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Systemic Absorption of the Sunscreens Benzophenone-3, Octyl-Methoxycinnamate, and 3-(4-Methyl-Benzylidene) Camphor After Whole-Body Topical Application and Reproductive Hormone Levels in Humans

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Recent *in vitro* and animal studies have reported estrogen-like activity of chemicals used in sunscreen preparations. We investigated whether the three sunscreens benzophenone-3 (BP-3), octyl-methoxycinnamate (OMC), and 3-(4-methylbenzylidene) camphor (4-MBC) were absorbed and influenced endogenous reproductive hormone levels in humans after topical application. In this 2-wk single-blinded study 32 healthy volunteers, 15 young males and 17 postmenopausal females, were assigned to daily whole-body topical application of 2 mg per cm^2 of basic cream formulation without (week 1) and with (week 2) the three sunscreens at 10% (wt/wt) of each. Maximum plasma concentrations were 200 ng per mL BP-3, 20 ng per mL 4-MBC, and 10 ng per mL OMC for females and 300 ng per mL BP-3, 20 ng per mL 4-MBC, and 20 ng per mL OMC for men. All three sunscreens were detectable in urine. The reproductive hormones FSH, LH were unchanged but minor differences in testosterone levels were observed between the 2 wk. A minor difference in serum estradiol and inhibin B levels were observed in men only. These differences in hormone levels were not related to sunscreen exposure.

Key words: sunscreens/systemic absorption/human/excretion/topical application/reproductive hormones
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Terrestrial solar radiation and particularly ultraviolet radiation are absorbed by chemical sunscreens. The use of sunscreens is increasing due to growing public concern about sunburn, photoaging, skin cancer, and to protect against photodermatoses.

A study (Schlumpf *et al*, 2001) recently reported an estrogen-like activity *in vitro* in MCF-7 breast cancer cells and *in vivo* in the immature rat uterotrophic assay of the most frequently used sunscreens. The orally most active compound 3-(4-methylbenzylidene) camphor (4-MBC) also increased uterine weight following topical application. The two other less active substances were benzophenone-3 (BP-3) and octyl-methoxycinnamate (OMC).

Our aim was to investigate whether the three compounds BP-3, OMC, and 4-MBC applied topically could be found in the plasma and urine and whether systemic uptake had any acute effect on the levels of endogenous reproductive hormones in humans.

Thirty-two healthy Caucasian volunteers: 15 males (aged 23–29, mean 26 y) and 17 postmenopausal females (aged 54–86 y, mean 65 y) were enrolled.

Postmenopausal women were selected to achieve a stable hormone level independent of ovarian-menstrual cycle. The subjects were only allowed to use a sunscreen-free moisturizer supplied by us 1 wk before the study. Physical exercise, sun bathing, intake of caffeine, nicotine, or alcohol were not allowed during the study.

The study lasted 2 consecutive wk; a control week followed by a treatment week. Hereby the study became single-blinded and the subjects served as their own control. In both weeks subjects met Monday–Friday in our Department of Dermatology. The time point for the first cream application and blood sampling was noted and thereafter performed at the same time each day. Morning urine was collected each day. Immediately after the 0 h blood sample basic cream formulation without sunscreens was applied by staff to the whole body every day for 4 d. The same procedure was used in the second week except that a daily prepared sunscreen formulation was used. We applied 2 mg per cm^2 of cream, corresponding to 40 g for an average body area of 2.0 m^2.

The basic formulation was an Essex cream (Schering-Plough, Brussels, Belgium). The active formulation consisted of Essex cream with 10% (wt/wt) of the three sunscreens: Eusolex 4360 (BP-3) (Merck), Eusolex 6300 (4-MBC) (Merck, Darmstadt, Germany), and Escalol 557 (OMC) (International Specialty Products, Waterfield, Tadworth, UK).

Serum inhibin B in males was measured in antibody enzyme immunometric assay (Oxford-bioinnovation, UK).

Abbreviations: BP-3, benzophenone-3; 4-MBC, 3-(4-methylbenzylidene) camphor; OMC, octyl-methoxycinnamate

Inhibin B was not measured in the woman as postmenopausal women all have undetectable inhibin B levels.

