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Frequent cellular phone use modifies hypothalamic–pituitary–adrenal axis response to a cellular phone call after mental stress in healthy children and adolescents: A pilot study

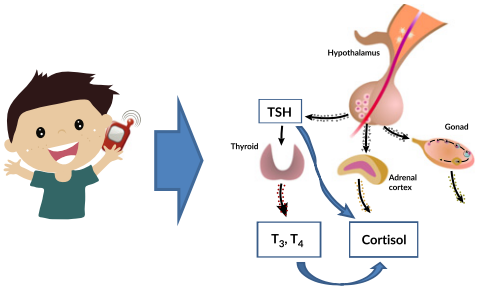
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HIGHLIGHTS

- We model two environmental stressors: a cellular phone call and a mental stress.
- Frequent versus sporadic cellular phone users demonstrate different HPA-axis response.
- Baseline thyroid hormones predict HPA response to environmental stressors in children.

GRAPHICAL ABSTRACT



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ABSTRACT

Objective: The hypothalamic–pituitary–adrenal (HPA) axis is the main "gate-keeper" of the organism's response to every somatic or mental stress. This prospective study aims to investigate the HPA-axis response to a cellular phone call exposure after mental stress in healthy children and adolescents and to assess the possible predictive role of baseline endocrine markers to this response.
Subjects and methods: Two groups of healthy school-age children aged 11–14 (12.5 ± 1.5) years were included in the study, the one comprising those who are occasional users of a cellular phone (Group A) while the second those who do regularly use one (Group B). Blood samples were obtained from all participants at 8.00 am after a 12-hour overnight fasting for thyroid hormone, glucose, insulin, and cortisol levels determination. The participants performed the Trier Social Stress Test for Children (TSST-C) (5 min oral task followed by 5 min arithmetic task). Salivary cortisol samples were obtained at baseline, 10′ and 20′ min after the TSST-C and 10′ and 20′ after a 5 minute cellular phone call.
Results: Significant changes in the salivary cortisol levels were noted between 10′ and 20′mins after the cellular phone call with different responses between the two groups. Baseline thyroid hormone levels seem to predict the cortisol response to mental stress mainly in group A, while HOMA had no impact on salivary cortisol response at any phase of the test, in either group.

Abbreviations: HPA, hypothalamic–pituitary–adrenal axis; TSST-C, Trier Social Stress Test for Children; T4, thyroxine; T3, tri-iodothyronine; TSH, thyroid stimulating hormone; BMI, body mass index; Hs-CRP, high sensitivity C-reactive protein; ACTH, adrenocorticotropic hormone; HOMA, homeostasis model assessment.

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Conclusions: HPA axis response to cellular phone after mental stress in children and adolescents follow a different pattern in frequent users than in occasional users that seems to be influenced by the baseline thyroid hormone levels.

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1. Introduction

Environmental insults have repeatedly been reported to have a negative impact on public health. Children and adolescents grow nowadays in a different environment in comparison to their parents since electromagnetic field exposure is, nowadays, diffuse and inevitable. The electromagnetic exposure to "close to the body" sources include cellular phone usage which is a common part of daily life even among children.

Potential endocrine effects from this technology expansion in pediatric and adolescent populations is suggested by the European Health Risk Assessment Network on Electromagnetic Fields Exposure in its 2012 report (EHFRAN, 2012; Sadetzki et al., 2014): "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".

Certain research groups sought to find possible endocrine effects of 900 MHz radiation exposure (Bortkiewicz, 2001; Djeridane et al., 2008; Eskander et al., 2012; Koyu et al., 2005; Mortavazi et al., 2009). In fact, there is growing evidence that people and animals exposed to 900 MHz radiation present thyroid function and morphology alterations (Djeridane et al., 2008; Riccioni et al., 2004; Bergamaschi et al., 2004; Eskander et al., 2012; Hackney and Gulledge, 1994; Koyu et al., 2005; Shahryar et al., 2009). The effect of cellular phone use on fasting glucose is established in the literature (Meo and Al Rubeaan, 2013), while scarce data exist on insulin secretion or insulin resistance to cellular phone call response.

