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Transdermal absorption of phytoestrogens

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The transdermal absorption of the isoflavones, daidzein and genistein, applied on the skin in olive oil was studied *in vivo*. The concentrations of the isoflavones and their metabolites were monitored in plasma and urine by GC-MS methods. It was found that the concentration of genistein in plasma was 3-fold higher than the plasma concentration of daidzein. In contrast, daidzein excretion was 2–3-fold higher than that of genistein in urine. The excretion rate of the studied phytoestrogens in urine and their concentration in plasma was significantly decreased after repeated transdermal application. The urinary recovery of administered daidzein and genistein after the first application was 15.9% and 7.7%, respectively and this dropped to 1.6% and 0.7% after the second application. The results obtained might suggest that daidzein and genistein are captured in the skin following repeated transdermal application.

1. Introduction

Phytoestrogens, isoflavones and lignans, are reported to alter sex hormone synthesis and metabolism or modulate events at the cellular level by influencing intracellular enzymes, protein synthesis, growth factor action, malignant cell proliferation and differentiation, angiogenesis, calcium transport, lipid oxidation and many other metabolic processes [1–3]. It has been suggested that they play a role as natural protective compounds in hormone-dependent cancers and other degenerative diseases, as such conditions are reportedly linked to sex hormones or sex hormone metabolism [4]. The recent research data and observations of habitual lifestyle show that phytoestrogens can influence the menstrual cycle, climacteric symptoms and osteoporosis, but have little effect on fertility in humans [5–9]. The main source of phytoestrogens in humans is food, particularly processed soy products [3, 10–12], therefore the metabolism of the perorally administered phytoestrogens has been extensively studied [13–17].

Transdermal application is an alternative form of administration, avoiding the metabolic processes associated with the gastrointestinal system and liver (so called first-pass metabolism). The ability of estrogens to be absorbed transdermally and their profound effect on the skin is well known. These effects include the multiplication of the mitotic cycle, epidermal proliferation, vascularisation, positive effect on collagen and elastin fibres and increased levels of hyaluronic acid in the dermis [18–26]. Transdermal estrogen patches are very useful tools for the hormone replacement therapy. To our knowledge, the transdermal penetration of phytoestrogens *in vivo* has never been studied.

The aim of the present study was to evaluate the ability of the soy isoflavones, daidzein and genistein, to penetrate skin structures by monitoring their plasma and urine levels.

2. Investigations and results

Phytoestrogens were applied to the skin as a suspension in olive oil, which is known to contain a small amount of isoflavones. The amount of isoflavones (μg/100 g) was determined in olive oil base prior to clinical experiments (average/SD: daidzein, 0.23/0.00, genistein 0.27/0.02, biochanin A, 0.21/0.04). However, the concentrations of daidzein and genistein were quite low, and therefore, not considered in the recovery counting. The solubility of daidzein and genistein in the lipophilic olive oil was not ideal and a suspension was formed. The amounts of daidzein and genistein remaining in the non-absorbed bases are summarised in Table 1.

Table 1: Urine recovery of phytoestrogens after transdermal absorption

Isoflavone	Daidzein			Genistein		
Experiment	A	B	C	A	B	C
Sum extracted (mg)	1.647	0.159	0.019	0.800	0.065	0.008
Baseline discounted (mg)	1.591	0.148	0.015	0.767	0.063	0.002
Residual base (mg)	0	0.606	0.803	0	0.596	0.885
Amount applied (mg)	10.000	9.394	9.197	10.000	9.404	9.115
Recovery (%)	15.91	1.57	0.16	7.67	0.66	0.02

In four clinical experiments A–D, the concentrations of transdermally applied isoflavones – daidzein and genistein, and the end metabolites of daidzein – equol and *O*-demethylangolensin (ODMA), were monitored in both urine and plasma. All experiments were carried out in the same subject (premenopausal woman).

Clinical experiment A was carried out as the first experiment in the sequence. Following transdermal administration of equal amounts of both isoflavones, the urinary concentration of daidzein was twice as high as that of genistein (Fig. 1). ODMA was excreted more rapidly than equol giving the peak concentration 2.5-times higher than this of equol. Experiment B followed one month later and peak concentrations of daidzein were consistently twice as high as that of genistein (Fig. 2). Surprisingly, the peak concentration of both isoflavones was approximately 10-fold lower and reached later than in previous experiment A. The concentration of equol was, in contrast to experiment A, 3-fold higher than the concentration of ODMA, however, both peaks were 3-fold lower compared to experiment A. Experiment C was conducted after 5-months washout. The urine level of daidzein was again twice as high as that of genistein; the concentration of equol was higher than ODMA (Fig. 3). The comparison of peak concentrations of daidzein and genistein in urine in experiments A (Fig. 1), B (Fig. 2), and C (Fig. 3) shows clearly that the urinary excretion of both studied isoflavones has decreased significantly within the repeated applications.