Serum FSH, LH, and SHBG were measured by time-resolved immunofluorometric assay (DELFIA, Wallac, Inc., Turku, Finland). Testosterone and estradiol were measured by radio immunoassay (RIA) (Coat-a-Count, Diagnostic Products, California and ImmunoDiagnostic Systems, Boldon, UK).

Essential Data

Systemic uptake In all plasma samples drawn before the first application of the active formulation BP-3, 4-MBC, and OMC were below the detection limit.

In the postmenopausal women maximum plasma levels of approximately 200 ng per mL BP-3, 20 ng per mL 4-MBC, and 10 ng per mL OMC were reached 3–4 h after application. In the young men maximum plasma concentrations of 300 ng per mL BP-3, 20 ng per mL 4-MBC, and 20 ng per mL OMC were reached 3 h after application.

The plasma concentrations of BP-3 at 24 and 96 h after the first application of the active formulation were not significantly different in neither the men nor in the women, indicating that no accumulation occurred during the treatment week. The same was true for OMC in the women. In the men the plasma concentrations of both OMC and 4-MBC were significantly higher after 96 h than after 24 h ($p < 0.001$ and $p = 0.03$, respectively; differences were compared by Wilcoxon's signed rank test).

In females urine approximately 60 ng per mL BP-3 and 5 ng per mL of 4-MBC and OMC, were found. In the males urine 140 ng per mL BP-3, 7 ng per mL 4-MBC and 8 ng per mL of OMC were found. In men there were no significant change in urine levels of the three sunscreen compounds from 24 to 96 h after the first application of sunscreen. The same was true for 4-MBC and OMC in women, but the concentration of BP-3 in urine increased from the 24 to the 48 h samples and remained constant thereafter.

Hormone levels There were no statistically significant differences in the levels of FSH, LH, and SHBG in neither the women nor in the men (Figs 1 and 2) in samples obtained at the same time points in the control week and the treatment week. This was also the case for estradiol in the women. A significant difference in mean ($p < 0.01$, differences were compared by paired *t* test) testosterone levels, however, was found in women between the control week and the treatment week at 0–3 and 24 h, but not at 4 and 96 h. As this difference was evident also in the 0-h samples when treatment with the active formulation had not yet started and as it was not consistent this observed difference most likely was a chance finding not related to the sunscreen compounds. Likewise, minor but statistically significant differences between the 2 wk in inhibin B levels in the 0–3-h male samples and in estradiol in the 2- and 4-h male samples ($p < 0.05$ and $p < 0.01$; differences were compared by paired *t*-test) most likely reflects chance findings. In the men a significant difference in mean testosterone levels ($p < 0.05$) between the 2 wk was also found in the 4-h samples, which might suggest a more steep diurnal decrease in testosterone during the treatment week compared with the control week. To further explore this a statistical model, which allowed for a general shift in hormone levels from 1 wk to the other and for different diurnal trends during the 2 wk, was used. In this model a significant decrease in both inhibin B and testosterone but not in estradiol levels was observed in the men from 0 to 4 h on day 1 in both weeks ($p < 0.001$). This is in accordance with the known decreasing levels during daytime. The trend in the daytime decrease in inhibin B and in testosterone, however, was not significantly different between the 2 wk ($p = 0.086$ and 0.31, respectively).

Discussion

In the present human study, we have demonstrated for the first time that the sunscreen compounds BP-3, OMC, and 4-MBC were absorbed through the skin and excreted in urine. The systemic presence of these three sunscreens, however, did not seem to have any influence on the levels of endogenous reproductive hormones in young men and postmenopausal women.

Absorption of BP-3 and OMC into the stratum corneum of Micro-Yucatan pigskin reaches a peak level within an hour and OMC had a lower skin penetration than BP-3, although this effect was depending upon formulation (Gupta *et al*, 1999). Evidence for percutaneous absorption of 4-MBC through human skin has been presented in an experimental model with very small skin areas of 12 subjects *in vivo* (Hagedorn-Leweke and Lippold 1995). In our study, BP-3 also gave a higher plasma concentration than 4-MBC and OMC, which indicates that the two latter have the same skin penetration abilities in this formulation as in the experimental setting.

A small sex difference in plasma concentrations might be explained by an age difference between the groups. Body fat increases from 33% to 45% in women with age, and increased body fat increases the volume of distribution for lipophilic drugs and may result in increased plasma elimination half-lives (Cusack and Evestal, 2000).

