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Metabolic effects of low-dose fluconazole in healthy female users and non-users of oral contraceptives

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1 Azole antifungal agents such as ketoconazole act by inhibiting cytochrome P-450 mediated sterol synthesis in the fungal cell membrane and thus have the potential to interfere with mammalian steroidogenesis. Fluconazole is a novel orally-effective antifungal triazole which has been reported to have more specific effects on the cytochrome P-450 enzymes involved in fungal sterol synthesis.

2 Due to the potential value of systemic antifungal agents in the treatment of infections commonly occurring in women, we assessed the effect of oral fluconazole on the metabolic profile of 18 healthy premenopausal women, 10 of whom were taking combined oral contraceptives (OC). Each woman acted as her own control, being studied both before and 21–28 days after fluconazole therapy (50 mg daily), in the luteal phase of consecutive menstrual cycles.

3 The endocrinological profile included measurement of serum oestradiol, progesterone, testosterone and sex hormone binding globulin (SHBG) concentrations, short tetracosactrin adrenal stimulation test and thyroid function tests. Carbohydrate metabolism was investigated by means of an oral glucose tolerance test with measurement of plasma glucose, insulin and C-peptide concentrations. Serum lipids, lipoproteins and apolipoproteins were analysed on samples taken after an overnight fast.

4 Minor biochemical changes associated with fluconazole treatment included increases in serum thyroxine and testosterone concentrations (but not in women taking OC as well as fluconazole) and in insulin and apolipoprotein B levels (but only in women taking OC as well as fluconazole). In general, these changes were small and of no clinical significance with the values remaining within the laboratory normal range. There were no adverse side-effects.

5 Oral administration of fluconazole (50 mg day^{-1}) was not associated with any consistent effect on the endocrinological profile or on lipid and carbohydrate metabolism. In this respect, fluconazole would appear to be a safe and specific agent for use in the control of fungal infections in women.

Keywords fluconazole antifungal oral contraception lipoproteins serum cortisol testosterone

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Introduction

The mode of action of the azole antifungal agents involves inhibition of a cytochrome P-450 mediated enzyme, lanosterol C-14 demethylase (Yoshida & Aoyama, 1986). The resultant inhibition in the conversion of lanosterol to ergosterol interferes with fungal cell wall metabolism. However, cytochrome P-450 enzymes are involved in many mammalian steroidogenic pathways and indeed ketoconazole, the first orally active antifungal azole, lacks appropriate selectivity and interacts with human steroidogenesis (Pont *et al.*, 1982b; Santen *et al.*, 1983; Pont *et al.*, 1984). In males, ketoconazole lowers serum testosterone levels and blunts the adrenal cortical response to ACTH challenge, although basal cortisol levels are unchanged (Pont *et al.*, 1982b; Trachtenberg & Pont, 1984). When considered together with reports of hepatotoxicity (Lewis *et al.*, 1984), the therapeutic applications of ketoconazole are limited to critical fungal infections or to conditions requiring suppression of gonadal or adrenal hormone production (Sonino, 1987).

Fluconazole is a novel triazole antifungal agent which in preliminary studies in animals and healthy male volunteers has shown marked advantages in potency and selectivity over ketoconazole. It is suitable for both oral and parenteral administration and has a long half-life (Humphrey *et al.*, 1985). Fluconazole given at 50 mg day^{-1} orally for 14 days does not affect the clearance of oral contraceptives (OC) in women (Purba & Black, 1986). In healthy male volunteers given fluconazole, plasma testosterone levels were unchanged (Shaw *et al.*, 1987) and the adrenal cortical response to corticotrophin challenge was unaltered, even at doses as high as 400 mg day^{-1} (Farrow, 1989).

Studies of the effects of fluconazole on steroid hormones in women, who are major users of antifungal agents for vulvovaginal candidiasis, are lacking. To ensure that the proposed use of this drug for such infections is not associated with any metabolic disturbance, we investigated the effects of fluconazole 50 mg once daily (the dose level recommended for treatment of superficial fungal infections) in 18 healthy premenopausal female volunteers, including users of combined oral contraceptives. In addition to gonadal hormones, we evaluated glucose and lipid metabolism, thyroid function, and the serum cortisol response to adrenal stimulation.

