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Natural occurrence of steroid hormones in food

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The natural occurrence of the sex steroid hormones progesterone, testosterone, 17β-estradiol and estrone in food was investigated in a survey of the German market basket. The main metabolic precursors, intermediates and metabolites (pregnenolone, androstenedione, hydroxyprogesterone, dehydroepiandrosterone (DHEA), dihydrotestosterone, androsterone, 17α-estradiol and estriol) were also included in the investigation. Particular attention was paid to DHEA, which is said to have anti-aging properties. Analysis was carried out by gas chromatography–mass spectrometry (GC–MS). The steroid patterns of pork, meat products, fish and poultry resemble those known for beef. Milk and milk products reflect the hormone profile of female cattle with high amounts of progesterone, which accumulates with increasing milk fat content. Milk products supply about 60–80% of ingested female sex steroids. Eggs are a considerable source of any of the investigated steroids and contribute to the nutritional hormone intake in the same order as meat and fish (10–20%). In vegetable food no estrogens could be detected. Plants supply testosterone in the same order as meat and milk products (20–40%) though. They contain considerable amounts of hormone precursors as well (contribution to DHEA supply: about 80%). In comparison to the human daily production of steroid hormones the nutritional supply (about 10 $\mu g\,d^{-1}$ progesterone, 0.05 $\mu g\,d^{-1}$ testosterone, 0.1 $\mu g\,d^{-1}$ estrogens, 0.5 $\mu g\,d^{-1}$ DHEA) is insignificant.

INTRODUCTION

Contents of naturally occurring steroid hormones in beef and veal have been frequently reported because of their misuse as anabolic performance enhancers in cattle production (Henricks *et al.*, 1983; Scippo *et al.*, 1993). Nevertheless, it is known that steroid hormones also occur in tissues of non treated cattle (CEC, 1989; Hartwig *et al.*, 1997), pigs (Claus *et al.*, 1989) and poultry (Cantoni *et al.*, 1993, 1994). However, mammals and birds are not the only organisms that synthesize steroid hormones. The existence of steroids is widespread in both the animal and the plant kingdom. The occurrence of steroid hormones in fish plasma has been frequently reported (So *et al.*, 1985; Pavlidis *et al.*, 1994). Animal products such as milk and milk products contain steroid hormones as well (Hoffmann *et al.*, 1975*a*; Ginther *et al.*, 1976; Cantoni and d'Aubert, 1995). The presence of steroid hormones, especially of estrogens, in plants is controversial. Whilst some authors have detected steroidal estrogens in plants (Geuns, 1978; Young *et al.*, 1978), others deny their existence and their function in plants (van Rompuy and Zeevaart, 1979). Even single-cell organisms like yeast are said to produce steroidal hormones, which commonly function as chemical messengers (Feldman *et al.*, 1984).

The aim of this study was to give a survey of the natural occurrence of steroid hormones in human nutrition. The samples were selected according to German habits of consumption (Adolf *et al.*, 1994), taking all literature references concerning the presence of steroid hormones into consideration. The contribution of meat (particularly beef and veal, to which most attention is paid) to the nutritional hormone supply in comparison to other foodstuffs was evaluated. A further particular concern of the study was the influence of food processing on the hormone content. The average alimentary intake of hormones was estimated, taking into consideration hormone amount and average consumption of the different foodstuffs.

Above all, not only the potent hormones but also their most characteristic precursors and metabolites were investigated to obtain knowledge on their biochemical pathways in food producing animals and plants. The steroids investigated were selected with regard to their physiological potency and the human steroid metabolism (Fig. 1). The basic precursor for any steroid hormone is pregnenolone. The biosynthesis of androgens, which are protein anabolics and dominate in male animals, follows either the Δ4- (via progesterone

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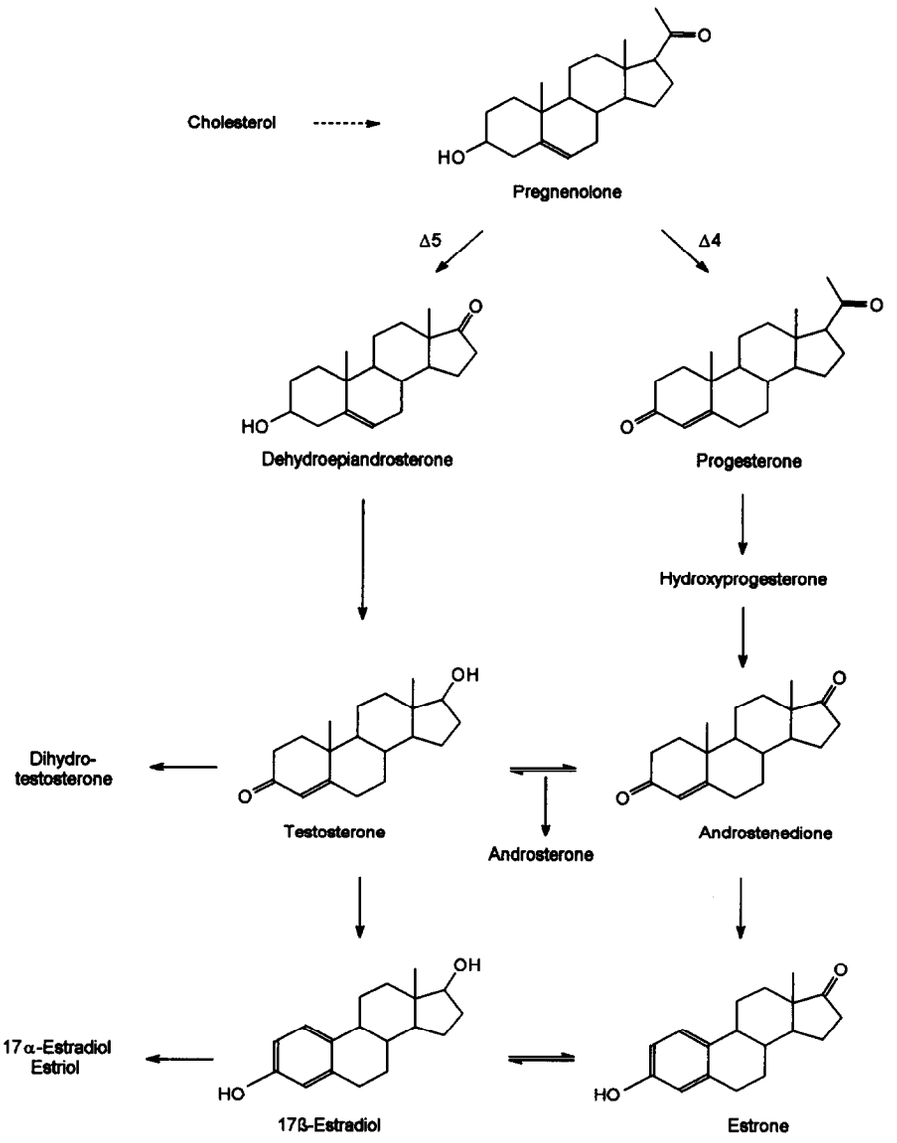

Fig. 1. Biosynthetic relationships between the investigated steroids.

and its metabolite 17α-hydroxyprogesterone) or the Δ5-pathway (via dehydroepiandrosterone). The estrogens, which are fat anabolics and are regarded as typical female hormones, are formed from the androgen testosterone or the weakly androgenic intermediate androstenedione. Another female hormone is progesterone. In male organisms, however, it does not show any hormonal action and functions as a metabolic intermediate. Not only the sex steroids have physiological effects; the intermediate dehydroepiandrosterone (DHEA) is also said to have biological significance (Regelson and Kalimi, 1994). It has an antagonistic effect to corticosteroids. It may also act as estrogen or androgen depending on the hormonal state of the individual (Ebeling and Koivisto, 1994). Like pregnenolone, it is considered as a neurosteroid. Due to its gradual decrease with advancing age (Birkenhäger-Gillesse *et al.*, 1994), DHEA is also of interest as a possible 'anti-aging pill'.

