# Intravenous Lipid and Heparin Infusion-Induced Elevation in Free Fatty Acids and Triglycerides Modifies Circulating Androgen Levels in Women: A Randomized, Controlled Trial

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**Background:** The polycystic ovarian syndrome (PCOS) is characterized by hyperandrogenism and associated with obesity and impaired glucose metabolism. Despite the high prevalence of PCOS and the considerable clinical impact, the precise interplay between metabolism and hyperandrogenemia is not entirely clear.

**Objective:** The objective of the study was to analyze the effects of iv lipid and heparin infusion on circulating androgen levels in healthy women.

**Design:** This was a randomized, controlled, crossover trial.

**Setting:** The study was conducted at an endocrinology center.

**Patients:** Patients included 12 healthy young women during the early follicular phase of two subsequent cycles.

**Intervention:** After an overnight fast, a 20% lipid/heparin or a saline/heparin infusion was administered in random order for 330 min.

**Main Outcome Measures:** A detailed characterization of androgen metabolism was performed.

**Results:** Elevations in free fatty acids and triglycerides, induced by lipid/heparin infusion, elevates the levels of androstenedione, dehydroepiandrosterone (DHEA), dehydroepiandrosterone sulfate (DHEAS), testosterone, 5$\alpha$-dihydrotestosterone, estrone, and 17$\beta$-estradiol. Urinary excretion of DHEA, DHEAS, 5-androstene-3$\beta$,17$\beta$-diol, and the sum of urinary excreted DHEA and its 16-hydroxylated downstream metabolites, 16$\alpha$-hydroxy-DHEA and 5-androstene-3$\beta$,16$\alpha$,17$\beta$-triol, were reduced.

**Conclusion:** The mechanism of iv lipid and heparin infusion-induced elevation of circulating androgens described here might contribute to the development of hyperandrogenism in women with PCOS and suggests that lowering of hyperlipidemia might be a potential therapeutic target in patients with PCOS to treat hyperandrogenemia. **(*J Clin Endocrinol Metab* 93: 3900–3906, 2008)**

The syndrome of polycystic ovaries (PCOS) is among the most common endocrinological disorder in women and is characterized by dysmenorrhea, polycystic ovaries, and hyperandrogenism (1). About 40% of women with PCOS have been described to have a metabolic syndrome (2). Insulin resistance, a central feature of the metabolic syndrome, has been suggested to

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Abbreviations: An, Androsterone; AUC, area under the concentration-time curve; DHEA, dehydroepiandrosterone; DHEA+Metabs, DHEA and 16-hydroxylated metabolites; DHEAS, DHEA sulfate; DHT, 5$\alpha$-dihydrotestosterone; Et, etiocholanolone; PCOS, polycystic ovarian syndrome.

be a major driver of hyperandrogenemia in these subjects (3, 4). However, although there is no doubt that a considerable amount of data link insulin resistance and androgen production, other mechanisms affecting circulating androgen levels may exist.

Hyperlipidemia due to lipid infusion induces insulin resistance and has also been found in women with PCOS (5, 6). A recent study demonstrated that short-term induced elevation of free fatty acids (FFAs) and triglycerides leads to increased levels of adrenal androgen precursors in healthy young men (7). Androgen changes occurred within 2 h after start of lipid infusion, whereas no changes of insulin sensitivity were observed at that time (7, 8). Thus, insulin-independent effects of elevated FFAs and triglycerides on adrenal androgen levels may exist. Although those data in males suggested that dysregulation of FFAs and triglycerides, both of which will be increased by lipid/heparin infusion (9–11), might also contribute to the development of hyperandrogenemia in women with PCOS, potential mechanisms linking androgens and hyperlipidemia remained unclear.

We therefore evaluated the effects of a short-term induced elevation in FFAs and triglycerides on androgen metabolism in a randomized, controlled, crossover trial in healthy young women.

