemaker located at the sinoatrial node of the heart (Billman 2011; Task Force of the European Society of Cardiology and the North American Society of Electrophysiology 1996). When individuals are in a relaxed or resting state (supine position), the heart rate is expected to be less frequent and less consistent (more variability between beats), reflecting greater parasympathetic activity that facilitates social interaction (Porges 2007). In contrast, when individuals are faced with challenging or stressful events, a reduction in vagal influence on the sinoatrial node inhibits parasympathetic nervous system activity (in other words, sympathetic predominance and more beats with less variability), preparing the person for a "flight or fight" response (Porges 2007; Montano et al. 1994; Mourot et al. 2004). The components of heart rate variability (either parametric (time or frequency domain) or nonparametric) are illustrated in Table 1.

The ratio of low frequencies to high frequencies (LF/HF) is proposed as a measure of sympathovagal balance in health and disease (Pagani et al. 1986, 1984). Albeit criticisms voiced (Billman 2013), it is widely accepted that an increase of the ratio reflects "sympathetic dominance," whereas a decrease of the ratio reflects "parasympathetic dominance." The ANS can be affected by 900 MHz exposure, as demonstrated by Braune et al. (Braune et al. 2002) in a cross-sectional study of the hypothalamic-pituitary-adrenal axis. A meta-analysis on an attempt to evaluate well-being concluded to a Hedges' g effect size of CPC exposure on HRV of 0.03 (Augner et al. 2012).

The purpose of the present study is (i) to detect and quantify the radiofrequency radiation acute effect on heart rate variability as a measure of the autonomic nervous system response to cellular mobile phone use and (ii) to identify possible predictors/confounders that may modify this effect.

Table 1 Frequency and time domain parameters measured in the included studies

	Parameter	Unit	Description	ANS branch
Time domain	SDNN	ms	Standard deviation of all NN intervals	Mixed
	SDANN	ms	Standard deviation of the average of NN intervals for each 5 min	Mixed
	NN50	ms	Adjacent cycles greater than 50 ms apart	Vagally mediated
	pNN50	%	Percentage of the adjacent cycles that are greater than 50 ms apart	Vagally mediated
	RMSSD	ms	Root mean sum of the squares of successive differences in milliseconds that reflect the vagal influences	Vagally mediated
Frequency domain	HF	ms^2	High frequency (HF) (0.15–0.4 Hz)	Vagally mediated, related to ventilation
	LF	ms^2	Low frequencies (0.04–0.15 Hz)	Mixed, related to baroreflexes
	LF/HF	–	Balance	Mixed
	VLF	ms^2	Very low frequencies (VLF) (0.003–0.04 Hz)	Mixed
	ULF	ms^2	Ultralow frequencies (ULF) (<0.003 Hz)	Mixed related to renin-angiotensin system
Non Linear	NRAD		The absolute distance between two ranks	Complexity of HRV
	DFa-1		Detrended fluctuation analysis 1	Complexity of HRV
	DFa-2		Detrended fluctuation analysis	Scaling properties of HRV
	R		Regularity	Regularity
	ApEn		Natural logarithm of the relative prevalence of repetitive patterns	Predictability of time series
	SD1		Poincaré plots the ith RR interval plotted against the RR_{i-1}	Dynamics
	SD2		Poincaré plots	Dynamics

2 Methods

2.1 Literature Search (Data Collection)

Databases as Scopus and PubMed (The National Library of Medicine) were searched using the keywords “mobile/cellular phones and heart rate variability” or “radiofrequency fields and heart rate variability” or “GSM and heart rate variability.” The literature search was conducted in February 2015, and the articles meeting the eligibility criteria were evaluated in the aid of the PRISMA approach (Whitlock et al. 2010; Moher et al. 2009; Cohen 1960).