On the other hand, the hypothalamic–pituitary–adrenal (HPA) axis, which constitutes the stress response system of the organism, when activated, as documented by altered salivary or plasma cortisol levels, may affect several systemic responses including those of the nervous, cardiac and immune systems (Chrousos and Gold, 1992; Heim et al., 2000; Hench et al., 1950; Holsboer, 1989; Raison and Miller, 2003; Sternberg et al., 1992; Stratakis and Chrousos, 1995; Tsigos and Chrousos, 1994). Salivary cortisol constitutes a non invasive and sensitive stress biomarker (Aardal-Eriksson et al., 1998; Laudat et al., 1988; Safar Zadeh et al., 2005; Schmidt, 1998; Vining et al., 1983a, 1983b) that is repeatedly used in Trier Social Stress Test for Children (TSST-C) (Beetz et al., 2012; Pesonen et al., 2012; Sherzai et al., 2012). Trier Social Stress Test is a standardized experimental tool for stress assessment independent of individual's confounding factors such as personality, education, income, etc. (Buske-Kirschbaum et al., 1997; Gunnar et al., 2009; Kudielka and Wust, 2010). It consists of a brief preparation period (3 min) followed by a test period in which the subject has to deliver a free speech (5 min) and perform a mathematical task (5 min) in front of an audience. With this, the total exposure time adds up to no more than 13 min (Kudielka et al., 2007a, 2007b). As already reported, stress has repeatedly been demonstrated to trigger metabolic and systemic cascades affecting the function of the nervous, cardio-respiratory or immune system, while little is known concerning the impact of other hormones besides the HPA axis, namely of the thyroid hormones, on the HPA axis response to mental stress. On the other hand, little is known on the HPA axis response to cellular phone call after mental stress The aim of the current study was to investigate how HPA axis response changes over time in every day stimuli, in other words, the HPA response to cellular phone call exposure after mental stress in healthy children and adolescents (this generation is the first to be exposed since conception) as well as the possible predictive role of baseline endocrine and biochemical markers such as thyroid hormones levels, glucose and insulin levels to this HPA response.

2. Subjects and methods

2.1. Study population

Twenty eight healthy primary school graders and high school attendants aged 11–14 (12.5 ± 1.5 years), were recruited from schools from January 2011 to February 2011 for participation in the study. The exclusion criteria included the presence of an infection during the previous month, cardiac or other chronic disease, obesity or chronic medication. They were divided into two groups: those who do not possess and regularly use a cellular phone (occasional users) (n = 16) (Group A) and those who do possess and regularly use (n = 12) (Group B). The participants were asked to give more details about their behavior towards the cellular phone use irrespective of whether they possessed a cellular phone or not: a. Daily frequency of cellular phone use and b. Minutes of use per day.

The participants health status was confirmed by a careful clinical examination (including weight, height,) and personal history data collection including intrauterine life events. Venous blood sampling was then undertaken after an overnight fasting at 8.00 am for tri-iodothyronine (T3), thyroxin (T4), thyroid stimulating hormone (TSH), insulin and glucose determination. The insulin resistance was assessed by the calculation of the homeostasis model assessment-insulin resistance index according to the initial formula [HOMA-IR = insulin (expressed in mU/L) glucose (expressed in mg/dL) / 405] (Levy et al., 1998; Wallace et al., 2004). The study was conducted at the Biomedical Research Foundation of the Academy of Athens.

2.1.1. Ethical issues

The study protocol adhered to the Helsinki Guidelines of Good Clinical Practice and was approved by the Ethics Committee of "Aghia Sophia" Children's Hospital. Written informed consent was obtained from the parents/guardians of all participants before the children were enrolled in the study.

2.2. Methods

2.2.1. Mental stress: Trier Social Stress Test for Children

Details on the procedure are previously described by Buske-Kirschbaum et al. (1997), consisting of public speaking and mental arithmetic tasks in front of an audience with two modifications: the investigator was leaving the room during intervals as modified by Dedovic et al. (2005). b. The story details (but not the concept) were adapted to the Greek children's habits and temperament. Upon arrival in the laboratory, the participants rested in calm for 20 min. At time + 15 min the researcher re-entered the room asking the participant to introduce himself. The child was told that he/she should complete the following story as exciting and plausible as possible — better than all other children. "You have decided to make a Sunday trip to a forest with your friends. The sun was shinning and you climbed the mountain Taygetos (a mythical Greek mountain with dangerous itineraries and unexplored forests). In the afternoon just before you decided to return, clouds appeared in the sky, and soon afterwards, a tremendous rain began. The pathways disappeared under the unexpected rainfall and you