From our experimental design, we did not intend to estimate the amount absorbed of the three compounds. It has been estimated, however, that over a 10-h period the amount of BP-3 being absorbed through the skin was 1%–2% of the topically applied amount (Hayden *et al*, 1997). Thus the systemic uptake of BP-3 may be in the order of milligrams per application.

The plasma levels of 4-MBC increased from 24 to 96 h in both genders and the same was true for OMC in the young men, indicating accumulation after repeated daily applications. The excretion in urine did not show a similar increase. In women, however, BP-3 in urine increased from 24 to 48 h, which is in line with the findings of Gustavson Gonzalez *et al* (2002). However, as we collected only morning spot urines, the concentrations measured in urine should be interpreted with caution.

Both BP-3 and OMC are approved as a cosmetic ingredient in Europe and an over-the-counter drug in the US by the FDA. The highest approved concentration in Europe for OMC and BP-3 is 10%. Although the highest

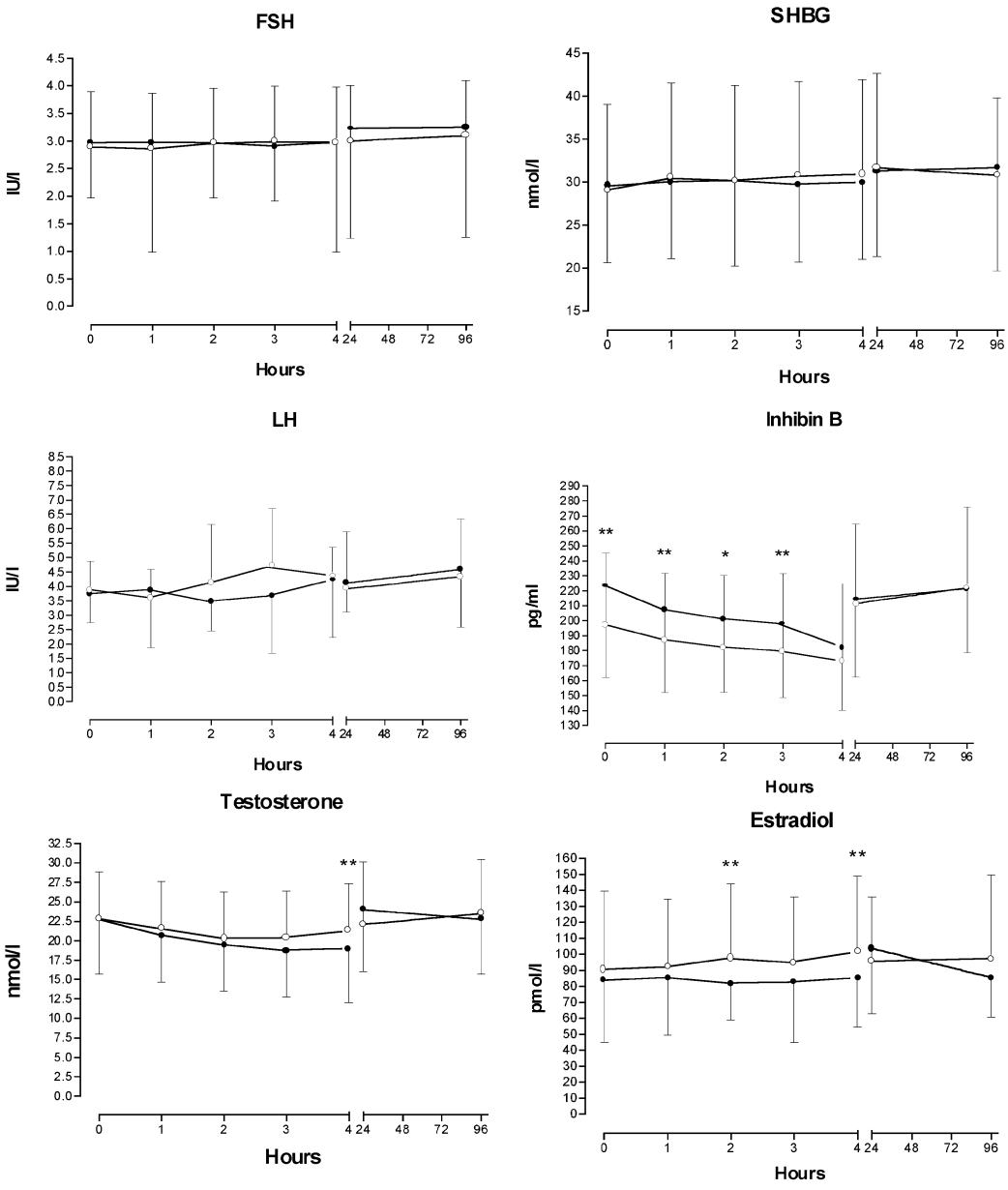

Figure 1
Mean serum concentrations of hormones for males during the week of treatment with control cream (○) and during the week of treatment with cream containing sunscreens (●). All values are means ± SD. Significant differences in hormone levels at the same time points between the 2 wk are indicated by *$p<0.01$, **$p<0.05$. Differences are compared by a paired *t* test.

approved concentration for 4-MBC in Europe in commercial formula is 4%–6%, the active formulation with 10% (wt/wt) of all was specifically made for this study (Gagliardi *et al*, 1987; Lowe, 1998).