Analysis of steroids was based on the official method in the Federal Republic of Germany for the determination of hormonally active agents in meat, liver, kidney and fatty tissue (Bundesgesundheitsamt, 1989), after adaptation on the different matrices. Gas chromatography–mass spectrometry (GC–MS) permits the simultaneous determination of a multiplicity of steroids. However, sample preparation is complex and time-consuming. Therefore only a few selected foodstuffs could be investigated, to give an overview of the occurrence of steroid hormones in human nutrition. If available, data from the literature were brought in to support the determined values.

MATERIALS AND METHODS

Food samples

The foodstuffs investigated for the market basket survey were selected according to the German nutritional study (Adolf *et al.*, 1994). The samples were purchased in local shops or supermarkets. They included meat, fish, animal

products (including fermented ones), fats (vegetable and animal), plants, alcoholic drinks and yeast. 48 samples were investigated altogether. They were subdivided as follows:

- *Beef and veal*: roast beef (bull, steer, heifer), liver
- *Milk and milk products*: milk, cream, butter, yoghurt, cheese (fresh, ripened)
- *Pork and meat products*: chops (barrow, gilt), liver, bacon (raw), ham (cooked), frankfurter (sausage), salami (20% beef, 50% pork, 20% bacon),
- *Poultry and eggs*: chicken (breast), turkey (steak), laying hen (whole), goose-fat, hen's eggs (whole)
- *Fish*: herring (whole fish), carp (filet)
- *Plants*: potatoes (steamed), wheat (whole meal), rice (parboiled, ground), soybeans (whole meal), haricot beans (dried), mushrooms, olive oil (native), safflower oil (native), corn oil (refined)
- *Yeast and fermented alcoholic beverages*: beer, wine (white), bakers' yeast

Steroids

Twelve steroids were investigated altogether according to Hartwig *et al.* (1995):

- the main hormone precursors pregnenolone (5-pregnene-3β-ol-20-one), dehydroepiandrosterone (DHEA, 5-androstene-3β-ol-17-one) and androstenedione (4-androstene-3,17-dione),
- progesterone (4-pregnene-3,20-dione) and its metabolite 17α-hydroxyprogesterone (4-pregnene-17α-ol-3,20-dione),
- the androgens testosterone (4-androstene-17β-ol-3-one) and 5α-dihydrotestosterone (5α-androstane-17β-ol-3-one), the androgen metabolite α-androsterone (5α-androstane-3α-ol-17-one),
- the estrogens 17β-estradiol (estra-1,3,5(10)-triene-3,17β-diol) and estrone (estra-1,3,5(10)-triene-3-ol-17-one) and their metabolites 17α-estradiol (estra-1,3,5(10)-triene-3,17α-diol) and estriol (estra-1,3,5(10)-triene-3,16α,17β-triol).

The biosynthetic relationships between the investigated steroids are illustrated in Fig. 1, in which main steroids and their precursors are presented along with their structural formulas. Testosterone and progesterone were obtained from Serva (Heidelberg, FRG), all other reference compounds were from Sigma (Deisenhofen, FRG). Testosterone-3,4-$^{13}C_2$ (Cambridge Isotope Laboratories) and 17β-estradiol-16,16,17-D_3 (MSD Isotopes, IC Chemikalien, Munich, FRG) served as internal standards.

Reagents

All solvents used were analytical grade (obtained from Merck, Darmstadt, FRG). Derivatization reagents were from Fluka (Neu-Ulm, FRG); solid phases from Serva (Amberlite XAD-2) and Merck (neutral Al_2O_3 90, Celite). β-Glucuronidase/arylsulfatase (EC 3.2.1.31/EC 1.6.1, from *Helix pomatia*, glucuronidase activity: 100 000 Fishman units ml^{-1}, sulfatase activity: 800 000–1 000 000 Roy units ml^{-1}) was from Serva.

Analysis

Generally 25 g of the foodstuffs were submitted to analysis. The sample size was increased for liquid foods (milk, yoghurt: 50 g, alcoholic beverages: 100 g) and reduced for dry foods (cereals, legumes: 10 g). Sample sizes of foods rich in milk fat (therefore expected to contain high amounts of progestogens) were also reduced (butter: 5 g; cheese: 10 g; cream: 15 g).

Sample preparation depended on the foodstuffs' matrix and the occurrence of conjugated steroids. To obtain the sum of all steroids present in food, hydrolytic liberation of steroids was carried out if the presence of conjugated steroids was expected. Food samples with known amounts of glucuronidated and/or sulfated steroids (all offal, milk and milk products) as outlined by Hoffmann and Rattenberger (1977) and CEC (1989) were incubated with their specific cleaving enzymes. Food samples with unknown conjugates or conjugates other than sulfates or glucuronides, e.g. glycosides in plants (Kalinowska, 1994), were treated with mineral acid to liberate any conjugates. Samples with more than 30% fat had to be dissolved first in lipophilic solvents to allow the extracting agent (methanol) to penetrate through the sample.

Sample pretreatment

After homogenizing the samples $^{13}C_2$-testosterone (2.0 ng μl^{-1} methanol) and D_3-17β-estradiol (1.0 ng μl^{-1} methanol) were added as internal standards to eliminate preparation losses.

- Liver, meat and milk products and eggs were treated with β-glucuronidase/arylsulfatase overnight at 37°C in 25 ml acetate buffer (0.04 mol litre^{-1}) at pH 5.1–5.3 (Bundesgesundheitsamt, 1989).
- Plant samples underwent an unspecific acid hydrolysis (Curtius and Müller, 1967). Homogenized material was suspended with 50 ml water and heated under reflux. After addition of 5 ml concentrated HCl the mixture was boiled for 15 min. The suspension was then neutralized with NaOH and centrifuged.
- Fat and fatty tissues were first dissolved in 30 ml hexane at 40°C (van Look *et al.*, 1989).

The different sample preparations were checked by analysing the recovery of spiked samples.

Isolation and purification of the steroids (Bundesgesundheitsamt, 1989)

To extract the liberated steroids, the samples were homogenized with 90 ml methanol and 0–25 ml water (depending on the original water content of the

foodstuff and the sample pretreatment) and centrifuged (10 min at *ca* 2000 *g*). The supernatant was extracted with 2×40 ml hexane to remove fat. The methanol/water layer was then extracted three times with dichloromethane (70, 40 and 30 ml). The crude extract was purified on an Amberlite XAD-2 column followed by a fractionation in phenolic and neutral steroids through a Celite/KOH column coupled to an Al_2O_3 column as described by the Bundesgesundheitsamt (1989).