found yourself wet, lost from the rest of the group, without guiding pathways or signs with no possibility to lodge or protect yourself. Darkness dropped around you and huge rolling rocks falling from the top of the mountain appeared here and there. The blowing wind and thunders did not allow you to hear your colleagues voices you are lost". The investigator quits the room again and returns 2 min later to hear the narration of the child. This constituted the narrative part of the test. Afterwards, the investigator asked the participant to count down from a large prime number (1492) in decrements of 13 as quickly and accurately as possible. On every failure the child had to restart at the beginning number with the investigator prompting:"error — restart". Five minutes later, the task was completed and the investigator quitted the room. A calming period lasting 20 min followed the arithmetic task before the cellular phone call.

2.2.2. Cellular phone call exposure

After the completion of the TSST as described above and a relaxation period, the participants were then instructed to talk to their own/parental cellular phone for 5 min without interruption, with the phone placed at the left ear. The participants were placed in supine posture (on a typical metallic bed with cotton mattress, whose distance from each wall of the room was 0.7 m × 0.2 m × 3.47 m × 0.95 m). A second similar bed and a small metallic table (with experimental instruments) had been also placed in the room. The cellular phones were of second and third generation (2G and 3G) of mobile networks. The field characteristics were those of a typical use (under the ICNIRP limits (ICNIRP, 2009)) during daily cellular phone use (Table 1). Thus, the participants' body was exposed to the complicated and intense near field, which is extended no more than 50 cm from the radiation antenna, with the adding of some low intensity values from the far field reflections. In other words, the hypothalamus, the pituitary, the thyroid and the salivary glands of the participants were exposed to the near field, whereas the adrenal gland of tall participants might be exposed to the far field of the cellular phone antenna. Therefore, we kept the radiofrequency exposures approximately identical for all participants to avoid differences of the Specific Absorption Rate (SAR) values in children of the same age and the same anthropometric characteristics profile communicating for 5 min with a 2G or 3G cellular phone.

2.2.3. Hormonal and biochemical markers

2.2.3.1. Blood samples. Twenty minutes after venipuncture and blood samples collection in EDTA tubes, the samples were centrifuged at 2000 *g* for 20 min in 4 °C. The blood aliquots were then stored at −80 °C in the Biomedical Research Foundation of the Academy of Athens until analysis. Hormonal and biochemical measurements, including plasma glucose, insulin, T3, T4 and TSH, were performed in the Endocrinology Laboratory of the Eugenideion Research Institute. The method used for hormonal assessments was the Chemiluminescence assay (Immulite 2000 Siemens). Glucose was measured with a photometric method.

2.2.3.2. Salivary cortisol samples. The 28 participants after the baseline blood sampling at 8.00 am rested for 30 min until calmness was assured and then underwent the two investigations: First, the TSST, as described above, followed by a relaxation period, and afterwards, a cellular phone call of 5 min duration (Fig. 1). Between the two stressors a 20 min break took place. Five salivary cortisol samples were collected during the whole experiment (Fig. 1). The first salivary cortisol sample was collected upon the completion of the first relaxation period (baseline), the second one 10 min after the mental/arithmetic task, the third one 20 min after the arithmetic task, the fourth one 10 min after the cellular phone call and the fifth 20 min after the cellular phone call, so as to reflect hypothalamic–pituitary–adrenal axis response to each one of them (Fig. 1).

Table 1
Participants main exposure, anthropometric and laboratory characteristics.