4-MBC is only approved in Europe. At the moment the Danish EPA is advising not to use 4-MBC for children younger than 12 y because of the insufficient safety profile for its use in children.

Schlumpf *et al* (2001) concluded that sunscreens are potential endocrine disrupters and should be investigated more closely, in particular penetration through human skin. They also suggested that the benefits of extensive sunscreen use may have to be reconsidered from a medical and ecological perspective.

We observed no biologically significant effects on hormone levels indicating that the amount of sunscreen compounds absorbed were not capable of disturbing the homeostasis of endogenous reproductive hormones in adults. Minor but statistically significant differences in hormone levels between the control week and the treatment week was observed for testosterone, estradiol, and inhibin B at some time points. These differences, however, did not seem to be related to the exposure to sunscreen compounds and at least some of these statistically significant differences between the 2 wk may be chance findings due to mass significance. Normal biological variations in hormone levels may have contributed to some of the differences. This is supported by the fact that for inhibin B in men and testosterone in women significant differences were found between the 0-h samples of the 2 wk, although both samples were drawn before the first application of the active formulation. Earlier reports substantiate that humans use less sunscreens than intended by the regulators, under natural conditions (Deutshes Institut für Normung, 1985; Bech-Thomsen and Wulf, 1992–1993; Stender *et al*, 1996a, b; Wulf *et al*, 1997). This indicates that in real life humans are exposed to lower concentrations. Thus, with a whole-body dermal application of 2 mg cream per cm^2 of

