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REPLY

Intracellular receptor regulation of adipose metabolism by the isoflavone genistein

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In the last few lines of his letter, Dr. Chirumbolo concludes that the complex action of genistein in the modulation of adipose metabolism and growth is dependent on several factors among which the most relevant are its plasma concentration, its nuclear receptor-mediated signaling and the gender. I would add that the dietary background is itself a factor that may shift the variable effect of genistein on adipose metabolism from negative (inhibitory control) to positive (stimulation), influencing the gut microbiota and intestinal metabolization, absorption and bioavailability.

To investigate the effect of genistein on the adipose metabolism, experimental laboratory models have been established to reproduce the consumption of amounts of genistein as they are occurring in populations eating moderate-to-high quantity of soy or soy-derived products [1]. Dietary amounts of genistein are present in standard soy-containing rodent diets at average concentrations of 200 ppm which produce serum levels of about 70 ± 9 nM. This falls between the serum concentrations obtained in mice fed genistein at the nutritional doses of 500 and the 5,000 μg/kg/day on an estrogen-free dietary background (66 ± 7, 74 ± 7 nM). At these doses, genistein induces a significant increase in the epididymal and renal fat pads of male mice. The same doses do not affect either the abdominal or the renal fat pads of intact females, thus indicating gender-specific effects of this isoflavone on adipos metabolism. Higher serum levels of genistein produced by feeding doses higher than 50,000 μg/kg/day inhibit adipose deposition [2, 3] in male and female mice, and at 200 mg/kg/day, it shows an inhibitory effect comparable to that observed with the natural estrogen estradiol [4].

In the attempt to interpret the dose-dependent variations in adipose weight as are described by a Gaussian curve in mice fed genistein in the range of 50–200,000 μg/kg/day [4, 5], we may look for a correlation with the dose-dependent transcriptional regulation curve that is driven in vitro by ligand-activated nuclear receptors [6]. Genistein is an affinity ligand for a few NRs (ERs, AR, PPARg, LXRs and PXR and membrane-bound ERs and AR) [7–10], and the dose of this isoflavone together with its different affinity for different NRs is a critical element for its ability to regulate receptor-mediated cellular processes. Among the mentioned receptor systems which recognize genistein, the most characterized beyond the ERs, is PPARγ. PPARγ is a central switch for adipose metabolism. It does its work also by regulating estrogen-dependent modulatory functions through a negative cross-talk with the ERs [11]. Through regulating both the ERs and PPARγ, genistein can alter cell functions and modulate systemic homeostatic controls [12]. Experimental in vivo data indicate that the ERs and PPARγ-mediated transcriptional regulation of genistein depends on the dose and the tissue [4–6] and the different affinity for these receptors. Studying the adipose tissue in mice we have observed that at low doses, genistein induces enzymes directly involved in lipid storage such as the lipoprotein lipase (LPL), an effect that it is likely to be produced through the activation of PPARγ, a regulator of LPL. At higher supra-nutritional or pharmacological doses, it strongly inhibits the same enzymes, including LPL through a mechanism likely involving the ERs since its effects are very similar to that elicited by estradiol although not targeting the same promoters ([4], and the paper under discussion

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from Zanella et al.). This would suggest that genistein is alternatively a promoter and/or an inhibitor of fat accumulation, depending on the extent of intake, effects that are themselves modulated by the fat concentration of the background diet.

There are solid evidences that PPARγ activated by adipogenic signals may counteract estrogen signaling and that estrogens regulate PPARγ action [11, 13]. We believe that this is a critical point for the balanced action of genistein through these NRs. The ER–PPARγ interference has been shown to occur through different mechanisms. First, activated PPARγ can bind to the ERE on estrogen target promoters. Through this DNA binding, which depends on the structure of the ERE in target promoters, PPARγ interferes with the ERs and the expression of ER-regulated genes. The PPARγ transcription complex (PPARs/RXRs) can bind several ERE-related palindromic response elements containing AGGTCA half-sites, but does not activate transcription due to a non-permissive promoter structure [14]. Inhibitory action of PPARγ versus ERα has been observed in different cell systems. In MCF-7 breast cancer cells, Suzuki et al. [15] showed by DNA microarrays analysis that PPARγ activation by agonists such as 15d-PGJ2, GW1929 or ciglitazone, suppresses estrogen-dependent transactivation by estradiol in a dose-dependent manner, an effect observed in the presence of constant cellular levels of ERα. Conversely, inhibitory actions of estrogens on PPARγ signaling have also been evidenced. ERα can bind to PPAR-responsive elements and can repress PPARγ transactivation through physical and functional interactions which involve the p85 regulatory subunit of PI3 K causing an opposite effect on the regulation of the PI3/AKT transduction cascade [16]. Cooperative cross-talks have also been found between the ERs and PPARγ where the PPARγ/RXR complex activates ERE-dependent transcription in CV-1 cells [17].