The chosen sample preparation proved to be suitable for most foodstuffs. Interferences were effectively removed and so chromatograms which were very suitable for interpretation were obtained. Matrix problems arose with rice and yeast and with liver and sausage, although the method was recommended for analysis of offal and meat products by the Bundesgesundheitsamt (Bundesgesundheitsamt, 1989).

Gas chromatography–mass spectrometry

Both fractions were analysed separately by GC–MS. The steroids were derivatized with 50 μl *N*-methyl-*N*-trimethylsilyltrifluoroacetamide/trimethyliodosilane/dithioerythritol (1000:2:2) at 60°C for 15 min (according to Smets *et al.*, 1993). GC–MS conditions: GC: Varian 3400 (column: DB-5 MS, 30 m×0.25 mm, 0.25 μm film), MS: Finnigan INCOS 50 B (EI, electron energy: 70 eV, ion source temperature: 180°C). Masses for selected ion monitoring: α-androsterone, 5α-dihydrotestosterone, $^{13}C_2$-testosterone: 434, 419; dehydroepiandrosterone, testosterone: 432, 417; androstenedione: 430, 415; pregnenolone: 460, 445; progesterone: 458, 443; 17α-hydroxyprogesterone: 546, 441; 17α-/17β-estradiol: 416, 326, estrone: 414, 399; estriol: 504, 414; D_3-17β-estradiol: 419, 339.

The determination limit of this GC–MS method was about 0.01–0.3 $\mu g\,kg^{-1}$ depending on the hormone and matrix.

RESULTS AND DISCUSSION

In the following tables and paragraphs only the main biologically active steroids (progesterone, testosterone, 17β-estradiol and estrone) are presented together with their main precursors (the basic precursor pregnenolone, the weak androgenic intermediate androstenedione and the ambivalent intermediate dehydroepiandrosterone) which were detectable in most samples in considerable amounts. Minor steroids, which appear in only a few foodstuffs, are discussed at the end of this paper.

Steroids in beef and veal

The amounts of naturally occurring steroid hormones in the tissues of calves, bulls, steers and heifers have been extensively investigated. Therefore, just one sample of bulls', steers' and heifers' meat each and one liver were analysed. The hormone content of the latter could not be quantified, however, because of interferences in the chromatogram. The most comprehensive data concerning androgens, progestogens and their precursors and metabolites in meat of bulls, steers and heifers have been published recently by Hartwig *et al.* (1997). Further information is available on the content of testosterone, progesterone and estrogens in veal and beef in a review published by the European Communities (CEC, 1989), and on androstenedione and testosterone in tissues of calves, bulls and heifers (Gaiani and Chiesa, 1986), on testosterone and estrogens in veal (Scippo *et al.*, 1993) and on estrogens and progesterone in tissues of steers and cows (Tsujoka *et al.*, 1992). Existing data are summarized in Table 1.

Tissues from adult cattle can reach higher testosterone and progesterone concentrations than calves. However, calves show comparatively high amounts of estrogens. These values are only exceeded by pregnant cows with up to 0.7, 0.3 and 5.4 $\mu g\,kg^{-1}$ estrone and up to 0.9, 1.4 and 0.2 $\mu g\,kg^{-1}$ 17β-estradiol in muscle, liver and fat, respectively (CEC, 1989). The hormone patterns of male and female cattle obviously differ with heifers showing high levels of progesterone (about 20 $\mu g\,kg^{-1}$) but lower levels of testosterone than male animals. Hormone levels in cattle liver resemble those in muscle tissue, whereas fatty tissues accumulate lipophilic hormones.

Residues of steroid hormones in the tissues of calves, steers and heifers treated with estradiol and/or testosterone or progesterone are in the same order as in untreated cattle (Henricks *et al.*, 1983; Tsujoka *et al.*, 1992).

Steroids in milk and milk products

It is known that steroid hormones pass the blood–milk barrier. This effect has been used for diagnosis of pregnancy in cattle by analysing the progesterone content in milk. The mechanism of transport (active or passive) is discussed in the literature as well as the synthesizing and metabolizing potential of the mammary gland (Erb *et al.*, 1977; Gaiani *et al.*, 1984). As expected, most information is available about the progesterone content in milk and milk products (Hoffmann *et al.*, 1975*a*; Ginther *et al.*, 1976; Cantoni and d'Aubert, 1995). Concentrations range from 1.4 $\mu g\,litre^{-1}$ in skim milk to about 10 $\mu g\,litre^{-1}$ in whole milk and to about 300 $\mu g\,kg^{-1}$ in butter depending on the fat content (Table 2). A strong correlation of the progesterone level with the milk fat content has been proved, which is due to the hormone's fat solubility. In distribution studies with ^{3}H-progesterone, milk fat contained 80% of the labelled progestogen, casein made up 19% (also indicating some protein binding) and whey made up 1% (Heap *et al.*, 1975). The protein binding property of progesterone can also be observed in protein concentrated milk products like dried milk (dried skim milk: 17 $\mu g\,kg^{-1}$, dried whole milk: 98 $\mu g\,kg^{-1}$).

Table 1. Steroid concentrations ($\mu g\,kg^{-1}$) in beef and veal (mean and standard deviation)