Parameter	Group A mean ± SD	Group B mean ± SD
Age (years)	11–12	13–14
n	16	12
Males	13	8
Females	3	4
Height**	153.36 ± 10.78	165.28 ± 13.36
Height z-score*	0.26 ± 1.2	1.3 ± 0.77
Weight**	40.07 ± 8.82	54.01 ± 5.01
Weight z-score*	0.11 ± 1.12	1.1 ± 0.58
BMI*	18.15 ± 2.5	21.11 ± 2.49
BMI z-score**	0.37 ± 0.79	0.54 ± 0.61
ACTH (pg/mL)	15.7 ± 7.84	18.1 ± 0.7
Plasma cortisol (μg/dL)	9.1 ± 5.3	9.09 ± 3.68
Glucose (mg/dL)	78.2 ± 5.5	76.7 ± 9.4
Insulin (miU/mL)	5.04 ± 1.59	7.1 ± 4.4
HOMA-IR	0.97 ± 0.31	1.37 ± 0.91
hsCRP (SIU mg/L)	1.9 ± 2.9	0.7 ± 0.4
TSH (miU/dL)	1.9 ± 0.69	2.68 ± 1.77
T3 (pg/mL)	1.4 ± 0.19	1.44 ± 0.39
T4 (μg/dL)	8.43 ± 148	7.3 ± 1.3
SAR (W/kg)	0.268–102	0.571.02

* Limited statistical significance $p = 0.05$.
** Statistical significance $p < 0.005$.

The salivary cortisol samples were collected in salivette saliva collection cotton pieces (Stardsted, Norbrecht, Germany). Immediately, after saliva collection, the salivettes were centrifuged at 2400 *g* for 20 min in 4 °C and aliquots were stored at −80 °C in the Biomedical Research Foundation of the Academy of Athens. After completion of the tests, the samples were transported to the Choremeion Endocrinology Laboratory of "Aghia Sophia" Children's Hospital (Athens, Greece) on dry ice and were then analyzed by an Immunochemiluminescence assay in a Roche COBAS E411 analyzer.

3. Statistical analysis

Results are presented as mean value ± standard deviation in Table 1. Salivary cortisol concentrations were analyzed with Shapiro–Wilks test in order to test the normality of their distribution. The hormonal and biochemical concentrations of the two groups were compared with *t*-test. Two way ANOVA analysis for repeated measurements was used to evaluate the fluctuation of the salivary cortisol levels reflecting the HPA response. Pearson correlation coefficient was also applied in each group, in order to find possible hormonal or biochemical predictors of salivary cortisol activation at each time point of the study. Contrast evaluations with repeated contrasts were performed in order to

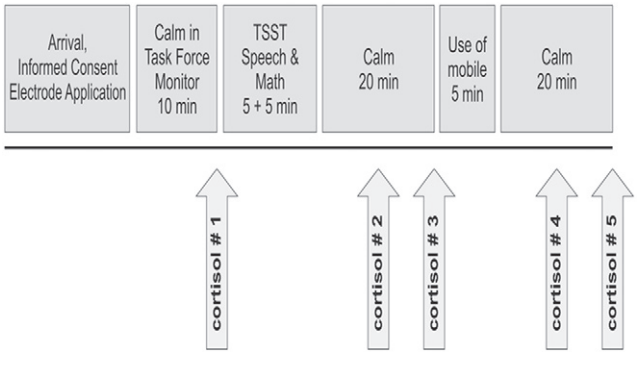


Fig. 1. Design of the sampling schedule for salivary cortisol of the study.

estimate alterations between the time points of the procedure. Greenhouse-Geisser correction was applied to account for the violation of the sphericity assumption. We further calculated effect sizes and power of the analysis in SPSS. The Statistical significance p value was adjusted at <0.05 for the ANOVA and <0.01 for the (2-tailed) Pearson correlation. Statistical analysis was performed by SPSS Version 17 Software.

4. Results

In order to evaluate whether children/adolescents who do not possess a cellular phone have a different response to cellular phone use after mental stress in comparison to those who possess a cellular phone, the 28 participants have been sub-divided in the cellular phone non-owners — primary school pupils, who are not allowed by parents and/or school teachers to carry a cellular phone (Group A), and the high school attendants, who possess a cellular phone (Group B). The participants' anthropometric, hormonal and biochemical characteristics are shown in Table 1.

The hormonal and biochemical concentrations in the two groups did not differ $t(11) = 1.65$, $p > 0.05$. The BMI and BMI z-score differed between the two groups ($p \le 0.05$), as expected by the older age of the participants of group B. The mean values of salivary cortisol assessments in each group are illustrated in Table 2.

