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Estradiol Does Not Influence Lipid Measures and Inflammatory Markers in Testosterone-Clamped Healthy Men

Ferdinand Roelfsema,[1] Rebecca J. Yang,[2] and Johannes D. Veldhuis[2]

[1]Department of Internal Medicine, Section Endocrinology and Metabolism, Leiden University Medical Center, 2333ZA Leiden, Netherlands; and [2]Endocrine Research Unit, Mayo School of Graduate Medical Education, Center for Translational Science Activities, Mayo Clinic, Rochester, Minnesota 55906

Context: Experimentally controlled studies of estrogenic regulation of lipid measures and inflammatory cytokines in men are rare.

Objective: To delineate the effect of estradiol (E_2) on lipids and inflammatory markers.

Design: This was a placebo-controlled, single-masked, prospectively randomized study comprising experimentally degarelix-downregulated healthy men [n = 74; age 65 years (range, 57 to 77)] assigned to four treatment groups: (1) IM saline and oral placebo; (2) IM testosterone and oral placebo; (3) IM testosterone and oral anastrozole (aromatase inhibitor); and (4) IM testosterone, oral anastrozole, and transdermal E_2 for 22 ($\pm$1) days.

Results: Mean mass spectrometry–quantified serum E_2 concentrations ranged from 1.2 to 82 pg/mL in the four treatment groups. E_2 extremes did not alter total cholesterol, triglyceride, low-density lipoprotein (LDL) cholesterol, high-density lipoprotein cholesterol (HDL-C) , non–HDL-C, apolipoprotein B, lipoprotein (a), IL-6, or high-sensitivity C-reactive protein (hsCRP) concentrations. Higher E_2 concentrations elevated both sex hormone-binding globulin and prolactin as positive controls. LDL cholesterol, adiponectin, and leptin were higher in hypogonadal subjects without testosterone or E_2 addback (P = 0.018, 0.039, and 0.023, respectively). Abdominal visceral fat area by CT (independent variable) correlated negatively with HDL-C (P = 0.017), and positively with triglycerides (P = 0.004), hsCRP (P = 0.005), and leptin ($P < 0.0001$).

Conclusion: In this placebo-controlled prospectively randomized study, wide variations in circulating E_2 did not influence lipid measures and inflammatory markers when testosterone concentrations were controlled experimentally. However, medically induced central hypogonadism in older men was accompanied by increased LDL cholesterol and metabolic cytokines, adiponectin and leptin. Abdominal visceral fat correlated strongly and positively with triglycerides, hsCRP, and leptin, but negatively with HDL.

Freeform/Key Words: age, estradiol, human, inflammation, lipids, testosterone

Sex steroids are used extensively in estrogen treatment of menopausal symptoms, androgen supplementation in men with low serum testosterone (T) levels, and androgen or estrogen administration to transsexual patients [1–4]. Clinical concerns are potential cardiovascular complications (myocardial infarction and cerebrovascular accidents), venous thromboses,

Abbreviations: ApoB, Apolipoprotein B; BMI, body mass index; CV, coefficient of variation; E_2, estradiol; HDL-C, high-density lipoprotein cholesterol; hsCRP, high-sensitivity C-reactive protein; LDL-C, low-density lipoprotein cholesterol; Lp(a), lipoprotein (a); SHBG, sex hormone–binding globulin; T, testosterone; TC, total cholesterol; TG, triglyceride.

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and hormone-related cancers (breast, uterine, and prostatic) [5–7]. Investigation of the lipid spectrum is one tool for assessing the safety of hormone treatment. Many studies have been published on side effects of estrogen replacement in menopausal women, but less attention has been devoted to potential drawbacks of estrogen repletion or deprivation in men. To assess direct effects of sex steroids on lipid profiles, short-term studies are required, before any substantial change in body composition occurs [7–9]. Nonetheless, many clinical sex-hormone treatment studies are focused on long-term results [10–12]. In women, the choice of a particular estrogen, its dose and route of administration, and concomitant use of progestin with more or less intrinsic androgenic effects can confound the interpretation of metabolic results [13].

The degree to which estrogen regulates lipid or metabolic outcomes in men is unclear [7, 14–16]. Prior investigations have been handicapped by pathological and/or pharmacologic settings and interventions, such as (diabetic) hypogonadism or surgical castration, high-dose estrogen or human chorionic gonadotropin administration or mixed antiestrogen/partial estrogen agonist treatment [5, 9, 15–19]. In most models, there are profound concurrent changes in T availability, thus confounding interpretation of any estrogen effect *per se*. Under such circumstances, low estrogen milieus tend to result in lower high-density lipoprotein (HDL) concentrations, and conversely for high-estrogen milieus. Moreover, as suggested by others, longer term interventions also alter body composition, typically lowering HDL whenever abdominal visceral fat increases as under prolonged T deprivation [8, 20].