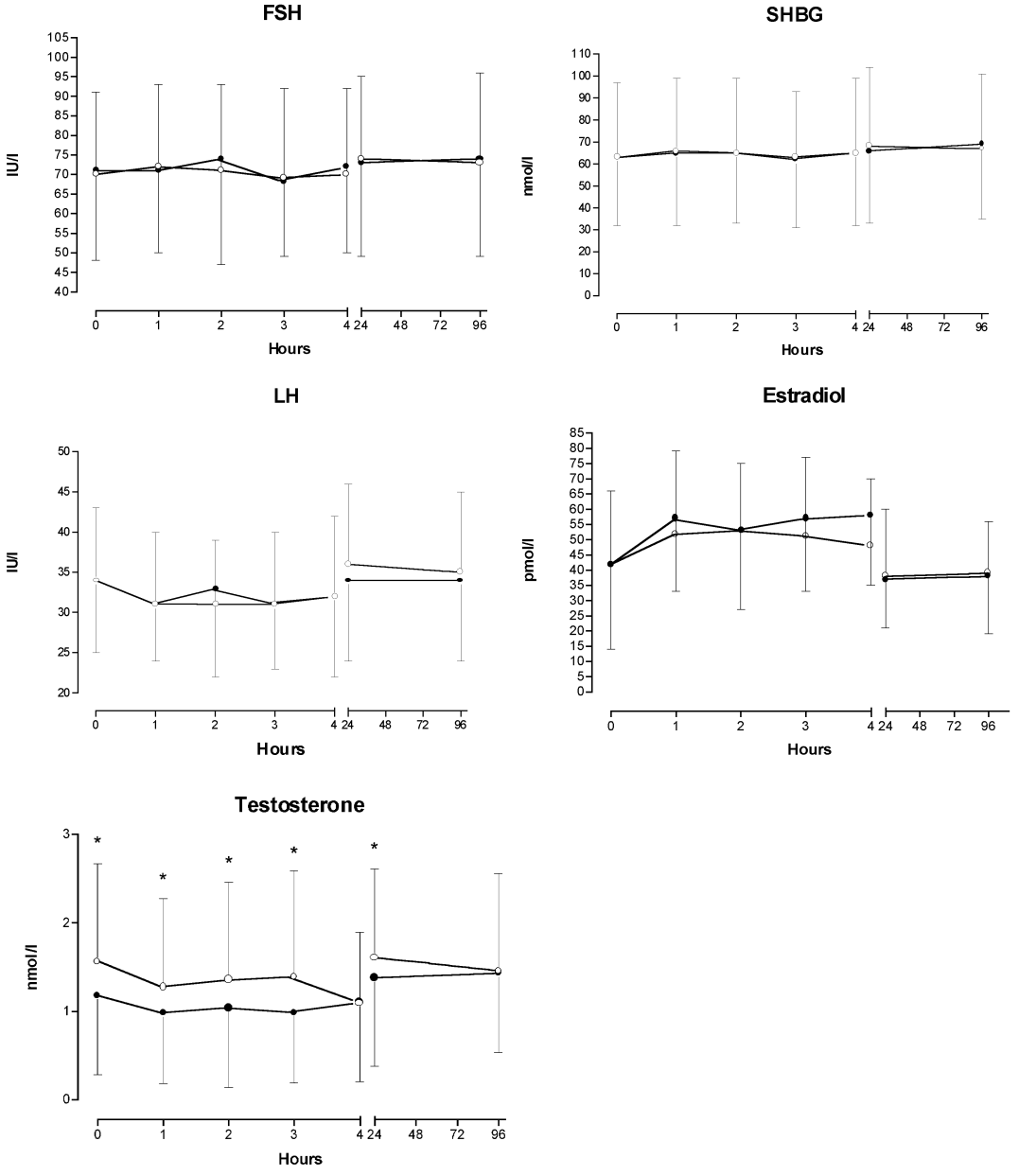

Figure 2
Mean serum concentrations of hormones for females during the week of treatment with control cream (○) and during the week of treatment with cream containing sunscreens (●). All values are means ± SD. Significant differences in hormone levels at the same time points between the 2 wk are indicated by $^{*}p < 0.01$. Differences are compared by a paired *t* test.

the three sunscreens in the concentration of 10% (wt/wt) each we intended to reach maximum exposure.

We applied all three sunscreen compounds simultaneously and might expect an additive or possibly even a synergistic effect. The above findings may indicate that either adult humans have a high threshold for regulation of reproductive hormone levels (which is in accordance with the fact that treatments of postmenopausal women with high levels of very potent estrogens only have moderate effects on the gonadotropin levels (Lind *et al*, 1979; Castelo-Branco *et al*, 1993) or the tested sunscreen compounds have a very low potency for hormone disruption.

The study was designed as single-blinded because we did not know the baseline hormone level of each subject, and secondly, if there was a sunscreen effect due to treatment we did not know how long it would last. Thus, the control week provided us with the baseline hormone levels for our subjects.

In conclusion, this study showed that with an whole-body dermal application of 2 mg cream per cm^2 with BP-3, 4-MBC, and OMC used as sunscreens in the concentration of 10% (wt/wt) each, we were able to detect all three compounds in their parent forms both in plasma and urine, showing that there is a substantial skin penetration, systemic uptake, and urinary excretion of all three compounds in humans. We cannot, however, rule out that data from this study may not reflect how a single or dual combination of these three sunscreens may penetrate human skin. The systemic concentrations achieved of the "estrogenic" sunscreen compounds tested in this short-term study did not seem to interfere with the hypothalamic–pituitary–gonadal axis as the endogenous levels of reproductive hormones were unaffected. Prepubertal children, however, are considered to be more sensitive to low levels of hormone action due to their low levels of endogenous reproductive hormones. Young children have less devel-

oped elimination of drugs and larger surface area per body weight than adults leading to a possible higher uptake and bio-accumulation. Thus, our results cannot exclude that a sunscreen treatment similar to the treatment given in this study might have adverse effects in children.

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