## Patients and Methods

### Setting and participants

Twelve healthy young women with regular menstrual cycles and no signs of hirsutism (12) participated in this study. Briefly, women were 25.5 ± 1.0 yr old, body mass index was 21.9 ± 0.8 kg/m$^2$, waist to hip ratio was 0.76 ± 0.01, and body surface area (13) was 1.71 ± 0.04 m$^2$. Ferriman and Gallway score was 2.6 ± 0.7. None of the participants had taken any medication for at least 3 months before study. All participants were initially screened for any systemic disease or biochemical evidence of impaired hepatic or renal function. Subjects with a history of hypertension, type 2 diabetes, renal or liver disease, dyslipidemia, heart failure, or a family history of diabetes or any other endocrine disorder were excluded from this study. Body weights were stable for at least 2 months before the study. Informed written consent was obtained from each participant. The study protocol was approved by the Institutional Review Board of the Charité-Universitätsmedizin Berlin, Campus Benjamin Franklin.

### Design overview

Subjects were studied in random order during the early follicular phase of two subsequent menstrual cycles (d 4–6). Therefore, a randomization list was created using block randomization (14). To avoid interactions between the study procedures, the study was performed at two subsequent cycles. After a 10-h overnight fast, a short polyethylene catheter was inserted into an antecubital vein for infusion of test substances at 0800 h. Another catheter was placed into the contralateral forearm vein for blood sampling.

### Randomization and intervention

Eligible participants were randomly assigned to receive either lipid/heparin or saline/heparin infusion. The trial itself was not blinded, but all biochemical measurements were blinded. A 0.9% saline infusion plus heparin (0.4 U/kg·min) or Lipovenös 20% (Fresenius Kabi, Bad Homburg, Germany; contents in 1.000 ml: soybean oil 200 g, glycerol 25.0 g, egg phospholipids 12.0 g, oleate 0.3 g) plus heparin (0.4 U/kg·min) were infused at a constant rate of 1.5 ml/min for 330 min.

On both days blood samples were collected before and 1, 2, and 4 h after start of the lipid/heparin or saline/heparin infusion. To assess overall daily secretion of the adrenal androgens, urine was collected for 24 h on the day of saline/heparin and lipid/heparin infusion from 0800 to 0800 h the next morning, respectively. There were no adverse events during these studies among study participants.

### Laboratory tests

Blood was immediately chilled on ice and centrifuged at 4 C. Subsequently aliquots were frozen at −80 C until assay. Metabolic parameters and circulating and urinary androgens were determined as described previously (7, 15, 16). Circulating parameters included testosterone, dehydroepiandrosterone (DHEA), dehydroepiandrosterone sulfate (DHEAS), androstenedione, 5$\alpha$-dihydrotestosterone (DHT), SHBG, estrone, 17$\beta$-estradiol, progesterone, 17-hydroxyprogesterone, LH, FSH, ACTH, insulin, FFAs, triglycerides, and glucose. Glycerol was determined by free glycerol kit (BioVision, Mountain View, CA).

Urinary steroid included DHEA, DHEAS, 4-androstene-3,17-dione (androstenedione), androsterone (An), etiocholanolone (Et), 5-androstene-3$\beta$,17$\beta$-diol (androstenediol), 16$\alpha$-hydroxy-DHEA, and 5-androstene-3$\beta$,16$\alpha$,17$\beta$-triol (androstenetriol). Androstenedione and DHEAS were measured by specific in-house RIAs established at the Steroid Laboratory of the University of Heidelberg, using tritiated steroids (Amersham Biosciences, Freiburg, Germany) and antibodies, raised and characterized in the laboratory, as described elsewhere (17, 18). Androstenedione was extracted from urine samples before RIA, and DHEAS was measured directly in diluted urine samples. The intraassay coefficients of variation were less than 10%, and interassay coefficients of variation were less than 15%, respectively. The other urinary steroids were determined using gas chromatographic-mass spectrometric analysis as described previously (16, 19).

### Statistical analysis

Total androgen secretion (C19) was determined as the sum of An, Et, androstenediol, DHEA, 16$\alpha$-hydroxy-DHEA, and androstenetriol. The sum of the latter three steroids, *i.e.* DHEA and the 16-hydroxylated metabolites (DHEA+Metabs), was calculated to estimate the major metabolites of DHEA and DHEAS (19). Androstenediol as the direct metabolite of DHEA was determined to assess the urinary excretion of DHEA. In addition, the sum of DHEA+Metabs and androstenediol was calculated as a marker of the total excretion of adrenal androgens. The sum of urinary excretion of An and Et was assessed to estimate both gonadal and adrenal activity. Furthermore, the 5$\alpha$-reductase activity was determined by the urinary steroid metabolite ratio An to Et (19). Free testosterone was calculated as previously described (20). The activity of the hepatic sulfotransferase was assessed by calculating the DHEAS to DHEA ratio.