A total urine sample was also collected during the experiments, which enabled the calculation of total excretion. The mean recoveries of daidzein and genistein decreased

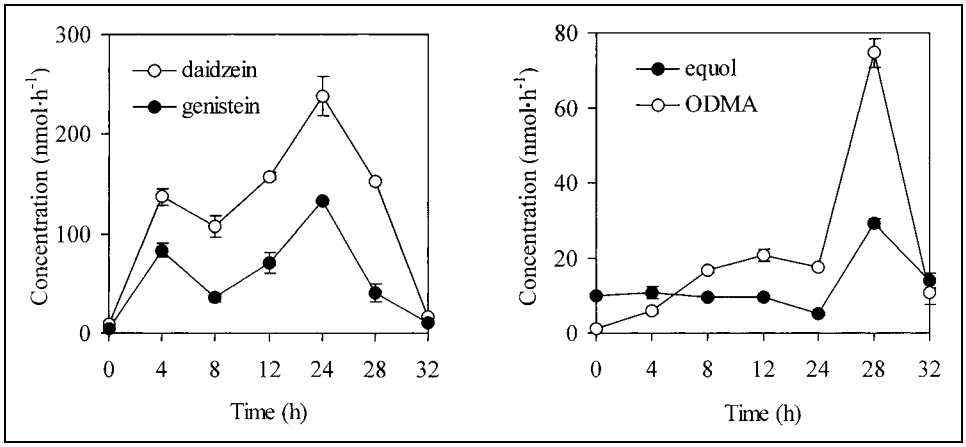

Fig. 1:
The kinetic curves of the excretion of phytoestrogens and metabolites in urine – experiment A

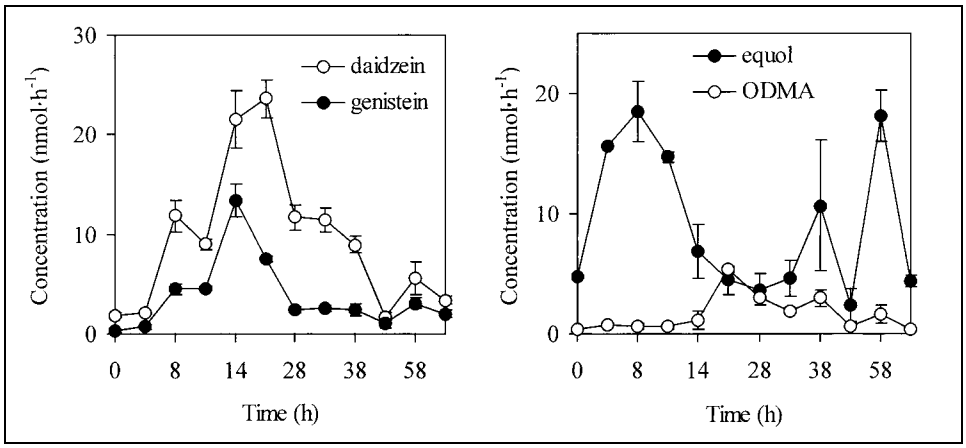

Fig. 2:
The kinetic curves of the excretion of phytoestrogens and metabolites in urine – experiment B

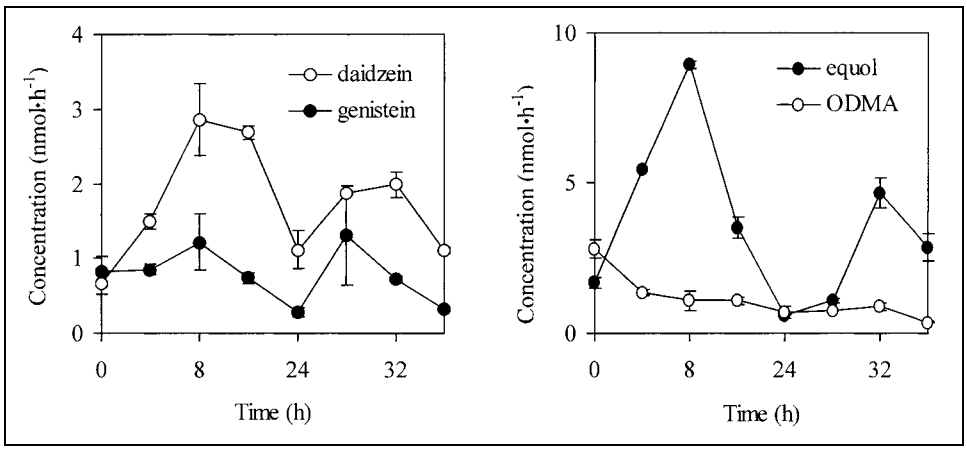

Fig. 3:
The kinetic curves of the excretion of phytoestrogens and metabolites in urine – experiment C

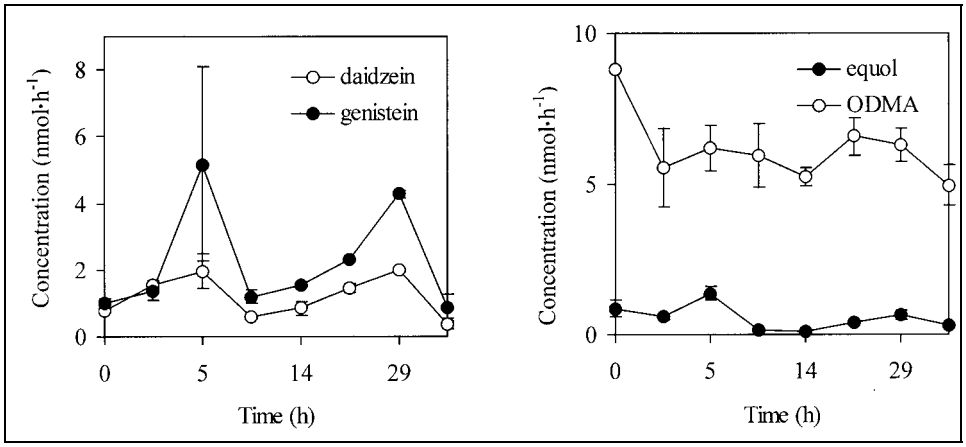

Fig. 4:
The kinetic curves of the excretion of phytoestrogens and metabolites in urine – experiment D

significantly (Table 1). The recovery of daidzein was higher than that of genistein in all trials.