2.2 Data Selection

The inclusion criteria were (a) English language, (b) mobile/cellular phone emitting at 900 MHz, (c) healthy subjects, (d) recording before and/or sham exposure and during exposure, and (e) HRV recording in supine position,

Further exclusion criteria are as follows: (a) reviews and meta-analyses are excluded from this study, and (b) investigations examining HRV during or after sleep are also excluded. From the 29 investigations retrieved, we excluded 2 as non-English publications, 6 conference papers, 2 systematic reviews and meta-analyses, 2 referring to exposure during sleep, 1 referring to patients, 10 referring to recordings in sitting position, and 2 referring to recordings in supine position, but during and after exposure, only 4 met the inclusion criteria.

Two investigators (SG and DC) blindly searched and screened the articles and consented to articles' quality: Cohen's kappa for inter-rater agreement was 90% agreement (0.676) for the abstract selection, but 100% (1.0) for the full study inclusion (Cohen 1960). Non-exposure is identified as baseline relax recording (stress recordings are excluded) or sham exposure recordings. As exposure, the recordings of the first exposure (in case of repeating measurements) that should be in relax (and not stressed state) with the phone placed to the ear (not the hip or the chest) were included, herein. Two exposure markers have been proposed in literature: time and/or specific absorption rate (SAR). The latter has been widely discussed in terms of accuracy and credibility Geronikolou et al. 2014), and the recently published guidelines proposed time or total power of the antenna, as preferable markers for EMF exposure measurements (Belyaev et al. 2016). No information regarding the total power was available in the detected for this work literature, so time was chosen as validated marker for exposure.

2.3 Statistical Analysis

The Comprehensive Meta-Analysis (CMA; Borenstein, Hedges, Higgins, & Rothstein 2009) software was used to transform results of individual studies into the common effect sizes of Hedges' g. Hedges' g is a measure of the standardized difference between intervention and control condition that corrects for biases associated with small sample sizes and can be interpreted in the same way as Cohen's d, whereby 0.2 represents a small effect, 0.5 a medium effect, and 0.8 a large effect. A single overall meta-analysis was conducted to acquire an effect size for HRV during CPC exposure relative to baseline. For studies that reported more than one outcome, the effects were combined with formulas provided by Gleser and Olkin (Geronikolou et al. 2014). Positive effect sizes point to higher means during exposure. The sample sizes are small; the number of the studies is also small. Thus, the assumption of a common population effect was tested with the I^2 – the percentage of total variation across studies that is due to heterogeneity – rather than chance

$I^2 = 100\% \times (Q - \mathrm{df})/Q$, *where* χ^2 is distributed homogeneity test Q and df is degrees of freedom. An estimate of the between-study variance in a random effects meta-analysis is known as tau squared (τ^2): if $\tau^2 > 1$ a substantial statistical between study heterogeneity is present (Belyaev et al. 2016). Furthermore, moderator analyses were conducted (with CMA software) to isolate the possible role of age and/or duration (minutes) of CPC exposure in heart rate variability.

3 Results

From the 29 research articles retrieved in the search machines, 5 studies from 3 countries met the inclusion criteria (Cohen 1988; Gleser & Olkin 2009; Borenstein et al. 2009; Barutcu et al. 2011), published between 2007 and 2014.

The (Barutcu et al. 2011) study contains data on separate age groups which are assumed as two separate studies in this meta-analysis. These studies comprised a total of 124 subjects. Sample sizes ranged from 20 to 26 (24.8 ± 2.6) with a median of 26.

