Background

The incidence of cancer among Asians who consume diets rich in soy (~1.5 mg/kg/day) is significantly lower than that of Americans whose diet contains minute or no amount of soy (~0.2 mg/kg/day) (1,2,3). However, second-generation Asians who consume less soy products after immigrating to the U.S. have cancer rates similar to those of Americans, suggesting that soy consumption may be cancer-preventive (4). Soy foods have been known for their other potentially beneficial uses in our health, including their role in osteoporosis treatment and prevention of cardiovascular diseases (5). Therefore, the public has perceived soy products as safe, despite the accumulating scientific data that challenges this perception (6). For several years, scientists have been concerned about the potential risks of early soy exposure on reproductive health (1,2,6,7,8), particularly in light of a recent study that determined the plasma concentration of soy proteins to be alarmingly high in soy-fed infants (9).

Most infants in the U.S. are fed formulas by 2 months old, and the number of infants consuming soy formulas has doubled in the last decade (10). Although soy has been part of Asian infants' diet for centuries, the use of soy foods for infants in the U.S. began only in the early 1900s (10). Commonly, infants are fed soy formulas because their mothers cannot breast-feed them and/or they cannot consume cow milk (lactose-intolerance or allergy). The use of soy formulas for these infants was recommended by the American Academy of Pediatrics (AAP) as "safe and effective alternatives," because several studies have found soy formulas to be as effective as maternal breast milk and cow milk in supporting normal growth and development (10,11,12). Despite these studies, the high amount of phytoestrogens present in soy formula has cast doubts over the safety of its use as a main source of nutrients for infants and its effect on their reproductive health (1,2,6,7,8).

Isoflavones in soy formulas

Phytoestrogens are heat-stable compounds and can survive through the usual processing methods that extract soy protein isolates (SPI). Genistein and daidzein, the major isoflavone phytoestrogens in SPI, can exist in either unconjugated (aglycones) or conjugated (glycosides) forms. Aglycones are biologically active whereas glycosides are less so. Although aglycones make up < 10% of the isoflavone content in soy formulas (5,9), they can be hydrolyzed into active forms by the intestinal bacteria present in the stomach of 4 month-old infants (13) or even younger infants (the latter has not been confirmed yet). Infants can efficiently metabolize and absorb isoflavones, as evidenced by isoflavones found in the plasma and urine of soy-fed infants (7). It was recently determined that the total isoflavone consumption by soy-fed infants is 28-47 mg/day or 4.5-8 mg/kg/day (7). This dosage/body weight is several times larger than the amount that has been shown to cause changes in women's menstruation cycles (14). In addition, the total plasma concentration of isoflavones in soy-fed infants averages at ~1000 ng/mL, which is not only much higher than that found in Japanese adults who consume soy-rich diets, but also 13,000 to 22,000 times higher than that of plasma estradiol in early life (9). In humans, high concentrations of estrogen is not seen until after puberty, but even the

plasma estrogen level achieved during the estrogen surge in the menstrual cycle is 3000 times less than the plasma concentration of isoflavone found in soy-fed infants (2). The phenolic ring in isoflavones (a structural characteristic also found in estrogen) allows isoflavones to bind to estrogen receptors (ER) and to mimic estrogen-like activities (5). Although isoflavones have a lower affinity for ER (genistein is 1000 times less potent than 17b-estradiol in binding to ER), the high plasma concentration of isoflavones circulating in soy-fed infants does not rule out their potential ability to exert significant physiological effects. Depending on their concentration, affinity to ER, target tissue types, and other conditions, isoflavones can also have anti-estrogenic effects (5,15,16). However, the focus has been on the ways in which isoflavones may influence health via their estrogenic-like activities, such as improving health in estrogen-deficient conditions (menopause) and/or inhibiting estrogen-dependent developments that lead to cancer by competing with endogenous estrogen (5,17). Similarly, their estrogenic behavior may exert harmful health effects if human exposure to them is abnormally high and/or occurs during inappropriate developmental periods. The possibility for the latter is supported by a well-known case in which millions of women who were given large doses of diethylstilbestrol (DES, a potent synthetic estrogen) during pregnancy gave birth to offspring that developed reproductive abnormalities/disorders later in life (18). This case illustrates that biochemical events occurring during critical periods of development can have long-lasting consequences that may not be expressed until later in life. The same can be speculated about the potential effects exerted by the high levels of isoflavones in soy-fed infants. The remainder of this paper will examine the human data and animal studies on the effects of early isoflavone exposure, with an emphasis on reproductive health.

Soy consumption and reproductive health in humans

Scientists who argue against the association between life-long soy consumption and its harmful effects on human reproduction often cite the lack of adverse reproductive effects seen in Japanese populations (2). However, this evidence does not exclude the possibility that early exposure, such as during months after birth, to isoflavones may have detrimental reproductive consequences because most Japanese infants are breast-fed between birth and weaning and would have lower exposure to soy proteins than soy-fed infants (2). Due to a recent study that demonstrated the ability of isoflavones to be transported from the maternal to the fetal compartment (19), a hypothesis was raised regarding the harmful effects of prenatal soy exposure. However, scientists challenging this hypothesis often referred to the lack of soy-food related adverse effects seen in newborns born to Japanese women as evidence for their position (2). It should be recognized that the amount that a Japanese fetus is exposed to prenatally is small in comparison to that ingested by soy-fed infants (plasma concentration of 225 nmol/L in amniotic fluid vs. 7000 nmol/L in soy-fed neonates) (9,19). Therefore, the amount of isoflavones transported to the fetal compartment may have been insufficient to elicit adverse effects in Japanese fetus, and the effects of prenatal exposure cannot be ruled out for a fetus of a pregnant women who ingests larger quantities of soy foods than a typical Japanese woman because of a belief that soy proteins could provide health benefits for her future child.