In the final experiment D, only genistein was applied transdermally in ethanol solution. In this case, the rate of the transdermal absorption was expected to be the highest, as ethanol is a known penetration-enhancing agent [27]. This expectation was unconfirmed by the concentration measurements of monitored compounds (Fig. 4). As genistein was the only isoflavone transdermally administered in experiment D, the concentration of genistein was about 2.5-fold higher than the concentration of daidzein and as expected, the concentration of ODMA was very low, actually equal to baseline level. Equol was found in a 7-fold higher concentration than ODMA.

The results obtained by monitoring the urinary excretion of isoflavones after transdermal administration of daidzein and genistein can be shortly summarised by the following:

- the elimination of isoflavones was completed approximately 50 h after application,
- daidzein is eliminated faster than genistein as indicated by its higher urinary recovery,
- the concentration of daidzein in urine is about 2–3 fold higher than the concentration of genistein,
- the excretion rate of daidzein and genistein in urine is significantly decreased by repeated dermal application,
- the total isoflavones to metabolite ratio decreased from 3.8 (experiment A) to 1.9 (experiment C).

In experiment A, daidzein and genistein reached the blood circulation 2 h after transdermal administration (Fig. 5). In contrast to urinary excretion, the concentration of genistein in plasma was higher than the concentration of daidzein. Concentrations of equol and ODMA were low and the curves followed opposite patterns. In the experiment B, the kinetic curves of genistein and daidzein were analogous to those in experiment A (Fig. 6). The significant difference was the 3-fold lower concentrations of both isoflavones. The concentrations of ODMA and equol were very low. Only the kinetics of daidzein and genistein were determined in experiments C and D (Fig. 7). In both experiments, the concentration of genistein in plasma was higher than the concentration of daidzein. The concentration of genistein in plasma was not capable of reaching the high values of experiment A, even when enhancing the transdermal absorption by ethanol in the experiment D.

Based on the concentration changes of transdermally administered daidzein and genistein in plasma, the following conclusions can be made:

- the concentration of genistein was higher than the concentration of daidzein,
- an increase in concentration of both isoflavones was observed during the first 2 hours following transdermal administration,
- the concentration of genistein and daidzein in plasma was rising after 10–20 h following transdermal administration,
- after repeated administration of studied isoflavones a decrease in concentration was observed in the tested subject.

In experiment B, the faecal excretion of isoflavones and lignans was analysed to assess the recovery of phytoestrogens in faeces (Fig. 8). Among the monitored compounds, daidzein and equol were excreted in faeces, with the highest concentrations being found 48 h after transdermal application. The faecal recovery of daidzein was 0.26%. In

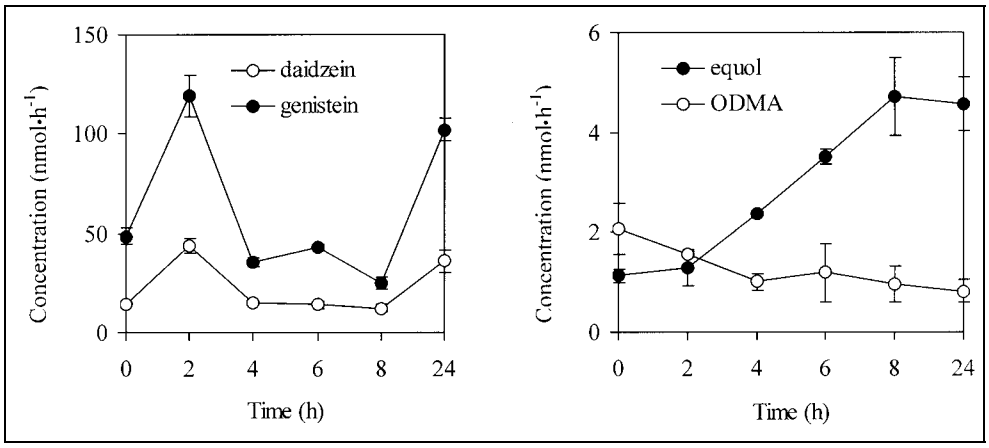

Fig. 5:
The kinetic curves of the excretion of phytoestrogens and metabolites in plasma – experiment A

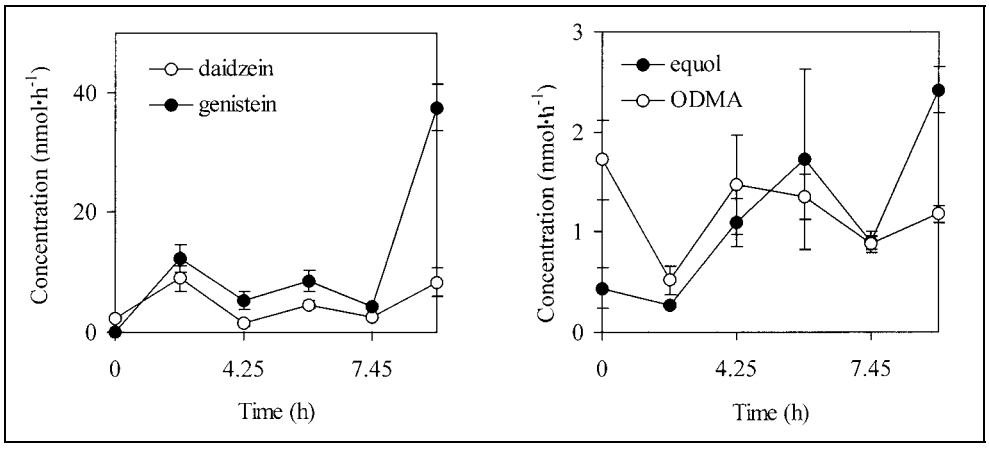

Fig. 6:
The kinetic curves of the excretion of phytoestrogens and metabolites in plasma – experiment B