Methods

Subjects

We studied 18 healthy female volunteers (16 Caucasian, 1 Asian and 1 Negroid) between the ages of 19 and 40 years. All were premenopausal, non-pregnant and within 25% of their ideal body weight. Ten women used OC; nine used OC containing levonorgestrel (four monophasic and five triphasic) and one used a monophasic OC containing desogestrel. The protocol was approved by the local Ethics Committee and informed consent was obtained in accordance with the Helsinki Declaration. All women were instructed to continue their current form of contraception during the course of the study and the importance of avoiding pregnancy was stressed. Women were excluded who were known to be sensitive to imidazole antifungal agents, who had significant clinical abnormalities likely to affect steroidogenesis, whose menses were irregular, who had impaired renal or hepatic function or who were taking regular medication other than OC. Those with a history of alcohol abuse or who were hepatitis B positive were also excluded.

Baseline evaluation of subjects prior to entry into the study included a clinical examination, routine haematology and biochemistry and hepatitis B surface antigen screen. A pregnancy test was carried out immediately before starting fluconazole treatment.

Study design

The women acted as their own controls, with metabolic studies being performed on two visits (baseline and after 21–28 days of fluconazole treatment) in the luteal phase of consecutive menstrual cycles. On the first day of menstruation following the baseline visit, the subjects were instructed to take one 50 mg fluconazole capsule daily until the day before the second visit. Concurrent medication (other than OC) was not encouraged, although antacids and analgesics were permitted.

Haematological and biochemical parameters (including hepatic and renal function tests and serum electrolytes) were monitored on a weekly basis. All subjects were asked to report any side effects while taking fluconazole and were regularly questioned about any unusual symptoms. The subjects kept a diary of menstrual loss,

discomfort and intermenstrual bleeding (if any) during the study and for a further 2 months. Compliance was checked at weekly intervals by taking blood samples for assay of fluconazole by gas liquid chromatography.

The subjects were instructed to consume at least 200 g carbohydrate/day for the 3 days prior to each visit and were seen at 09.00 h after an overnight (12–16 h) fast. Carbohydrate metabolism was assessed by means of an oral glucose tolerance test (OGTT) using 1 g glucose kg^{-1} body weight with half-hourly sampling for 3 h. In addition, fasting serum samples were taken for analyses of lipids, lipoproteins and apolipoproteins. Following the OGTT, blood samples were taken for gonadal hormone and sex hormone binding globulin (SHBG) assays and for thyroid function tests. Adrenal cortical function was assessed after intramuscular injection of 0.25 mg tetracosactrin ('Synacthen', CIBA, Horsham). Blood samples for serum cortisol estimation were taken at 0, 30, 45 and 60 min post-injection.

Plasma and sera were prepared by low-speed centrifugation. Glucose, lipid and lipoprotein analyses were performed on fresh samples; samples for other tests were stored at −20° C before analysis.

One woman was inadvertently given an erroneous glucose dose; her OGTT results were discarded. High baseline cortisol values in three subjects indicated they were already under strong ACTH stimulation, possibly due to anxiety, and the results of their provocation tests were rejected.

Laboratory methods

Serum oestradiol, progesterone and testosterone concentrations were measured by radioimmunoassay using extraction procedures. Sex hormone binding globulin concentrations were measured using the method of Iqbal & Johnson (1977). Thyroxine concentrations were measured by radioimmunoassay (Radcliffe *et al.*, 1974); T_3 uptake by a Sephadex binding technique and TSH using the immunoradiometric kit supplied by Boots-Celltech, Slough. Plasma cortisol was measured by radioimmunoassay.

Plasma glucose was determined by a glucose oxidase method (Trinder, 1969). The radioimmunoassay procedure of Albano *et al.* (1972) was used to determine plasma insulin concentrations. Plasma C-peptide was assayed using the radioimmunoassay kit provided by Guildhay Ltd, Sussex. Total and incremental area under the glucose, insulin and C-peptide curves (AUC) were derived using the formula of Wynn & Doar (1966).

Serum cholesterol and triglycerides were determined by fully enzymatic procedures using reagents supplied by Boehringer Mannheim, West Germany. High density lipoprotein cholesterol was determined after precipitation of other lipoproteins with heparin and manganese ions (Warnick & Albers, 1978). Further precipitation with dextran sulphate (Gidez *et al.*, 1982) allowed determination of HDL3 cholesterol and estimation of HDL2 cholesterol by difference. Low density lipoprotein cholesterol was calculated using the formula of Friedewald *et al.* (1972). Serum apolipoproteins AI, AII and B were estimated by 'rocket' immunoelectrophoresis (Laurell, 1972) using antisera and calibrating materials supplied by Immuno Ltd, Kent.

