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Review

Absorption, distribution, metabolism, and excretion of isoflavonoids after soy intake

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ABSTRACT

Soy is the major source of dietary exposure to isoflavonoids (IFLs). Accumulating evidence supports a role for soy and IFLs in the protection against many chronic diseases including cancer. After soy intake we found a biphasic IFL appearance pattern in plasma as well as in urine that we suggest to be due to IFL absorption in the small intestine (ca. 10%) during the first 2 h after intake and IFL absorption in the large intestine (ca. 90%) 4–6 h after intake. While each IFL disappears from the circulation at different times excellent correlations between urinary and circulating IFL values were discovered and algorithms to convert urinary excretion values into circulating levels were established. We suggest the term 'apparent bioavailability' when using urinary data to describe IFL exposure. The IFL bioavailability was found to be influenced by gut bacteria, oral antibiotic treatment (OABX), and an individual's age and health status. While daidzein (DE) and genistein start to be absorbed minutes after intake, equol (EQ) appears in plasma only after a minimum of 8 h following soy intake owing to the required transit time of DE to the colon where the conversion of DE to EQ takes place by intestinal microbiota. We have also shown that the apparent IFL bioavailability is higher in children than adults, higher in healthy versus non-healthy individuals, and decreased in children but increased in adults during OABX. Finally, we propose to use a urinary EQ/DE ratio of 0.018 with a DE threshold to identify EQ producers. With this cutoff definition we observed that EQ production is inconsistent over time in 5–30% of both premenopausal and postmenopausal women.

Introduction

Isoflavonoids (IFLs) are suggested to protect against many chronic diseases (including coronary heart diseases, subclinical atherosclerosis, type 2 diabetes), to decrease the risk of certain types of cancer such as breast and prostate cancers, and to improve bone health [1–3].

IFL exposure occurs mainly through the dietary intake of soy products which typically contain 0.01–0.3% total IFL and are composed mainly of the glycosides genistein (GE), daidzein (DE) and glycitein (GLYE) [4–6] (Fig. 1). Equol (EQ) is a metabolite formed by gut bacteria during digestion through the chemical reduction of DE [7,8] and is proposed to play a major role in the health benefits of soy and IFLs. Strong evidence supports a protective effect for soy intake against breast cancer in adulthood [9] and particularly when soy is consumed at an early age [10–14]. The pharmacologic effects of soy intake are thought to be due to the structural similarity of IFLs to steroidal estrogens and the potent binding of GE to the estrogen receptor beta [15,16]. We found that children have a higher IFL bioavailability compared to adults [17] and that the cardio protective effects of IFLs are independent from lipid profiles [18]; these findings strengthen the hypothesis that IFLs play an important role in the biological effects of soy intake [19]. Understanding the basis of the health benefits derived from IFLs requires a detailed knowledge on the absorption, distribution, metabolism, elimination (ADME)[1] and bioavailability of these phytoestrogens. Here, we have summarized findings from us and others regarding ADME studies on IFLs.

Use of extinction coefficient (ε) to determine stock solutions of standards for external calibration of analytical instruments

To assure accuracy of analyses for compounds of interest accurate calibration of analytical instruments remains of utmost

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[1] *Abbreviations used:* ADME, absorption, distribution, metabolism, elimination; DE, daidzein; EP, equol producer; EQ, equol; GE, genistein; GLYE, glycitein; ICC, intraclass correlations; IFL, isoflavonoid; IP, inconsistent producer; MBP, mechanical bowel preparation; NP, non-equol producer; OABX, oral antibiotic treatment; OU, overnight urine; PL, plasma; SU, spot urine; UIER, urinary isoflavonoid excretion rate.

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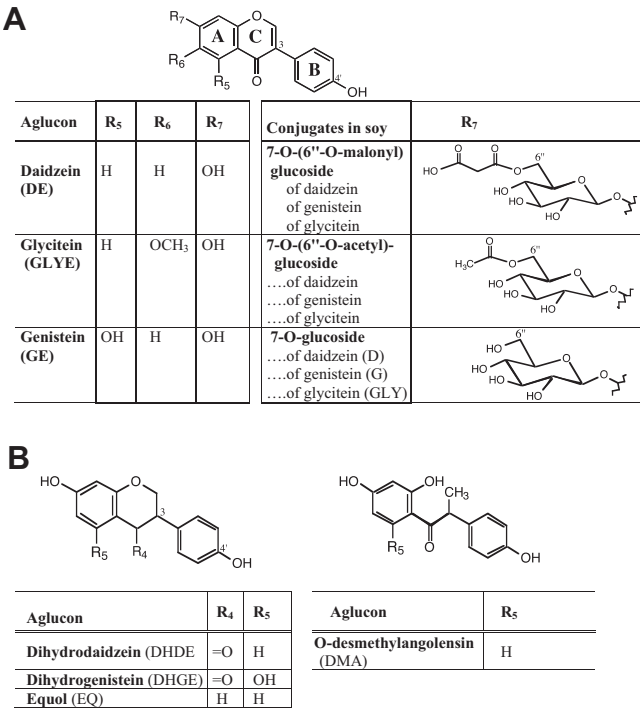

Fig. 1. Molecular structure of unmetabolized (A) and metabolized (B) isoflavonoids and their conjugates. Molecules shown in A occur in foods and in B in body fluids as metabolites after formation in the gut by intestinal bacteria (EQ derives from DE). In body fluids only EQ occurs predominantly as aglucon while the other aglucons occur predominantly glucuronated and sulfated.