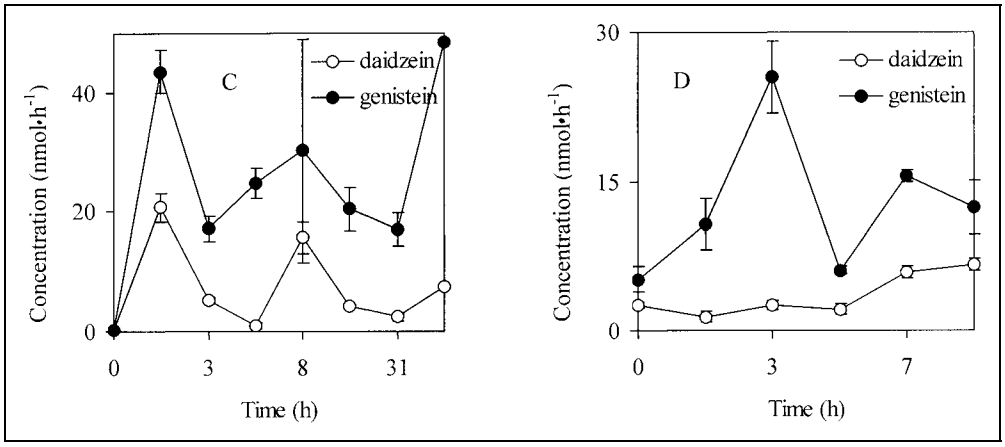

Fig. 7:
The kinetic curves of the excretion of phytoestrogens in plasma – experiment C and D

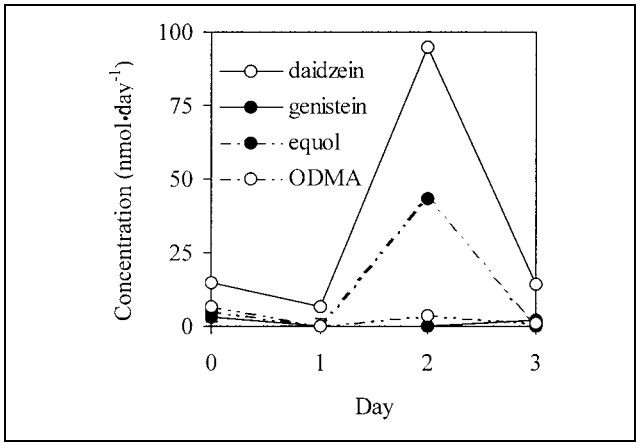

Fig. 8: Faecal excretion of isoflavonoids and metabolites – experiment B

contrast, genistein and ODMA were not noticably excreted by the faecal route. As the urine/faeces excretion ratio is believed to be constant [28], no further analysis of faeces was carried out.

3. Discussion

As no data on transdermal application of isoflavones are available we are only able to compare our findings with the results achieved by either transdermal absorption of estradiol and flavones or by peroral administration of isoflavones.

The comparison of the plasma levels of estradiol, daidzein and genistein reached by transdermal administration proved very interesting. In women, the concentration of estradiol in plasma varies during the menstrual phases as well as in postmenopausal years. In premenopausal women, the estradiol levels of $65 \pm 10\ ng \cdot l^{-1}$ (0.25 nM), $140 \pm 40\ ng \cdot l^{-1}$ (0.5 nM) and $110 \pm 40\ ng \cdot l^{-1}$ (0.4 nM) are considered to be normal baseline for the follicular phase, ovulation and luteal phase, respectively [29]. In postmenopausal women the estrogen levels drop by 60%, to $10–20\ ng \cdot l^{-1}$. The concentration of unconjugated estradiol in plasma required for physiological effect is about $40–60\ ng \cdot l^{-1}$ [24]. The dose recommended for transdermal administration is 1.5 mg/day and in postmenopausal women leads to plasma levels of $50–60\ ng \cdot l^{-1}$ relieving climacteric symptoms. In comparison, a single dose (10 mg) of genistein in olive oil resulted in a peak concentration of $32.5 \times 10^3\ ng \cdot l^{-1}$ (120 nM) as shown in experiment A carried out within the follicular phase. As genistein is reported to exert an estrogenic effect ranging from approximately $10^{-3}–10^{-5}$ that of estradiol [30], the concentration might be sufficient for mild estrogenic effect.

A previous *in vivo* skin penetration pharmacokinetic study employed the flavones; apigenin was found to exert a higher flux than luteolin [31]. Based on the amount of daidzein and genistein remaining unabsorbed in residual base, it seems that the rates of skin penetration are rather similar for both isoflavones (experiment – daidzein absorbed (%)/genistein absorbed (%): A – 100/100, B – 94.0/93.4, C – 91.2/92.0). Probably the most significant influence of the structure on a flux could be attributed to the lipophilicity of a single phenolic ring bearing one free hydroxyl group in the case of apigenin, daidzein, and genistein.