		Pregnenolone	DHEA	Androstenedione	Progesterone	Testosterone	17β-Estradiol	Estrone
Bulls	Roast beef	2.4	0.9	0.8	0.3	0.4	< 0.03	< 0.02
(intact males)	Meat	1.7(0.8–5.0)[a]	0.3(0.2–0.5)[a]	0.6(< 0.2–1.2)[a]	0.3(< 0.3–0.4)[a]	0.5(< 0.2–2.8)[a]		
	Muscle			0.37 ± 0.05[b]		0.73 ± 0.10[b]		0.01 ± 0.00[d]
						0.78 ± 0.73[c]		
	Liver					0.75 ± 0.41[c]		0.01 ± 0.00[d]
	Fat			10.31 ± 0.88[b]		5.26 ± 0.66[b]		0.04 ± 0.00[d]
						10.95 ± 8.68[c]		
Steers	Roast beef	1.4	0.1	0.2	0.1	< 0.02	< 0.03	< 0.02
(castrated males)	Meat	1.7(0.5–5.9)[a]	< 0.2(< 0.2–0.7)[a]	< 0.2(< 0.2–2.5)[a]	0.4(< 0.3–1.7)[a]	< 0.2[a]		
	Muscle				0.27 ± 0.33[e]		< 0.01[e,k]	< 0.01[e,k]
					3.89 ± 0.77[k]		0.01 ± 0.00[f]	0.00 ± 0.01[f]
	Liver				0.26 ± 0.07[e]		< 0.01[e]	< 0.01[e]
					0.35 ± 0.06[k]		0.01 ± 0.00[f,k]	0.01 ± 0.01[f,k]
	Fat				2.48 ± 1.61[e]		< 0.01[e,k]	< 0.01[e]
					4.55 ± 0.79[k]		0.01 ± 0.01[f]	0.01 ± 0.02[f,k]
Heifers/cows	Roast beef	1.1	0.5	0.4	21.5	< 0.02	< 0.03	< 0.02
(females)	Meat	2.8(1.0–6.5)[a]	< 0.2(< 0.2–0.6)[a]	0.3(< 0.2–4.3)[a]	18.9(5.8–43.7)[a]	< 0.2[a]		
	Muscle			0.13 ± 0.01[b]		0.07 ± 0.01[b]	0.01 ± 0.01[e]	0.03 ± 0.00[d]
					22.7 ± 4.7(cl)[k]	0.09 ± 0.03[c]	0.01 + 0.02[f]	< 0.01[e]
					3.78 ± 1.00(cf)[k]	0.02 ± 0.01[e]	0.00(cl,cf)[k]	0.01 ± 0.00(cl,cf)[k]
	Liver				1.50 ± 0.32 (cl)[k]	0.19 ± 0.10[c]	< 0.01[e]	0.02 ± 0.00[d,k]
					0.79 ± 0.25(cf)[k]	0.01 ± 0.00[e]	0.04 ± 0.00[f]	< 0.01[e]
	Fat			2.56 ± 0.42[b]		0.61 ± 0.07[b]		0.03 ± 0.01[d]
					16.7 ± 16.8[i]	0.25 ± 0.06[c]	0.01 ± 0.00[f]	0.04 ± 0.03[f]
					37.9 ± 5.5(cl)[k]	0.3 ± 0.01[e]	0.01 ± 0.01[e]	0.01 ± 0.00(cl)[e,k]
					17.4 ± 7.3(cf)[k]		0.02 ± 0.00[k]	0.02 ± 0.00(cf)[k]
Calves	Muscle				0.25 ± 0.09[h]	0.02 ± 0.01[c]		
(male/female)				0.44 ± 0.04(m)[b]		0.16 ± 0.02(m)[b]	0.11 ± 0.14[g]	0.08 ± 0.04[g]
						0.03–0.77(m)[j]	0.00–0.03[j]	0.02–0.08[j]
				0.22 ± 0.04(f)[b]		0.08 ± 0.01(f)[b]		
						0.01–0.42(f)[j]		
	Liver				0.27 ± 0.11[h]	0.04 ± 0.02[c]	0.07 ± 0.16[g]	0.20 ± 0.09[g]
						0.10–0.32(m)[j]	0.00–0.05[j]	0.17–0.20[j]
						0.01–0.12(f)[j]		
	Fat				5.8 ± 2.5[i]	0.18 ± 0.12[c]		
				17.45 ± 2.77(m)[b]		3.57 ± 0.64(m)[b]	0.13 ± 0.06[g]	0.28 ± 0.08[g]
						0.27–3.88(m)[j]	0.00–0.05[j]	0.08–0.09[j]
				2.32 ± 0.60(f)[b]		0.49 ± 0.03(f)[b]		
						0.02–0.17(f)[j]		

[a]Hartwig *et al.* (1997): retail cuts, median and range.
[b]Gaiani and Chiesa (1986).
[c]Hoffmann and Rattenberger (1977).
[d]Henricks *et al.* (1983).
[e]Kushinsky (1983).
[f]Henricks (1980).
[g]Hoffmann *et al.* (1975*b*).
[h]Heinritzi (1974).
[i]Hoffmann (1978).
[j]Scippo *et al.* (1993): range.
[k]Tsujioka *et al.*, (1992).
[c–i]Reviewed by CEC (1989).

(m): male.
(f): female.
(cl): cow, luteal stage.
(cf): cow, follicular stage.

Table 2. Steroid concentrations in milk ($\mu g\,litre^{-1}$) and milk products ($\mu g\,kg^{-1}$), published data

	Fat (%)	Androstenedione	Progesterone	Testosterone	17β-Estradiol	Estrone
Skim milk			2.1 ± 0.6^b			
	0.1		1.4^c			
unprocessed			4.6 ± 0.4^b			
Milk			5.8 ± 0.4^b			
(fat reduced)	1.5		6.0^c			
Whole milk			9.5 ± 0.5^b			
	3.5		11.8–12.5^c			
unprocessed		0.1–3.5^a	11.3 ± 0.6^b	0.02–0.12^a	0.03 ± 0.00^e	0.01 ± 0.00^e
				0.05–0.15^d	0.01–0.06^f	0.03–0.12^f
Buttermilk			4.7 ± 0.8^b			
	1.0		6.5^c			
Sour milk (fat reduced)	1.5		4.2^c			
Condensed milk	10		12.3^c			
Dried milk (skim)	1.5		17.1^c			
Dried milk (whole)	25		98.4^c			
Ricotta			1.7–2.0^g	$<0.1^g$	0.01^g	
Cheese (fresh)			3.0–4.0^g	$<0.1^g$	0.01^g	
Cheese (half ripened)			2.0–3.5^g	$<0.1^g$	0.02–0.03^g	
Cheese (ripened)			<1.0–3.3^g	$<0.1^g$	0.01–0.03^g	
Cheese (propionic fermentation)			5.9–10.5^g	0.07–1.41^g	<0.01–0.03^g	
Cream			72.7 ± 5.8^b			
unprocessed	32		43.0^c			
			58.7 ± 5.3^b			
Butter			132.9 ± 5.1^b			
	82		300.0^c			

[a]Gaiani *et al.* (1984).
[b]Ginther *et al.* (1976).
[c]Hoffmann *et al.* (1975*a*).
[d]Hoffmann and Rattenberger (1977).
[e]Erb *et al.* (1977).
[f]Hoffmann (1977).
[g]Cantoni and d'Aubert (1995).

The main estrogen in milk is the biologically inactive 17α-estradiol (about $0.16\,\mu g\,litre^{-1}$), followed by estrone (about $0.03\,\mu g\,litre^{-1}$) and 17β-estradiol (about $0.01\,\mu g\,litre^{-1}$) (Erb *et al.*, 1977). In accord with these values, Cantoni and d'Aubert (1995) detected only traces of 17β-estradiol in cheese (<4–$30\,ng\,kg^{-1}$). Only few reports deal with androgens in milk. Contents reported are 0.02–$0.15\,\mu g\,litre^{-1}$ testosterone (Hoffmann and Rattenberger, 1977; Gaiani *et al.*, 1984), and 0.1–$3.5\,\mu g\,litre^{-1}$ androstenedione (Gaiani *et al.*, 1984), depending on state of pregnancy. The ratio between free testosterone and conjugated testosterone in milk is about 1:1 (Hoffmann and Rattenberger, 1977). Published values are presented in Table 2.

Food processing does not seem to influence the amounts and ratios of the investigated hormones. An interesting observation was made by Cantoni and d'Aubert (1995), however, who could not detect testosterone in any cheese (ricotta, fresh cheese, half-ripened or ripened cheese) but did detect it in propionic fermented cheese (like Leerdammer, Gruyère, Emmenthal) in concentrations of 0.07–$1.41\,\mu g\,kg^{-1}$.

