FACULDADE DE MEDICINA
UNIVERSIDADE DO PORTO

MESTRADO INTEGRADO EM MEDICINA

2012/2013

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Human Infertility:
are Endocrine Disruptors to blame?

março, 2013

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Mestrado Integrado em Medicina

Área: Endocrinologia

Trabalho efetuado sob a Orientação de:
Prof. Doutor Davide Maurício Costa Carvalho

Trabalho organizado de acordo com as normas da revista:
Hormones: international journal of endocrinology and metabolism

março, 2013

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UNIDADE CURRICULAR
PROJETO DE OPÇÃO
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UNIDADE CURRICULAR PROJETO DE OPÇÃO DISSERTAÇÃO / MONOGRAFIA

Projeto de Opção do 6.º ano – DECLARAÇÃO DE REPRODUÇÃO

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Título da Monografia:

Human Infertility: are Endocrine Disruptors to blame?

Orientador:

Prof. Doutor Davide Maurício Costa Carvalho

Ano de conclusão: 2013

Designação da área do projeto:

Endocrinologia

Faculdade de Medicina da Universidade do Porto, 18/03/2013

Assinatura: André Filipe Marques Pinto

Às estrelas que me iluminam
na mais escura hora
e no mais claro dia.

Que lhes mereça sempre a luz.

Human Infertility: are Endocrine Disruptors to blame?

Endocrine Disruptors and Human Infertility

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Key words: Endocrine disruptors, environment, infertility, reproduction, male, female

Grants or fellowship supports/conflict of interests: The authors have nothing to declare.

Abstract

Over the last decades, epidemiological studies have been reporting worrisome trends in the incidence of human infertility rates. Extensive detection of industrial chemicals in human serum, seminal plasma, and follicular fluid has led the scientific community to hypothesize these compounds may disrupt hormonal homeostasis, leading to a vast array of physiological impairments that may cause developmental disorders, infertility and other detrimental effects. The main route of exposure to these ubiquitous chemicals is the ingestion of contaminated food and water. This review analyses the major scientific developments on the topic of human infertility associated with exposure to endocrine disruptors, incorporating evidence from epidemiological and experimental studies. Current data suggest endocrine disruptors can affect human fertility and reproductive function. They may easily disturb the intrauterine development of the reproductive system and central nervous system, in both genders, and they may also induce transgenerational effects. Endocrine disruptors may therefore be blamed for the rising incidence of human reproductive disorders and constitute a serious Public Health problem that should not be overlooked. The exposure of pregnant women and infants to endocrine disruptors is of great concern. Precautionary avoidance of exposure to endocrine disruptors is a prudent attitude.

Abbreviations

AFP: α-fetoprotein

AGD: anogenital distance

AhR: aryl hydrocarbon receptor

AR: androgen receptor

BPA: bisphenol A

DDT: dichlorodiphenyltrichloroethane

DES: diethylstilbestrol

ED: endocrine disruptors

EE: ethinyl oestradiol

ER: oestrogen receptors

FSH: follicle-stimulating hormone

GnRH: gonadotropin-releasing hormone

HPG axis: hypothalamus-pituitary-gonadal axis

INSL3: insulin-like factor 3

IVF: *in vitro* fertilization

kg-bw: kilogram of body weight

LH: luteinizing hormone

LOAEL: lowest observed adverse effect level

MOF: multiple-oocyte follicles

MXC: methoxychlor

NSAID: nonsteroidal anti-inflammatory drugs

ODS: ovarian dysgenesis syndrome

PBDE: polybrominated diphenyl ethers

PCB: polychlorinated biphenyls

PCOS: polycystic ovary syndrome

PG: prostaglandin

POF: premature ovarian failure

SHBG: sex hormone-binding globulin

TCDD: 2,3,7,8-tetrachlorodibenzo-*p*-dioxin

TDS: testicular dysgenesis syndrome

TGF-β: transforming growth factor β

VCZ: vinclozolin

Introduction

Infertility, which is currently defined as the inability to conceive after one year of unprotected intercourse, has a global prevalence of 9%, ranging from 3.5% to 16.7% in developed countries, compared to 6.9-9.3% in developing countries.[1] A multi-centre study conducted by the World Health Organization among infertile couples shown that the cause was predominantly female in 38% of the cases and primarily male in 20%, while 27% of couples had both male and female abnormalities, and no evident cause was identified as for the remaining 15%.[2] Since the mid-twentieth century, a cumulative number of studies report an increasing incidence of human reproductive diseases and consequent decline in reproductive function, in several locations and amongst different populations.[3] Given this relatively short time frame, genetic changes may not explain the rising infertility rates. Thus, environmental substances may be one of the factors responsible for the observed geographical and populational differences in the incidence of infertility.[4,5]

Following the economic development brought forward by the beginning of the industrial era, which inexorably led to massive production and use of a wide variety of synthetic substances, drastic lifestyle changes have occurred. Both human and wildlife have ever since been exposed to numerous potentially hazardous chemicals that are released by industries into the environment at an alarming rate. These substances may have significant adverse reproductive outcomes attributable to their ubiquitous presence in the environment and multiple routes of exposure.[6]

One of the most significant landmarks in Endocrinology of the past century is the recognition that some of these chemicals are able to disrupt the endocrine system, being named Endocrine Disruptors (ED).[7] Consistent detection of ED residues in human serum, seminal plasma, and follicular fluid raised concern that environmental exposure to ED is affecting human fertility.[8] Human exposure to some ED seems to be able to impair fertility without any other clinical signs of toxicity, suggesting that the main target of ED is the homeostasis of sex steroids.[9,10] Though ED are not considered major teratogens, reproductive function – from gamete production and fertilization through intrauterine and post-natal development of the offspring – is believed to be particularly susceptible to endocrine disruption, triggering morphological and functional abnormalities, and even reproductive tract cancers.[11,12] Still, there is much to be studied in order to substantiate an unequivocal relationship between ED exposure and reproductive and developmental disorders.

The main purpose of this paper is to review and summarize the major scientific developments on the topic of human infertility associated to ED exposure, integrating evidence arising from human epidemiological studies and experimental studies in model organisms. Examples of well-known and hypothetical ED are selected to highlight the rationale for the potential effects of ED on human fertility, identifying future research directions.