ER–PPARγ cross-talk has also been shown where genistein acts as a selective ligand for ERß, through which it reduces body weight, cholesterol, leptin and glucose [13]. Another study reported that ERß engages a negative cross-talk with PPARγ which involves the abrogation of PPARγ coactivation by PPAR-gamma coactivator 1-alpha (PGC-1a) and other key factors [18]. Former works also show a competition between the ERs and PPARγ through a direct involvement of the ER DNA-binding domain [19]. The existence of the ER–PPARγ antagonism may justify the hypothesis that the recruitment of both receptor systems in fat cells is functional to their response to genistein. Our in vitro results suggest also a productive cross-talk exerted by genistein-activated ERß and PPARγ on cell differentiation probably through binding of ERß to the transcription regulatory complexes at the PPARE. Mechanistically, the competitive or cooperative cross-talk between the two receptor systems may involve the squelching or condivision of tissue-specific coregulators which are differently espressed in differentiated versus undifferentiated cells, influencing ER and PPARγ functions on lipid metabolism. Based on the available data, we interpret our results in mice by hypothesizing that low doses of genistein are adipogenic through the involvement of PPARγ and that only at higher doses, PPARγ is counteracted by the activation of the ERs which take over in their competition with PPARγ and inhibit adipogenesis. This was not reproduced in vitro in the paper from Zanella et al. because of the limited-dose range of genistein used in 3T3-L1 cells, although recent data from our laboratory show an inverted bell-shaped curve on PPARE and ERE reporters if we extend the dose range of genistein.

We may also try to connect the observations related to the doses of genistein to its sex-dependent effects in mice and deduce that genistein in females is less efficient due to the presence of higher circulating levels of estradiol that, through the ERs, counteracts genistein's action on adipogenic genes. Transferring these observations to humans, we may expect that blood levels of phytoestrogens in combinations, following diets rich in isoflavones, lignans, coumestans, stilbenes, etc., create a ER-low affinity estrogenic load that may be relatively inefficient during fertile age because of high estradiol levels, while it might be more efficient when estradiol decreases at menopausal age or in males, contributing to a healthy metabolism. However, the effect of dietary phytoestrogens can be different in ERs rich tissues such as the endometrium, the breast tissue, etc., where these compounds may provide tissue-specific estrogenic activity in addition to estradiol at all ages due to a higher concentration of intracellular receptors.

To this picture, our recent work in vitro adds the evidence that genistein effects on lipid accumulation and hormonal response in 3T3-L1 cells is dependent on the state of cell differentiation, being very different in adipocytes versus fibroblasts. A key factor for both these actions appears to be ERß, for which genistein shows higher affinity than for ERα [20]. The transcriptional activation of a consensus PPARE and the ap2 promoter by genistein requires ERß in 3T3-L1 adipocytes, and in the same differentiated cells, ERß also upregulates an ERE reporter, while it is not active in undifferentiated cells. This would suggest that the adipocyte is better responsive to the estrogenic action of genistein than fibroblasts. In mature adipocytes, the action of genistein differs from that of E2 which instead is not active at the tested doses, while activates both ERα and ERß only in preadipocytes. It appears that the activation of the ERs in 3T3-L1 cells is dependent on the state of cell differentiation and the specific ligand since, at least in this model, the mature adipocyte somehow loses or decreases its responsiveness to estradiol while gaining responsiveness to genistein. More data from experiments

investigating ligand-dependent transcription activation of ERs and PPARγ relating to receptor co-regulators specifically expressed/repressed during cell differentiation should be produced to clarify the mechanisms involved.

In conclusion, amounts of isoflavones as low as those that are present in soy-containing diets have the potential to regulate fat development and deposition through mechanisms that involve NRs, and since our recent data [21] show that this effect is not associated with alterations of metabolic parameters, it may reflect physiological changes of a healthy development.

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