Table 3 shows the results of our market basket survey. Milk products can be considered as a rich source of steroids. The hormone pattern resembles that of meat from female cattle. For all milk products the sum of conjugated and free hormones was determined. The values for estrogens, progesterone and androstenedione are in accordance with the literature data. The amounts of lipophilic hormones depend on the fat content of the milk product. Not only progesterone but also pregnenolone, androstenedione and estrone increase with the fat content. Food processing, such as heating or churning, appears to have no effect on the hormone patterns although cheese ripening does. Testosterone was not detectable in milk or any other milk product, so the reported values by other researchers for testosterone in milk (Table 2) could not be confirmed. An exception is cheese. In fresh cheese ($n=2$) as well as in ripened cheese (Gouda) testosterone was detected (0.1–$0.5\,\mu g\,kg^{-1}$), in contrast to the results of Cantoni and d'Aubert (1995). Probably not only propionic acid bacteria but also other fermenting bacteria or clotting enzymes are responsible for the formation of testosterone during the fermentation process. The metabolic intermediate androstenedione or even the androgen metabolite estrone may possibly be precursors for testosterone. These lipophilic compounds are conspicuously low in Gouda cheese, in spite of its high fat content (which is comparable to cream, that shows

Table 3. Steroid concentrations ($\mu g\,kg^{-1}$) in milk and milk products

	Fat (%)	Pregnenolone	DHEA	Androstenedione	Progesterone	Testosterone	17β-Estradiol	Estrone
Milk	3.5	2.09	0.13	0.21	9.81	<0.01	<0.02	0.13
Cream	30	12.2	0.31	1.25	48.6	<0.03	<0.03	0.26
Cream	30	7.80	0.14	2.10	41.8	<0.03	n.d.	n.d.
Butter	85	49.6	1.15	5.98	141	<0.05	<0.03	1.47
Yoghurt	3.0	3.01	0.11	0.56	13.3	<0.01	<0.02	0.16
Fresh cheese	11	5.11	0.26	0.94	21.5	0.15	n.d.	n.d.
Fresh cheese	11	5.48	0.18	1.82	30.3	0.13	n.d.	n.d.
Gouda cheese	29	12.0	0.17	0.77	44.2	0.48	<0.03	0.17

n.d., not determined.

equivalent amounts of other lipophilic hormones such as progesterone and pregnenolone). It may, therefore, be concluded that testosterone can be formed from precursors, which are present in milk (like androstenedione or estrone), during cheese making or ripening by fermenting enzymes. However, yoghurt, another fermented milk product, does not show a differing hormone pattern in comparison to milk.

Steroids in pork and meat products

In pig tissues a similar steroid pattern as in ruminants was observed, with a predominance of the metabolic intermediates (Table 4). The concentrations of hormonally active steroids in pork are comparatively low. Liver and bacon show equivalent hormone contents to those in muscle tissues (chops). In contrast to cattle, no accumulation of hormones in fat was found. Between gilts (female pigs) and barrows (castrated males) no remarkable differences were found. Tissues of boars (intact males) were not analysed as they are not routinely marketed in Germany, due to the high incidence of the urine-like boar odour caused by the steroid 5α-androstenone, which is highly correlated to androgen synthesis.

In the literature only limited information is available about the concentrations of steroid hormones in pork: Claus *et al.* (1989) analysed 3 hormones (17β-estradiol, estrone, testosterone) in various tissues of pigs, including boars (Table 5). The values for barrows and gilts are in accordance with our data. Boar tissues show comparatively high concentrations of both estrogens and androgens.

Cooked ham and salami (80% pork) show similar hormone patterns (Table 4) as other pig tissues, whereas frankfurters that contained predominantly beef, show a

Table 4. Steroid concentrations ($\mu g\,kg^{-1}$) in pork and meat products

	Pregnenolone	DHEA	Androstenedione	Progesterone	Testosterone	17β-Estradiol	Estrone
Chop (gilt)	0.37	0.14	0.11	1.76	<0.02	<0.03	<0.02
Chop (gilt)	0.27	0.01	0.19	1.10	<0.02	<0.03	<0.02
Chop (barrow)	0.33	<0.02	0.12	0.76	<0.02	<0.03	<0.02
Chop (barrow)	0.10	<0.02	0.17	0.35	<0.02	<0.03	<0.02
Liver	1.78	0.22	0.12	1.85	<0.02	<0.03	?
Bacon	0.41	<0.02	0.65	0.71	<0.02	<0.03	<0.02
Ham	0.64	0.64	0.39	0.96	0.05	<0.03	<0.02
Ham	0.34	0.24	0.09	1.51	0.04	<0.03	<0.02
Frankfurter	1.16	<0.02	<0.02	6.82	0.07	?	?
Salami	0.20	<0.02	<0.02	0.79	0.05	<0.03	<0.02

?, not interpretable due to interferences.

Table 5. Mean, minimum and maximum concentrations ($\mu g\,kg^{-1}$) of testosterone, 17β-estradiol and estrone in pig tissues (Claus *et al.*, 1989)

		Testosterone	17β-Estradiol	Estrone
Boars	Muscle (diaphragm)	3.71 (0.18–8.40)	0.91 (0.16–2.45)	0.15 (0.02–0.33)
	Backfat	11.96 (1.26–20.34)	0.43 (0.12–0.78)	0.59 (0.09–1.38)
	Liver	1.20 (0.25–2.42)	9.67 (0.31–16.90)	3.33 (0.60–6.59)
Barrows	Muscle (diaphragm)	0.04 (0.00–0.16)	0.03 (0.00–0.07)	0.08 (0.01–0.16)
	Backfat	0.10 (0.00–0.22)	0.03 (0.00–0.06)	0.03 (0.00–0.10)
	Liver	0.04 (0.00–0.10)	0.08 (0.02–0.17)	0.15 (0.04–0.28)
Gilts	Muscle (diaphragm)	0.09 (0.00–0.23)	0.06 (0.00–0.20)	0.03 (0.00–0.16)
	Backfat	0.07 (0.00–0.32)	0.03 (0.00–0.12)	0.05 (0.00–0.20)
	Liver	0.04 (0.00–0.14)	0.21 (0.08–0.32)	0.32 (0.15–0.44)

female-cattle-like-profile with high progestogen concentrations. Food processing steps, such as cooking, smoking and fermenting, appear to have little effect on the steroid patterns. However, all investigated meat products had higher testosterone concentrations than the common ingredients (meat and fat from gilts, barrows and female cattle). This may indicate the partial use of boar meat for meat products although estrogens, which in that case would be expected (Table 5), could not be detected. Another explanation for the occurrence of testosterone in sausages might be a conversion of DHEA and androstenedione during manufacture, as these testosterone precursors were not detectable in salami and frankfurters.

Steroids in poultry and eggs

Hormones have been used in some countries for broiler fattening since the thirties (Abdalla *et al.*, 1992). However, reports about the contents of steroid hormones in poultry tissues are rare. Abdalla *et al.* (1992) investigated residues of 17β-estradiol in chicken carcasses by TLC. They found physiological levels of 20 $\mu g\,kg^{-1}$ in muscle and 30 $\mu g\,kg^{-1}$ in fat of nontreated birds. These values for 17β-estradiol using GC–MS could not be detected in chicken or turkey (Table 6). Only in goose-fat did detectable amounts of 17β-estradiol occur (up to 0.73 $\mu g\,kg^{-1}$). These values correspond to the results of Cantoni *et al.* (1993, 1994), who measured estradiol contents up to 0.02 $\mu g\,kg^{-1}$ in muscle tissues of chickens and laying hens and up to 0.004 $\mu g\,kg^{-1}$ in turkey with radioimmunoassay. In chicken liver they could not detect any 17β-estradiol. The discrepancy with the results of Abdalla *et al.* (1992) is probably due to the comparatively unspecific analytical technique that they used. Their values are, therefore, not presented in the table. With regard to estrone only information about plasma contents is available. Senior (1974) reported estrone concentrations similar to those for estradiol in plasma of hens (about 0.1 $ng\,ml^{-1}$). According to his observations the occurrence of estrone in goose-fat (0.51 $\mu g\,kg^{-1}$) was of the same order of magnitude as 17β-estradiol.

