Increases in serum estrogen levels during major illness are caused by increased peripheral aromatization

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Spratt, Daniel, R. Morton, Robert S. Kramer, Sara W. Mayo, Christopher Longcope, and Calvin P. H. Vary proposed that the elevated rate of aromatization is caused by induction of the aromatase gene by interleukin-6 (IL-6) as potential modulators of aromatase expression (33). An increased rate of aromatization would indicate that increased estrogen production is not merely an epiphenomenon of adrenal activation but a discrete, previously unreported response to critical illness. Increased aromatase mRNA expression would indicate that the increased peripheral aromatization is caused by induction of the aromatase gene by factors involved with critical illness.

METHODS

Patients. Peripheral metabolism of sex steroids was evaluated in 11 men and three postmenopausal women (age 42–69 yr). Adipose tissue biopsies were obtained from six other men. All patients were scheduled for elective coronary artery bypass graft surgery (CABG) and were ambulatory prior to surgery with New York Heart Association classification 3 or 4, with ejection fraction >40%. Weight varied from 76 to 123 kg (mean 94 ± 5), with BMI 22.8–35.7 (mean 29 ± 1.4). Patients with other factors known to affect the reproductive axis were excluded: 1) hepatic or renal dysfunction (abnormal bilirubin or AST or serum creatinine >1.5 mg/dl); 2) intracranial disease; 3) diseases of the hypothalamus, pituitary, adrenal, thyroid, or testes; 4) history of alcohol or drug abuse; or 5) administration of steroid drugs <6 mo or opiates <1 wk prior to admission. Patients with concomitant valvular surgery or previous cardiac surgery were also excluded.

Surgery was begun between 0800 and 1300. The time of day was not anticipated to influence hormone values because diurnal variations

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Isolation of total RNA and generation of mutant aromatase standard. Human total RNA was extracted and isolated from frozen adipose tissue biopsies using the Ultraspec RNA Isolation procedure (Biotexc, Houston, TX). This method is derived from a previously described guanidium-thiocyanate phenol chloroform extraction protocol. (42). An internal RNA standard was generated from human RNA using a mutating primer whose product length was the same as our wild-type RT-PCR product, containing a difference of only one point mutation.

RT-PCR and Southern hybridization. Competitive RT-PCR was carried out as previously described using a constant amount of the standard RNA for each RT-PCR reaction. Synthesized oligonucleotide primers and alkaline phosphatase-labeled synthetic DNA probes were employed (44). Southern hybridization took place under stringent conditions, with site-specific probes used to specify wild-type and mutant standard cDNA. Subsequent image analysis of the alkaline phosphatase-exposed film was performed using the public domain National Institutes of Health image program.

Changes in adipose tissue P450 aromatase mRNA content were analyzed by comparing densities of bands for wild-type aromatase on exposed film. To further refine this comparison, ratios of wild-type density to internal standard density were calculated. In addition, GAPDH mRNA was measured for additional standardization on five of six patients. In the sixth patient, sufficient adipose tissue sample was not available for GAPDH mRNA analysis.

Statistics. The rate of peripheral aromatization was reflected in the fraction of androgen administered that was converted to a specific estrogen conjugate in the urine ([\(H\)]An/\([14C]\)Es). This fraction was calculated from:

\[
\frac{([14C]E2)_{\text{M}}}{([14C]E1)_{\text{M}}}
\]

where ([\(H\)]Es/\([14C]\)Es)M is the ratio of radioactivities in the specific estrogen conjugate measured in the urine and ([\(H\)]An/[\(14C]\)Es]M) is the ratio of radioactivity administered.

Comparisons of metabolic parameters (aromatization, PRs, fractional conversion ratios, and clearance rates) before and after surgery were undertaken with both paired t-tests and Wilcoxon signed rank tests since the number of subjects was too small to conclusively assess normality of distribution of values. Because significance levels were similar with either method, only results of signed rank tests are listed. Differences between serum levels of hormones on different days were assessed with one-way analysis of variance. Mean hormone levels are listed as means ± SE.

Correlations between different parameters were assessed by linear regression analysis. Because postoperative changes in aromatization rates of A4 to E1 (in 8 men) and T to E2 (in 3 men) were qualitatively similar, aromatization rates were considered together in the group of 11 men to improve the power of our analyses. Similarly, production rates of E1 and E2 were considered together as estrogen production rates to improve statistical power. Mean values comprised of three individual values (all means in women and mean T production and clearance rates in men) were not evaluated for levels of statistical significance because of the low statistical power.