Shapiro–Wilks test for normality showed that salivary cortisol levels distributions are normal ($p > 0.05$). Levene's test for equality of variances showed that the participants of groups A and B do not violate the assumption of homoskedacity $F = 3.56$ $p = 0.069$. Mauchly's test indicated that the assumption of sphericity had been violated ($p < 0.001$), therefore degrees of freedom were corrected using Greenhouse Geisser estimates of sphericity. Eta-squared (η^2) as a measure of the effect size is used (Borenstein et al., 2005; Lakens, 2013).

The power of the repeated measure analysis was calculated as 0.705 which is convenient for a pilot study.

At baseline, participants of Group B had higher salivary cortisol levels than their counterparts ($t(11.3) = 2.1$, p-value < 0.05). Their response to mental stress (times 2 and 3) or to cellular phone call (times 4 and 5) did not differ. After both the mental stress and the cellular phone call, the participants of Group A demonstrated a statistically sound decrease of their salivary cortisol levels in comparison to their baseline salivary cortisol concentrations (Fig. 2). The within subjects differences in this group showed that these participants responded in a significantly different way, 10 min after mental stress (time 2) $F(2.12) = 6.93$, $p = 0.014$, $\eta^2 = 0.28$, 20 min after mental stress (time 3) $F(2.36) = 6.609$, $p = 0.016$, $\eta^2 = 0.20$ and 20 min (time 5) after a 5 min cellular phone call: $F(2.129) = 3.98$, $p = 0.022$, $\eta^2 = 0.38$ (Fig. 2).

The Pearson correlation coefficient approach showed that salivary cortisol concentrations of the five time points of the experiment were not associated with gender in either group studied. Baseline thyroxine levels are inversely correlated with salivary cortisol levels from 10 to 20 min after the cellular phone call in the non-owners group $r(14) = -0.625$, $p < 0.001$, while no such effect was noted in the owners' group between the relevant time points. Moreover, non-owners showed a positive correlation of the salivary cortisol levels with T_3 between time 4 and 5 $r(14) = 0.58$, $p < 0.001$ (Fig. 2). A positive correlation was also noted between TSH levels and salivary cortisol levels in the same time point $r(14) = 0.536$, $p = 0.003$. All reported results did not change when daily cellular phone use, calculated as minutes per day, was taken into consideration. As expected, those who do not possess a cellular phone had an occasional use of it whereas those who possess one were frequent users.

5. Discussion

In the current study we aimed to investigate the HPA axis response to cellular phone typical use after mental stress in healthy children and adolescents of different groups, the one group comprising cellular phone owners and the second one those who do not possess a cellular phone. The participants were exposed to the operating on the neighboring radiofrequencies 2G and 3G bands of the cellular phones. Yet, the GSM 2G networks support and complement in parallel the 3G wide band, in other words, the transmission of information via voice signals is served by the 2G technology in cellular phones of both generations. We kept the exposure to field characteristics identical as in typical use (under the ICNIRP limits (ICNIRP, 2009)). The dielectric properties vary over age, tissue of interest, body mass index (Christ et al., 2010; Christopoulou and Nikita, 2008; Davis et al., 2013; Gandhi, 2002; Martens, 2005; Morgan et al., 2014; Peyman, 2011; Wiart et al., 2011). Thus, it was assumed that the variability of the participants' anthropometric characteristics and the plurality of cellular phone models used as well as the surrounding reflecting objects were close to real life conditions and would represent realistic observations.

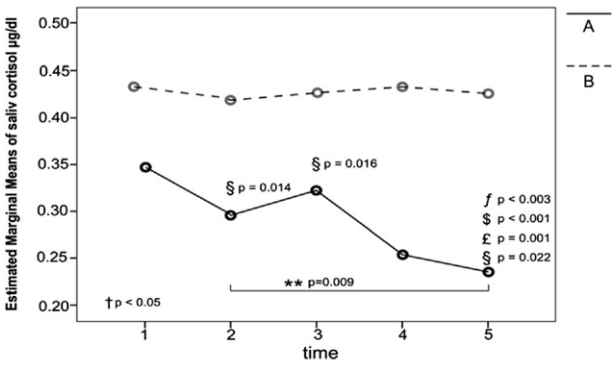
For ethical reasons, we avoided exposure to prolonged cellular phone call.