However, not only estrogens are accumulated in fat. The levels of steroid precursors and progestogens reach high levels in goose-fat and the meat of laying hens (approx. 20% fat) too (Table 6). Comparative data only exist about progesterone in plasma of hens (Kappauf and van Tienhoven, 1972; Furr *et al.*, 1973). The concentrations range from 0.5–20 $ng\,ml^{-1}$ dependent on age and cycle. These values are reflected in the tissue concentrations of 0.2 $\mu g\,kg^{-1}$ in chicken to 7.8 $\mu g\,kg^{-1}$ in the laying hen.

Androgens can hardly be detected in poultry. Testosterone could not be detected by GC–MS in any sample except for the purchased goose-fat. By radioimmunoassay Cantoni *et al.* (1994) were able to determine testosterone in male broilers at concentrations up to 0.03 $\mu g\,kg^{-1}$ and in turkey toms at levels up to 0.02 $\mu g\,kg^{-1}$.

A conspicious difference was observable between authentic goose-fat (melted from a Christmas goose) and goose-fat that was purchased from a butchers' (10% pig fat declared). The latter contained less than 20% of the female hormones compared to the authentic fat. On the other hand, testosterone was detected at 0.06 $\mu g\,kg^{-1}$. This leads to the assumption that either the percentage of goose-fat in the purchased sample was less than stated (that means that more than 10% pig fat were added) or that the fat was obtained from a gander.

Generally, it can be concluded that the metabolic pathways in poultry are equivalent to those of mammals. Similar steroid patterns (depending on gender) can be observed. Birds seem like cattle to accumulate more lipophilic hormones in fatty tissues with increasing age.

Table 6. Steroid concentrations ($\mu g\,kg^{-1}$) in poultry and eggs

	Pregnenolone	DHEA	Androstenedione	Progesterone	Testosterone	17β-Estradiol	Estrone
Chicken	0.59	< 0.02	< 0.02	0.24	< 0.02	< 0.03	< 0.02
Chicken					< 0.004–0.030[a]	< 0.004–0.020[b]	
Chicken liver						< 0.004[b]	
Turkey	0.25	0.05	0.06	8.18	< 0.02	< 0.03	< 0.02
Turkey					< 0.004–0.023[a]	< 0.004–0.004[a]	
Laying hen	1.06	0.62	0.62	7.78	< 0.02	< 0.03	0.16
Laying hen						< 0.004–0.015[b]	
Goose-fat[c]	8.96	1.01	0.63	31.85	< 0.02	0.73	0.51
Goose-fat[d]	1.66	0.30	0.09	3.83	0.06	0.03	< 0.02
Egg	85.3	0.06	9.27	25.9	0.49	n.d.	n.d.
Egg	103	0.26	5.96	31.2	0.30	< 0.03	0.18
Egg	143	0.05	2.14	12.5	0.04	0.18	0.35
Egg	118	1.76	7.34	43.6	0.25	0.22	0.89
Egg	83.3	0.07	1.83	21.7	0.15	n.d.	n.d.

[a]Cantoni *et al.* (1994).
[b]Cantoni *et al.* (1993).
[c]Melted from goose.
[d]Obtained from butchers' (10% pork fat declared).
n.d., not determined.

To our knowledge no information exists about the steroid hormone content of eggs. In our investigation high amounts of the basic precursor pregnenolone were detected (up to 140 $\mu g\,kg^{-1}$, Table 6). The biosynthetic intermediates, including the female steroid progesterone with up to 44 $\mu g\,kg^{-1}$ and androstenedione with up to 9.3 $\mu g\,kg^{-1}$, showed high levels, too. This could be expected, as eggs are produced directly in the hens' ovaries (a hormone synthesizing gland). Besides, eggs are known for their high cholesterol content, the precursor of pregnenolone. The estrogenic female hormones, 17β-estradiol with up to 0.2 $\mu g\,kg^{-1}$ and estrone with up to 0.9 $\mu g\,kg^{-1}$ were also found in considerable amounts in eggs. In addition, testosterone was determined in amounts up to 0.5 $\mu g\,kg^{-1}$. Eggs are, therefore, a considerable source of hormonally active steroids and their precursors.

Steroids in fish

Steroid genesis in fish follows a pathway differing from that in mammals, and the steroids show partly different functions (Bern, 1967; Ng and Idler, 1980; Fostier *et al.*, 1983). In addition to the classical mammalian steroid hormones and precursors (testosterone, progesterone, 17β-estradiol, estrone, 17α-hydroxyprogesterone, pregnenolone, 17α-hydroxypregnenolone, dehydroepiandrosterone and androstenedione) fish specific hormones (like 11β-hydroxyandrostenedione, 11-ketoandrostenedione and 11-ketotestosterone) have also been detected in plasma of trout, salmon, plaice and tilapia. 11-ketotestosterone is the main androgen of some fish, e.g. the flounder (Ng and Idler, 1980). Testosterone, on the other hand, can reach higher concentrations in females than in males (Campbell *et al.*, 1980; Pavlidis *et al.*, 1994). Progesterone has no progestogenic activity in fish as it is just a biosynthetic intermediate. Therefore, no differences between male and female fish can be detected (Campbell *et al.*, 1980). The plasma contents of steroid hormones in fish vary widely dependent on the season and reproductive stage. Basal concentrations are about 0.5–3 $ng\,ml^{-1}$, peak concentrations reach 100 and more $ng\,ml^{-1}$ (at time of spawning) (Wingfield and Grimm, 1977; van Bohemen and Lambert, 1981; Baynes and Scott, 1985; So *et al.*, 1985; de Monès *et al.*, 1989). Formation of 5α-dihydrotestosterone has been observed in skin and muscle of trouts (Fostier *et al.*, 1983). Most of the results were obtained by relatively unspecific radioimmunoassays following a TLC fractionation. They were rarely confirmed by mass spectrometry.

To our knowledge no articles dealing with contents of natural hormones in the tissues of fish exist. Our investigations show considerable amounts of androgen-like steroids in the herring (Table 7), even the potent metabolite 5α-dihydrotestosterone (0.37 $\mu g\,kg^{-1}$, see section Minor steroids). The C_{21}-steroids pregnenolone and progesterone are in the same order in both herring and carp. Estrogens were not detectable. It can be observed that the order of magnitude of steroid hormones in fish tissue reflects the basal concentrations determined in fish plasma. The levels also resemble those of mammals' tissues.

Steroids in plants

It is known that plants can possess hormonal activity which gives rise to visible effects on grazing animals. Responsible for estrogenic activities are mainly isoflavones and coumestanes. They occur in plants in the order of $mg\,kg^{-1}$ to $g\,kg^{-1}$ (Franke *et al.*, 1994). However, the occurrence of steroidal estrogens has been proposed by several authors, too, e.g. in *Graminae* (wheat, rice, oats), *Leguminosae* (beans) and *Palmae* (Farnsworth *et al.*, 1975). An influence of steroidal hormones on growth, sex expression and development of plants has also been observed. Hewitt *et al.* (1980) reviewed positive detections and discussed the possible function of these compounds in plants. However, confirmation of the results, which were frequently obtained with unspecific techniques (e.g. TLC) after insufficient purification of plant extracts, and unequivocal identification of the estrogenic principles have to date rarely been obtained. Investigations with modern analytical techniques are therefore deemed necessary (Price and Fenwick, 1985; Jones and Roddick, 1988), above all on basic foodstuffs such as potatoes or cereals.