Although estrogen administration is a straightforward procedure for the assessment of estrogen effects on the lipid profile in women, this is not the case for men. A major confounding action of administered estrogen in men is downregulation of the gonadotropic axis, resulting in decreased T production. Methods for increasing T in older men are more complicated. Treatment with aromatase blockers causes T levels to increase and estradiol (E_2) levels to decrease, whereas exogenously administering T elevates not only T but also E_2 concentrations [21]. Thus, for valid mechanistic studies of estrogen action *per se*, both T and E_2 levels must be controlled experimentally. This goal was accomplished here rapidly and precisely by: (1) downregulation of the gonadotropic axis using a potent and selective GnRH antagonist; (2) concomitant addback of placebo or T; (3) treatment with placebo or an aromatase inhibitor to decrease endogenously generated E_2 from administered T; and (4) administration of E_2 along with the aromatase inhibitor and T in a clamp model [22]. This fourfold strategy allowed us to investigate the effects of E_2 under experimentally controlled T levels. The purpose of this explorative study was to evaluate potential short-term effects of sex steroids (especially E_2) under controlled (T-clamped) conditions on lipids and selective inflammation markers in 74 healthy older men.

1. Material and Methods

The study was designed to address whether E_2 levels at fixed T availability affect lipid concentrations and inflammatory markers measured in fasting blood obtained after 3 weeks of the endocrine clamp.

A. Subjects

Seventy-four healthy, ambulatory, community-dwelling older men [mean age, 65 years (range, 57 to 77)] participated in the overnight clinical unit–based study. The mean body mass index (BMI) was 26.9 kg/m^2 (range, 20 to 36). Volunteers were recruited by newspaper advertisements, local posters, the Clinical Trials Center Web page, and community (general and minority) bulletin boards. In this single-blind, prospectively randomized, placebo-controlled study, all qualifying volunteers underwent gonadotropin downregulation using the GnRH antagonist, degarelix (Ferring Pharmaceuticals, NY). The primary randomization was to IM T *vs* IM saline, and transdermal E_2 *vs* no E_2, and oral placebo *vs* anastrozole, an aromatase inhibitor (AstraZeneca Pharmaceuticals, Wilmington, DE). Thereby,

the sex-hormone clamp comprised: (1) degarelix 80 mg (given as two subcutaneous injections of 40 mg) once (called day 1) (Ferring Pharmaceuticals, Parsippany, NY); (2) T enanthate or T cypionate (Cardinal Health, Hudson, WI) 100 mg or placebo IM given on day 1 and repeated on days 8 and 15, range ± 1 day; (3) oral placebo or anastrozole (AstraZeneca Pharmaceuticals, Wilmington, DE) 2.0 mg once daily for 23 days; and (4) no patch or an E_2 patch calibrated to deliver 0.05 mg/d E_2 transdermally (Novartis, Morris Plains, NJ) beginning on day 1 and changed every 3 days through day 22. Statistical comparison was among the four resulting groups: (1) degarelix/T/placebo/no patch; (2), degarelix/T/anastrozole/no patch; (3) degarelix/T/anastrozole/E_2 patch; and (4) degarelix/placebo/placebo/no patch.

Individuals arrived in the clinical research unit at or before 6:00 PM to permit placement of bilateral forearm IV catheters for overnight fasting and blood sampling. A blood sample was obtained at 8:00 AM for sex-hormone, lipids, inflammatory markers, and peptide measurements. The study was embedded in a protocol in which the influence of sex steroids on nocturnal GH secretion and the effect of GHRH on GH response were assessed (unpublished). Ambulation was allowed to the lavatory. The volunteer was allowed to sleep. To reduce nutritional confounds, subjects were given a prescribed meal to ingest at 6:00 PM on the evening before. Men received a standardized 10 kcal/kg meal (vegetarian or nonvegetarian) with a macronutrient composition of 20% protein, 50% carbohydrate, and 30% fat. Participants then remained fasting for 12 hours overnight (except for allowable intake of noncaloric and noncaffeinated liquids).

The protocol was approved by Mayo Institutional Review Board. Witnessed voluntary written informed consent was obtained before study enrollment. A complete medical history, physical examination, and screening tests of hematological, renal, hepatic, metabolic, and endocrine function were normal. Subjects underwent a single-slice CT of the abdomen, level L3-L4, as an exploratory test of the impact of relative visceral adiposity on lipid responses.