Methods

The influence of ED exposure on human fertility reported in the PubMed database is reviewed. The PubMed database was used to search for articles published until December 31st 2012, using the following MeSH keyword-based query: endocrine AND [(disruptors OR disrupters) OR (disrupting AND (compounds OR chemicals))] AND (fertility OR infertility OR fecundity). Only studies using the English language were considered. Altogether, 332 papers were retrieved. The abstract of every article was

read. The leading review criterion was human epidemiological studies in which a link between ED exposure and infertility was evaluated. Owing to the scarcity of human experimental studies, animal models were also considered in this review, as it has been shown that humans are at least as sensitive to ED as the most sensitive animal species, and the reliability of experimental animal data regarding reproductive disruption has been well established.[3] The full texts of the 214 selected articles were retrieved and read. Furthermore, the bibliographies from 41 selected review articles were also analysed, resulting in the inclusion of 153 other papers. In addition, 19 articles were included by authors' suggestion. Overall, 249 articles out of the 386 read full texts were deemed relevant and included in this review.

ED and Infertility

From a physiological standpoint, an ED is a compound, either synthetic or natural, that, through environmental exposure, disrupts the hormonal and homeostatic systems, which allow organisms to interact with the environment and are responsible for developmental processes and reproduction, through a closed feedback loop.[7] The group of known ED is extremely heterogeneous and embraces ubiquitous synthetic substances used as industrial lubricants and solvents, and their by-products: polychlorinated biphenyls (PCB),[13] polybrominated diphenyl ethers (PBDE),[14] and dioxins, such as 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD);[15] plastics: bisphenol A (BPA);[16] plasticizers: phthalates;[17] pesticides: atrazine,[18] cypermethrin,[19] dichlorodiphenyltrichloroethane (DDT),[20] dieldrin,[21] methoxychlor (MXC),[20] and vinclozolin (VCZ);[22] and drugs: diethylstilbestrol (DES),[23] ethinyl oestradiol (EE),[24] and nonsteroidal anti-inflammatory drugs (NSAID) and acetaminophen.[25] Natural chemicals such as phytoestrogens,[26] including genistein, and heavy metals[27] can also have endocrine-disruptive effects.

Although there has been an effort to list and rank all possible ED, the number of evaluated chemicals remains limited, in some cases because of technical restrictions regarding their detection.[28,29] Therefore, most studies have focused on a small number of ED, for which reliable biomonitoring data are available, as proxies for the total exposure.[30] The possible contribution of ED to many reproductive disorders has not been fully assessed because of scientific and ethical limitations. Moreover, as the interpretation of epidemiological studies is biased by many confounding factors, such studies must be supported by experimental research in animal models.[3]

Many documented ED are substances with oestrogenic or anti-androgenic activity, by interfering with the oestrogen receptors (ER) or the androgen receptor (AR) (see Table 1). Given the complexity of the endocrine system as a whole, the mechanisms of action of ED are difficult to unravel. So far, endocrine disruption is known to ensue changes of hormone-regulated mechanisms arising from ED binding, as either agonists or antagonists, to nuclear receptors and membrane receptors (comprising nuclear sex steroid receptors, non-nuclear sex steroid receptors, nonsteroidal receptors, such as neurotransmitter receptors, and both nuclear and membrane orphan receptors), thus interfering with gene expression and cell signalling pathways. The relative influence of nuclear, membrane-associated, and cytosolic receptors to the vast array of detrimental effects arising from ED exposure remains unclear. Current data suggest ED (such as BPA) may have more potent effects through non-nuclear receptors.[45] Some ED are also capable of modifying hormone bioavailability by interfering with its secretion and transport, as well as disrupting the enzymatic pathways involved in hormone synthesis and metabolism.[46-48] Yet, one should keep in mind ED probably do not reproduce nor abolish hormonal actions entirely, as most of them are imperfect ligands to endogenous targets.[49] Most ED are supposed to act through several mechanisms, which may have synergistic or antagonistic outcomes.[50]

Proper gonadal differentiation, maturation, and function rely on both steroidal and non-steroidal signalling. A relationship between ED exposure, impaired fertility and altered gonadal TGF-β superfamily signalling has been suggested in alligators.[51]

Apart from sex steroid receptors, the most studied receptor for interaction with ED is the aryl hydrocarbon receptor (AhR). This orphan receptor acts as a transcription factor for detoxifying enzymes and is thought to have evolved in response to sustained exposure to countless environmental chemicals.[52] Moreover, the cross-talk between AhR and nuclear sex steroid receptors modifies gene transcription.[53] Dioxins and some PCB exert their endocrine-disruptive effects through binding to AhR and impairing the usual gene transcription response.[54] Additionally, owing to the ubiquitin-ligase activity of this receptor, AhR ligands enhance the degradation of substrate proteins such as ER and AR.[55]

Traditionally, androgens are considered the male sex hormones and oestrogens, the female sex hormones. However, in either gender, androgens give rise to oestrogens, through aromatase, so together they play a vital role for homeostatic regulation in males and in females.[56,57] Some ED interfere with aromatase (atrazine[58] stimulates its activity,

while DDT and phthalates[47] inhibit it), hence disrupting the delicate androgen-oestrogen balance required for proper reproductive function. Recently, many of the ED that lessen virilisation in experimental studies, such as phthalates and BPA, have been found to be powerful cyclooxygenase inhibitors, reducing prostaglandin (PG) synthesis, and this might be the foremost mechanism by which they exert their anti-androgenic effects.[59]

The ability of an exogenous substance to modify hormonal actions is now regarded as a clear, intrinsic predictor of negative outcomes, which vary according to dose, time of exposure and the potency of that substance.[49] Still, and despite the build-up of epidemiological human studies reporting higher infertility rates among those exposed to ED, the principle of endocrine disruption has always been controversial, mostly regarding the definition of the lowest observed adverse effect level (LOAEL) and whether these are likely to be found *in vivo*.[60] Present postulated LOAEL for most ED are outdated.[61] For instance, BPA has been found to induce detrimental reproductive effects in levels several-fold below current LOAEL, which is 50 mg/kg of body weight (kg-bw)/day.[62,63] Perhaps expectably, there is a sharp division between those who are able to find significant effects of ED at low, environmentally relevant concentrations – mostly academic experts – and those who appear unable to show harmful effects of ED at any concentrations – industry corporations.[64] Still, current data overall supports that minute environmental ED levels may have more potent effects than higher levels, and that ED may have nonlinear, biphasic, inverted U or U-shaped dose-response curves, owing to the blending of the outcomes of different mechanisms, that may change independently, accordingly to dose.[61] Therefore, the existence of a threshold cannot be presumed.[49] However, assuming equivalent exposures, the incidence of detrimental reproductive effects of some ED may be significantly higher in vulnerable individuals, owing to several factors, such as genetics, developmental stage when exposure occurs and pre-existing disease. Nonetheless, these issues remain controversial.[65]