The mean age ranges from 15.3 to 28,4 years (24.3 ± 5.2) with a median of 25.54. Two studies targeted to male subjects (Barutcu et al. 2011), whereas the rest of the studies included both sexes (Cohen 1988; Borenstein et al. 2009; Parazzini et al. 2013a). The recordings took place in the supine position in all selected studies. The phone in all included experiments was placed on the ear. All included measurements refer to not exposure (baseline and/or sham exposure) versus exposure to CPC strictly: Choi 2014 (relevant Table 3 teenagers), (Barutcu et al. 2011) (relevant Table 3 adults), Barutcu 2011 (Table I, II baseline versus calling mode), Parazzini 07 (relevant Tables 1, 2 rest), and Parazzini 13 (relevant Table 1 rest). The exposure (calls) duration ranged from 5 minutes to 32 minutes with a median 26 and mean 21.6 ± 12.09 min. One study was double blind (Parazzini et al. 2007a). The participants of the included studies have been either sham and really exposed (Gleser & Olkin 2009; Borenstein et al. 2009; Barutcu et al. 2011) or only really exposed (Cohen 1988). Four publications reported both frequency and time domain parameters (Cohen 1988; Barutcu et al. 2011; Parazzini et al. 2007a), while one attempted the nonlinear approach of measuring dynamics of the cardiac function with NRAD, DFA-a1, DFA-a2, approximate entropy (Apen), SD1, and SD2 (Gleser & Olkin 2009).

The results of the meta-analyses for all measurements (combined) as well as the results for the LF/HF ratio effect are summarized in Table 2.

The effect size 33 for all measures is not quite homogeneous, nor is it for the LF/HF ratio ($p < 0.05$ for Q statistics for both meta-analyses; see Table 2). Furthermore, the I^2 values verified that most of the variability across studies (63.2% for all outcomes and 65.2% for LF/HF) is due to a moderate heterogeneity rather than chance. Indeed, there is variation in age groups and/or duration of exposure. Thus, we conducted four meta-regressions for identifying possible confounders: two separate meta-regressions with moderator age or minutes of exposure for all outcomes and

Table 2 Meta-analytical results for recordings in supine position before/sham and during exposure (mobile phone call)

	Study	*N*	Mean age	min call	Hedges *g*	P_g	Variance	S.E.	CI 95%	*Q*	P_Q	I^2	τ^2
Overall outcomes	Choi 10a	26	15.3	32	0.235	0.22	0.037	0.193	[−0.143,0.613]				
	Choi 10b	26	28.4	32	−0.552	0.007	0.042	0.205	[−0.954, −0.151]				
	Barutcu	20	27	5	0.294	0.89	0.051	0.215	[−0.148, 0.737]				
	Parazzini 07	26	25.54	26	−0.164	0.78	0.195	0.190	[−0.545, 0.218]				
	Parazzini 13	26	25.5	13	−0.038	0.847	0.039	0.198	[−0.426, 0.350]				
	Fixed effect	124	24.3	21.6	−0.52	0.566	0.008	0.09	[−0.23,0.125]	10.86	0.028	63.2	0.07
	Random effect				−0.048	0.747	0.022	0.149	[0.528, 1.582]				
LF/HF	Choi 10a	26	15.3	32	0.235	0.22	0.037	0.193	[−0.143, 0.613]				
	Choi 10b	26	28.4	32	−0.552	0.007	0.042	0.205	[−0.954, −0.151]				
	Barutcu	20	27	5	0.029	0.89	0.046	0.215					
	Parazzini 07	26	25,54	26	0.053	0.78	0.036	0.190					
	Fixed effect	98			−0.048	0.635	0.010	0.10	[−0.244, 0.149]	8.614	0.035	65.2	0.26
	Random effect				−0.55	0.745	0.029	0.170	[−0.388, 0.278]				

p_g significance level of Hedges' g, *S.E.* standard error, p_Q significance level of Cochrane Q, I^2 I squared; τ^2 tau squared
The summary HRV effect as indexed by the combination of all measurements random effect does not change $g = -0.048$ with precision 95% [−0.341, 0.245], nor does the LF/HF ratio random effect $g = -0.055$ with 95% precision [−0.388, 0.278] (Fig. 1)

Table 3 Meta-regression results with moderators *minutes of call* and/or *age* for all (combined) outcomes and LF/HF ratio separately