RESULTS

Serum hormone concentrations. Figure 1 displays the heterogeneity of changes in serum levels of estrogens following surgery in three representative men. A striking rise in both E1 and E2 is evident in Fig. 1A, moderate rises in both E1 and E2 in Fig. 1B and a mild rise in E1 and no rise in E2 in Fig. 1C. An abrupt decline in serum T levels is evident in all men. Changes in serum A4 were variable and did not always parallel changes in serum E1 levels.
the variability in changes of serum A4 concentrations between patients. The change in serum E1 from baseline to postoperative day 2 was highly correlated with the change in serum A4 ($r = 0.89, P < 0.001$). In four men, no postoperative change (>0.2 ng/ml) in serum A4 levels was observed, and in one man A4 decreased postoperatively by 0.26 ng/ml. Despite the lack of an increase in serum A4 concentrations in these five men, serum E1 concentrations rose from 34.2 to 69.8 pg/ml postoperatively ($P < 0.02$). Serum T levels decreased markedly in all men from a mean of 4.5 ± 0.4 ng/ml preoperatively to 1.2 ± 0.2 ng/ml on the second postoperative day ($P < 0.001$).

In the three women evaluated, mean serum E1 rose from 26.7 ± 3.2 pg/ml preoperatively to 75.3 ± 34.4 pg/ml postoperatively. Mean serum A4 increased from 1.2 ± 0.4 to 2.1 ng/ml. Mean serum E2 remained constant (28.3 ± 10.4 pg/ml before and 30.3 ± 10.9 pg/ml after CABG), as did mean serum T (0.38 ± 0.27 and 0.42 ± 0.03 ng/ml).

Mean serum LH levels decreased from 9.8 ± 1.1 IU/l preoperatively to 4.0 ± 0.3 IU/l on the first postoperative day ($P < 0.0001$). Mean LH remained low on postoperative day 2 (3.7 ± 0.2 IU/l) and day 3 (3.5 ± 0.3 IU/l), consistent with hypogonadotropic hypogonadism.

Mean SHBG levels decreased by the third postoperative day (26.7 ± 4.6 to 19.1 ± 3.7, $P = 0.01$). Morning serum cortisol values rose from 13.6 ± 1.7 before to 19.9 ± 2.9 µg/dl after surgery ($P < 0.002$). Serum IL-6 levels increased markedly from 6.8 ± 1.0 preoperatively to 139.8 ± 14.9 on the second postoperative day ($P < 0.001$).

Increase in peripheral aromatization rates. Percent conversion of A4 to E1 via aromatization increased strikingly postoperatively in all eight men studied with postoperative increases ranging from 61 to 329% over baseline values ($P < 0.005$; Fig. 3, left). Aromatization of T to E2 rose in all three men studied by 211, 275, and 556% (Fig. 3, right).

The mean PRs of A4 in the eight men were not significantly different before and after CABG (6.2 ± 1.2 and 7.9 ± 1.7 mg/d, $P = 0.20$). In the four men in whom A4 PR remained the same or decreased (mean 5.4 ± 1.8 to 4.4 ± 1.9 mg/d), serum E1 levels more than doubled by the second postoperative day (40.0 ± 11.9 to 89.8 ± 17.5 pg/ml). T production decreased markedly in two men (2.4 to 1.1 and 4.0 to 1.0 mg/day) and remained the same in the third man (3.9 and 4.3 mg/day). In the third man, MCR of T markedly increased (1,298 to 2,527), and serum T levels decreased (3.0 to 0.8 ng/ml) despite the constant T production rate.

MCRs of E1 and E2 almost doubled postoperatively (2,201 ± 306 to 4,209 ± 654 liters/day, $P = 0.01$; Fig. 4). Trends toward increased MCRs of A4 and T were also observed, although the statistical power with 11 men was too low for definitive analysis (2,764 ± 505 to 4,078 ± 867 liters/day, $P = 0.09$). Initial analysis suggested a trend toward decreased fractional conversion of A4 to T and E1 to E2 postoperatively (0.41 ± 0.20 to 0.24 ± 0.06% and 0.12 ± 0.03 to 0.08 ± 0.02%). However, when single outliers were removed from each group, no trend was evident.

In women, percent conversion of A4 to E1 via aromatase tripled from 3.1 ± 0.9 to 9.4 ± 1.2% (Fig. 5), whereas production rates of E1 also increased sharply (64 ± 11 to 240 ± 70 µg/day). Mean metabolic clearance rates of E1 increased markedly (2,379 ± 196 to 4,242 ± 528 liters/day).