All statistical procedures were performed using SPSS version 15.0 (SPSS Inc., Chicago, IL). Data were compared by paired Student's *t* test for normally distributed data and Wilcoxon test for skewed data. In addition, profiles of FFAs, insulin, androstenedione, DHEA, DHEAS, testosterone, DHT, estrone, LH, 17$\beta$-estradiol, FSH, ACTH, progesterone, and 17-hydroxyprogesterone were compared by repeated-measures ANOVA. The area under the concentration-time curve (AUC) was calculated by using the trapezoidal integration. Results were considered to be significant, if the two-sided $\alpha$ was less than 0.05. Data are presented as the mean ± SEM unless otherwise mentioned.

## Results

There was no baseline difference of FFAs before lipid/heparin and saline/heparin infusion (0.45 ± 0.05 *vs.* 0.49 ± 0.09 mmol/liter; $P$ = n.s.). Lipid infusion increased FFAs to 4.32 ± 0.44 mmol/liter at 1 h, 6.59 ± 0.42 mmol/liter at 2 h, and 6.75 ± 0.46 mmol/liter at 4 h ($P < 0.005$ *vs.* baseline, respectively). Due to the fasting situation, there was also a small increase in FFAs during

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saline/heparin infusion (1 h: 0.79 ± 0.06 mmol/liter; 2 h: 0.88 ± 0.08 mmol/liter; 4 h: 0.94 ± 0.08 mmol/liter, $P < 0.005$ for each time point *vs.* baseline). In conclusion, there were substantially higher levels of FFAs during lipid/heparin infusion, compared with saline/heparin infusion at 1, 2, and 4 h ($P < 0.005$). Accordingly the triglyceride levels rose during lipid/heparin infusion (baseline: 0.79 ± 0.04 mmol/liter, 1 h: 3.75 ± 0.34 mmol/liter, 2 h: 6.78 ± 0.69 mmol/liter, 4 h: 9.58 ± 1.48 mol/liter; $P < 0.05$ *vs.* baseline) and showed a small decrease during saline/heparin infusion (baseline: 0.80 ± 0.09 mmol/liter, 1 h: 0.53 ± 0.03 mmol/liter, 2 h: 0.58 ± 0.06 mmol/liter, 4 h: 0.66 ± 0.06 mol/liter; $P < 0.05$ *vs.* baseline), leading to higher triglyceride levels during lipid/heparin infusion at 1, 2, and 4 h, compared with saline/heparin infusion ($P < 0.05$). Due to the induction of lipolysis by heparin, the glycerol increased during lipid/heparin infusion (baseline *vs.* 2 h: 0.026 ± 0.003 *vs.* 0.683 ± 0.026 nmol/liter; $P < 0.05$) and in a smaller amount during saline/heparin infusion (baseline *vs.* 2 h: 0.025 ± 0.003 *vs.* 0.043 ± 0.005 nmol/liter; $P < 0.05$). This resulted in higher glycerol values during lipid/heparin infusion ($P < 0.05$). Glucose levels were unchanged during both, lipid/heparin and saline/heparin infusion (baseline *vs.* 4 h: 4.5 ± 0.1 *vs.* 4.5 ± 0.1 and 4.4 ± 0.1 *vs.* 4.4 ± 0.0 mmol/liter, respectively; $P$ = n.s.). There was a progressive decline in insulin concentration during saline/heparin infusion, reflecting circadian rhythm of insulin sensitivity (5.47 ± 0.60 *vs.* 4.12 ± 0.51, 3.67 ± 0.32, and 2.81 ± 0.32 mU/liter; $P < 0.05$), whereas no change in insulin levels was detected during lipid/heparin infusion up to 2 h (1 h: 4.18 ± 0.60 and 2 h: 5.15 ± 0.69 *vs.* baseline: 5.14 ± 0.59 mU/liter; $P$ = n.s.), and only a small decrease in insulin could be observed after 4 h (4.22 ± 0.63 mU/liter; $P < 0.05$), compared with baseline. Thus, insulin levels were higher after 2 and 4 h of lipid, compared with saline infusion ($P < 0.05$).