Human exposure to ED

Populations are exposed to ED in air, water, food, and in a variety of industrial products, including personal care goods. Most individuals have traceable amounts of these substances in their serum or urine.[3,30] For instance, over 90% of the North American population has measurable amounts of urinary BPA, and children have the highest levels, perhaps because they are the most exposed subpopulation to BPA and other ED.[66]

The major route of human exposure to ED is supposed to be ingestion of food and beverages contaminated with deliberately added compounds, including additives, processing aids, anabolic steroids used in food production, and monomers leaking from food containers;[16,67] and also side products, impurities and other inadvertently added substances, such as pesticide residues and other pollutants,[68] as well as heavy metals.[27] Current data suggest there is chronic and sustained exposure to ED such as BPA through inhalation, skin contact, and other routes as well.[69] Direct occupational exposure to ED, such as pesticides and other industrial chemicals, is a known risk factor for reproductive disorders.[11,70-72]

Humans and wildlife are exposed to fluctuating levels of ED, depending on production and utilization trends. The mixture of ED that leaches into soil and waterbodies (e.g. pesticides, contraceptive pills, and other industrial chemicals from urban and agricultural waste) accumulates in the environment and in animals higher up the food chain, due to the lipophilicity of these compounds and their resistance to biodegradation.[6,73] Therefore, removal of these substances from the environment is neither simple nor cheap.[74,75] Numerous ED are continuously detected in drinking water and food supplies, including meat, fish, dairy products and vegetables, and may therefore cause endocrine disruption in both humans and wildlife, long after their release into the environment.[6,7,11] Indeed, some ED that have been banned decades ago, such as DDT and PCB, are still found in human serum.[8]

Human development has been proven susceptible to disruption, predominantly throughout decisive stages, such as the periconceptional, embryonic, foetal, infant and pubertal periods.[76-78] The developmental and physiological plasticity (partially owing to epigenetic modification of gene expression) that prevails in the perinatal period in response to changes in the intrauterine environment is evolutionarily beneficial, as it allows foetuses to adapt to prenatal and postnatal life. Yet, intrauterine exposure to ED may easily disrupt the foetal endocrine system, resulting in permanent changes that may lead to immediate outcomes as well as long-term adverse effects on reproduction and development, which may only become apparent during puberty or even later in life.[79] The timing of exposure may explain this differences:[80] if ED exposure occurs during critical windows, adverse effects may be very drastic, including congenital abnormalities; on the other hand, if it occurs during sensitive, non-critical windows, detrimental outcomes may still arise, though possibly with reduced magnitude, including adult onset diseases and mild functional deficits. Supporting this hypothesis,

neonatal exposure to BPA for instance has been shown to have significant effects on spermatogenesis but adult exposure has not.[81]

The prenatal period has become a significant research topic of ED exposure for two primary reasons: (a) the placenta does not protect the developing foetus from damaging chemicals, which may be biomagnified in some cases.[82,83] BPA has low binding affinity to serum proteins such as the sex hormone-binding globulin (SHBG) or α-fetoprotein (AFP), which stop maternal sex hormones from crossing the placenta and affecting foetal development.[84] Therefore, BPA and other ED may have access to hormone-sensitive foetal tissues and disrupt their proper development.[85] (b) Since programming of the hypothalamus-pituitary-gonadal (HPG) axis occurs during this period, ED exposure at this stage may determine fertility in adulthood.[81] It is worth mentioning millions of children are conceived by women while on contraceptive pills containing EE. Albeit most do not show conspicuous congenital abnormalities, long-term reproductive consequences may ensue in adulthood.[85] Indeed, developmental exposure of both male and female rats to EE in doses similar to those found on contraceptive pills cause permanent reproductive abnormalities, including impaired fertility.[86]

Breastfeeding is another significant source of exposure to ED, such as BPA.[87] As many ED accumulate in fat-rich tissues such as the breast, breastfeeding exposes both the mother and the foetus to relatively high levels of these substances.[88,89] In most reported cases, detrimental effects in the offspring occurred at doses that produce minimal or no effects in the maternal organism.[11] For this reason, women of childbearing age, and specifically those who are pregnant or breastfeeding, constitute a population group of utmost importance regarding ED exposure.[90] Likewise, children deserve special consideration, as they have proportionally higher exposures to ED, due to a higher food and water intakes than adults, leading to a potentially higher body burden of such chemicals.[91]

Recent studies concluded that plastic packaging is an additional important source of ED, such as phthalates and BPA, in the average human diet. Repeated exposure of food-contact materials to UV light, heat, and acidic or alkaline contents may cause polymers to breakdown into monomers, which then leach into food and beverages.[92] For instance, bottled water contains a complex mixture of ED, including those that leach from the bottle itself. Chronic intake of ED from bottled water is believed to have adverse effects on human fertility, particularly among sensitive population groups such

as infants and pregnant women.[67] Food-contact materials can also be a relevant source of ED in dry foods.[90] Examples include the reported migration of phthalates from recycled paperboard and plastic packaging into infant food.[67,93] So far, there is no consistent total estimation of ED leaching from food-contact materials.