Outcome	Predictor		Estimate	S.E.	*Z*	CI (95%)	*p*-value	τ^2
Overall	Call (min)	Slope	−0.01305	0.0087	−1.5	[−0.03011, 0.004]	0.1336	0.07519
		Intercept	0.2405	0.21487	1.1191	[−0.18067,0.662]	0.26208	
		Model		$Q_M = 2.249$			0.1336	
		Residual		$Q_R = 8.6107$			0.0349	
	Age	Slope	−0.0369	0.01892	−1.9512	[−0.074, 0.0002]	0.05103	0.05663
		Intercept	0.8372	0.46457	1.8022	[−0.0733, 1.7478]	0.07151	
		Model		$Q_M = 3.807$			0.051	
		Residual		$Q_R = 7.5352$			0.0702	
LF/HF	Call (min)	Slope	−0.00548	0.00947	−0.5789	[−0.0240,0.01307]	0.523	0.12065
		Intercept	0.0866	0.25243	0.3433	[−0.40810,0.5814]	0.7314	
		Model		$Q_M = 0.3351$			0.5626	
		Residual		$Q_R = 8.2805$			0.0159	
	Age	Slope	−0.0399	0.01907	−2.0924	[−0.07728,−0.0025]	0.0364	0.04581
		Intercept	0.9016	0.46450	1.9410	[−0.00882,1.8120]	0.0523	
		Model		$Q_M = 4.378$			0.0364	
		Residual		$Q_R = 4.23759$			0.1202	

S.E. standard error, *CI 95%* confidence intervals 95%, Q_M model of sum of squares compare to chi-distribution with p-1 degrees of freedom, Q_R model of residual sum of squares compare to chi-square distribution with k-p-1 degrees of freedom (k is the number of studies; p is the number of predictors in the model), τ^2 tau squared

two meta-regressions with moderator age or minutes of exposure for the LF/HF ratio effect. The results of the meta-regression with moderator age are illustrated in Figs. 2a and 3a while presented in Table 3.

The results of the meta-regression with moderator min of exposure are illustrated in Figs. 2b and 3b while presented in Table 3. In meta-regression plots, each study is represented by a circle, proportional to its weight to the analysis, pointing out which studies have the greatest impact on the slope of the regression line. The CMA software provides *z*-values for regression coefficients. In this case, age rather than minutes of exposure significantly influences the Hedges' g mixed effect of HRV variation. Q_M model of fit in meta-regression models sum of squares compare to chi-distribution with *p*-1 degrees of freedom (*p* is the number of predictors in the model) and reflects the dispersion explained by the moderators. In our study, Q_M for all outcomes is calculated to 3.81 with 1 degree of freedom and $p = 0.05$ which means that the relationship between HRV and age is limited and the variance may be attributed to contributing factors that have not been considered in this study and/or the analysis should be repeated as soon as more publications allow the effort. Factors that have not been considered in this study (because there has been no available data) are (a) the exposome of each subject for each study and (b) detail exposure doses and absorption rates (SAR). For the LF/HF ratio, Q_M is calculated to 4.378 with 1 degree of freedom and significance 0.03. This means that the quoted age effect on sympathovagal balance is stronger than we could expect by chance.

Accordingly, the results of z-value and Q_M modeling show that the predictor "minutes of exposure" has no effect on heart rate variability. Q_R is the residual sum of squares compared to chi-square distribution with *k-p*-1 degrees of freedom (*k* is the number of studies; *p* is the number of predictors in the model) and reflects the distance of studies from the regression line. Thus, for all outcomes $Q_R = 7.04$ for age effect with 3 degrees of freedom and significance 0.07, which means that some of the between-study variance might not be explained by age alone. For LF/HF ratio, the $Q_R = 4.24$ for age effect with 2 degrees of freedom and significance 0.12, which implies that some of the between-study variance was strongly predicted by age.