Baseline values of DHEA, DHEAS, androstenedione, testosterone, DHT, free testosterone, estrone, 17β-estradiol, progesterone, 17-hydroxyprogesterone, LH, FSH, and SHBG were within the physiological range. Androstenedione levels were significantly elevated during lipid/heparin infusion, compared with saline/heparin infusion (AUC: 546 ± 44 *vs.* 471 ± 44 ng/ml·min; $P < 0.05$). AUCs of DHEA and DHEAS were elevated during lipid/heparin infusion (DHEA: 3,631 ± 610 *vs.* 2,341 ± 284 ng/ml·min; $P < 0.05$ and DHEAS: 538,275 ± 100,611 *vs.* 420,300 ± 63,471 ng/nl·min; $P < 0.01$). Although basal testosterone levels were slightly higher in the saline group, the lipid/heparin infusion resulted in a strong and significant increase of testosterone, DHT, and 17β-estradiol levels, compared with saline/heparin infusion (AUCs: 1,030 ± 112 *vs.* 412 ± 62 nmol/l·min, $P < 0.005$; DHT: 57,771 ± 5,640 *vs.* 46,636 ± 3,974 pg/ml·min, $P < 0.005$; 17β-estradiol: 23,505 ± 2,840 *vs.* 11,462 ± 2,811 pg/ml·min, $P < 0.05$) (see also Fig. 1). Concordant to testosterone levels, the calculated free testosterone was higher during lipid/heparin infusion (1 h: 60.1 ± 9.5 *vs.* 22.8 ± 2.7 pmol/liter; 2 h: 77.4 ± 10.1 *vs.* 23.0 ± 3.3 pmol/liter; 4 h: 64.0 ± 7.3 *vs.* 31.5 ± 7.9 pmol/liter; $P < 0.005$), whereas SHBG levels and the corresponding AUCs were unchanged and not different during lipid/heparin and saline/heparin infusion. The estrone levels slightly increased during saline/heparin infusion ($P < 0.05$), but a substantially stronger increase was observed during lipid/heparin infusion at 2 and 4 h ($P < 0.01$). Accordingly elevated estrone levels were found during lipid/heparin infusion (2 h: 126.3 ± 8.7 *vs.* 54.2 ± 5.6 pg/ml, 4 h: 150.2 ± 16.0 *vs.* 55.3 ± 7.4 pg/ml; AUC: 27074 ± 1819 *vs.* 12869 ± 1527 pg/ml·min; $P < 0.05$).

Progesterone levels showed the physiological circadian decline ($P < 0.01$ *vs.* baseline) during saline/heparin and lipid/heparin infusion (2 h: 0.84 ± 0.07 *vs.* 0.66 ± 0.06 ng/ml; 4 h: 0.72 ± 0.05 *vs.* 0.66 ± 0.09 ng/ml; $P$ = n.s.). A comparable decrease was observed in 17-hydroxyprogesterone during saline/heparin and lipid/heparin infusion (2 h: 0.79 ± 0.09 *vs.* 0.62 ± 0.04 ng/ml and 4 h: 0.73 ± 0.08 *vs.* 0.72 ± 0.12 ng/ml, $P < 0.01$; $P < 0.01$ *vs.* baseline). Accordingly, AUCs of progesterone and 17-hydroxyprogesterone did not differ between lipid and saline infusion (202 ± 18 *vs.* 225 ± 14 and 201 ± 16 *vs.* 216 ± 22 ng/ml·min, respectively; $P$ =n.s.).