The average diet exposes humans to a wide variety of synthetic ED. However, it also contains natural ED, such as phytoestrogens, which are nonsteroidal compounds that possess strong oestrogen-like biological activity.[26,35] The potential health benefits of phytoestrogens and seeming absence of major long-term adverse effects have led to an increased consumption of these substances, mainly through soy-based food.[94,95] Yet, high phytoestrogen intake, either chronically or during critical developmental periods, may trigger endocrine disruption.[96] Studies have revealed that infants ingesting soy-based formulas may have a phytoestrogen serum concentration 13.000-22.000 times higher than endogenous oestrogen levels,[97] and tenfold that of the average adult phytoestrogen levels, leading to concerns about the possible adverse effects of phytoestrogens on brain development and infertility later in life.[10,98]

ED and the Central Nervous System

Successful human reproduction requires appropriate morphological and physiological development of both male and female reproductive system. Gonadal hormones play a vital role in the development of these structures and are crucial for fertility. ED may modify endogenous steroidogenesis via negative and positive feedback, both locally and through the HPG axis, which works a delicate balance to maintain the homeostatic parameters required for reproduction.[7]

Human HPG axis is active *in utero* and during the first year of life.[99] Afterwards, gonadotropin secretion is reduced until puberty, when sequential endocrine changes in the secretion of gonadotropin-releasing hormone (GnRH), gonadotropins, and sex steroids set in motion the development of secondary sexual characteristics that will lead to sexual maturation, and hence the possibility to reproduce.[100] Some PCB have been shown to alter GnRH synthesis[101] and to decrease GnRH release by disrupting serotoninergic pathways.[102] On the other hand, DDT and BPA stimulate glutamate-induced GnRH release.[103]

Kisspeptin is broadly recognized as a fundamental activator of the HPG axis, at the onset of puberty.[104] As many ED have been reported to alter sexual behaviour, puberty, and fertility, the kisspeptinergic system became an obvious object of research.[100] In rats,

neonatal exposure to oestrogenic ED, such as BPA and genistein, induced variable degrees of kisspeptin suppression, possibly by reducing hypothalamic kisspeptin mRNA levels.[105,106] The relative importance and prospective interaction of endocrine disruption at both these reproduction-controlling brain networks (GnRH and kisspeptin neurons) remains unclear.

Numerous chemical signals regulate the development of the central nervous system. Particularly, sex steroids have prominent roles in the differentiation of several dimorphic neural circuits and behaviours, in addition to the development of primary and, later, secondary sexual characteristics.[107,108] The developing brain is protected from excessive exposure to maternal sex hormones by proteins such as SHBG and AFP.[109] As BPA and other ED bypass this control mechanism, they may cross the immature blood-brain barrier[16] and therefore disrupt the differentiation of sexually dimorphic areas (such as the dopaminergic, nitrergic, and kisspeptinergic systems), reversing the neurochemical phenotype, which adversely affects fertility and sexually differentiated behaviours in adulthood.[110] Developmental exposure to BPA, MXC, and VCZ has been shown to produce gender-inadequate adult behaviours,[111] possibly by disrupting specific neural pathways (eg. nitrergic fibres) that influence complex functions and behaviours such as those related to reproduction.[112]

Intrauterine exposure to ED may impair the development and function of the HPG axis as a whole.[110] In rats, perinatal exposure to environmentally relevant BPA doses, below the LOAEL, disrupted the HPG axis maturation, by inducing defective GnRH pulses and low serum gonadotropin levels up to adulthood, leading to infertility.[113] Long-lasting reproductive disorders induced by developmental ED exposure may be more likely to arise from a dysfunctional HPG axis than from impairments at reproductive organs.[114] In fact, current evidence suggests the primary target of developmental ED exposure might be the hypothalamus and the pituitary gland rather than gonads themselves.[108]

ED and the Male Reproductive System

Epidemiological studies taking place over the last decades have reported an ominous growth in the incidence of male infertility, accompanied by decreasing sperm quality.[115] A large review of international studies shown that, over fifty years, the global average sperm count dropped by half (from 113 to 66 million/mL), reflecting an average yearly decrease of 1%, and sperm morphology and motility abnormalities significantly

increased.[116] Amongst semen quality parameters, sperm motility is the most valuable independent predictor of male fertility.[117] A subsequent larger study confirmed the declining sperm concentration at a yearly rate of 1.5-3%.[118] However, those results are still questioned due to concerns about the study design.[119]

Studies comparing male reproductive disorders amongst the Nordic-Baltic countries, which are geographically and socially closely related, have reported an east-west gradient showing higher reproductive tract abnormalities and infertility rates in Denmark as compared to Finland.[120,121] Although genetic differences cannot be excluded, ED may more likely explain this geographical trend. Actually, significantly higher levels of ED were found in the breast milk of Danish women as compared to Finnish women.[88] Moreover, several epidemiological studies have found an association between increased urinary and serum levels of ED, such as phthalates,[122] PCB,[123] PBDE,[124,125] and BPA,[126] and inferior semen quality parameters, suggesting ED may be a contributing factor to male infertility.

Male infertility is closely related to low semen quality (reflecting impaired spermatogenesis), congenital abnormalities of the reproductive system, and testicular cancer.[96] Epidemiologic studies have shown a correspondence between temporal and geographic trends in semen quality and the incidence of cryptorchidism, hypospadias, and testicular cancer.[127] These disorders have been regrouped under the term Testicular Dysgenesis Syndrome (TDS),[128] as they are thought to arise from intrauterine disruption of proper testicular development and function, possibly through epigenetic modifications,[129] as a result of individual genetic susceptibility influenced by ED exposure.[130] In the mildest, most common cases of TDS, men have slightly decreased testicular volume in addition to poor semen quality and relatively low testosterone levels, while in the more severe cases there is cryptorchidism or hypospadias and an increased risk of testicular cancer.[131] A subtle TDS may thus explain some of the idiopathic infertility cases, which constitute approximately half the men presenting at infertility clinics.[132] ED exposure has been suggested to have triggered the escalation of mild to moderate TDS cases, whereas the most severe forms of this syndrome are undeniably related to genetic factors.[133]

Current epidemiological data suggest that human developmental exposure to environmental levels of ED, such as phthalates, PCB and pesticides, is indeed connected to an increased risk of hypospadias and cryptorchidism.[89,134-137] Hypospadias, a condition in which the urethral meatus is not at the tip of the glans but on the ventral

side of the penis, affects about 0.4% of males at birth and has been reported to have increased significantly over the last decades.[138] ED are regarded as a contributing factor,[4] since several of these substances, such as VCZ[139] and phthalates[140] consistently induce hypospadias in laboratory animals.