B. Exclusion Criteria

Exclusion criteria were acute or chronic systemic diseases, HIV positivity by medical history, anemia, endocrine disorders (except hypothyroid subjects who were biochemically euthyroid on replacement), psychiatric illness, alcohol or drug abuse, deep venous or arterial thromboses, cancer of any type (except localized basal or squamous cell cancer of the skin treated surgically without recurrence), recent use (within 6 weeks) of anabolic steroids or glucocorticoids, history of stroke, myocardial infarction or angina, allergy to sex steroids used in the study; substantial recent weight change (loss/gain of ≥6 lb over 6 weeks), transmeridian travel (exceeding 3 time zones within the preceding 3 weeks), current or recent night shift work, systemic drugs, abnormal renal, hepatic or hematologic function, concomitant sex-hormone replacement, and unwillingness to provide written informed consent.

C. Assays

Total cholesterol (TC), triglycerides (TG), and HDL cholesterol (HDL-C) were measured using the Roche Cobas c311 Chemistry Analyzer (Roche Diagnostics, Indianapolis, IN) with interassay coefficients of variation (CV) of 2.2%, 0.8%, and 0.6% at 249, 178, and 51 mg/dL, respectively. Low-density lipoprotein cholesterol (LDL-C) was calculated by the Friedewald equation. Apolipoprotein B (ApoB) was measured immunoturbimetrically on the Hitachi Chemistry Analyzer using the DiaSorin ApoB SPQ II Reagent Set (DiaSorin Inc., Stillwater, MN) with an interassay CV of 4% at 94 mg/dL. Lipoprotein (a) [Lp(a)] was measured by a turbidimetric immunoassay on the Roche Cobas c311 Chemistry Analyzer. The interassay CV was 4.3% at 21 mg/dL. Interleukin-6 was measured by a high-sensitivity two-site enzyme-linked immunoassay from R&D Systems (Minneapolis, MN). The interassay CV was 3.6% at 3.88 pg/mL. Leptin and adiponectin were measured by specific immunoassays (Linco Research, Inc., St. Louis, MO). The interassay CVs were 11% and 4.7% at 20.4 ng/mL and 29.9 ng/mL, respectively. High-sensitivity C-reactive protein (hsCRP) was measured by a

high-sensitivity immunoturbimetric assay on the Roche Cobas c311 Chemistry Analyzer. The interassay CV was 1.7% at 0.17 mg/dL. E_2 and total T were measured using liquid chromatography-tandem mass spectrometry (Agilent Technologies, Inc., Santa Clara, CA). Intraassay CVs were: E_2, 10.8% at 0.29 pg/mL and 5.1% at 32 pg/mL; and T, 8.9% at 0.69 ng/dL, 4.0% at 45 ng/dL, and 3.5% at 841 ng/dL. Sex hormone–binding globulin (SHBG), IGF-I, and IGFBP3 were quantified by solid-phase chemiluminescent assay on the Siemens Immulite 2000 Automated Immunoassay System (Siemens Health Care Diagnostics, Deerfield, IL). Intraassay CV for SHBG was 4.0% at 5.4 nmol/L and 5.9% at 74 nmol/L; for IGF-I, 4.9% at 37 μg/L and 5% at 225 μg/L; and for IGFBP3, 4% and 3.9% at 1.0 and 4.3 mg/L. IGFBP1 was determined by a two-site immunoradiometric assay (Diagnostic Systems Laboratories, Webster, TX). Interassay CVs were 10.2% at 0.49 μg/L and 6.7% at 4.5 μg/L. The interassay CV was 1.7% at 0.17 mg/dL. Insulin was measured by a two-site immunoenzymatic sandwich assay on the Roche e411 (Roche Diagnostics). Intraassay CVs were 3.3%, 2.8%, and 2.5% at 18, 61, and 172 mU/L. Prolactin, FSH, and LH were measured by two-site chemiluminescent sandwich immunoassays on a DXL 800 automated immunoassay system (Beckman Instruments, Chaska, MN). Intraassay CVs for prolactin were 3.7%, 2.1%, and 4.8% at 6.1, 16.4, and 34.5 μg/L; for FSH, 3.6%, 3.2%, and 4.7% at 6.5, 16.7, and 58.0 IU/L; and for LH, 9.3%, 6.0%, and 6.0% at 1.4, 15.6, and 48.8 IU/L, respectively.

D. Statistics

Data were analyzed by one-way ANOVA for the four randomly assigned treatment groups. In case of nonnormal distribution, the Kruskal-Wallis nonparametric ANOVA test was used. This was followed by the Dwass-Steel-Critchlow test for pairwise comparisons. Linear regression analysis was applied to identify concentration-dependent effects of T and/or E_2, and effects of abdominal visceral fat. The statistical power of this study was 80% based upon a change of 20% of mean lipid concentrations with a standard deviation of 20% when studying 12 to 15 subjects in each group.