4 Discussion

This work investigates the autonomic nervous system response to a single CPC exposure/stimulation. Health is associated to ANS balance, whereas ANS disturbance has been linked to discomfort and/or even disease (Task Force of the European Society of Cardiology and the North American Society of Electrophysiology 1996). The autonomic nervous system, herein, is indexed by heart rate variability measurements, expressed by (a) combined parameters effect sizes and (b) the sympathovagal balance (LF/HF ratio). We estimated a lower Hedges' g effect size for combined outcomes than the one that Augner et al. (Augner et al. 2012) had estimated. This might be attributed to their protocol which had not considered posture. Regardless of the effect size, our results confirm the finding of a previous meta-analysis: CPC

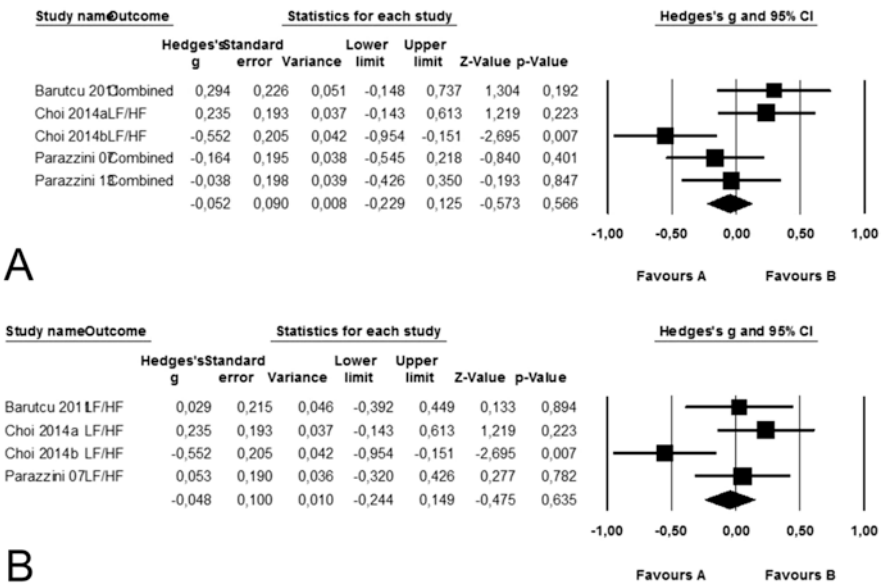

Fig. 1 (**a**) Forest plot for overall outcomes. (**b**) Forest plot of sympathovagal balance outcomes

has minimal effects on the ANS. This might be interpreted as health thrift (Fig. 1), if a variance $I^2 = 63.2\%$ for all outcomes and $I^2 = 65.2\%$ for sympathovagal balance had not been identified. Consequently, further research is warranted for ANS physiology and/or electromagnetic impact elucidation.

Furthermore, although the LF/HF ratio has been criticized (Billman 2011) for its accuracy on quantifying sympathovagal balance, being considered to be influenced by stress (Choi et al. 2014; Parazzini et al. 2013b; Parazzini et al. 2007b) and posture (Porges 2007; Parazzini et al. 2007b), it is the only proposed measure available. Posture could not have an impact on our results since all investigations included had been conducted in the same (supine) position. For recordings in supine position, stress could be considered as a confounder in longer than 15 minutes recordings, when drowsiness and discomfort from the required immobility may appear (Parazzini et al. 2007b).

The *minutes of exposure* effect on Hedges' g effect is null in all meta-regressions for mobile phone calls lasting up to 32 minutes. Thus, the duration of recording identical to the duration of experimental exposure to a CPC is not responsible for the heterogeneity identified $I^2 = 63.2\%$ and $I^2 = 65.2\%$ accordingly (Figs. 2b and 3b).

On the contrary, the effect of *age* is higher in younger ages (adolescence) and decreases as age increases (Figs. 2a and 3a). This result supports the caution already expressed that the risk for children and adolescents from radiofrequencies exposure needs to be extensively investigated (Akselrod et al. 1981; Hjortskov et al. 2004). Our finding may be either explained in terms of development or in terms of accumulated exposure of each individual to electromagnetic fields even since conception (exposome). Indeed, potential health effects from this technology expansion in