FSH concentrations declined during saline/heparin infusion, but this effect was more pronounced during lipid/heparin infusion, resulting in higher FSH levels at 1, 2, and 4 h and a higher AUC of FSH (1 h: 4.95 ± 0.33 *vs.* 4.31 ± 0.35U/liter, 2 h: 5.19 ± 0.42 *vs.* 4.50 ± 0.38 U/liter, 4 h: 5.21 ± 0.41 *vs.* 4.19 ± 0.34 U/liter and 1079 ± 87 *vs.* 1246 ± 93 U/liter·min, respectively; $P < 0.05$). Lower LH levels were detected at baseline and after 1 h during lipid infusion. This resulted in slightly different AUCs of LH, which was decreased during lipid/heparin infusion (803 ± 96 *vs.* 1046 ± 140 U/liter·min; $P < 0.05$). However, no significant difference in LH levels was observed at 2 and 4 h (4.61 ± 0.67 *vs.* 3.62 ± 0.43 U/liter and 4.08 ± 0.61 *vs.* 3.39 ± 0.45 U/liter, respectively; $P < 0.05$). ACTH levels did not differ between saline/heparin and lipid/heparin infusion (data not shown).

Urinary excretion of DHEA, DHEAS, and androstenediol, a direct metabolite of DHEA, which can be considered as a marker of the urinary excretion of the adrenal secreted DHEA, was decreased during lipid/heparin infusion (Fig. 2). Comparably, the urinary excretion of the sum of DHEA+Metabs was reduced after lipid infusion. No significant difference of the urinary excretion of androstenedione (5.6 ± 0.7 *vs.* 4.9 ± 0.7 μg/d; $P$ = n.s.), its metabolites An and Et (1776.8 ± 247.8 *vs.* 1506.9 ± 254.4 and 1704.0 ± 210.4 *vs.* 1447.0 ± 252.0 μg/d, respectively; $P$ = n.s.), and the total androgen secretion (C19) (6202.2 ± 758.5 *vs.* 4681.3 ± 639.3 μg/d, $P$ = n.s.) was detected during lipid/heparin compared with saline/heparin infusion. Urinary volume was not different during lipid/heparin compared with saline/heparin infusion (1761 ± 238 *vs.* 1954 ± 290 ml; $P$ = n.s.).

The activity of the hepatic sulfotransferase, assessed by the ratio of DHEAS to DHEA, was not different between saline/heparin and lipid/heparin infusion at baseline (130 ± 11 *vs.* 131 ± 15), 1 h (186 ± 15 *vs.* 217 ± 32), 2 h (196 ± 16 *vs.* 191 ± 26), and 4 h (156 ± 26 *vs.* 198 ± 22) ($P$ = n.s.). Calculated 5α-reductase activity was also unchanged (1.03 ± 0.10 *vs.* 1.05 ± 0.09; $P$ = n.s.).

Adding various amounts of lipid solution [Lipovenös 20%; Fresenius Kabi; 0, 0.5, 1.5, and 2.5% (vol/vol)] to human plasma samples resulted in measured triglyceride concentrations of 1.28,