Several attempts to confirm the occurrence of animal hormones in plants failed (van Rompuy and Zeevaart, 1979). The precursors pregnenolone and progesterone have unequivocally been isolated from higher plants (see review by Geuns, 1978; Deepak *et al.*, 1989). Furthermore, it is known that exogenous animal steroids can be metabolized by many plants. This indicates that the required enzyme systems are present or easily induced in plants (Geuns, 1982). However, currently only the presence of 17β-estradiol and estrone in French beans (*Phaseolus vulgaris*) has been proved, with gas chromatography–mass spectrometry (Young *et al.*, 1978; Hewitt *et al.*, 1980). Amin and Bassiouny (1979) detected estrone ester in olive oil and estrone in corn oil

Table 7. Steroid concentrations ($\mu g\,kg^{-1}$) in fish

	Pregnenolone	DHEA	Androstenedione	Progesterone	Testosterone	17β-Estradiol	Estrone
Herring	1.00	0.60	0.29	0.51	0.07	< 0.03	< 0.02
Carp	0.28	0.18	0.06	< 0.1	< 0.02	< 0.03	< 0.02
Carp	1.41	0.16	0.03	0.2	0.03	< 0.03	< 0.02

(confirmation with TLC, UV, NMR and IR spectroscopy) but not in sesame, coconut, lettuce, linseed, palm, arachis, cottonseed or soybean oil. Few reports are available on androgens. Testosterone and androstenedione have been detected in pollen of *Pinus sylvestris* and *P. nigra*; in the latter dehydroepiandrosterone and androsterone have also been detected (see review by Jones and Roddick, 1988).

In our study almost no steroidal estrogens were detectable (Table 8). Only in olive oil could small amounts (0.02 $\mu g\,kg^{-1}$) of estrone be determined, which lay significantly below the values stated by Amin and Bassiouny (1979) of 9 $\mu g\,kg^{-1}$. The occurrence of estrogens could not be confirmed in cereals, corn oil or beans. Steroid precursors and intermediates, however, could be detected in all analysed plants except for mushrooms. In mushrooms none of the investigated steroids could be determined. Pregnenolone was highest in haricot beans (5.6 $\mu g\,kg^{-1}$), corn oil (3.4 $\mu g\,kg^{-1}$) and wheat (up to 2.5 $\mu g\,kg^{-1}$), followed by potatoes, rice, soybeans and safflower oil. The two samples of *Leguminosae* showed, in addition to pregnenolone, only the presence of DHEA. Progesterone was highest in wheat (up to 2.9 $\mu g\,kg^{-1}$) and potatoes (5.1 $\mu g\,kg^{-1}$). These main foodstuffs also contained DHEA and androstenedione. In wheat even testosterone could be detected (0.1–0.2 $\mu g\,kg^{-1}$). This androgen occurred in native safflower oil and refined corn oil as well, together with progesterone. The intermediate androstenedione could not be detected, however.

It can be concluded from these observations that plants show a similar but not identical steroid metabolism to that of animals. Whereas the basic precursor was present in almost all investigated samples and the metabolic intermediates were present in most of the samples, the compounds, which are hormonally active in humans and animals, were less often detectable in plants. Generally, plants synthesize a variety of plant specific steroids, for example cardenolides, digitanoles or alkaloids. Their biosynthesis may also start from pregnenolone proceeding partly via the same steps as in animals (Heftmann, 1971). On the other hand plant specific steroids may function as precursors for the animal steroids (Geuns, 1978).

Yeast and fermented alcoholic beverages

In 1982 Feldman and co-workers found high affinity estrogen receptors in *Saccharomyces cerevisiae*. In addition they identified a substance that displaces labelled estradiol from mammalian estrogen receptors and that exhibits estrogenic activity in mammalian systems, like the female sex hormone 17β-estradiol (Feldman *et al.*, 1984). Identification was carried out by GC and HPLC retention times, UV absorbance, mass spectrometric fragmentation pattern and two radioimmunoassays after seven chromatographic purification steps. Minimum quantities of 0.5 $\mu g\,kg^{-1}$ were detected. As *S. cerevisiae* is the common bakers' and brewers' yeast, the question arose if people ingest steroidal hormones when they are drinking alcoholic beverages, for example.

In our survey none of the investigated hormonally active steroids could be detected. Estrogens could be detected in neither wine nor beer (detection limit approx. 0.01 $\mu g\,kg^{-1}$). In these fermented alcoholic beverages only the metabolic intermediates dehydroepiandrosterone (0.02 and 0.10 $\mu g\,kg^{-1}$, respectively) and androstenedione (0.02 and 0.05 $\mu g\,kg^{-1}$, respectively) were determined in small amounts. They probably come from the vegetable ingredients. In yeast none of the investigated steroids could be detected, nor could any estrogens. The supposed 17β-estradiol in yeast has now been proved to be the plastic monomer bisphenol-A (Krishnan *et al.*, 1993). This estrogenic substance is released from polycarbonate flasks (which were used to cultivate the yeast) during autoclaving.

Minor steroids

Some of the investigated steroids were hardly detected in food. They are therefore not presented in the tables.

The metabolite of testosterone and androstenedione, α-androsterone, occurred in pig and cattle tissues (0.05–0.18 $\mu g\,kg^{-1}$) and in meat products (0.03–0.31 $\mu g\,kg^{-1}$), above all if considerable amounts of androstenedione were determined. This is in accordance with the results of Hartwig *et al.* (1997) who reported levels of α-androsterone for tissues of bulls from < 0.2–0.2 $\mu g\,kg^{-1}$

Table 8. Steroid concentrations ($\mu g\,kg^{-1}$) in plants

	Pregnenolone	DHEA	Androstenedione	Progesterone	Testosterone	17β-Estradiol	Estrone
Potatoes	1.30	3.09	0.05	5.07	< 0.02	< 0.03	< 0.02
Wheat	2.50	0.67	0.48	2.86	0.09	< 0.07	< 0.05
Wheat	0.96	0.15	0.10	0.60	0.19	< 0.07	< 0.05
Rice	2.35	0.35	?	0.38	?	< 0.07	< 0.05
Soybeans	1.29	0.31	< 0.05	< 0.3	< 0.05	< 0.07	< 0.05
Haricot beans (dry)	5.58	0.51	< 0.05	< 0.3	< 0.05	< 0.07	< 0.05
Mushrooms	< 0.1	< 0.02	< 0.02	< 0.1	< 0.02	< 0.03	< 0.02
Olive oil	0.45	0.04	< 0.02	0.08	< 0.02	< 0.03	0.02
Corn oil	3.39	0.32	?	0.31	0.05	< 0.03	< 0.02
Safflower oil	1.17	< 0.02	< 0.02	0.71	0.21	< 0.03	< 0.02

?, not interpretable due to interferences.