Within- and between-batch coefficients of variation are given in Table 1. Between-batch values are not given where assays were carried out in a single batch.

Statistical analyses

Mean values (with ranges) are given for all results where paired data (on-treatment *vs* pre-treatment) was available. Triglyceride and insulin related values were log transformed to normalise distributions and the means presented are those calculated after transformation. Comparisons of paired data were made on untransformed data using the two-tailed Wilcoxon signed-ranks test.

Data from users and non-users of OC were analysed separately where baseline values differed due to known metabolic effects of OC.

Results

The age and body weights of the subjects are given in Table 2. Women not taking OC showed a small (600 g) reduction in body weight after fluconazole treatment. The mean plasma fluconazole concentrations, approximately 2 h post-dose after 2 weeks of administration, was 2.41 (s.d. 0.58) mg l^{-1}, range 1.49–3.72 mg l^{-1}, and all were in the expected range, indicating that compliance was satisfactory.

Side effects were reported by five women. Other than somnolence, dizziness and fatigue (commonly reported in volunteer studies) there were only two reports which were regarded as related to fluconazole administration; one of headache for a part of every day and one of nausea. No symptoms necessitated discontinuation of treatment. Increased appetite was reported by several subjects although this was not

Table 1 Analytical coefficients of variation

	Within-batch (%)		*Between-batch (%)*	
	Low level	*High level*	*Low level*	*High level*
Oestradiol	13.0	12.1	—	—
Progesterone	6.5	5.2	—	—
Testosterone	11.2	7.8	—	—
SHBG	6.7	7.6	—	—
T_4	10.7	7.1	—	—
T_3 uptake	2.6	—	—	—
TSH	3.6	8.9	—	—
Cortisol	8.6	7.8	—	—
Glucose	2.4	2.2	2.8	2.9
Insulin	5.5	4.2	—	—
C-Peptide	7.5	7.0	—	—
Total cholesterol	1.1	1.1	2.1	3.0
Triglycerides	2.0	1.5	2.7	3.9
LDL-cholesterol	3.5	—	4.1	—
HDL-cholesterol	4.1	—	5.3	—
HDL2-cholesterol	10.2	—	11.0	—
HDL3-cholesterol	6.4	—	10.0	—
Apolipoprotein AI	4.2	—	—	—
Apolipoprotein AII	3.4	—	—	—
Apolipoprotein B	3.0	—	—	—

Table 2 Age and body weights in women during low-dose fluconazole treatment

	All subjects (n = *18*)		*OC users* (n = *10*)		*OC non-users* (n = *8*)	
Time on treatment (months)	*0*	*1*	*0*	*1*	*0*	*1*
Age (years)						
Mean	26.0		24.6		27.8	
Range	(19–40)		(19–34)		(19–40)	
Body weight (kg)						
Mean	63.0	61.7	61.3	61.1	65.2	64.6*
Range	(52–81)	(52–80)	(52–71)	(52–71)	(54–81)	(53–80)
% ideal body weight						
Mean	104.9	103.7	103.1	102.5	107.1	105.3*
Range	(79–123)	(79–119)	(79–119)	(79–119)	(88–123)	(88–119)

*$P < 0.05$ compared with pre-treatment value.

reflected in any weight gain during the study. None of the volunteers showed any elevation in hepatic function tests. No menstrual disorders were reported and no volunteers withdrew from the trial or were withdrawn.