We have chosen to conduct a repeated measures design rather than a case–control study, as the repeated measure design reduces the variance of estimates of exposure effects, allowing statistical inference to be conducted with limited number of participants while further allowing researchers to monitor how participants' characteristics change over time, both long- and short-term situations (Gravetter and Wallnau, 2009; Howell, 2009; Mitchell and Jolley, 2009). We performed all experiments at the same morning time so that circadian variations would be identical in all participants. At baseline, cellular phone owners had higher salivary cortisol levels than non-owners, younger participants, probably demonstrating higher levels of arousal and anxiety in this group. This observation has already been discussed in the literature and associated with pubertal development (Aronow, 2012; Sherzai et al., 2012). During both the mental stress and the cellular phone call participants who possess a cellular phone did not demonstrate a statistically significant decrease in their salivary cortisol levels in comparison to baseline, while non-owners did demonstrate a statistically significant decrease. In the international literature a down-regulation of the HPA axis response to mental stress has been described to be common in bullying victims (Beetz et al., 2012; Dorn et al., 2003). It has furthermore been associated to lower income and parental unemployment (Kraft and Luecken, 2009; Ockenfels et al., 1995) and/or insecure attachment (Beetz et al., 2012; Dorn et al., 2003; Gunnar et al., 2009). Moreover, it may reflect the adaptivity of the HPA-axis (Gunnar et al., 2009; Helmreich et al., 2005) or the increased sensitivity of the HPA axis (Koyu et al., 2005). Children in Greece, nowadays, do experience

Table 2
Mean salivary cortisol levels ± SD (μg/dL) at each sampling time point.

Group	Baseline	Stress + 10′	Stress + 20′	Call + 10′	Call + 20′
B	0.445 ± 0.39	0.42 ± 0.355	0.423 ± 0.3	0.434 ± 0.29	0.429 ± 0.41
n = 12	95% [0.208, 0.78]	95% [0.101, 0.795]	95% [0.101, 0,51]	95% [0.224, 0. 466]	95%[0.109, 0.533]
A	0.37 ± 0.198	*0.293 ± 0.189	*0.326 ± 0.15	0.249 ± 0.145	*0.228 ± 0.37
n = 16	95% [0.172,0.54]	95% [0.145, 0,46]	95% [0.167, 0.474]	95% [0.15, 0.475]	95% [0.12, 0.669]

Group A: occasional cellular phone users, Group B: frequent cellular phone users.
* $p < 0.05$.



Group A: younger children, occasional cellular phone users
Group B: older adolescents, frequent cellular phone users
Time: 1: baseline, 2: TSST+10′, 3: TSST+20′, 4: call +10′, 5: call+20′
** Statistical Significance between different time points in the same group
§ Significance between successive time points in the same group
† Statistical Significance of the total experiment in the same group
$ Significant correlation of thyroxine
£ Significant correlation of triiothothyronine
ƒ Significant correlation of TSH

Fig. 2. Anova estimated marginal repeated measures of salivary cortisol levels in subgroups.

insecure circumstances and/or parental unemployment due to the current socioeconomic crisis that the country is going through. Although the participants' families belonged to the mid socioeconomic class, beginning to be affected by the socioeconomic crisis at the time of the experiment, the insecurity concerning their own future may have influenced their response. Moreover, it is well established that lack of change in the cortisol response indicates that the applied stressor is no longer a novelty (Petrowski et al., 2012), as is the case for older adolescents concerning both cellular phone use and arithmetic task.

Those who did not possess a cellular phone responded significantly differently to mental stress in comparison to those who owned a cellular phone: probably, the TSST-C protocol adopted is adequate to assess mental stress in the first group (younger, non-owners) but not in the older, owners group, where a stressful video would have been more adequate to assess mental stress.

To our knowledge this is the first study to evaluate the effect of baseline hormones, such as baseline thyroid hormones on the salivary cortisol response to a common stressor — the cellular phone call after mental stress. This successive model of stress applied in this protocol was developed to mimic daily life in a way that the two stressors' effects would not interfere. It is still unclear how long the interval between stimuli might be so as to avoid an interference of the first stressor to the subsequent (Petrowski et al., 2012).