Cryptorchidism is generally regarded as the failure of one or both testicles to descend into the scrotal sac and is the most common congenital abnormality in male children, affecting 2-4% full-term born males.[120] One should keep in mind that the definition of cryptorchidism differs between authors, and this might influence the reported prevalence of this condition.[141] Nonetheless, epidemiological studies conducted over the last decades suggest the incidence of cryptorchidism is on the rise.[142] In adulthood, these men are frequently infertile due to disrupted spermatogenesis, and commonly show reduced testicular volume that correlates to reduced sperm quality and lower serum testosterone.[143] Cryptorchidism currently embodies the best characterized risk factor for infertility and testicular cancer in adulthood.[141] Testicular migration is a complex, two-stage process. ED exposure, *in utero* and after birth may explain the reported increasing incidence of cryptorchidism, due to a (a) reduction in insulin-like factor 3 (INSL3) expression in Leydig cells, disturbing the development of the gubernaculum, which is vital for transabdominal testicular migration;[144] and (b) suppression of gonadotropin production, and impairment of steroidogenic enzymes in Leydig cells, resulting in relative testosterone deficiency, which is essential for transinguinal testicular descent.[130] Exposure to some ED, such as PBDE, through breastfeeding has been correlated to cryptorchidism in new-borns.[83] There have also been reports of an association between higher maternal urinary phthalate metabolites and higher testicular position in infant boys but not with actual cryptorchidism.[134]

Testicular dysgenesis may result in early effects (e.g. hypospadias and cryptorchidism) as well as late consequences (e.g. infertility and testicular cancer).[145] Delayed effects may be difficult to predict. Though, there are some signs that may indicate an increased risk of testicular cancer, such as low testicular volume and evidence of testicular microcalcifications on ultrasound.[146] Over the last decades, testicular cancer rates have greatly increased in many developed countries, probably owing to ED exposure.[147] As cancer registers are usually more accurate than those for congenital abnormalities, the former may be regarded as a proxy for fertility problems in the general population.[148]

A large epidemiological study in adult Danish men found that infertile men had significantly lower serum testosterone/luteinizing hormone (LH) ratio than proven

fertile men, suggesting compromised Leydig cell function.[149] Besides, those changes were more obvious among men with history of cryptorchidism. Consequently, decreased Leydig function (that may be secondary to Sertoli cell dysfunction) is presumably the main trait of TDS, either causally or as a consequence of developmental ED exposure, being accountable for androgen insufficiency, reduced genital volumes and impaired spermatogenesis in the adult.[133,145]

The differentiation of the male reproductive system is entirely dependent on foetal testicular androgen production.[150] As a transitory peak in testosterone levels is critical for appropriate male differentiation,[151] disruption of androgen activity by ED during the virilisation period (around 8-14 weeks of human foetal development), will perhaps cause TDS.[152] In experimental studies examining the effects of exposure to anti-androgenic ED, reduced anogenital distance (AGD) is an important reproductive endpoint,[46] reflecting ineffective perineal virilisation.[153] In animal models, developmental exposure to phthalates results in a syndrome of reproductive anomalies encompassing, cryptorchidism, testicular injury, reproductive tract malformations and shorter AGD.[140] This pattern of effects, which parallels TDS, has been called the "phthalate syndrome" and its severity is dose-dependent.[154] Similar to rodents, human male infants exhibit twice as long an AGD than female, and this distance can be used as a reliable marker of human virilisation.[155] Developmental exposure to phthalates at environmental doses seems to cause reduced AGD in male infants, and severe forms of TDS are expected at higher doses of exposure.[134,156] Recently, a study on children with hypospadias and cryptorchidism indicated that these conditions are associated with reduced AGD.[157] Furthermore, in adults, shorter AGD correlates to poorer sperm quality parameters and therefore may be considered a predictor of male infertility.[158]

Other anti-androgenic ED can induce TDS in animals: exposure to different doses of VCZ during critical periods produces a wide spectrum of reproductive disorders.[159] In a study, all male rats exposed *in utero* to 20-100 mg/kg-bw/day of VCZ shown shorter AGD, hypospadias, and minute sperm counts.[139] However, these ED exert their effects through different mechanism, as VCZ acts through AR antagonism, while phthalates inhibit *INSL3* expression as well as other genes involved in testosterone synthesis.[160] Interestingly, chronic oral VCZ exposure may also alter the levels of several sperm proteins which are vital for fertilization.[161] It is believed VCZ can induce such abnormalities in humans as well.[22] Though average human ED exposure levels may be lower than those customarily used in animal studies, certain population clusters may be

exposed to higher levels.[162] Actually, occupational pesticide exposure has been connected to male infertility owing to lower sperm quality, consequent to lower testosterone levels.[135,163,164] In a recent epidemiological study, NSAID or acetaminophen consumption during pregnancy was shown to be directly related to a higher risk of cryptorchidism in male infants, and possibly other TDS traits, particularly if intake had taken place during more than one week or if there had been simultaneous ingestion of more than one of those drugs.[25] Rats exposed to 150 mg/kg-bw/day of acetaminophen during foetal development, had AGD reductions comparable to that induced by phthalates.[25]

Besides androgens, oestrogens also contribute to proper male reproductive system differentiation and fertility in adulthood,[56,165] since both androgens and oestrogens reciprocally affect AR and ER expression throughout the male reproductive system.[166] A disproportionate oestrogenic exposure early in foetal life may therefore disturb the delicate androgen-oestrogen balance, leading to infertility.[167]

ED may disrupt spermatogenesis by interfering with germ cells and spermatogenesis-supporting cells.[168] Phthalate exposure is associated with male germ cell apoptosis in rats,[168] as well as in humans.[169] Furthermore, neonatal exposure to DES and EE increases the apoptosis rate throughout spermatogenesis, in rats.[170] Testicular androgenic signalling may be compromised through several mechanisms that deregulate Leydig cell development and function.[171] Many ED, including phthalates,[77] PCB,[172] cypermethrin,[19] and EE,[24] hamper the steroidogenic response of Leydig cells by disrupting its enzymatic activity. PCB are also thought to decrease AR gene expression.[173] Phthalates may disrupt hormone synthesis by down-regulating AR, ER, and steroidogenic enzymes via an AhR-dependent pathway.[77] Some NSAID and acetaminophen lessen testosterone synthesis supposedly by reducing PG levels or through other unclear mechanisms.[174] The link between phytoestrogens and semen quality parameters is still debated.[26] Nevertheless, phytoestrogen exposure is thought to disrupt the late stages of spermatogenesis by interfering with the AR pathway.[96] Some ED are thought to be Sertoli cell toxicants. Rats exposed to DES or EE *in utero* have a reduced Sertoli cell population in adulthood.[170]. Additionally, prenatal and neonatal mice exposed to BPA lessen the expression of Sertoli cell junctional proteins, a significant component of the blood-testis barrier, which may lead to germ cell loss within the seminiferous epithelium and consequently to infertility in adulthood.[81,175] Disruption of the HPG axis, consequently reducing testicular androgen production and

signalling, was demonstrated in the rat, following exposure to an array of ED, such as DES,[170] PCB,[101] and atrazine.[18] Additionally, exposure of foetal human testis *in vitro* to dieldrin at doses far below average human body burden significantly impairs the development and function of Leydig cells, by antagonizing LH action.[21]