Oral contraceptive users had higher SHBG and thyroid hormone levels and lower oestradiol and progesterone levels than did non-users (Table 3). Fluconazole treatment induced small but statistically-significant rises in serum testosterone and thyroxine in women not taking OC. These changes were minor when compared with the differences between OC-users and non-users and the results remained within the laboratory normal range. The serum cortisol response to tetracosactrin provocation is shown in Figure 1. Baseline cortisol values were higher in OC users than in non-users. There was no alteration in

Table 3 Sex hormone and sex hormone binding globulin concentrations and thyroid function tests in women during low-dose fluconazole treatment

	OC users (n = *10)*		*OC non-users* (n = *8)*	
Time on treatment (months)	*0*	*1*	*0*	*1*
Oestradiol (pmol l^{-1})				
Mean	19.9	9.8	267.1	229.3
Range	(0–37)	(0–29)	(84–562)	(88–400)
Sex hormone binding globulin (nmol l^{-1})				
Mean	99.7	101.1	46.1	44.9
Range	(55–197)	(50–197)	(29–59)	(32–63)
Progesterone (nmol l^{-1})				
Mean	1.13	0.64	10.55	8.83
Range	(0.3–3.8)	(0.3–1.3)	(0.6–21)	(0.6–19)
Testosterone (nmol l^{-1})				
Mean	0.98	1.03	1.24	1.43*
Range	(0.6–1.4)	(0.6–1.5)	(0.9–1.8)	(1.1–2.0)
Thyroxine (nmol l^{-1})				
Mean	105.8	100.4	64.4	69.5*
Range	(67–161)	(62–118)	(56–82)	(51–89)
T_3 uptake				
Mean	107.5	107.2	98.0	97.5
Range	(95–112)	(99–112)	(95–101)	(93–105)
Thyroid stimulating hormone (iu l^{-1})				
Mean	2.05	1.68	1.33	1.38
Range	(1.0–3.5)	(0.7–2.6)	(0.4–2.6)	(0.5–2.0)

*$P < 0.05$ compared with pre-treatment value.

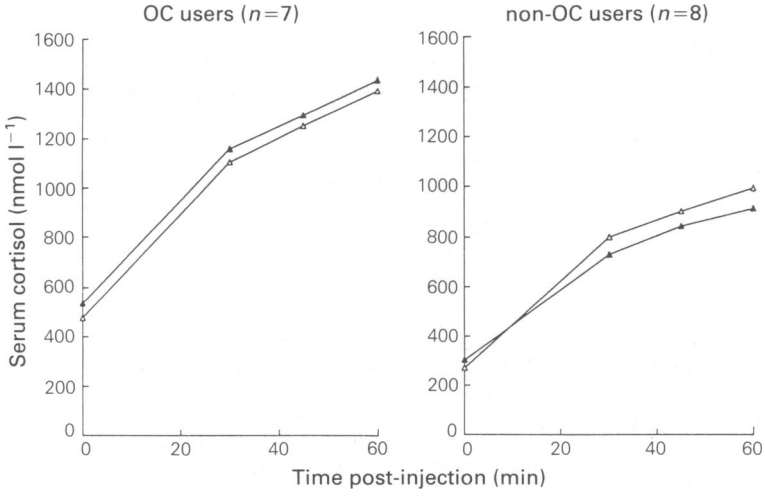

Figure 1 Serum cortisol response to adrenal corticol provocation test in women treated with low-dose fluconazole. ▲ pre-treatment, △ post-treatment.

adrenal cortical response in women given fluconazole, regardless of OC use. Plasma glucose levels during the OGTT were unchanged by fluconazole treatment (Table 4). However, the fasting insulin levels were higher during fluconazole treatment when the women were also taking OC. The insulin AUC in OC users was increased with fluconazole treatment whereas the incremental AUC was not significantly increased, suggesting that the increase in the insulin AUC is due solely to the raised fasting level and not to stimulation of insulin secretion. This change in insulin levels was not reflected in the C-peptide concentrations and is unlikely to be of clinical significance. There was no change in insulin or C-peptide concentrations in women taking fluconazole who did not take OC.

Serum lipid and lipoprotein levels were not affected by fluconazole treatment (Table 5). In contrast, apolipoprotein B, the protein component of LDL, was significantly raised, but only in women taking both OC and fluconazole. Apolipoproteins AI and AII, the major protein components of HDL, were unaffected by fluconazole.