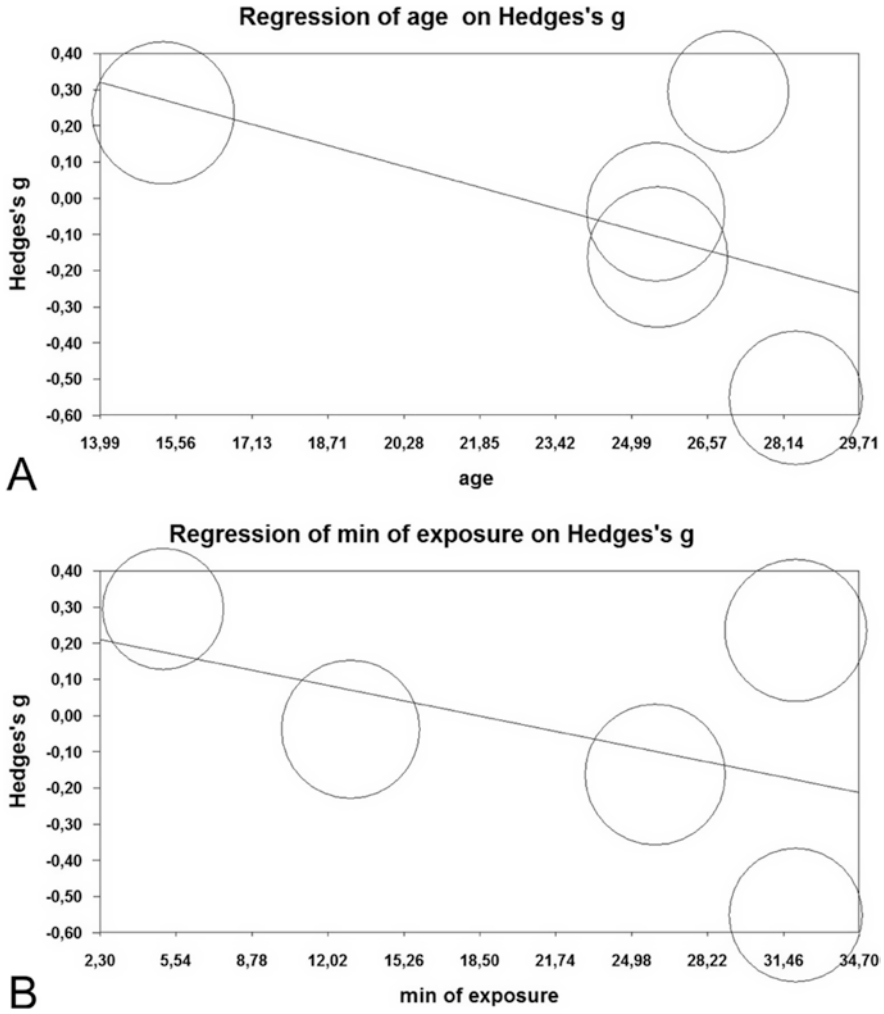

Fig. 2 (**a**) Meta-regression of age on Hedge's g (overall outcomes). (**b**) Meta-regression of min of exposure on Hedge's g (overall outcomes)

pediatric and adolescent populations are suggested by the European Health Risk Assessment Network on Electromagnetic Fields Exposure in its 2012 report (Nam et al. 2011; Morgan et al. 2014a): "These groups represent the first generation of Europeans to be exposed to diffuse EMF fields since conception and birth, thus, are expected to be more sensitive to these fields."

Additionally, it has, already, been reported that the child's brain is deeper penetrated from electromagnetic field radiation than the adult's brain (Sienkiewicz et al. 2010; EHFRAN 2012; Sadetzki et al. 2014; Peyman et al. 2009). The absorption includes areas responsible for intellectual development – a function still in the