Deleterious effects of ED may be more severe in individuals with some genetic susceptibility, assuming the same exposure circumstances. There are AR and ER-α genetic polymorphisms that cause mild functional impairments[176,177] and are expected to lead to infertility, and possibly more severe forms of TDS, when combined with ED exposure.[130] Indeed, a correlation has been reported between cryptorchidism and specific, ED-vulnerable ER-α polymorphisms.[178] Furthermore, despite current epidemiological evidence does not support a unequivocal association between PCB and DDT and TDS features,[179] among European men with measurable serum levels of these substances, those having particular AR polymorphisms were found to have significantly inferior sperm quality.[180]

ED and the Female Reproductive System

Female fertility depends upon a functional reproductive system, capable of producing fertilizable oocytes through folliculogenesis, a multi-stage process which requires specific endocrine signals.[181] Both oestrogens and androgens are essential for maintaining HPG homeostasis, which is crucial for follicular development and ovulation, and ultimately female fertility.[182] Proper differentiation of the female reproductive system is regulated by sex hormones, particularly oestrogens, but it proceeds even in their absence, being regarded as the default developmental pathway.[183] Nevertheless, oestrogen signalling pathway overstimulation during critical developmental periods is known to result in several irreversible abnormalities.[23,184] All components of the female reproductive system are prone to ED action particularly during the developmental period.[185] The array of female reproductive disorders where ED have been implicated is fairly large, comprising endometriosis, disorders of the uterus, as well as disorders of the ovary, such as premature ovarian failure (POF), oocyte aneuploidy and polycystic ovary syndrome (PCOS), all of which may result in infertility.[11,186] Since these disorders may arise from impaired ovarian development and function, the term Ovarian Dysgenesis Syndrome (ODS) has recently been suggested as the female form of TDS.[187] The incidence of ODS traits is growing.[76]

Data concerning ED effects on female reproductive system and fertility are scant, when compared to the male counterpart, in both human epidemiological studies and experimental literature.[188] Still, a correlation between developmental ED exposure and long-term effects is suggested.[185] For instance, women whose mothers had high maternal serum concentration of DDT during pregnancy were found to have a significantly higher risk of infertility.[189] In adult women, high serum concentration of ED, such as BPA, is associated with a variety of female reproductive system disorders, including endometrial hyperplasia, PCOS, and infertility.[190] Occupational exposure to ED such as pesticides, plastics, and other industrial chemicals is a risk factor for female infertility.[191]

Many ED can potentially hamper the differentiation of the female reproductive system, both *in utero* and after birth, by disrupting the appropriate expression of oestrogen-regulated genes.[185] The development of the female reproductive system is regulated by the differential expression of *Hox* genes in the Müllerian duct.[192] Disruption of the precise chronological regulation of *Hoxa10* by ED, that either increase (e.g. BPA) or down-regulate (e.g. DES and MXC) the expression of this gene, has been shown to lead to uterine abnormalities and infertility.[193,194] In extreme cases, Müllerian agenesis may occur.[195] DES has also been found to contribute to uterine abnormalities by reducing the expression of other developmental genes, such as the *Wnt7* or *Msx2* genes.[196] Intrauterine exposure to genistein disrupts the proper differentiation of the female reproductive system, in a dose-dependent response.[197]

Endometriosis is accountable for one third of infertile women, affecting up to 10% of women of childbearing age and causing infertility in about half those women.[198] This condition is undeniably related to hormonal disturbances, and ED have recently been proposed as a possible contributing factor for the development and exacerbation of this disorder.[188,199] Indeed, a significantly higher BPA[200] and phthalate[201] serum concentration has been found in women with endometriosis. Women exposed to DES *in utero* may have an 80%-higher risk of endometriosis than unexposed women.[202] This condition can be regarded as a developmental disorder. Experimental studies support this hypothesis, as intrauterine exposure of mice to BPA[203] or TCDD[204] produces an endometriosis-like adult uterine phenotype. Even though several epidemiological studies have failed to prove an association between elevated serum levels of TCDD or PCB and the development of endometriosis,[205] a recent study shown that women with endometriosis have significantly higher concentrations of these ED in peritoneal fluid

than healthy women,[206] possibly leading to chronic inflammation, which may result in greater oestrogen synthesis, and stimulation of endometrial cells derived from retrograde menstruation.[207]

There are growing concerns about the reproductive outcomes of ovarian exposure to ED during foetal development and after birth.[208] It is largely agreed that ED can damage the testis and the ovaries in distinct ways, consistent with their differing intrinsic properties: unlike male germ cells, female germ cells are a fixed population. Therefore, exposure of hormone-responsive, primordial and preantral follicles to ED may lead to impaired folliculogenesis or even to depletion of follicular reserves, which may ultimately result in POF.[209] This is a syndrome consequent to impaired ovarian function before the age of 40, affecting about 1% of women.[210] Folliculogenesis-supporting cells, such as granulosa and theca cells, which are crucial for ovarian steroidogenesis and oocyte development, are also a target of ED.[211,212] Current data suggest chronic exposure to TCDD at environmental levels (lower than 1 ng/kg-bw/day) may induce POF in female rats, leading to low serum oestradiol levels without a substantial reduction of preantral follicles.[15] This is thought to occur chiefly through the inhibition of follicle-stimulating hormone (FSH)-stimulated LH receptor expression,[213] and the inhibition of genes responsible for steroidogenesis.[214] Some ED, such as atrazine, have been shown to disrupt folliculogenesis in adult rats, inducing follicular atresia and the development of multiple-oocyte follicles (MOF).[215] Developmental exposure to PCB has been found to negatively affect oocyte development, to induce follicular atresia and to impair oocyte quality in mice[13] and in farm animals.[216] Neonatal mice exposed to environmental doses of genistein show MOF in adulthood.[217,218] Genistein exposure in adulthood inhibits the transition of primordial to primary follicles, impairing ovulation.[219] Postnatal exposure to high doses of BPA has also been shown to increase the number of MOF in mice.[220] Furthermore, intrauterine BPA exposure has been linked to gross meiotic aberrations, leading to aneuploidy and follicular atresia.[221] Actually, an inverse correlation between BPA serum levels and oocyte quality has been suggested in women undergoing *in vitro* fertilization (IVF) procedures.[222] MOF oocytes from DES-exposed mice are less likely to be fertilized through IVF, suggesting DES adversely affects oocyte quality.[223] DDT metabolites and MXC have been shown to disrupt the early steps of steroidogenesis in granulosa cells.[224] Additionally, MXC inhibits folliculogenesis, possibly by stimulating the production of anti-müllerian hormone directly.[225] Phthalates induce oestrogen metabolism, thus may disrupt follicular differentiation.[226]