importance. The determination of a standard stock solution concentration is preferably performed through absorbance readings at the maximum wavelength (λ_{max}) using molar extinction coefficients (ε) after purity is assured via HPLC. The concentration of the final standard stock solution is then calculated using the λ_{max} reading adjusted for the purity. This method of calibration avoids inaccuracies connected with weighing (due to hygroscopy, balance miscalibration, or other causes), allows repeated checking of existing stock solutions when necessary, and assures most of all simple inter-laboratory comparability. The estimation of a standard solution's molar concentration from its measured absorbance is easy and fast performed. ε represents a physical constant that does not change and measures accurately how strongly a sample absorbs light at a given wavelength. However, ε varies depending on the solvent, temperature and of course the selected wavelength and, therefore, these variables have to be taken into account and reported if absorbance readings are used for standard concentration determinations (Table 1). A great advantages of using absorbance readings is that it serves as a reliable reference point whereby final results can be corrected if the used ε values should require adjustments [20].

Elucidation of the IFL biphasic absorption pattern

Metabolic investigations by us [30,31] and others [32–34] have consistently observed a biphasic IFL appearance pattern in plasma and urine of humans after consumption of soy or purified IFL preparations with peak levels occurring 1–2 h and again 4–6 h after intake (Fig. 2). We elucidated this pattern in more detail *in vivo* using a simultaneous soy challenge and oral antibiotic treatment (OABX) combined with mechanical bowel preparation (MBP), the latter being the only modality to drastically reduce intestinal bacteria [35].

Our results indicated separate and preferred locations of IFL absorption during digestion. The time at which the first IFL peak occurred (1–2 h after intake) suggested that IFL uptake occurred in the proximal small intestine. OABX + MBA did not remove peak 1; therefore, we concluded that the intestinal gut bacteria could

Table 1
Molar extinction coefficients (ε) of isoflavonoids and common flavonoids as a function of wavelength and solvent.

Compound	Solvent	λ (nm)	ε (L/mol/cm)	References
Daidzein	96% EtOH	250	20893	[21]
	100% EtOH	262	24739	[22]
	n.a.	249	31563	[23]
	MeOH	248–249	27100–27200	a*
	80% MeOH	250	27542	[24]
Genistein	96% EtOH	263	37154	[21]
	100% EtOH	262	35842	[22]
	n.a.	263	35323	[23]
	EtOH	263	35000–38400	a*
	96% EtOH	262.5	37291	b*
	n.a.	261	33113	[25]
	85% MeOH	261	24435	[26]
Glycitein	Alcohol	256	22387	
	n.a.	256	25388	[23]
Daidzin	n.a.	249	26830	[23]
	n.a.	n.a.	23749	c*
	MeOH/Water	250	28561	d*
Genistin	MeOH/Water	262.5	39129	d*
	n.a.	262.5	35323	[27]
	85% aq. EtOH	262	39000–40000	a*
Glycitin	n.a.	259	26713	[23]
Acetyldaidzin	n.a.	256	29007	[23]
Acetylgenistin	n.a.	261	38946	[23]
Acetylglycitin	n.a.	260	29595	[23]
Malonyldaidzin	n.a.	258	26830	[23]
Malonylgenistin	n.a.	260	29895	[23]
Malonylglycitin	n.a.	260	26313	[23]
Dihydrodaidzen	n.a.	277	13600	e*
Dihydrogenistin	n.a.	290	18300	e*
DMA	n.a.	280	12023	e*
Equol	n.a.	281	6761	e*
Formononetin	n.a.	256	29512	[21]
Biochanin-A	n.a.	263	27542	[21]
Kaempferol	96% EtOH	273	15849	[28]
Myricetin	MeOH	378	19498	[29]
Quercetin	96% EtOH	373	20892	[28]

λ (nm) = wavelength of absorbance maximum in nanometer; EtOH = ethanol; MeOH = methanol; ε = molar extinction coefficient in L/mol/cm; DMA = O-desmethylangolensin; formononetin = 4′-O-methyl daidzein; Biochanin-A = 4′-O-methyl genistein; n.a. = not available.

* Private communications.
[a] Sigma–Aldrich.
[b] Merck Index 10th ed.
[c] Nigel Botting 2004 (University of St Andrews, UK).
[d] Purina/Nestle 2006.
[e] Kristiina Wahala 1998 (University of Helsinki, Finland).

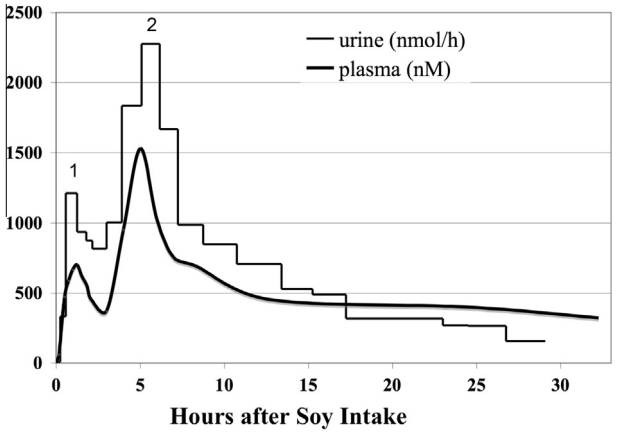

Fig. 2. Typical biphasic IFL appearance pattern in urine and plasma after soy intake. 1 = first peak 1–2 h after soy intake; 2 = second peak 6–8 h after soy intake. From [35] with permission.