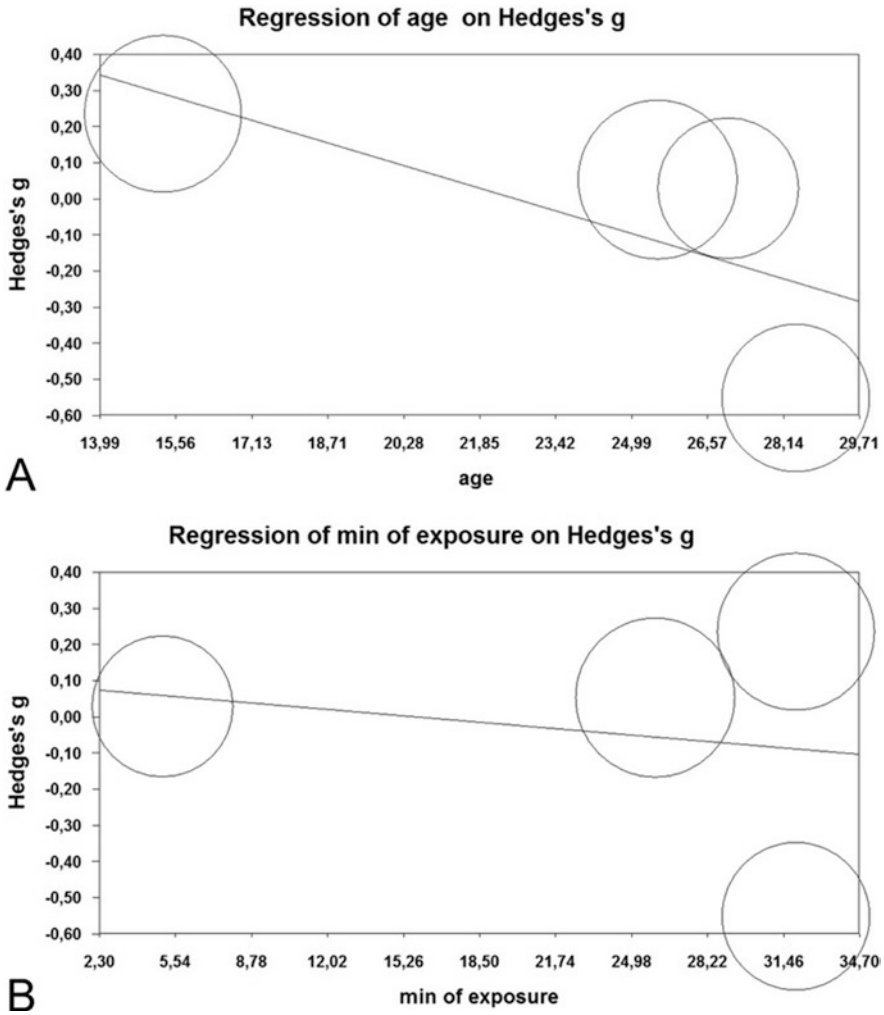

Fig. 3 (**a**) Meta-regression of age on Hedge's g (sympathovagal balance). (**b**) Meta-regression of min of exposure on Hedge's g (sympathovagal balance)

maturation process in children and teenagers and thus more vulnerable to environmental insults and hazards (Peyman 2011; Morgan et al. 2014b; Redmayne & Johansson 2015). Yet, no effects of GSM exposure have been reported on cognitive function by several investigators (Grigoriev et al. 2011; Kramarenko & Tan 2003; Kheifets et al. 2005). In this meta-analysis, we estimated both random and fixed effect sizes as we anticipated systematic differences between the results of different studies (heterogeneity). Standard random effects methods for meta-analysis may provide unduly precise estimates of effect, as they assume that the observed

distribution of effects is the true distribution – an assumption that may not be valid in sparse data (Belyaev et al. 2016; Kwon et al. 2010; Haarala et al. 2005).

Conclusively, our analysis suggests that (a) *minutes of exposure* (minutes of speaking on the mobile phone) do not affect the autonomic nervous system of the heart or its sympathovagal balance, (b) *age* synergizes with other variables (exposome, SAR) so as to explain combined HRV parameter effects, and (c) the sympathovagal balance is strongly predicted by *age*, implying that adolescents' autonomic nervous system seems to be more vulnerable to the CPC exposure. Additionally, risk assessment analysis of the additive effects of continuous exposure (exposome) is strongly suggested.

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