PCOS, which consists mainly of chronic anovulation and hyperandrogenemia that often lead to infertility, is a common endocrine disorder affecting 5-8% of women of childbearing age.[227] ED that stimulate hyperandrogenemia may elicit PCOS in women who have genetic susceptibility.[228] Higher serum BPA levels have been reported in women with PCOS, as compared to healthy women.[190,229] In lambs, developmental exposure to BPA and MXC leads to smaller and hindered LH surges, and consequently to anovulatory cycles, as well as ovarian abnormalities, and metabolic disorders related to PCOS.[230]

Transgenerational effects of ED

ED have been shown to disrupt the development of the human reproductive system, impairing fertility not only in the directly exposed offspring but also in subsequent generations. During the mid-twentieth century, DES was extensively prescribed for preventing miscarriages. However, DES use in pregnant women was banned in the 1970s because of increased frequency of vaginal carcinoma and uterine abnormalities later found in the daughters of those women.[23] Since then, a vast array of reproductive system abnormalities leading to infertility, among other adverse effects, have been reported in both male and female offspring of women treated with DES, and validated in experimental studies.[98,231] Recently, a French epidemiologic study has shown that pregnant women who were exposed to DES gave birth to children with reproductive system abnormalities.[232] Furthermore, grandchildren born to daughters with Müllerian abnormalities had higher incidence of reproductive abnormalities. Other ED may have transgenerational effects: the offspring of TCDD-exposed mice was found to have fertility disorders up to third non-exposed generation.[204] It has been reported that perinatal exposure to low PCB levels induces more severe outcomes in the following generation.[233] Indeed, both male and female mice exposed *in utero* to environmental levels of PCB presented impaired gamete quality and permanent morphological abnormalities of the reproductive system, and so did the following two generations.[13]

Epigenetic mechanisms may play an important role in the transgenerational effects of ED. The epigenome refers to changes made in gene expression by altering DNA structure without changing the actual genomic sequence. Epigenetic processes include DNA methylation, posttranslational histone modifications, and chromatin remodelling.[234] These changes can have temporary or durable – even transgenerational – effects.[235] ED capable of modulating genomic imprinting may induce persistent,

transgenerational changes.[236] Epigenetic changes of some genes, such as the AR gene, transmitted through female germ line, could explain the transgenerational effects of DES.[232] BPA, phthalates, and VCZ have been shown to alter gene expression and imprinting patterns in mouse embryos.[237] In experimental studies, epigenetic modifications of gamete DNA at imprinted loci triggered by ED exposure affected the non-exposed following generations, through either the male or the female germ line. Transient developmental exposure of male rats to VCZ and MXC during the epigenetic-reprogramming stage was shown to induce shorter AGD and to adversely affect spermatogenesis, leading to infertility; these effects were transmitted through the male germ line to almost every male up to the fourth generation.[235] More recently, an industry-sponsored research group failed to report transgenerational effects of VCZ.[238] Perinatal exposure to BPA can induce the down-regulation of AR and ER gene expression, as well as co-regulator genes, leading to impaired spermatogenesis; these modifications may be imprinted on the germ line, causing abnormal reproductive function up to the following two generations, which were not exposed to BPA.[239]

Current data suggest epigenetic modifications induced by ED may have drastic adverse effects on human fertility, as the entire reproductive system is very sensitive to these compounds.[185,234] In particular, male germ cells are the most vulnerable cells, as they have distinctive methylation patterns and epigenetic markers.[234] So far, no major progresses have occurred in the medical management of poor sperm quality. As the current assisted reproductive techniques such as IVF and intracytoplasmic sperm injection do not necessarily address the underlying problem, its escalating use, regardless of cause, may accidentally convey serious genetic anomalies.[9] Thus, through epigenetic changes, ED can perhaps have negative evolutionary effects, by permanently altering DNA structure, hence facilitating genetic mutations.[240]

Synergistic effects of ED

In order to study the detrimental effects in the development of both male and female reproductive system, most experimental studies have used a single ED during short exposure periods, frequently during critical developmental stages, in doses that sometimes exceeded environmental levels and human expected body burdens. However, humans and wildlife are constantly exposed to countless ED simultaneously, generally at low levels, which may act synergistically to produce endocrine disruption at doses far below individual LOAEL, if there is enough overall exposure.[241] Indeed, sheep exposed

to sewage sludge, which contains many known ED, shown impaired GnRH expression.[242] Additionally, a study addressing the adverse effects of developmental exposure of rats to a mixture of diverse-acting anti-androgenic ED, including VCZ and phthalates, has shown cumulative synergistic effects regarding the incidence of hypospadias and cryptorchidism.[243] Furthermore, it has been shown that more than half the mice exposed *in utero* to a mixture of AR antagonists, including VCZ, developed hypospadias at individual drug doses that would not have caused hypospadias at all.[244]

In view of recent evidence, a number of brief high-dose exposures to customary drugs, as NSAID or acetaminophen,[25,174] on top of the potential long-lasting hormonal disruption by ubiquitous ED, particularly during critical developmental periods, are suspected to have a serious impact on human reproductive health. Additionally, it is hypothesized that even low levels of oestrogenic ED, particularly phytoestrogens, may be capable of altering cell responsiveness to other ED, such as anti-androgenic ED, thereby inducing wider negative effects when there is concomitant exposure.[141] In this matter, a mixture of genistein and other ED has also been found to elicit a quicker capacitation and acrosome loss in human spermatozoa *in vitro*, rather than each compound alone, rendering them unable to complete fertilization.[245]