The epidemiological studies monitoring phytoestrogens in human fluids after peroral intake of various diet patterns exhibit clearly the variation in the excretion of isoflavones and their metabolites among various ethnic groups [16, 32, 33]. This high variability is likely due to the dependence of phytoestrogen absorption on the gut microflora. Nevertheless, most of the ethnic groups excreted daidzein in urine at a higher rate than genistein [32, 33–37]. The substantially higher urinary recovery of daidzein compared with genistein has been uniform observation also in the pharmacokinetic studies on soybean isoflavones [14, 17, 37–40]. Interestingly, our results indicate the higher urinary excretion of daidzein compared to genistein even after transdermal absorption avoiding the gastrointestinal tract. Thus, the maximum recoveries of daidzein and genistein in urine were 15.9% and 7.7%, respectively, in the first experiment A (Table 1). These values are somewhat lower than those reported, moreover, the recoveries show large variations between the different studies. The mean recoveries range from 16% [28] to 66% [40] for daidzein and from 9% [28, 37] to 22% [14, 40] for genistein depending mainly on the nature of the soy food and the time of exposure. Women were found to excrete more isoflavones in urine than men do [39]. Furthermore, a progressive decrease in urinary recoveries of both daidzein and genistein after repeated transdermal administration was observed (Table 1). In addition, the daidzein to equol ratio of peak concentrations dropped successively from 8.2 (Experiment A) through values of 1.3 (Experiment B) to 0.5 (Experiment C). Whilst the first dermal application of daidzein resulted in its prevailing degradation into ODMA, repeated treatment probably resulted in the ability to produce and excrete larger amounts of equol. This findings are consistent with those reported from chronic soy exposure of women but not of men [39, 40]. Total amounts excreted and peak levels were decreased over 4 weeks of

daily soy ingestion by 42% for genistein and 31% for daidzein. Simultaneously, increased production and elimination of equol were established. Authors concluded that metabolism and disposition of the ingested isoflavones are altered during chronic soy exposure in women, perhaps from increased metabolic degradation into non-isoflavone metabolites. It could be assumed that the repeated dermal application of soy isoflavones exhibit typical characterisation of a long-term consumption in some respects. Urinary excretion of daidzein and genistein showed two or more peaks over 48 h after transdermal application (Figs. 1–4). This biphasic elimination pattern has been already observed in urine [38], plasma, and milk [17] after soy intervention. It has been generally accepted that this phenomenon is due to the enterohepatic circulation. This process may be involved in transdermal absorption of isoflavones as well although one could expect its lower significance.

The recovery of isoflavones in faeces (0.26% of daidzein with trace genistein) after dermal application was low in comparison to peroral soy intake. In this case, the reported faecal excretion varied from 1–2% [37] to 4% [38] or 6–7% [38]. In accordance with the urinary elimination patterns, faecal isoflavones peaked again indicating the presence of enterohepatic circulation (Fig. 8).

In contrast, the plasma level of genistein was found to be higher than that of daidzein in agreement with several intake studies [14, 38], although other studies revealed high variability in genistein to daidzein ratios [17] or approximately equal plasma concentrations [37]. There are several possible conditions that may have contributed to this variation: relative concentrations of isoflavones in soy food employed, the use of processed soy food, the composition of gut microflora, or the dosage and duration. In general, plasma elimination patterns after peroral intake of soy isoflavones is similar to that form urine with peak concentrations occurring 6 h [38], 6.5 h [37], 7–8 h [14], or 10 h [17] after ingestion. As expected, the transdermal administration of daidzein and genistein resulted in a significant decrease of the time required to reach peak plasma concentrations (Figs. 5–7); they peaked 2 h after application. This suggests that an interval between the consumption of soy isoflavone glycosides and their gastrointestinal absorption takes several hours. In comparison, the synthetic isoflavone aglycone, ipriflavone, gave the peak concentration 1.3 h after peroral administration [41].

Isoflavones were completely eliminated from plasma 24 h [37], 35 h [14], or 60 h [17] after intake of a single soy dose. Log transformation of the plasma concentrations of the isoflavones over ca. 40 h yielded single slopes, with elimination half-lives for daidzein 4.7 h [14], or 6.0 h [38], and for genistein 5.7 h [14] or 11.8 h [38]. In this regard, a remarkable difference between peroral intake and transdermal administration can be seen. In all our experiments, an increase of the phytoestrogen levels was observed during the corresponding period 10–24 h (Fig. 5 and 6) or 31–49 h (Fig. 7). Furthermore, the genistein concentration was rising slightly faster than that of daidzein. This observation, as well as the low urinary and faecal recoveries, might imply that the isoflavones can be captured within the human body, probably in the skin. The stratum corneum can act as both a reservoir and a principal diffusion barrier [24]. This suggestion can be supported by the investigation of the binding ability of daidzein, but not genistein, to phospholipids [42]. Finally, the role of enterohepatic circulation cannot be omitted as well. However, we are aware of the fact that only one subject was employed and the experimental data must therefore be interpreted with caution. Care should be also taken in generalising the present findings because target tissue concentrations of isoflavones are still unknown.