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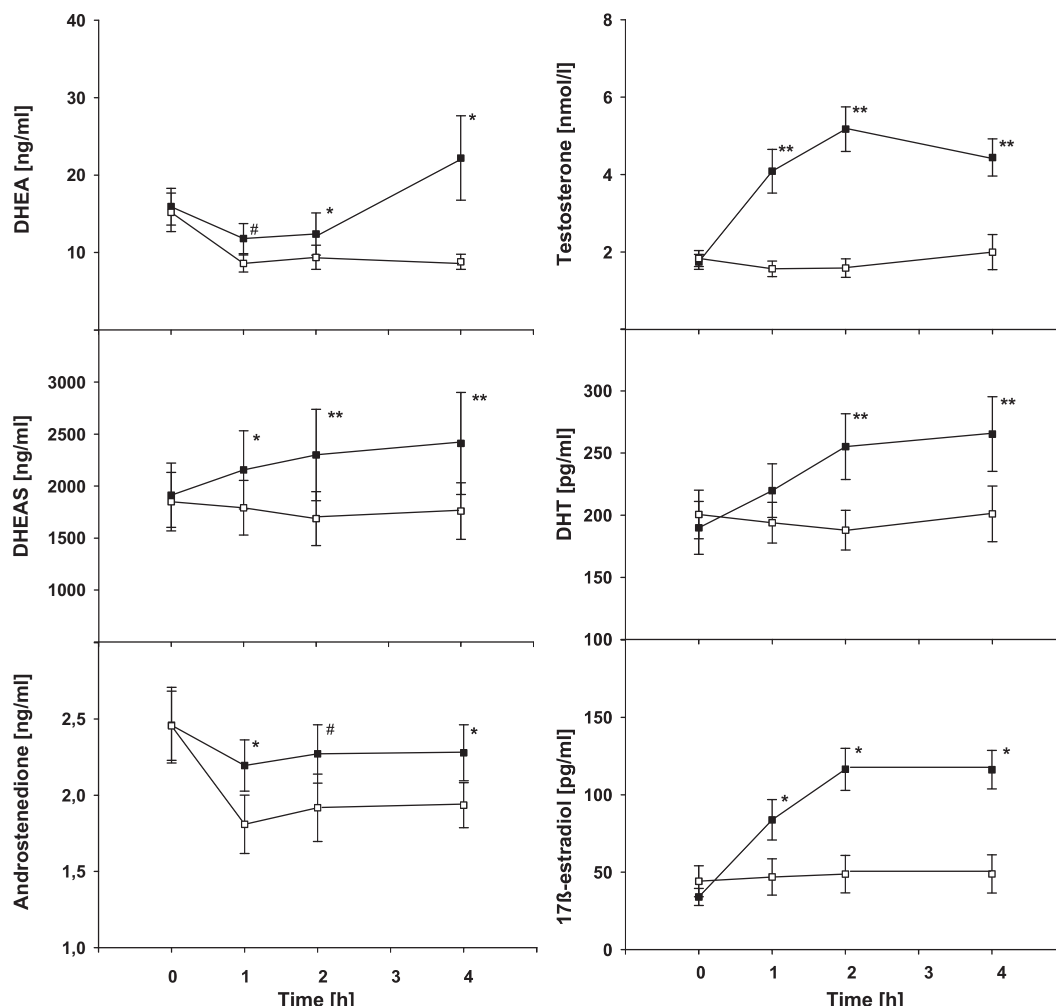


**FIG. 1.** DHEA, DHEAS, androstenedione, testosterone, DHT, and 17$\beta$-estradiol during lipid/heparin infusion (*filled squares*) *vs.* saline/heparin infusion (*open squares*); *, $P < 0.05$; **, $P < 0.01$; x, $P = 0.117$; #, $P = 0.136$ *vs.* saline/heparin infusion. Results are expressed as means ± SEM.

3.56, 8.09, and 12.69 mmol/liter. Correspondingly measured androgen and estrogen values were 587, 555, 588, and 533 ng/ml for DHEAS; 20.1, 20.0, 27.5, and 20.0 pg/nl for estradiol; and 0.92, 1.07, 0.97, and 0.80 nmol/liter for testosterone. Furthermore, we added 6 mmol/liter oleic acid to these 0, 0.5, 1.5, and 2.5% (vol/vol) triglyceride dilutions, resulting in FFA concentrations comparable with those in plasma samples during lipid infusion (5.94–7.31 mmol/liter). The subsequently measured DHEAS values were 577, 587, 578, and 485 ng/ml. The measured estradiol and testosterone values were 22.2, 20.0, 22.3, and 20.0 pg/ml and 1.11, 0.91, 0.87, and 0.86 nmol/liter, respectively. Therefore, neither the triglyceride nor the FFA concentrations as observed *in vivo* in the present study interfered with the measured concentrations of DHEAS, estradiol, and testosterone.

## Discussion

We demonstrated in this controlled, randomized, crossover trial an increase of the adrenal androgen precursors DHEA, DHEAS, androstenedione, and the androgens testosterone and DHT during lipid infusion in healthy young women. These data imply a novel physiological mechanism linking fat metabolism and regulation of circulating androgens. Clinically the association between impaired metabolism and hyperandrogenemia is well known in women with PCOS. The novel findings presented here suggest that elevated FFAs and triglycerides might be a causal factor linking metabolism to circulating adrenal androgen precursors and androgen levels.