Controlled studies on the delayed effects of early soy exposure in humans have been very scarce. One such study interviewed 811 young adults who participated in a formula feeding study as infants (7). Data was collected on 30 measurements, such as self-reported pubertal maturation, menstrual and reproductive history, pregnancy outcomes, etc. Only a few significant differences were found between the cow-milk and soy-formula groups: soy-fed females experience "slightly longer duration of menstrual bleeding" and "greater discomfort with menstruation." Other reproductive measurements such as pregnancy outcomes and reproductive function were not significantly different between the two groups. However, the authors' conclusion that there is "no systematic cause for concern" over the safety of soy formula seems premature, because there are several problems with this study that can potentially confound their findings. For example, there was an imbalance between the two groups with respect to age (the soy-formula group consisted of the youngest and oldest participants). The two significant results reported may actually reflect more serious conditions such as endometriosis or uterine fibroids, and the study may not have comprehensively assessed fertility (e.g. more sensitive measurements such as time to pregnancy and use of infertility treatments were not used) (8). Other pregnancy outcomes that did not reach significance may be important and require re-evaluation (e.g. slightly more preterm or stillborn deliveries and multiple births in soy-formula group). Lastly, the sample size was too small and the participants were too young to draw any solid conclusions regarding fertility and soy consumption in infancy, which leaves the question regarding the safety of soy-formula consumption still unanswered.

Animal studies

Many animal studies have investigated the relationship between isoflavone exposure and reproduction, but the results have been inconsistent across the studies. Interestingly, early and life-long exposure to isoflavones have been found to be cancer-preventive in rats, but the treatments also caused an earlier onset of puberty (vaginal opening occurring 1 day sooner) while other reproductive developments such as reproductive organ weight remained normal (2,3). Acceleration of puberty was also found in another study in which rats were exposed to genistein pre- and postnatally until puberty (20). In another set of studies, large doses of genistein injected into mouse neonates exhibited cancer-preventive potential, but caused the mice to have abnormal ovarian follicular development and estrous cycle (21).

Isoflavones may exert different effects in male- and female-reproductive development. Adverse changes in female reproductive processes were observed in a study in which pre- and postnatal exposure to genistein reduced the ovaries and uterus of female rats (22), but the same treatment did not adversely affect the gametogenic function in male rats (e.g. normal testis weight and testicular sperm count) (23). Similarly, a recent study (but genistein exposure was limited to days 1-5 postnatally) showed "reproductive toxicity" in females, such that irregular estrous cycle and changes in the tissue of ovaries/uterus were observed in female rats, whereas no reproductive effects were seen in male rats (1). Although Jefferson et. al. demonstrated the ability of genistein to affect female reproductive development, uterine-weight increase rather than decrease was found in female rats that were given genistein subcutaneously for three consecutive days as

immature rats (24). The conflicting results in uterine-weight changes between the studies may be due to the difference in the duration of genistein exposure (pre- and postnatal vs. just postnatal alone) (22,24). Another important finding in Jefferson et. al.'s study is that the presence of multi-oocyte follicles in the ovary is greater in the genistein-treated animals than those treated with DES, which can be accounted for by their different affinities for the different types of ER (24).

Conclusion

Most animal studies have shown that early exposure to isoflavones can affect reproductive development, but the effects found are often inconsistent and complicated. One reason for such inconsistency may be that the parameters that can alter the effects of isoflavone in animals were not always employed similarly across these animal studies. Examples of such parameters include the time when the animals are exposed to isoflavones (e.g. prenatal, neonatal, or both), the exposure duration, the type of isoflavones used, the dosage given, and the administration method. Since human infants and animal neonates used in research may not be at the same stages of development, these animals may be a poor estrogen model for human infants (25). Not surprisingly, it is difficult to apply the results of animal studies to human infants and to draw any solid conclusions based on these studies.

Soy-based infant formulas have been on the market for only 30-40 years, which means the children who consumed these formulas are starting to have children now (24). Perhaps ,the long-term effects from early soy consumption are beginning to surface. Although there are some human data supporting the safety of soy-based infant formulas (e.g. normal weight gain and development), the biochemical mechanisms involved and the effects of early soy consumption specifically on reproductive health are still unclear. Soy formulas are unquestionably lifesaving alternatives for infants whose nutritional needs cannot be met by cow- and breast-milk. However, more research is needed to take us a step closer to resolving the conflicting data and reaching a consensus on the safety of soy formula consumption by infants. As Dr. Lynn Goldman aptly writes, "Just as scientists should avoid insupportable allegations, they should also avoid absolute declarations of safety in areas whose risks have yet to be assessed" (8). Until more conclusive data is available, parents should be cautioned against using soy-based formulas as the main source of nutrient for their babies who are able to receive nutrients from cow- and/or breast-milk.

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