Calculations were performed with Systat 13 (Systat Software, Inc., San Jose, CA). $P < 0.05$ was construed as significant for the overall study.

2. Results

Demographic and anthropomorphic data of the volunteers are presented in Table 1. Statistically, the four subject groups were balanced before any treatment protocol started.

Table 1. Baseline Demographic and Endocrine Data in 74 Healthy Older Men

Variable	D/T(−)	D/T(+)	D/A/E_2(−)/T(+)	D/A/E_2(+)/T(+)	ANOVA *P*
Number of subjects	16	18	20	20	
Age (y)	64 ± 0.8	66 ± 1.0	65 ± 1.0	65 ± 1.2	0.69
BMI (kg/m^2)	28.1 ± 1.1	27.7 ± 0.8	25.4 ± 0.5	26.9 ± 0.9	0.12
Visceral fat (cm^2)	193 ± 25	194 ± 22	144 ± 13	176 ± 23	0.29
HOMA-IR	1.28 ± 0.19	1.28 ± 0.20	1.00 ± 0.15	1.30 ± 0.26	0.67
LH (U/L)	5.0 ± 0.9	4.0 ± 0.4	5.5 ± 0.8	4.0 ± 0.4	0.23
FSH (U/L)	9.1 ± 1.0	6.6 ± 0.7	9.9 ± 1.3	6.8 ± 0.6	0.12
T (ng/dL)	430 ± 30	430 ± 21	480 ± 22	500 ± 28	0.11
E_2 (pg/mL)	22.6 ± 2.5	21.4 ± 1.6	27.2 ± 1.3	24.7 ± 1.3	0.08
SHBG (nmol/L)	45 ± 3.3	43 ± 3.2	42 ± 2.7	50 ± 3.3	0.35
TSH (mU/L)	2.33 ± 0.2	2.76 ± 0.3	2.83 ± 0.3	2.90 ± 0.3	0.44
PRL (μg/L)	9.2 ± 0.9	8.4 ± 0.6	8.7 ± 0.7	8.3 ± 0.6	0.81

Data are shown as the mean ± SEM. *P* values were estimated by one-way ANOVA across the four study groups. Abbreviations: A, anastrozole; D, degarelix; E_2(–), no 17β estradiol; E_2(+), 17β estradiol; T(−), no testosterone addback; T(+), testosterone addback.

Interventions were well tolerated and there were no dropouts. Serum concentrations of IGF-I and its binding proteins IGFBP3 and IGFBP1 were similar after treatment in the four groups. Total T and (calculated) free T levels were low in subjects with downregulated gonadotropins without T addback, whereas T addback in the other three groups yielded statistically comparable T concentrations (Table 2). E_2 concentrations were lowest in subjects treated with anastrozole, which inhibits the conversion of T in E_2, followed by the control group with no T addback. Serum SHBG concentrations in E_2-depleted men (group C) were lower than in men with E_2 addback (group D); namely, 30.0 ± 2.6 and 38.0 ± 2.7 nmol/L, respectively ($P = 0.03$). Serum prolactin was higher in subjects treated with E_2 compared with men treated with placebo (8.0 ± 0.7 μg/L *vs* 6.2 ± 0.3 μg/L, $P = 0.02$). Furthermore, serum gonadotropin concentrations were higher in the two groups with low E_2 concentrations (*post hoc* contrasts for LH and FSH, $P < 0.0001$), consistent with known negative feedback by E_2 on gonadotropin secretion. However, likely because of differences in sensitivity to degarelix between individuals, LH and FSH were not completely suppressed in all subjects. This is obviously important for subjects in group A, whereas the other groups received testosterone addback. Nevertheless, subjects of group A were all hypogonadal with 80% decrease of free T levels. Addback of E_2 or T or the combination had no influence on serum lipids, including TC, HDL-C, LDL-C, non-HDL-C, TG, ApoB, and LP(a). Adiponectin and leptin concentrations were higher in men treated with degarelix only (group A) compared with the other three groups, who received addback of one or both sex steroids (Table 3). Absolute values of the inflammatory markers, hsCRP and IL-n-6, were higher in subjects treated with degarelix only (group A). However, differences with men who received E_2 and/or T addback were not important.