Table 9. Estimated daily intake of steroid hormones (calculation based on nutrition tables of Adolf *et al.*, 1994)

	Progesterone ($\mu g\,d^{-1}$)				Testosterone ($\mu g\,d^{-1}$)				Estrogens (17β-estradiol + estrone) ($\mu g\,d^{-1}$)				DHEA ($\mu g\,d^{-1}$)			
	Meat, fish	Milk products	Eggs	Vegetable food	Meat, fish	Milk products	Eggs	Vegetable food	Meat, fish	Milk products	Eggs	Vegetable food	Meat, fish	Milk products	Eggs	Vegetable food
Men	0.63	8.18	0.92	0.86	0.02	0.02	0.01	0.02	0.02	0.06	0.02	0.00	0.05	0.06	0.02	0.49
Women	0.45	7.06	0.76	0.71	0.01	0.02	0.01	0.02	0.01	0.05	0.02	0.00	0.03	0.05	0.01	0.37
Boys (prepubertal)	0.37	7.27	0.62	0.61	0.01	0.02	0.01	0.01	0.01	0.06	0.01	0.00	0.03	0.06	0.01	0.31
Girls (prepubertal)	0.33	6.57	0.60	0.57	0.01	0.02	0.01	0.02	0.01	0.05	0.01	0.00	0.02	0.06	0.01	0.29
Relative contribution	*ca* 5%	*ca* 80%	*ca* 10%	*ca* 10%	20–30%	30–40%	15–20%	20–40%	15–20%	60–70%	15–20%	< 10%	*ca* 7%	10–15%	*ca* 3%	*ca* 80%

(androstenedione: <0.2–$1.2\,\mu g\,kg^{-1}$), steers from <0.2–$0.5\,\mu g\,kg^{-1}$ (androstenedione: <0.2–$2.5\,\mu g\,kg^{-1}$) and heifers from <0.2–$1.9\,\mu g\,kg^{-1}$ (androstenedione: <0.2–$4.3\,\mu g\,kg^{-1}$). A correlation of α-androsterone with the concentration of testosterone was not observable. Positive detection of α-androsterone was also achieved in fish (herring: $0.6\,\mu g\,kg^{-1}$, carp: 0.02–$0.07\,\mu g\,kg^{-1}$) and milk products (0.06 (milk) up to 2.3 (butter) $\mu g\,kg^{-1}$), increasing with the fat content.

Also rarely detected was the potent androgen 5α-dihydrotestosterone, which is formed in peripheral tissues from testosterone. Traces were found in tissues of bulls ($0.05\,\mu g\,kg^{-1}$), herring ($0.37\,\mu g\,kg^{-1}$) and fermented milk products (0.07–$0.28\,\mu g\,kg^{-1}$). Hartwig *et al.* (1997) determined $\leqslant 0.2\,\mu g\,kg^{-1}$ dihydrotestosterone in bulls' tissues.

The estrogenic metabolites estriol and 17α-estradiol were detected in goose-fat (0.60 and $0.78\,\mu g\,kg^{-1}$, respectively) and hens' eggs (up to $0.25\,\mu g\,kg^{-1}$ each). 17α-estradiol is the main epimer of estradiol in milk (Erb *et al.*, 1977). In our studies it was determined in concentrations from 0.03 (milk) to 0.16 (cheese) $\mu g\,kg^{-1}$.

The progesterone metabolite and androgen precursor 17α-hydroxyprogesterone was detectable in some beef and pork samples and meat products (0.08–$0.88\,\mu g\,kg^{-1}$), herring ($0.48\,\mu g\,kg^{-1}$), eggs (up to $0.36\,\mu g\,kg^{-1}$) and milk products (up to $0.72\,\mu g\,kg^{-1}$). Highest amounts appeared in safflower oil ($3.28\,\mu g\,kg^{-1}$).

Estimation and evaluation of daily intake of hormones

Residues of hormones in meat are frequently the first concern of consumers (compared to other health related issues like saturated fatty acids, cholesterol) in Europe and North America (Sundlof, 1994). In Germany, 76–83% of men and 70–87% of women (percentage depending on age) consider hormones in meat a high or very high risk (Heseker *et al.*, 1992). This is probably a result of a number of scandals where the misuse of anabolic hormones in cattle fattening was exposed (Santarius, 1985; David, 1989) and by the association of synthetic substances which have an effectiveness as estrogens, above all diethylstilbestrol (DES), with carcinogenic effects (Waltner-Toews and McEwen, 1994). DES was used as an abortion preventing drug from 1948–1971 and has been illegally administered to veal calves and beef cattle. In addition to health damaging effects by synthetic compounds, consumers fear hormonal effects caused by consumption of meat.

In contrast to the consumers, scientists consider the occurrence and the use of natural hormones as safe (Hapke *et al.*, 1991). Residues of applied hormones rarely exceed the physiological levels of non-treated animals. However, natural steroid hormones may act as tumor promoting agents in certain target tissues, but only at levels exceeding the no-hormonal-effect-level (NOEL). They do not exert genotoxic effects (Waltner-Toews and McEwen, 1994).

Up to now only animal derived food has been considered in order to estimate exposure to steroid hormones (Hapke *et al.*, 1991). But as seen in this survey, a number of foods contain hormonally active substances at concentrations exceeding those found in meat. The daily intake of hormones by nutrition is estimated on the basis of the German nutritional study (Adolf *et al.*, 1994). Data of the average intake for prepubertal boys and girls and for men and women are presented in Table 9. Meat does not play a dominant role in the daily intake of steroid hormones. Meat, meat products and fish contribute to the hormone supply according to their proportion in human nutrition (average about one quarter). The main source of estrogens and progesterone are milk products (60–80%). Eggs and vegetable food contribute in the same order of magnitude to the hormone supply as meat does.

These values are far exceeded by the human steroid production (Table 10). Children, who show the lowest production of steroid hormones, produce about 20 times the amount of progesterone and about 1000 times the amount of testosterone and estrogens that are ingested with food on average per day. It has further to be taken into consideration that about 90% of the ingested hormones are inactivated by the first-pass-effect of the liver. This leads to the conclusion that no hormonal effects, and as a consequence no tumor promoting effects, can be expected from naturally occurring steroids in food.

The concentration of DHEA in adult human plasma is about 3–$6\,\mu g\,litre^{-1}$. The conjugate dehydroepiandrosterone sulfate, from which DHEA can be released, reaches concentrations of 1–2 mg litre^{-1} (Regelson *et al.*, 1994). In clinical studies $40\,(mg\,kg^{-1})\,d^{-1}$ of DHEA were administered (Regelson and Kalimi, 1994) to achieve health protecting effects. The supply by nutrition can therefore be disregarded, too. The daily intake

Table 10. Daily production of steroid hormones in humans (Kushinsky, 1983) compared to total daily intake

	Progesterone		Testosterone		Estrogens (17β-estradiol + estrone)	
	Daily production ($\mu g\,d^{-1}$)	Daily intake ($\mu g\,d^{-1}$)	Daily production ($\mu g\,d^{-1}$)	Daily intake ($\mu g\,d^{-1}$)	Daily production ($\mu g\,d^{-1}$)	Daily intake ($\mu g\,d^{-1}$)
Men	420	10.6	6480	0.07	140	0.10
Women	19600	9.0	240	0.05	630	0.08
Boys (prepubertal)	150	8.9	65	0.05	100	0.08
Girls (prepubertal)	250	8.1	32	0.04	54	0.07

is about $0.5\,\mu g\,kg^{-1}$ (Table 9), to which plants contribute the most part (about 80%).

More effects on human beings can be expected from exposure to phytoestrogens, which occur in plants in high amounts, or by environmental chemicals with hormonal or hormone blocking activity such as some pesticides, polychlorinated biphenyls or dioxines, which are widespread in food and water.

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