In summary, the first *in vivo* study on transdermal application of phytoestrogens implies that both daidzein and genistein penetrate the skin and reach systematic circulation. Excretion patterns seem to be analogous to those after peroral administration whilst plasma levels and the urinary recoveries might indicate an accumulation of isoflavones in skin structures followed by a slow desorption. The plasma concentration of transdermally administered phytoestrogens, each of them in a dose of 10 mg, reached but not maintained levels useful for therapeutic or preventive medicinal purposes within the experiment A only. Further work is therefore required because of a growing interest in the transdermal application of phytoestrogens and because their use for the prevention and treatment of macular degeneration [43] and spider veins [44] has been already claimed.

4. Experimental

4.1. Clinical experiments

The design of the pharmacokinetic study was approved by the Head of the Department of Clinical Chemistry, Meilahti Hospital, Finland and all procedures of the protocol followed were in accordance with the ethical standards of the Helsinki Declaration of 1975, as revised in 1983. The experiments are named chronologically A–D and were carried out on one subject (woman, aged 26, weight 68 kg, BMI 22.2). Prior to experiments the "zero diet" in lignans and isoflavones was held for four days to assure that only the transdermally administered isoflavones were excreted. During the study, the participant maintained phytoestrogen-free diet consisting mainly of yoghurt, quark, fat-free milk, fish, vegetables, and coffee twice a day. The normal lifestyle including regular showering twice a day and no protection for the application site was continued. "Zero plasma" sample was taken prior to the experiment. The "zero urine" sample was collected for the 24 h period prior to the initiation of the experiment, to avoid the circadian differences in excretion of isoflavones.

On the day of application (A, 09.01.1996; B, 08.02.1996; C, 24.07.1996) daidzein and genistein (10 mg each) suspended in extra virgin olive oil (1 g) were applied on a marked area of the ventral forearm (5×10 cm) on each hand. In experiment D, (29.08.1996) transdermal administration of 10.3 mg of genistein dissolved in ethanol (2 ml) was followed immediately by rinsing of the application area with ethanol (2 ml). After 1 h, the application site was covered with 1 g of extra virgin olive oil.

4.2. Sample collection and analysis

The urine samples were collected in bottles containing ascorbic acid (1–2 g). 0.1% NaN_3 was added immediately after collection to minimise possible bacterial degradation. The volume of urine collected was measured and the samples were stored at −20 °C until analysed. The blood samples were collected into the EDTA or heparin tubes. The two tubes of blood were allowed to cool at room temperature, centrifuged twice and stored in a freezer (−20 °C).

The determination of phytoestrogens in urine was carried out according to the quantitative method originally developed by Adlercreutz [45, 46]. The method included fractionation and purification by ion-exchange chromatography and analytical measurements by GC-MS in the selective ion-monitoring mode. The quick method employed for determination of isoflavones in plasma was modified from the quantitative method for the determination of phytoestrogens in plasma published by Adlercreutz et al. [47, 48].

4.2.1. Quick method for plasma analysis

To the centrifuged plasma sample (2.0 ml) radioactive recovery standard 3H-E1-Gl (about 15000 cpm) was added and the sample was incubated for 20 min at room temperature. After incubation 800 µl of 1.5 M acetate buffer pH 5.0, 1200 µl of distilled H_2O and 4 ml of 0.5 M triethylamine, pH 5.0 was added and the mixture incubated for a further 20 min at room temperature. After incubation, the sample was passed through a Sep-Pak C18 cartridge, primed by washing with 6 ml of CH_3OH, and 10 ml of distilled H_2O. The cartridge was subsequently washed with 3 ml of 0.15 M acetate buffer (pH 5.0) and the sample eluted with 4 ml of 70% CH_3OH and, under a gentle stream of nitrogen, evaporated to dryness. Ethylacetate and conc. HCl were added to the sample in amounts of 2 ml and 2 µl, respectively, and the sample was incubated overnight at +37 °C. After incubation the sample was evaporated to dryness under a gentle stream of

Table 2: Intra-assay imprecisions of both methods at different concentration levels

Compd.	No. of replicates	Average (nM)	SD (nM)	RSD (%)
Daidzein[a]	3	1.24	0.35	29.8
Daidzein[a]	4	4.13	0.53	12.8
Daidzein[b]	4	43.74	7.80	17.9
Genistein[a]	3	1.44	0.36	25.0
Genistein[a]	4	2.80	0.44	15.7
Genistein[b]	4	101.3	13.68	13.5
Equol[a]	3	5.86	0.75	12.8
Equol[b]	2	14.48	0.78	19.2
ODMA[a]	5	6.47	0.29	4.5
ODMA[b]	2	11.25	2.13	18.9

[a] the published method for plasma [47], [b] the quick method for plasma

nitrogen. The dry sample was dissolved in 1 ml of acetate buffer 0.15 M, pH 5.0, containing the β-glucuronidase enzyme (*Helix pomatia*, Sigma Chemicals Co.,) 0.1 $U.ml^{-1}$, and the mixture incubated for 1 h at +37 °C. After incubation the sample was extracted twice with 3 ml of diethyl ether. A suitable amount of deuterated internal standards (synthesised by Drs. T. Hase and K. Wähälä, Department of Chemistry, University of Helsinki, Helsinki, Finland) was added, the sample evaporated to dryness and dissolved in 500 µl of methanol. The 1/10 part (50 µl) was taken for radioactive recovery counting. Before counting in the B-Counter, 3 ml of scintillation liquid was added. The remaining sample (450 µl) in CH_3OH was applied on the QAE-CO^{3-}-Sephadex column (batch 4 cm placed into a Pasteur pipette) and eluted with 1.5 ml of methanol followed by 6 ml of 80% CH_3OH. The first fraction (containing estrogens) was discarded. The second fraction (containing daidzein and genistein) was eluted with 7 ml of 0.1 M CH_3COOH in 80% CH_3OH. The isoflavone fraction was evaporated to dryness under a gentle stream of nitrogen, dissolved in 0.1 ml of silylation reagent (pyridine, hexamethyldisilazan, trimethylchlorsilanen in a 9 : 3 : 1 ratio) and incubated for a minimum period of 30 min at room temperature. The sample was subsequently evaporated to dryness under a gentle stream of nitrogen and dissolved in 100 µl of *n*-hexane (containing approximately 3% vol. of Silyl-8). The sample (1–2 µl) was then subjected to GC-MS.

