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Estrogenic endocrine disruptors present in sports supplements. A risk assessment for human health

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ABSTRACT

Sports supplements are becoming a regular dietary addition for consumers who view such products as a means of improving their health and performance. Previously estrogenic endocrine disruptors (EDs) were detected in 80% of 116 sports supplements investigated by biological *in vitro* reporter gene assays (RGAs). The aim of this study was to quantify the hormonal activity in 50 of these sports supplement samples using a validated estrogen RGA and perform an exposure and risk assessment for human health.

Results showed that 17β-estradiol equivalent levels were higher than those reported as being present in the typical human omnivore diet in 33 of the sports supplements and higher than the acceptable daily intake (ADI) in 13 of these products. The highest activity samples presented a potential to influence the human daily exposure to 17β-estradiol like activity in various risk groups with a predicted hormonal impact of greatest concern in young boys and postmenopausal women.

In conclusion, consumers of sports supplements may be exposed to high levels of estrogenic EDs.

1. Introduction

Estrogens are important biologically and physiologically as they play an essential role in reproduction, cardiovascular health, bone integrity, cognition, behaviour and gastrointestinal systems (Deroo & Korach, 2006; JECFA, 2000; Rosen & Marcelle, 2007). The direct role of 17β-estradiol, the most potent naturally occurring estrogen, is the growth and development of the reproductive tract and female secondary sex characteristics (Rosen & Marcelle, 2007). The implication of estrogens in the development or progression of numerous diseases, such as cancer, osteoporosis and cardiovascular disease, has also been reviewed (Burns & Korach, 2012; Deroo & Korach, 2006).

An endocrine disruptor (ED) is any compound that interacts with and disturbs the endocrine system. This occurs through various mechanisms, such as mimicking natural hormones or blocking their receptors (Tabb & Blumberg, 2006). Exposure to EDs may lead to adverse health effects (Bergman et al., 2012).

The Joint FAO/WHO Expert Committee on Food Additives (JECFA) have evaluated 17β-estradiol using various toxicological studies and observations in humans, including epidemiological studies of women exposed to postmenopausal estrogen therapy and hormonal contraceptives (JECFA, 2000). Due to the specificity and affinity of 17β-estradiol to its nuclear receptors, the hormonal effects of 17β-estradiol were seen to occur at much lower doses than for the other toxicological responses. On this basis JECFA have established an ADI of 0–50 ng/kg bw. This evaluation takes into account a no-observed-adverse-effect-level (NOEL) of 0.3 mg/day (equivalent to 5 μg/kg bw/day) in studies of changes in several hormone-dependent parameters in postmenopausal women, safety factor of 10 to account for normal variation among individuals and an additional factor of 10 to protect sensitive populations.

Epidemiological studies, based on women receiving postmenopausal estrogen replacement therapy, reported an increased incidence of breast and endometrium cancers (Rossouw et al., 2002). Polycystic ovary syndrome, one of the leading causes of

Abbreviations: Abs, absorbance; ABW, average body weight; ADI, acceptable daily intake; bw, body weight; DDT, dichlorodiphenyltrichloroethane; DMEM, Dulbecco's modified Eagle medium; DMSO, dimethylsulphoxide; ED, endocrine disruptor; EEQ, 17β-estradiol equivalent; HLB, hydrophilic–lipophilic balance; HPLC, high performance liquid chromatography; HPLC–MS, high performance liquid chromatography–MS; JECFA, Joint FAO/WHO Expert Committee on Food Additives; LOD, limit of detection; MTT, thiazolyl blue tetrazolium bromide; PBS, phosphate buffered saline; RGA, reporter gene assay; SHBG, sex hormone binding globulin; SPE, solid phase extraction.

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female infertility, together with the timing of menarche has been considered as an adverse effect of estrogen exposure by the Institute for Environment and Health (2004) and Tsutsumi (2005). Infertile women commonly suffer from endometriosis which has been linked with exposure to xenoestrogens that are found in the environment (Heilier et al., 2005). The European Commission has also reported that exposure to EDs may result in fibrocystic disease of the breast, uterine fibroids and pelvic inflammatory disease (EC, 2004). Clinical and laboratory research summarised by Toft (2009) suggests that exposure to EDs results in a reduction of testosterone secretion and may influence semen quality and quantity (Zhang, Zheng, & Chen, 2006) as well as be responsible for testicular cancer, hypospadias and cryptorchidism in adulthood (Fowler et al., 2007).

Phytoestrogens are mainly known for their beneficial effects on different types of cancers. Adlercreutz (2002) suggested that a diet rich in phytoestrogens may protect against breast and prostate carcinoma. However, there is an increasing interest in the possible adverse effects of these estrogenic compounds of plant origin. The stimulation of breast tumour cell proliferation by phytoestrogens was reported by Hsie, Santell, Halsam, and Helferich (1998). Jefferson and Newbold (2000) reported that the action of estrogenic compounds present in soy induced detrimental effects on the foetal reproductive system and hypospadias in male offspring. The agonistic or antagonistic effects of the phytoestrogens may be caused by their different interaction with estrogen α and β receptors as described by Dip et al. (2008). Sakamoto, Horiguchi, Oguma, and Kayama (2010) reported that diverse dietary phytoestrogens may differentially effect, stimulate or inhibit cell growth, apoptosis and cell cycle.