PCOS is one of the most frequent endocrine disorders in women. Central features are hirsutism, polycystic ovaries, dysmenorrhea, infertility, obesity, and an elevated diabetes risk. Hypertriglyceridemia and elevated FFAs have been described in obese and diabetic individuals and have been shown to induce insulin resistance. Comparably, hyperlipidemia has also been found in women with PCOS (5, 6) and might thus mediate effects on androgen metabolism. Indeed, a recent study demonstrated that iv lipid and heparin infusion-induced elevation in FFAs and triglycerides increase adrenal androgen precursors (7). However, this study was restricted to men, although there is a well-known sexual dimorphism in terms of androgen metabolism. Additionally, a potential mechanism linking elevated FFAs/triglycerides and androgens was not presented. These data are now supported and extended by the results presented here, which strongly sug-

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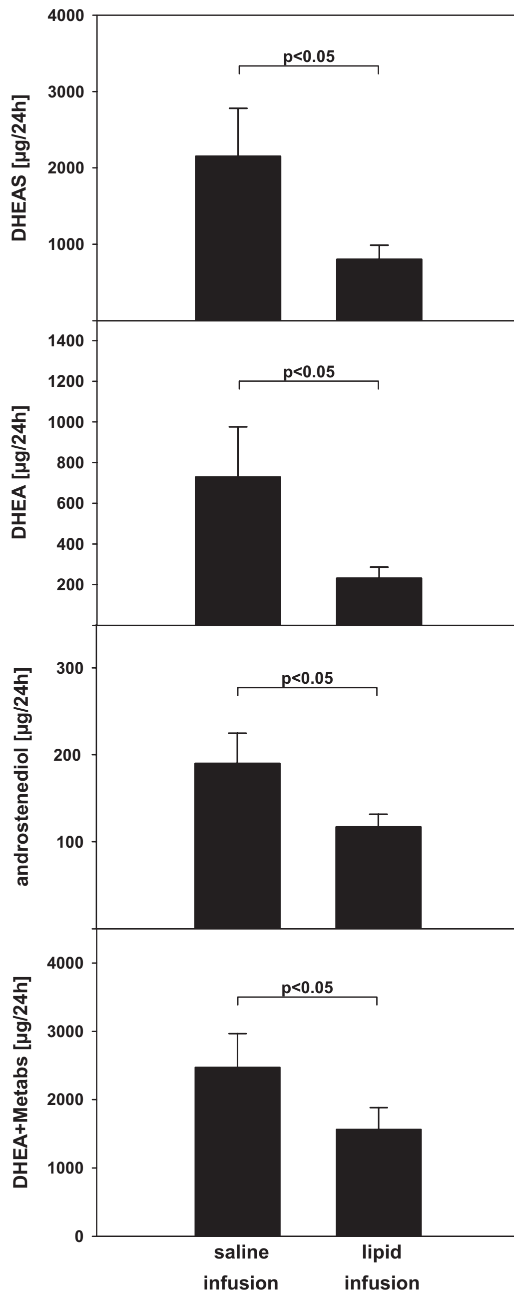


**FIG. 2.** Urinary excretion rates of DHEA, DHEAS, androstenediol, and DHEA+Metabs during lipid/heparin infusion and saline/heparin infusion. Results are expressed as means ± SEM.

gest that iv lipid and heparin infusion-induced elevation in FFAs and triglycerides links directly to the regulation of androgen precursors and androgens in healthy young women. Androstenedione is known to be the precursor of both testosterone and estrone in women. Indeed, the elevated androstenedione levels during lipid/heparin infusion resulted in an increase of testosterone and DHT. As expected, elevated levels of estrone and 17$\beta$-estradiol were also detected. This is in agreement with the well-described sexually dimorphic conversion pattern of DHEA in humans, with predominant conversion into estrogens in men (7, 21) and conversion into estrogens and androgens in women (22).

Whether this effect is independent of insulin resistance is not entirely clear, although it is suggested by the data presented here. Elevation in FFAs and triglycerides due to lipid infusion is generally known to induce insulin resistance, but this effect on insulin sensitivity is usually not detectable within the first hour of a lipid infusion (8). The lipid-induced increase of androgens was detected after 1 h, whereas no difference of insulin levels was found at this time point. Although this time course suggests that iv lipid/heparin infusion-induced effects on androgens may be independent of a subsequently induced peripheral insulin resistance, this interpretation should not be overemphasized. There is no doubt that a considerable amount of data suggests that insulin sensitivity directly affects synthesis of androgen precursors. Thus, future studies on this interrelation between hyperlipidemia, insulin sensitivity, and androgens are desirable.