4.2.2. GC-MS analysis

A GC-MS instrument HP 5995 equipped with a quadrupole mass spectrometer, Autoinjector 7673A and data system HP 59970 CMS Chem Station was used. The analyses were performed on a fused silica column (0.2 mm I.D. × 12.5 m) with bonded phase BP-1 (SGE, Ringwood, Victoria, Australia), film thickness 0.25 µm, and employed helium as a carrier gas. Temperatures of the transfer line, ion source and the analyser were 310 °C, 250 °C and 250 °C, respectively. The column temperature was programmed: initial temperature 150 °C, 30 °C · min^{-1} up to 280 °C. The relative retention times and ion pairs (nondeuterated, deuterated) used for the selected ion monitoring mode within ID-GC-MS-SIM are standard characteristics of each monitored compound [45].

4.2.3. Data evaluation

The sample was analysed twice repeatedly and the compound identified by the comparison of relative retention time with deuterated standards. In addition, the first separation steps in the plasma method were controlled by a radioactive recovery standard, counted afterwards in B-Counter. The calibration curve together with the control samples with high and low amounts of isoflavones were included in each sample series to determine accuracy. As the separation of phytoestrogens from biological fluids was complicated, long and quite expensive, additional statistical methods were not considered. The quick method employed in this study was faster and more convenient for the monitoring of the compounds in plasma. The RSD values representing the intra-assay imprecision of both methods at the different concentration levels are summarised in Table 2. The comparable intra-assay imprecision of the quantitative method for lower concentrations and the quick method for higher concentrations confirm the acceptability of the quick method for screening purposes.

4.3. Analysis of the unabsorbed transdermal base

The remaining unabsorbed transdermal base on the application spatula and mixing vessel were rinsed twice with methanol thoroughly and the liquid was centrifuged and stored in a freezer. The supernatant was evaporated to dryness, the residue silylated and later dissolved in n-hexane containing approximately 3.4% (v/v) cholestane as the internal standard. The analysis was carried out on a Hewlett Packard 5890 Series II Gas Chromatograph combined with a HP 7673A Automatic Sampler and coupled with a HP data system consisting of a HP 7673A Controller, a HP 9114B Disk Station and a HP 3396A Integrator. The instrument was equipped with a bonded phase BP-1 (SGE, Ringwood, Victoria, Australia) column (0.22 mm I.D. × 25 m), flame ionisation detector and operated with helium as the carrier gas.