Fig. 1. Changes in serum sex steroid levels following coronary artery bypass graft surgery (CABG) in 3 representative men, demonstrating considerable individual variation.

![Graph](https://example.com/graph.png)
Production rates of $A_4$ increased from $4.2 \pm 2.4$ to $10.0 \pm 2.1$ mg/day.

Adipose tissue aromatase P450 increased postoperatively. Aromatase mRNA was estimated by an internally standardized RT-PCR. In this approach, a sequence-encoded internal standard aromatase amplicon (Standard probe) was coamplified with DNA sample derived from mRNA by reverse transcription. Wild-type and internal standard alkaline phosphatase-labeled probes were used to detect the aromatase amplicon and the internal standard, respectively. As shown in Fig. 6, RT-PCR clearly demonstrates the increase in aromatase mRNA levels in two representative patients following CABG. Increases in band density of $>50\%$ were observed in four of the six men. Comparison of the mean density in relative light units (RLUs) prior to surgery ($1,973 \pm 564$) to density after surgery ($4,019 \pm 1,265$) without using the internal standard for normalization did not reach statistical significance ($P = 0.07$). This comparison was further refined by creating the ratio of wild-type density to internal standard density. A clear increase in this ratio was evident following surgery ($0.157 \pm 0.023$ to $0.746 \pm 0.095$, $P < 0.001$; Fig. 7). Data in Fig. 7, presented with additional normalization with respect to the levels GAPDH, are consistent with data presented in Fig. 6 and support the view that aromatase P450 mRNA levels are elevated postoperatively in CABG patients. No increase was evident in mean GAPDH band densities following CABG ($5,109 \pm 1,985$ RLU preoperative vs. $4,824 \pm 1,526$ RLU postoperative, $P = 0.76$). These results suggest that aromatase gene transcription is upregulated in this population.

**DISCUSSION**

Our data indicate that the increased serum estrogen concentrations reported with acute illness (3, 7, 27, 37) are caused primarily by increased peripheral aromatization rates due to increased expression of aromatase. The evidence for increased...
aromatization was consistent and pronounced across our patient population with marked increases in peripheral aromatization and estrogen production rates in all of our subjects, both men and women.

Our data argue against alternative explanations for increased circulating estrogen levels. MCRs were not decreased but were actually increased postoperatively. Estrogen precursor availability was not convincingly increased. In fact, serum levels of T (the androgen precursor to E2) were markedly decreased postoperatively. Mean A4 (the precursor to E1) was mildly increased postoperatively, although A4 production rates were not. Increases in serum E1 were observed in some men in the absence of increased serum A4 levels or production rates. Some previous studies (32) report mildly increased serum A4 accompanying increased serum estrogen levels in acute illness, whereas others (16, 22) do not. Still, the positive correlation with changes in A4 and E1 observed in our study suggests that increased androgen precursor availability may contribute to increased E1 production, even if increased aromatization rates are the primary mechanism.

Our prospective model is likely to be representative of other major acute illnesses with respect to endocrine response. We (36) have previously demonstrated that changes in the adrenal, thyroid, growth hormone, and reproductive axes are similar in CABG patients and in patients with nonsurgical critical illnesses. The elevations of circulating levels of cortisol and cytokines typical of critical illness were evident in our patients. Several items indicate that the anesthetic agents employed in surgery patients do not appreciably affect endocrine measurements performed the day after surgery. Volatile anesthetic agents, particularly halothane, have been reported to decrease hepatic blood flow and hepatic clearance rates. However, the inhaled agents in our patients, flurane and isoflurane, are short-acting agents and have negligible effects on hepatic blood flow and clearance following surgery (8, 13, 25). Furthermore, we observed increased rather than decreased hepatic

Fig. 4. Clearance rates of E1 (left) and E2 (right) before and after CABG in 11 men. Increased clearance rates were observed in most men and in the 3 women (not shown). The 2 men with decreased clearance rates did not have greater increases in serum estrogen levels than the other men (see text). MCR, metabolic clearance rate.

Fig. 5. Increases in %conversion of A4 to E1 via aromatase were observed in all 3 women following CABG.

Fig. 6. P450 aromatase gels in 2 representative patients. Increases in wild-type aromatase P450 band densities following CABG are evident in both patients. Increases in aromatase were confirmed by calculating ratios of wild-type to standard band densities (see text and Fig. 7).
metabolic clearance rates in our patients after surgery. Finally, narcotic anesthetic agents that can suppress hypothalamic-pituitary endocrine axes were discontinued well before our postoperative studies were performed. The narcotic-mediated suppression of the adrenal axis was no longer evident the day after surgery.