The intervention studied here is a well-described model to investigate lipid-induced effects (23–27). However, FFAs and triglycerides are both increased by lipid/heparin infusion (9–11); thus, the respective effects are difficult to separate. Given that triglycerides and FFAs are elevated in patients with PCOS, both a FFA- or a triglyceride-induced effect would be comparably relevant to the pathogenesis of elevated androgens in those women. In addition, we cannot entirely exclude an effect of glycerol or lecithin because Lipovenös is a mixture containing these substances.

Generally elevated circulating androgen levels can result from increased synthesis or reduced excretion of androgens and its precursors. Remer (28) suggested in a thoughtful recent comment that specifically urinary excretion might have a predominant role in this setting. Indeed, the urinary excretion rate of DHEA, DHEAS, and its specific metabolites was substantially reduced during lipid infusion. Interestingly, the urinary excretion of androstenedione and its metabolites, which were strongly affected by gonadal androgen secretion (29), was not substantially decreased, suggesting that the effect was predominantly existent for adrenal androgen precursors. These data imply a novel mechanism linking FFAs and triglycerides to increased serum androgen levels.

It is important to note that the rise in FFAs and triglycerides during lipid infusion was unphysiologically high in the setting of this study. Therefore, future studies need to demonstrate that a lowering of physiologically elevated lipids, specifically in women with PCOS, results in a reduction of androgen precursors. In addition to the reduced excretion of androgen precursors, changes in the hepatic DHEA sulfotransferase activity may also increase serum DHEA levels. However, the activity of hepatic DHEA sulfotransferase was not affected according to our data, as demonstrated by an unchanged DHEAS to DHEA ratio. Therefore, we did not find any implication of a modified hepatic androgen metabolism because the calculated 5$\alpha$-reductase activity was also not different. Given the unchanged urinary volume, the described differences of urinary excretion are most likely the result of a modified androgen clearance, although this was not directly investigated in our study.

Another relevant aspect may be a modification of central control mechanisms (30). Whereas ACTH levels were not affected by hyperlipidemia in this study, reduced FSH and LH levels were found. In accordance with these findings, reduced androgens

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after a low-fat diet were not mediated by inhibition of ACTH (31). Actually the decrease in gonadotrophin levels during lipid/heparin infusion may be the result of increased estrogen levels rather than a primary lipid-induced effect, although the nature of this study does not allow separation of these effects (32, 33). Nevertheless, reduced FSH and LH concentrations would result in lower levels of sexual hormones, thus suggesting that the effects of iv lipid and heparin infusion on circulating androgens and urinary excretion of androgen precursors may have been underestimated.

The mechanism linking elevated FFAs and triglycerides due to iv lipid and heparin infusion and urinary excretion of androgen precursors is yet unclear. As discussed by Remer (28), DHEA, DHEAS, and androstenedione are largely bound to albumin. Therefore, interaction of triglycerides and FFAs with the binding of circulating androgens to albumin may substantially modulate the free hormone concentration and thereby the metabolic clearance rate of these hormones. Future studies are desirable to investigate this question in more detail.

Clearly it is tempting to speculate that the mechanism described here might be of therapeutic relevance in women with PCOS. However, the implied therapeutic option cannot be directly transferred, on the basis of these results, to the treatment of patients with PCOS. Therefore, future intervention studies investigating this question are desirable. However, PCOS might be a heterogenous disorder, and given this scenario, the mechanism of FFAs and triglycerides-induced hyperandrogenemia presented here may be relevant at least in a subcohort of women with PCOS (5).

In summary, this is the first study presenting reasonable evidence that lipid/heparin infusion-induced elevation in FFAs and triglycerides increases adrenal androgen precursors and circulating androgens due to lowering their urinary excretion *in vivo* in healthy young women. Increased levels of DHEA subsequently result in hyperandrogenemia with elevated levels of testosterone and DHT. This novel mechanism linking fat metabolism and androgens might contribute to the development of hyperandrogenism in women with PCOS and suggests novel therapeutic targets to treat those patients.

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