Based upon linear regression analysis, TC, HDL-C, LDL-C, non-HDL-C, TG, ApoB, and Lp(a) were not related to serum E_2 or T concentrations (Table 4). Restricting analysis to subjects treated with anastrozole (groups C and D), serum E_2 and lipid concentrations also were not correlated (Table 4). On the other hand, CT-estimated abdominal visceral fat area correlated positively with TG ($R = 0.34$, $P = 0.004$), nonsignificantly with non-HDL ($R = 0.20$, $P = 0.07$) and negatively with HDL-C ($R = -0.28$, $P = 0.017$). Additionally, visceral fat area correlated positively with leptin ($R = 0.72$, $P < 0.0001$) and hsCRP (R = 0.33, $P = 0.005$) (Fig. 1). Serum SHBG concentrations were linearly related to Lp(a) ($R = -0.30$, $P = 0.011$) and leptin ($R = 0.37$, $P = 0.001$), but nonsignificantly to adiponectin ($R = 0.22$, $P = 0.06$).

3. Discussion

This prospectively randomized, placebo-controlled, single-masked study investigated separate and combined effects of E_2 and T addback in older healthy men under a sex-steroid clamp

Table 2. IGF-I, IGF-Binding Proteins, and Sex Hormones During Hormone Administration in 74 Older Men

	Group A	Group B	Group C	Group D	ANOVA
IGF-I (μg/L)	106 ± 8.4	111 ± 7.4	106 ± 6.6	114 ± 6.1	0.80
IGFBP1 (μg/L)	1.51 ± 0.19	1.31 ± 0.12	1.40 ± 0.15	1.14 ± 0.07	0.25
IGFBP3 (mg/L)	2.96 ± 0.19	2.84 ± 0.12	3.06 ± 0.14	2.89 ± 0.12	0.68
Estradiol (pg/mL)	9.4 ± 1.9^a	31.2 ± 3.5^b	1.21 ± 0.24^c	82 ± 18^d	**<0.0001**
Free testosterone (ng/dL)	3.1 ± 0.7^a	19.5 ± 1.9^b	19.9 ± 2.0^b	20.4 ± 1.8^b	**<0.0001**
Testosterone (ng/dL)	164 ± 35^a	760 ± 61^b	748 ± 71^b	845 ± 66^b	**<0.0001**
SHBG (nmol/L)	37 ± 3.3	33 ± 2.9	30 ± 2.4	38 ± 2.7	0.22
Prolactin (μg /L)	6.5 ± 0.6^a	10.4 ± 0.8^b	6.2 ± 0.3^a	8.0 ± 0.7^{a,b}	**<0.0001**
FSH (U/L)	4.0 ± 0.9^a	0.29 ± 0.04^b	5.02 ± 1.18^a	0.33 ± 0.06^b	**<0.0001**
LH (U/L)	2.4 ± 0.5^a	0.21 ± 0.1^b	1.50 ± 0.34^a	0.24 ± 0.09^b	**<0.0001**

Data are shown as mean ± SEM. Differing superscripts denote significant *post hoc* contrasts by multiple-comparison testing among the four treatment groups. Boldface values denote $P < 0.01$ level of significance.

Table 3. Lipid Profiles and Inflammatory Markers During Hormone Administration in 74 Older Healthy Men

Parameter	Group A	Group B	Group C	Group D	ANOVA
TC (mg/dL)	175 ± 7.1	166 ± 6.3	158 ± 7.1	166 ± 5.0	0.34
HDL-C (mg/dL)	42 ± 3.5	37 ± 2.8	42 ± 3.3	42 ± 2.6	0.62
LDL-C (mg/dL)	100 ± 6.3	91 ± 4.3	84 ± 5.8	90 ± 4.5	**0.018**[a]
Non-HDL-C (mg/dL)	133 ± 6.7	128 ± 6.2	116 ± 7.2	124 ± 4.9	0.31
Triglycerides (mg/dL)	161 ± 16.7	187 ± 20.9	163 ± 16.9	168 ± 19.0	0.77
Lp(a) (mg/dL)	27.0 ± 6.3	23.3 ± 5.3	21.9 ± 4.4	16.8 ± 4.1	0.55
ApoB (mg/dL)	0.88 ± 0.044	0.84 ± 0.043	0.78 ± 0.049	0.82 ± 0.029	0.34
Adiponectin (mg/dL)	9840 ± 1190	6120 ± 616	7690 ± 650	8250 ± 980	**0.039**[b]
HsCRP (mg/dL)	0.36 ± 020	0.17 ± 0.05	0.076 ± 0.012	0.11 ± 004	0.16
Leptin (ng/mL)	14.6 ± 2.1	9.6 ± 1.9	6.8 ± 0.7	10.2 ± 1.9	**0.023**[c]
Interleukin 6 (pg/mL)	5.2 ± 2.1	3.8 ± 0.6	3.4 ± 0.4	3.9 ± 0.7	0.67

Data are shown as mean ± SEM. Boldface values denote ANOVA $P < 0.05$.
Post hoc contrasts:
[a]Group A *vs* group C: $P = 0.029$.
[b]Group A *vs* groups B, C, D jointly: $P = 0.008$.
[c]Group A *vs* groups B, and C and D jointly: $P = 0.02$, group A *vs* group B; $P = 0.005$.

enforced after gonadal-axis downregulation with a potent, selective, and long-acting GnRH antagonist, degarelix. T was added back in some groups, and its conversion to E_2 was inhibited by the aromatase blocker, anastrozole, in two other groups. Placebo or E_2 was then added back under anastrozole block. Sex-steroid concentrations were quantified by mass spectrometry, yielding an absolute range of mean E_2 concentrations of 1.2 to 82 pg/mL across the four study groups.