Our observations suggest that the increase in serum estrogens may be a specific response to acute illness rather than merely an epiphenomenon of other events in acute illness, such as increased adrenal androgen precursor availability or decreased MCRs secondary to altered hemodynamics. This study did not intend to identify the cause of the increased aromatization rates. The rise in circulating cortisol or cytokine concentrations in critical illness may potentially contribute to an increase in aromatase expression. Glucocorticoids stimulate aromatase activity in vitro (12, 21). Although in vivo studies have not confirmed this effect (18, 28), a glucocorticoid response element has been identified on the aromatase gene, supporting a modulating role for glucocorticoids on aromatase activity (49). Tumor necrosis factor-α and IL-1, -6, and -10 are all elevated in acute illness and have been demonstrated to increase aromatase activity in vitro (33).

In contrast to the increased serum estrogen levels, serum T levels are markedly decreased during major illness or after surgery (2, 3, 6, 7, 23, 27, 32, 34, 35, 37, 41, 43, 47). This decrease may be caused by multiple factors, including suppressed gonadotropin-releasing hormone (GnRH) secretion, resulting in hypogonadotropism as well as decreased testicular response to gonadotropins in at least some patients (34). Our data also suggest that increased MCRs may contribute to decreased circulating T levels. Androgen MCRs were increased in 12 of our 14 men. In one man, decreased serum T concentrations were accompanied by an increased T MCR, although there was no decrease in the T production rate.

The clinical impact of elevated circulating estrogen levels in severely ill patients is not yet clear. Estrogens have acute nonreproductive physiological effects on cardiovascular function, the immune system, and hepatic protein production. Cardiovascular function is generally enhanced by estrogens. Estrogens promote arterial vasodilation (46). In vivo and animal studies, E2 protects against inappropriate vasoconstriction of coronary arteries and promotes coronary flow (46). E2 also enhances carotid artery blood flow, at least in pharmacological doses. Animal studies (29, 30, 48) report that E2 promotes, whereas androgens suppress, myocardial function after trauma/hemorrhage. One study in humans reported increased exercise tolerance in postmenopausal women immediately following E2 administration, implying improved coronary artery circulation (31). Whether or not enhanced estrogen production with severe illness promotes cardiac function and circulation remains to be studied.

T and estrogen also affect levels of tissue damage from hypoxia in animal models. T aggravates ischemic renal injury (26). Estrogen protects against and T exacerbates neuronal damage during hypoxia (10, 24). Estrogen administration or T receptor blockade mitigates gut injury and inflammation associated with shock and hypoxia in rats (11). If these animal data extend to humans, the acute increases in serum estrogen levels and decreases in androgen levels may be beneficial in protecting against hypoxic tissue damage in sepsis and other critical illnesses.

Sex steroids have complex interactions with the immune system (5). In animal models of trauma and sepsis, E2 promotes (14, 15) and T suppresses (1, 45) important aspects of immune function. These actions of E2 appear to enhance survival (14), whereas those of T appear to decrease survival (1). The effects of high levels of estrogen on immune function and survival in critically ill humans have not been studied.

Estrogens also enhance production of several hepatic proteins, including factors in the coagulation cascade and acute phase proteins, such as ceruloplasmin (4, 39). Many other factors also influence production and effects of these proteins during illness, and no data have been reported evaluating the relative role of sex steroids within these influences. With the present limited evidence, only general conjectures can be offered regarding potential effects of estrogens in modulating coagulation and enhancing antioxidant effects of ceruloplasmin in humans with severe illness (9).

In summary, the sex steroid responses to nonendocrine illness appear to be more complex than previously appreciated. We describe for the first time increased aromatase mRNA expression accompanied by increased peripheral aromatization rates of androgens to estrogens in acute illness. Decreased GnRH secretion and testicular responsiveness to gonadotropins have previously been described (34, 40) as mechanisms underlying the hypogonadotropic hypogonadism of acute illness.
Our data suggest that increased MCRs of androgens also contribute to this hypoandrogenemia. Several mechanisms appear to be induced by acute illness to produce a marked increase in the ratio of circulating estrogens to T. Thus these responses are unlikely to represent merely a suppression of reproductive activity to shunt energy to other more critically required physiological processes but may represent adaptive responses in other ways as well. Because animal studies (1, 10, 11, 14, 15, 24, 29, 30, 45, 48) suggest that this increased ratio may be clinically beneficial in many respects, additional investigation in humans is warranted.

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REFERENCES