Moreover, a study in rats shown chronic ingestion of a low-dose genistein and VCZ mixture (at 1 mg/kg-bw/day each), from conception through adulthood, brought forward more severe changes on the male reproductive system and lower semen quality compared to individual exposure to each of these ED, at the same dose, suggesting genistein may potentiate the effects of VCZ.[246] In contrast, there are studies reporting genistein may reduce the detrimental effects of VCZ on male germ cells, using the same experimental procedure from conception to birth.[247]

Altogether, different ED may have cumulative effects on the same physiological process or intensify the effects of one another through diverse and distinct mechanism of action, leading to more severe consequences than previously ascertained. Yet, their net effects may be reciprocally invalidated, depending on the exposure period (either chronically or during critical developmental stages), as well as individual and global exposure doses. All in all, chronic exposure to a mixture of ED may induce worse outcomes than a single high dose exposure, thus may seriously affect human reproductive system differentiation and contribute to human infertility.

Conclusion

This paper has reviewed and summarized the existing evidence regarding ED as contributing agents to the rising rates of human infertility. Although the number of studied and mentioned ED is not comprehensive, an adequate amount of data has accumulated demonstrating that ED may have deleterious effects on human fertility via numerous mechanisms. ED may be blamed for the rising incidence of human reproductive disorders, and may also explain some idiopathic infertility cases, both in men and women, although the influence of already well-known lifestyle-related aspects[248] (such as alcohol consumption during pregnancy as well as parental smoking) on human fertility should not be neglected.

Endocrine disruption is a serious Public Health problem that must not be ignored. Legislation concerning ED must take into account current scientific knowledge.[249] Additionally, regulatory authorities should endorse preventive measures regarding the exposure to ED, such as limiting the utilization of ED in industry worldwide. Meanwhile, the general population might reduce ED exposure by following some simple yet important advices, such as (a) choose glass over plastics, (b) avoid using the same plastic containers repeatedly or plastic wrapping to microwave food, (c) reduce consumption of fatty animal products, (d) prefer pesticide-free vegetables and fruits, and (e) avoid excessive utilization of cosmetics and other personal care items, particularly during pregnancy. Precautionary avoidance of exposure to well-known and putative ED is a prudent attitude.

Further research is needed to assess the use, persistency and latent risks of the known ED (even those banned decades ago), in addition to identify potential endocrine-disruptive activity in other chemicals aiming to discourage their continued use, in order to protect wildlife and humans, particularly developing foetuses, neonates and young children, from permanent effects on fertility. Prospective epidemiological studies on this subject should be performed. For instance, it would be important to examine adult fertility and hormonal parameters of infants fed soy-based formula or cow milk formula-fed using baby bottles made of different substances, as opposed to breastfed infants; as well as of infants inadvertently exposed to contraceptive hormones during the early foetal development.

Future research agenda should focus on assessing the dose-effect curve of known ED even at minute concentrations, and also on measuring the impact of mixed exposure of

low doses of different ED and their possible effects on future generations, relating to genetic polymorphisms, especially during gametogenesis and foetal development.

Acknowledgements

The authors wish to thank Paula Freitas, MD, PhD, for helpful comments on the manuscript.

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Apêndice

Table 1. Reported agonist and antagonist binding of several ED to ER and AR

ED	ER agonism	ER antagonism	AR agonism	AR antagonism
PCB	Takeuchi *et al.*, 2011[31]		Svobodová *et al.*, 2009[32]	Takeuchi *et al.*, 2011[31]
PBDE	Hamers *et al.*, 2006[33] Liu *et al.*, 2011[34]	Hamers *et al.*, 2006[33] Liu *et al.*, 2011[34]		Hamers *et al.*, 2006[33] Liu *et al.*, 2011[34]
BPA	Kuiper *et al.*, 1998[35]			Fang *et al.*, 2003[36]
Phthalates	Jobling *et al.*, 1995[37]			
Cypermethrin	Kojima *et al.*, 2004[38]			
DDT	Kuiper *et al.*, 1998[35] Kojima *et al.*, 2004[38] Lemaire *et al.*, 2006[39]			Kelce *et al.*, 1995[40] Kojima *et al.*, 2004[38] Lemaire *et al.*, 2006[39]
Dieldrin	Kojima *et al.*, 2004[38] Lemaire *et al.*, 2006[39]			Kojima *et al.*, 2004[38] Lemaire *et al.*, 2004[41]
MXC	Kuiper *et al.*, 1998[35] Gaido *et al.*, 2000[42] Kojima *et al.*, 2004[38] Lemaire *et al.*, 2006[39]	Gaido *et al.*, 2000[42] Kojima *et al.*, 2004[38] Lemaire *et al.*, 2006[39]		Gaido *et al.*, 2000[42] Kojima *et al.*, 2004[38] Lemaire *et al.*, 2004[41]
VCZ	Lemaire *et al.*, 2006[39]			Kojima *et al.*, 2004[38] Orton *et al.*, 2011[43]
DES	Kuiper *et al.*, 1998[35]			
Phytoestrogens	Kuiper *et al.*, 1998[35] le Maire *et al.*, 2010[44]			

AR: androgen receptor; BPA: bisphenol A; DDT: dichlorodiphenyltrichloroethane; DES: diethylstilbestrol; ED: endocrine disruptors; ER: oestrogen receptors; MXC: methoxychlor; PBDE: polybrominated diphenyl ethers; PCB: polychlorinated biphenyls; VCZ: vinclozolin

Anexo

INSTRUCTIONS TO AUTHORS
(http://www.hormones.gr/4/instructions-to-authors.html – 01/03/2013)

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ABSTRACT

For research papers the abstracts must be structured: OBJECTIVE, DESIGN, RESULTS, CONCLUSIONS. Not exceeding 200 words (not necessary for review articles)
A structured abstract is not necessary for review articles. Describe briefly the background, the aim of the study or the hypothesis tested, the methods used, the results and the conclusions.

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Evans WJ, Campbell WW, Nikolaou O, et al, 1993 Sarcopenia and Age-related changes in body composition and functional capacity. J Nutr 123: Suppl 2: 465-468.
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