The current study circumvents many earlier issues by experimentally fixing systemic T concentrations for 22 ± 1 days, whereas adjusting serum E_2 concentrations over a nearly 80-fold range verified by mass spectrometry to ensure accurate quantification of very low E_2 levels. We are unaware of any prior investigations constraining E_2 in men over a comparable range while fixing T concentrations. The short study interval of 3 weeks obviates major shifts in body composition otherwise observed over more prolonged intervals, although minor changes may occur short term in severe acute hypogonadism [23]. In the current study, only group A had reduced testosterone levels, whereas the other three groups had normal levels under T addback, limiting concerns about a possible (minor) change in body composition. Under the present conditions, marked E_2 variations did not detectably alter LDL-C, HDL-C, TG, TC, non-HDL-C, Lp(a), ApoB, hsCRP or IL-6 concentrations. In contrast, extensive

Table 4. Linear Regressions Among Serum Sex-Hormone Concentrations and Lipids, Lipoproteins, and Inflammatory Proteins, and Analogously for Visceral Fat in 74 Older Men

	T	Free T	E_2	E_2 in Anastrozole-Treated Subjects	Visceral Fat Area
Total cholesterol	−0.065/0.58	−0.10/0.39	+0.024/0.84	+0.052/0.75	+0.071/0.56
Triglycerides	+0.02/0.87	−0.00/0.99	+0.09/0.94	+0.023/0.89	**+0.34/0.004**
HDL-C	+0.008/0.95	−0.004/0.98	+0.052/0.67	+0.031/0.85	**-0.28/0.017**
LDL-C	−0.088/0.46	−0.015/0.31	−0.006/0.96	+0.028/0.87	+0.008/0.95
Non-HDL-C	−0.062/0.60	−0.10/0.39	+0.001/0.99	+0.038/0.82	+0.20/0.07
ApoB	+0.07/0.55	−0.109/0.36	+0.046/0.70	+0.023/0.89	+0.12/0.32
Lp(a)	−0.030/0.80	+0.066/0.58	−0.047/0.69	−0.045/0.78	+0.002/0.99
hsCRP	−0.14/0.23	−0.17/0.16	+0.061/0.06	−0.051/0.76	**+0.33/0.005**
Leptin	−0.078/0.51	−0.22/0.06[d]	+0.036/0.76	+0.010/0.52	**+0.72/<0.0001**
Adiponectin	−0.007/0.95	−0.063/0.60	−0.004/0.97	+0.020/0.87	−0.22/0.06
IL-6	−0.15/0.22	−0.17/0.15	−0.06/0.62	−0.078/0.64	+0.17/0.16

Data are the linear correlation coefficient (β) and P value. Boldface values denote that individual data are plotted in Figure 1.