Habituation presumes that stressful exposure is no more conceived as stress (Grissom and Bhatnagar, 2009; Wust et al., 2005).

A significant effect of age on HPA axis response to cellular phone call was observed with owners' group demonstrating a blunt HPA axis response after the first 20 min, while the non-owners group showed a further significant decrease in salivary cortisol response 20 min after the cellular phone call in comparison to their response to the mental stress and to baseline. This finding is consistent with the fact that usually parents of primary schools' attendants do not allow them to frequently use cellular phone (non-owners), while high school attendants carry and use their own cellular phone device and are practically assumed as frequent users. Yet, the salivary cortisol response was irrespective of the minutes per day of cellular phone use in both groups. This implies that frequently carrying the cellular phone close to the body is an important factor that should be more thoroughly studied.

In accordance to our findings, in a previous study, concerning adult cell phone users, no significant change in salivary cortisol levels was observed (Radon et al., 2001). Yet, two other studies, one investigating 8 female cellular phone users after 30 min exposure (Jarupat et al., 2003), and another 20 workers after 4 weeks (Djeridane et al., 2008) showed decrease in salivary cortisol levels. Boys reacted in the same way as girls in the current study. A recent publication, however, reported a sexually dimorphic different response between boys and girls of the same age like our participants, (Fu et al., 2012) but the participants of that study were obese, unlike ours. Previous studies had shown no gender variation in Trier Social Stress Test response (Kudielka et al., 2004) a finding that is confirmed by our study. Nevertheless, the limited number of our participants may have masked a possible difference that could have been statistically significant if a significantly larger number of participants had been included.

It is well established that stress modulates the hypothalamic–pituitary–thyroid (HPT) axis (Cremaschi et al., 2000; Maccari and Morley-Fletcher, 2007; Tsatsoulis, 2006). Growing evidence points that the HPT axis can be influenced by GSM radiation emitting at 900 MHz (Eskander et al., 2012; Djeridane et al., 2008; Koyu et al., 2005; Riccioni et al., 2004).

Yet, our study revealed a paradox: baseline thyroid hormones levels in cellular phone non-owners healthy children and adolescents, whose organism is still developing and who have very limited use of cellular phone in daily life due to their young age, have an effect of HPT axis on the HPA axis response to the cellular phone use, while such an effect was not found in the cellular phone owners' group. This observation certainly necessitates confirmation by a larger number of participants of different age groups. Our study might bring to light an internal cross-talk between the HPT and the HPA axis in children who do not have a cellular phone to carry near their body constantly, while such an observation is no more relevant in older subjects.

There was no effect of baseline glucose, insulin or HOMA on the HPA response of the participants in either group, while frequent users (Group B) presented a borderline, though not statistically significant, decreased fasting glucose level. The latter finding is also supported by literature data (Meo and Al Rubeaan, 2013).

Salivary cortisol response was age dependent, a finding that has already been reported (Kiess et al., 1995). More importantly, our study revealed an interesting finding: salivary cortisol response to a cellular phone call after stress depends on carrying or frequent use of a cellular phone. The molecular mechanisms involved in the HPA axis response to mental stress and cellular phone use need to be further investigated, in the light of electrophysiology, as the EFHRAN project in its 2012 report suggested that foetuses, pregnant women, infants, children and adolescents should be thoroughly studied as the dielectric properties of their tissues are different from adults (EHFRAN, 2012). Furthermore, the introduction of promising new theory and techniques such as quantum mechanics (QM), molecular simulations, systems biology and proteomics applied to EMF and endocrine tissues should be further studied. Detailed exposure assessment of each subject should be also investigated, in order to unravel lifestyle impact and/or exposure variation correlated to this phenomenon. The latter is enhanced by recent publications implicating oxidative stress with the extensive use of cellular phone (Hamzany et al., 2013; Lu et al., 2012). In conclusion, the current study provides evidence for different HPA axis response to cellular phone near and far field exposure after mental stress in children and adolescents of different cellular phone use profile and highlights the importance of large-scale studies in order to elucidate the impact of extended electromagnetic field exposure on future health of children and adolescents.

Declaration of interest

This research has been conducted with no funding. There has been no conflict of interest for any author.

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