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References

1 Messina, M. J.; Persky, V.; Setchell, K. D. R.; Barnes S.: Nutr. Cancer. **21**, 113 (1994)
2 Setchell, K. D. R.; Adlercreutz, H.: in: Rowland, I. (Ed.): Role of the Gut Flora in Toxicity and Cancer, p. 315, Academic Press, London 1988
3 Adlercreutz, H.; Mazur, W.: Ann. Med. **29**, 95 (1997)
4 Adlercreutz, H.: Environ. Health. Perspect. **103**, 103 (1995)
5 Baird, D.; Umbach, D.; Lansdell, L.: J. Clin. Endocrinol. Metab. **80**,1685 (1995)
6 Lock, M.: Lancet **337**, 270 (1991)
7 Cassidy, A.; Bingham, S.; Setchell, K. D. R.: Br. J. Nutr. **74**, 587 (1995)
8 Lu, L-J. W.; Anderson, K. E.; Grady, J. J.; Nagamani, M.: Cancer. Epidemiol. Biomarkers Prev. **5**, 63 (1996)
9 Phipps, W. R.; Martini, M. C.; Lampe, J. W.; Slavin, J. L.; Kurzer, M. S.: J. Clin. Endocrinol. Metab. **77**, 1215 (1993)
10 Mazur, W. M.; Wähälä, K.; Ojala, S.; Makkonen, A.; Hase, T.; Adlercreutz, H.: Br. J. Nutr. **79**, 37 (1998)
11 Dwyer, J. T.; Goldin, B. R.; Saul, N.; Gualtieri, L.; Barakat, S.; Adlercreutz, H.: J. Am. Diet. Assoc. **94**, 739 (1994)
12 Hutchins, A. M.; Slavin, J. L.; Lampe, J. W.: J. Am. Diet. Assoc. **95**, 545 (1995)
13 Kelly, G. E.; Joannou, G. E.; Reeder, A. Y.; Nelson, C.; Waring, M. A.: Proc. Soc. Exp. Biol. Med. **208**, 40 (1995)
14 King, R. A.; Bursill, D. B.: Am. J. Clin. Nutr. **67**, 867 (1998)
15 Lu, L-J. W.; Grady, J. J.; Marshall, M. V.; Ramanujam, V. M. S.; Anderson, K. E.: Nutr. Cancer **24**, 311 (1995)
16 Zeleniuch-Jacquotte, A.; Adlercreutz, H.; Akhmedkhanov, A.; Toniolo, P.: Cancer Epidem., Biomarkers Prev. **7**, 885 (1998)
17 Franke, A. A.; Custer, L. J.; Wang, W.; Shi, C. Y.: Proc. Soc. Exp. Biol. Med. **217**, 263 (1998)
18 Värilä, E.; Rantala, I.; Oikarinen, A.; Risteli, J.; Reunala, T.; Oksanen, H.; Punnonen, R.: Br. J. Obstet. Gynaecol. **102**, 985 (1995)
19 Castelo-Branco, C.; Pons, F.; Gratacós, E.; Fortuny, A.; Vanrell, J. A.; González-Merlo, J.: Maturitas **18**, 199 (1994)
20 Creidi, P.; Faivre, B.; Agache, P.; Richard, E.; Haudiquet, V.; Sauvanet, J. P.: Maturitas **19**, 211 (1994)
21 Schmidt, J. B.; Binder, M.; Macheiner, W.; Kainz, C.; Gitsch, G.; Bieglmayer, C.: Maturitas **20**, 25 (1994)
22 Punnonen, R.; Vaajalahti, P.; Teisala, K.: Ann. Chir. Gynaecol. **76**, 39 (1987)
23 Brincat, M.; Versi, E.; O'Dowd, T.; Moniz, C. F.; Magos, A.; Kabalan, S.; Studd, J. W. W.: Maturitas **9**, 1 (1987)
24 Sitruk-Ware, R.: Ann. Med. **25**, 77 (1993)
25 Jemec, G. B. E.; Serup, J.: Maturitas **11**, 229 (1989)
26 Goldzieher, J. W.; Baker, R. E.: J. Invest. Dermatol. **35**, 215 (1960)
27 Wendker, H.; Schafer, H.; Zesch, A.: Arch. Derm. Res. **256**, 67 (1976)
28 Xu, X.; Harris, K. S.; Wang, H-J.; Murphy, P. A.; Hendrich, S.: J. Nutr. **125**, 2307 (1995)
29 Cassidy, A.; Bingham, S.; Setchell, K. D. R.: Am. J. Clin. Nutr. **60**, 333 (1994)
30 Messina, M. J.; Persky, V.; Setchell, K. D. R.; Barnes, S.: Nutr. Cancer. **21**, 113 (1994)
31 Merfort, I.; Heilmann, J.; Hagedorn-Leweke, U.; Lippold, B. C.: Pharmazie **49**, 509 (1994)
32 Maskarinec, G.; Singh, S.; Meng, L.; Franke, A. A.: Cancer. Epidemiol. Biomarkers Prev. **6**, 613 (1998)
33 Xu, X.; Duncan, A. M.; Merz, B. E.; Kurzer, M. S.: Cancer. Epidemiol. Biomarkers Prev. **7**, 1101 (1998)
34 Franke, A. A.; Custer, L. J.: J. Chromatogr. **662**, 47 (1994)
35 Karr, S. C.; Lampe, J. W.; Hutchins, A. M.; Slavin, J. L.: Am. J. Clin. Nutr. **66**, 46 (1997)
36 Ingram, D.; Sanders, K.; Kolybaba, M.; Lopez, D.: Lancet **350**, 990 (1997)
37 Xu, X.; Wang, H.-J.; Murphy, P. A.; Cook, L.; Hendrich, S.: J. Nutr. **124**, 825 (1994)
38 Watanabe, S.; Adlercreutz, H.: ACS Symp. Ser. **702**, 198 (1998); C.A. **130**, 13534 (1999)
39 Lu, L.-J., W.; Anderson, K. E.: Am. Clin. Nutr. **68**, 1500 (1998)
40 Lu, L.-J. W.; Lin, S. N.; Grady, J. J.; Nagamani, M.; Anderson, K. E.: Nutr. Cancer **26**, 289 (1996)

41 Saito, A. M.: Jpn. Pharm. Ther. J. **13**, 7223 (1985)
42 Lehtonen, J. Y. A.; Adlercreutz, H.; Kinnunen, P. K. J.: Biochim. Biophys. Acta. **1285**, 91 (1996)
43 Zender, H.: DE 19654750 (1998); C.A. **129**, 90472 (1998)
44 Becker, P. E.; Doepker, M. L.: U.S. 5698206 (1998); C.A. **128**, 93012 (1998)
45 Adlercreutz, H.; Fotsis, T.; Bannwart, C.; Wähälä, K.; Brunow, G.; Hase, T.: Clinica. Chimica. Acta **199**, 263 (1991)
46 Adlercreutz, H.; Van der Wildt, J.; Kinzel, J.; Attalla, H.; Wähälä, K.; Mäkelä, T.; Hase, T.; Fotsis, T.: J. Steroid. Biochem. Molec. Biol. **52**, 97 (1995)
47 Adlercreutz, H.; Fotsis, T.; Lampe, J.; Wähälä, K.; Mäkelä, T.; Brunow, G.; Hase, T.: Scand. J. Clin. Lab. Invest. **53**, 5 (1993)
48 Adlercreutz, H.; Fotsis, T.; Watanabe, S.; Lampe, J.; Wähälä, K.; Mäkelä, T.; Hase T.: Cancer Detection and Prevention **18**, 259 (1994)

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