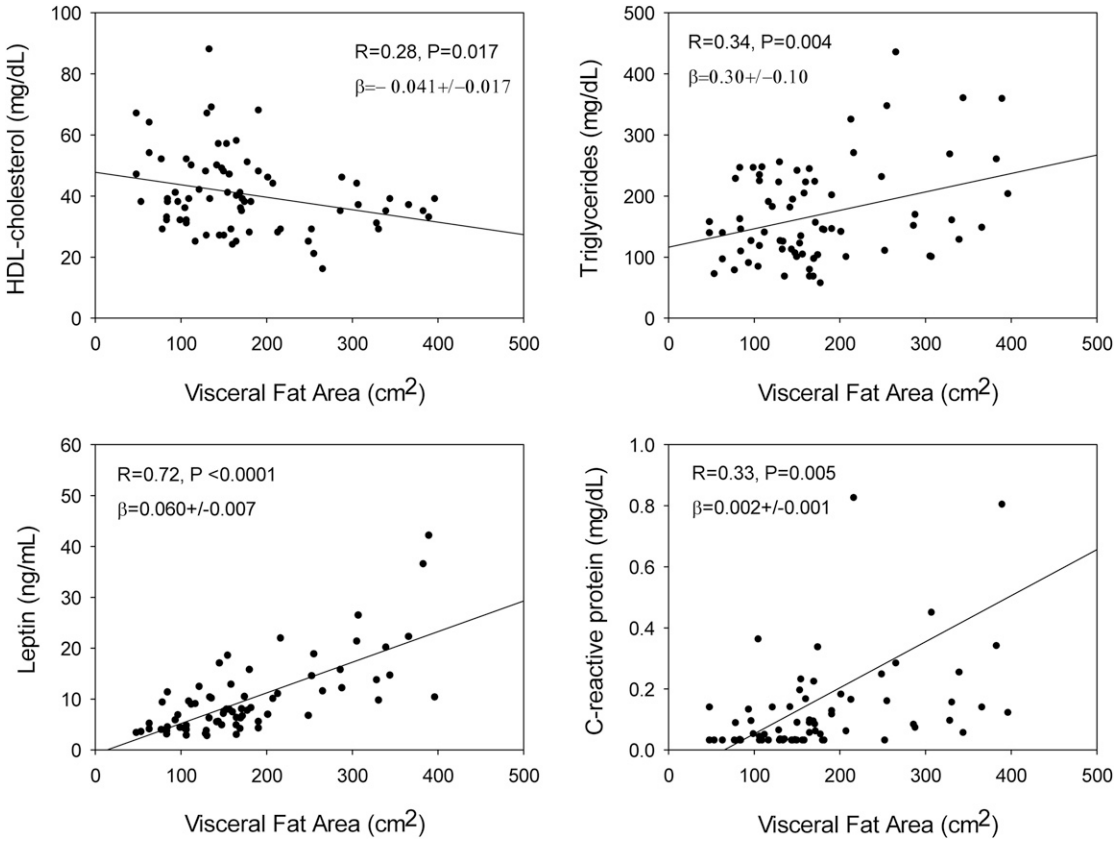

Figure 1. Linear regressions between abdominal visceral fat area and each of HDL-C, TG, leptin, or hsCRP in 74 healthy older men. *P* values are for the indicated slope (β coefficient) and *R* (correlation) values.

available literature establishes that nonaromatizable anabolic steroids and androgenic progestins consistently suppress HDL, often markedly [24], depending upon chemical structure, dose, route, and duration of exposure [14, 25].

Estradiol administration for 22 ± 1 day in the current study resulted in well-known stimulatory effects on serum prolactin and SHBG concentrations. This verifies adequacy of the E_2 clamp to modulate known endocrine targets of E_2. Furthermore, in the presence of low E_2 and/or low T levels, the competitively antagonistic effect of degarelix on gonadotropin secretion was partly disinhibited. Thus, expected hypothalamopituitary and hepatic effects of sex steroids were present in these sex steroid–clamped healthy subjects.

In the overall cohort of 74 men, no effects of either E_2 or T on the lipid profile could be demonstrated, including on TC, HDL-C, LDL-C, non-HDL-C, TG, ApoB, and Lp(a). The power of these observations was emphasized by the absence of important (linear) relationships between serum T and E_2 concentrations on one hand and lipid concentrations on the other. In women, the beneficial effects of endogenous estrogens including E_2 on lipids and cardiovascular risk are well accepted, inasmuch as premenopausal women are protected from cardiovascular mortality compared with age-matched men. After menopause, a less favorable lipid profile emerges, along with higher vascular risk. The menopausally defined low estrogen, androgen, and progesterone milieu is associated with increased TC, LDL-C, and TG, but decreased HDL-C and Lp(a) [26]. Administration of estrogens to postmenopausal women can improve the lipid profile, depending on the choice of estrogen; the dose, duration, and route of administration; and accompanying progestin [27].

In uncontrolled epidemiological studies, long-term effects of sex-steroid administration can introduce confounding factors (*e.g.*, changes in lifestyle and/or body composition), which secondarily alter lipoproteins and inflammatory markers. These drawbacks were avoided in a

recent placebo-controlled prospective short-term study in postmenopausal women [13]. The paradigm demonstrated that E_2 and natural progesterone modify lipids and inflammatory markers after 3 weeks of sex-steroid treatment. The current study exploits an analogous sex-steroid clamp strategy adapted to men, wherein systemic E_2 and T concentrations are experimentally controlled. The absence of estrogenic effects on the lipid profile in this paradigm in men raises the possibility that lipoproteins are less sensitive to estrogen in men than women, although mass spectrometry–quantified E_2 concentrations were numerically comparable in men (81 pg/mL) and women (99 pg/mL) in the two studies [13]. As positive controls, there were clear effects of administered E_2 on prolactin, SHBG, and gonadotropin concentrations in both women and men. Thus, the present clinical model of E_2 administration is sufficient to demonstrate expected E_2 regulation of well-known physiological endpoints.