When assessing the risk of estrogenic EDs to human health, it must be remembered that not only is the vulnerability of the consumer, the type of ED (natural or synthetic), the concentration, duration and frequency of exposure, its metabolism, distribution and elimination important critical parameters, but also that the mixture effect of different compounds must be considered (Kashuba & Nefziger, 1998).

Most of the documented adverse effects of ED exposure in humans relate to high level exposure of individual hormonally active contaminants. Much less data is available on the effects of "low level cocktails" (Rajapakse, Silva, & Kortenkamp, 2002). Recently however, studies assessing the effects of exposure to low levels of environmental endocrine disrupting pollutants have begun to emerge (Fowler et al., 2007). It is crucial that low level exposure to real life mixtures be further assessed.

Numerous routes of ED exposure exist for humans, including contaminated air, water and soil. However, it is widely accepted that one of the most important routes is through our diet (Connolly, 2009; Institute for Environment and Health, 2004).

Hormonal compounds in food may have different origins, including naturally occurring estrogens and phytoestrogens (found in milk, meat or plants). Xenoestrogens or environmental chemicals (bisphenol A, dichlorodiphenyltrichloroethane (DDT), diethylstilboestrol and phthalates) can also contaminate food through contact or bioaccumulation and biomagnification in the food chain (Thomson, 2009). Hormonal compounds can also be added accidentally or illegally to food products during the manufacturing process in facilities which also frequently produce hormonally active substances (Judkins, Teale, & Hall, 2010). It has also been suggested that hormonally active steroids may be formed as by-products during the synthesis of inactive prohormones, resulting in widespread steroid contamination of dietary supplements (Van Poucke, Detavernier, Van Cauwenberghe, & Van Peteghem, 2007). Recently, a number of studies have reported that health food supplements are a high risk foodstuff with the potential to contain hormonally active constituents (Geyer et al., 2004; Plotan, Elliott, Oplatowska, & Connolly, 2012; Plotan et al., 2011). The consumption of these products is progressively expanding among the general public and athletes (Koncic & Tomczyk, 2013; Van Thuyne, Van Eenoo, & Delbeke, 2006), due to their advertised health, physical appearance and performance improving potential. The use of supplements in the general population has been reported to be as high as 40% (Perko, Bartee, Dunn, Wang, & Eddy, 2000). Even greater use is reported for athletes, both amateur and professional, with 44–100% prevalence, dependent on age, gender, level of competition and the type of sport (Maughan, Depiesse, & Geyer, 2007). Therefore, the need to fully understand the potential risks as well as the benefits from consuming such food products for the consumer is essential.

The aim of the present study was to quantify estrogenic activity and perform a risk assessment on 50 sports supplements selected as the samples with the highest estrogenic activity observed from 116 sports supplement samples screened previously (Plotan et al., 2012, 2011). The risk assessment was undertaken by comparing the levels of exposure from the samples with the established 17β-estradiol ADI and estimated daily consumption of estradiol through drinking water and the omnivore diet. The study also aimed to predict the potential influence of the highest activity samples on the human daily exposure to 17β-estradiol like activity (as the endogenous production of 17β-estradiol and daily ingestion of exogenous 17β-estradiol) for various consumer groups (based on age and gender) through exposure to these levels.

2. Materials and methods

2.1. Reagents and chemicals

Reference standards including 17β-estradiol, genistein, daidzein and equol were supplied by Sigma (Poole, Dorset, UK). All cell culture reagents were obtained from Invitrogen Ltd (Paisley, UK). Falcon tissue culture flasks were supplied by BD Biosciences (Oxford, UK). A luciferase kit (Promega E1500) was obtained from msc Ltd, Ireland. Specialised white walled and clear flat bottomed 96 well plates used in the reporter gene assay (RGA) were supplied by Greiner Bio-One (Stonehouse, UK). Dispersive SPE citrate extraction tubes and PSA/ENVI-Carb SPE clean up tubes together with sodium acetate, glacial acetic acid, tert-butyl-methyl-ether and high performance liquid chromatography (HPLC) grade water were obtained from Sigma (Poole, Dorset, UK). Oasis hydrophilic–lipophilic balance (HLB) glass cartridges (5 ml volume; 200 mg sorbent) were supplied by Waters Chromatography Ireland (Dublin, Ireland). Methanol and acetone were obtained from BDH (Poole, Dorset, UK) and acetonitrile from Analab (Lisburn, UK).

2.2. Sports supplements

Previously 116 sports supplements were analysed for estrogenic activity (Plotan et al., 2012, 2011). The products were obtained from various retail outlets across the island of Ireland (including gyms, sport centres, supermarkets, health food shops, pharmacies and the internet) and included various forms such as powders, tablets, capsules, liquids, bars and injections. Samples included commonly used sports supplements such as creatine monohydrate, whey protein, DHEA, power and protein bars, iso energy drinks and body building tablets. Various ingredients were listed including proteins, carbohydrates, amino acids, vitamins, minerals, plant and herbal extracts.

2.3. Exposure assessment

Fifty of these products, presenting a positive estrogenic response and providing information regarding the recommended