Table 4 Oral glucose tolerance test glucose, insulin and C-peptide levels in women during low-dose fluconazole treatment

	All subjects (n = 17)		*OC users* (n = 10)		*OC non-users* (n = 7)	
Time on treatment (months)	*0*	*1*	*0*	*1*	*0*	*1*
Fasting glucose (mmol l^{-1})						
Mean	4.7	4.6	4.6	4.7	4.7	4.6
Range	(4.3–5.2)	(4.3–5.2)	(4.3–5.0)	(4.4–5.2)	(4.4–5.2)	(4.3–4.9)
Glucose AUC†						
Mean	39.2	38.3	41.7	40.9	35.6	34.6
Range	(32–48)	(30–45)	(38–48)	(35–45)	(32–43)	(31–38)
Glucose incremental AUC						
Mean	11.1	10.4	13.8	12.9	7.3	6.8
Range	(4–21)	(3–19)	(10–21)	(4–19)	(4–11)	(3–10)
Fasting insulin (μg ml^{-1})						
Mean	4.6	5.0	5.3	7.3*	3.8	3.0
Range	(1–15)	(1–24)	(1–15)	(2–24)	(1–13)	(1–10)
Insulin AUC						
Mean	246	280*	299	337*	186	215
Range	(74–674)	(119–706)	(143–674)	(132–706)	(74–411)	(119–352)
Insulin incremental AUC						
Mean	215	244	265	290	161	190
Range	(68–584)	(113–562)	(134–584)	(114–562)	(68–333)	(113–317)
Fasting C-peptide (pmol l^{-1})						
Mean	0.29	0.30	0.32	0.36	0.24	0.22
Range	(0.1–0.5)	(0.1–0.6)	(0.1–0.5)	(0.1–0.6)	(0.1–0.4)	(0.1–0.3)
C-peptide AUC						
Mean	5.07	4.90	5.61	5.49	4.29	4.08
Range	(3.2–9.9)	(2.4–8.8)	(3.4–9.9)	(2.9–8.8)	(3.2–5.8)	(2.4–5.8)
C-peptide incremental AUC						
Mean	3.35	3.12	3.71	3.34	2.83	2.79
Range	(2.0–6.9)	(1.2–5.0)	(2.5–6.9)	(2.0–5.0)	(2.0–4.3)	(1.2–4.4)

*$P < 0.05$ compared with pre-treatment value.
† AUC = Area under OGTT curve. Insulin related means are derived from log-transformed data.

Table 5 Serum lipid, lipoprotein and apolipoprotein concentrations in women treated with low-dose fluconazole

	All subjects (n = 18)		*OC users (n = 10)*		*Non-OC users (n = 8)*	
Visit	*1*	*2*	*1*	*2*	*1*	*2*
Total cholesterol (mmol l^{-1})						
Mean	4.2	4.3	4.4	4.7	3.9	3.8
Range	(3.1–5.8)	(3.0–6.2)	(3.6–5.8)	(3.9–6.2)	(3.1–4.7)	(3.0–4.7)
Triglycerides (mmol l^{-1})						
Mean	0.7	0.8	0.9	1.0	0.6	0.5
Range	(0.4–1.4)	(0.3–1.5)	(0.6–1.4)	(0.6–1.5)	(0.4–0.9)	(0.3–1.1)
LDL-cholesterol (mmol l^{-1})						
Mean	2.1	2.2	2.3	2.6	1.8	1.7
Range	(1.0–3.7)	(1.0–4.3)	(1.5–3.7)	(1.5–4.3)	(1.0–2.8)	(1.0–2.8)
HDL-cholesterol (mmol l^{-1})						
Mean	1.8	1.8	1.7	1.7	1.9	1.9
Range	(1.2–2.2)	(1.2–2.3)	(1.2–2.0)	(1.2–2.3)	(1.5–2.2)	(1.4–2.2)
HDL2-cholesterol (mmol l^{-1})						
Mean	0.7	0.7	0.6	0.6	0.9	0.8
Range	(0.3–1.4)	(0.3–1.2)	(0.3–0.9)	(0.3–1.2)	(0.4–1.4)	(0.5–1.2)
HDL3-cholesterol (mmol l^{-1})						
Mean	1.1	1.1	1.2	1.2	1.0	1.0
Range	(0.6–1.4)	(0.8–1.5)	(0.8–1.4)	(0.8–1.5)	(0.6–1.3)	(0.8–1.2)
Apolipoprotein AI (mg dl^{-1})						
Mean	142	139	145	147	137	128
Range	(123–166)	(91–170)	(128–166)	(118–170)	(123–155)	(91–163)
Apolipoprotein AII (mg dl^{-1})						
Mean	44	44	47	47	41	40
Range	(34–54)	(34–65)	(34–54)	(34–65)	(38–45)	(35–48)
Apolipoprotein B (mg dl^{-1})						
Mean	67	73*	78	89*	54	54
Range	(38–113)	(38–136)	(56–113)	(54–136)	(38–72)	(38–72)

*$P < 0.05$ compared with pre-treatment value.
Triglyceride means are derived from log-transformed data.

Discussion

Cytochrome P-450, a haemoprotein found throughout the plant and animal kingdoms, plays a fundamental role in the synthesis and degradation of endogenous fatty acids and steroids (Estabrook, 1984). It is also involved in the metabolic clearance of many drugs. Where fungal infections are to be controlled by inhibition of enzymes associated with cytochrome P-450, a high degree of selectivity is important. Ketoconazole, the first antifungal azole with significant oral activity, lacks such selectivity and has profound effects on steroidogenesis (Pont *et al.*, 1982b). Ketoconazole inhibits the synthesis of adrenal enzymes in mammals, including man (Pont *et al.*, 1982b) by inhibition of the cholesterol side-chain cleavage, steroid 17 hydroxylase and C17–20 lyase cytochrome P-450 enzymes (Pont *et al.*, 1982a). Ketoconazole has also been shown to prolong the elimination of antipyrine in man (D'Mello *et al.*, 1985), probably by inhibiting its cytochrome P450-mediated clearance, and therefore has the potential to interfere with the action of other drugs. In contrast, no such effect on antipyrine clearance was observed with fluconazole (Purba & Back, 1986).

The endocrine effects of ketoconazole have aroused considerable interest and have inspired clinical studies to assess its value in the management of such conditions as Cushing's syndrome (Sonino *et al.*, 1985) and carcinoma of the prostate (Van den Bossche *et al.*, 1987) in which suppression of gonadal or adrenal hormone production is beneficial. Ketoconazole has been reported to reduce serum oestrogen in rats (Watanabe & Menzies, 1985) by inhibition of aromatase, the cytochrome P-450 enzyme involved in the production of oestrogen (Mason *et al.*, 1985). Interpretation of the effects of ketoconazole on oestrogens is complicated by the fact that the synthesis of androgens, the precursors of oestrogens, is also blocked by the drug.

In the present study, fluconazole had no effect on serum oestradiol levels in healthy premenopausal women. The small increase seen in serum testosterone levels (but not in women using OC as well as fluconazole) is opposite to that expected from an inhibitor of mammalian cytochrome P-450 activity and the reverse of what is found with ketoconazole. Users of OC had higher baseline plasma cortisol levels than did non-users, as reported previously (Wild *et al.*, 1982). Fluconazole administration had no effect on the serum cortisol response during the provocation test, indicating that adrenal corticol function is unimpaired by fluconazole. The increase in serum thyroxine seen in fluconazole users not taking OC is small when compared with the variation between OC users and non-users and would not be considered to be of clinical significance.

Oral contraceptive usage was associated with alterations in the glucose, insulin and C-peptide values seen during the OGTT, as previously shown (Wynn & Godsland, 1986). The increase in fasting insulin levels seen in OC users taking fluconazole was not reflected in C-peptide concentrations and is unlikely to be of clinical significance. Overall, the glucose, insulin and C-peptide response to the OGTT indicates that fluconazole treatment has no effect on carbohydrate metabolism.

Similarly, serum lipid and lipoprotein metabolism appeared unaffected by fluconazole, aside from a small increase in apolipoprotein B in those women also using OC. Again, this is opposite to the effect seen in men with prostatic cancer given high-dose ketoconazole, in which LDL cholesterol and apolipoprotein B fall (Kraemer & Pont, 1986; Meittenen, 1988). The small increase in apolipoprotein B in women also taking OC may reflect an interaction between OC and the cytochrome P-450 system (reviewed by Gram & Gillette, 1969) and requires further study.

It is unlikely that these small changes are of significance at the relatively low doses and short durations of fluconazole treatment used for superficial mycoses. However, the long-term effects of fluconazole on metabolism and steroidogenesis need to be assessed when the drug is being used at higher doses for the treatment of life-threatening mycoses. There is an obvious advantage in an antifungal agent which is selective in its effects on fungal, but not mammalian, cytochrome P-450 mediated reactions. Fluconazole appears to be more specific in its action on steroidogenesis than does its predecessor, ketoconazole, and therefore could be a major advance in the management of fungal infections.

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