Investigations of the effects of estrogens on lipid concentrations in men are limited by cohort selection and clinical context (*e.g.*, male-to-female transsexuals and patients with prostatic carcinoma). In a recent study by Auer *et al.,* 24 previously untreated male-to-female transgender patients were studied at baseline and after 12 months of oral estrogen exposure. In response to estrogen, fat mass and the waist:hip ratio decreased, along with TG, TC, and HDL-C [11]. Another study of E_2 administration in men reported increased HDL-C and TG concentrations after 6 months [4]. Potential interpretational problems in these studies are concomitant changes in body composition, simultaneous exposure to antiandrogens and/or antiprogestins, and profound reduction in serum T concentrations. The combined factors do not allow facile interpretation of direct or exclusive E_2 effects on lipid measures and inflammatory markers in such individuals.

In men with prostatic carcinoma, orchiectomy with monthly injections of polyphosphate E_2 or daily oral administration of ethinyl E_2 decreased TC, LDL-C, and ApoB while increasing HDL-C [28]. Similar findings occurred during very high-dose transdermal E_2 treatment (0.6 mg/d), which is sixfold the menopausal dose [2]. In these settings, serum E_2 concentrations were at least fourfold greater than those in our study, whereas total and free T concentrations were in the hypogonadal or castrate ranges [2]. Some drawbacks of these studies were circumvented by a study using an early-generation GnRH antagonist along with parenteral T addback and a relatively nonspecific aromatase inhibitor, testolactone [29]. In the young men in whom E_2 levels decreased, HDL-C decreased by 8%, and apolipoprotein A by 6%. Concentrations of TC, LDL-C, and TG did not change. Unfortunately, mass spectrometry was not used to quantify T or E_2, E_2 was not added back, and no data on ApoB, Lp(a), or cytokine concentrations were reported. Collectively, these heterogeneous studies in very different populations suggest that, in the face of demonstrably normal adult male T levels, the effects of nonfeminizing concentrations of E_2 on LDL-C, HDL-C, and TG are limited.

Adiponectin and leptin concentrations were higher in hypogonadal men, defined here by low serum T, free T, and E_2 concentrations after 3 weeks. In chronically hypogonadal men, T repletion normalized initially elevated leptin levels [30]. Analogously, in healthy men, treatment with a GnRH-receptor antagonist reduced T levels in 7 days, and increased adiponectin levels. Concomitant addition of T prevented the rise of adiponectin [31]. Likewise, long-term (6 to 12 months) of T treatment in female-to-male transsexual patients decreased adiponectin [32] as well as leptin [11, 33]. Accordingly, T and/or its metabolites can diminish leptin and adiponectin concentrations. Because the same cytokines did not decrease in the current study after anastrozole's blockade of T-to-E_2 conversion, we infer that T's restoration of cytokine levels does not depend upon physiological amounts of E_2 derived from T's aromatization. Indeed, in another study, transdermal E_2 administration in men with prostate cancer for 8 weeks did not alter leptin levels [2]. On the other hand, short-term aromatase inhibition in young and elderly men resulting in increased T and decreased E_2 levels was accompanied by increased leptin, but unchanged adiponectin, concentrations [34]. Although available data do not agree on all points, overall observations on the regulation of serum leptin and adiponectin in sex-hormone controlled men are compatible with androgen and not estrogen effects on these adipokines.

Although *in vitro* studies have shown a direct negative effect of testosterone and dihydrotestosterone on leptin secretion by rodent adipocytes [35], a direct effect on adiponectin secretion in human adipocytes is not present [36]. Additional investigations have shown that humoral serum components of high molecular weight are involved in the secretion of various molecular forms of adiponectin [37].

The inflammatory markers, IL-6 and hsCRP, assessed in this study were not different among the four groups. In addition, their levels were not related to serum concentrations of E_2, T, and free T, suggesting that they are not modulated short-term by T or E_2 availabilities in men. Comparable conclusions were reached in other studies based upon T administration in older men, transdermal E_2 administration in patients with prostatic carcinoma [2], and anastrozole administration in elderly men with low T [38].

Limitations of the current analysis include the relatively small cohort (n = 74 men), shorter duration of observation (3 weeks), and absence of more exhaustive lipid fractionation by mass spectrometry, PAGE or other laboratory techniques, as well as the restricted age range evaluated. Thus, our outcomes do not necessarily apply to populational data, or reflect expected outcomes after lipid subfractionation, or in young adult cohorts. Likewise, our study does not address cardiovascular outcomes *per se*, which have been reviewed by others recently [8, 39].

In summary, this clinical investigation delineates the absence of influence of a very wide range of near-physiological concentrations of E_2 under fixed T concentrations on lipid measures and inflammatory markers in older healthy men over 22 days. Acutely induced central hypogonadism in men is associated with increased metabolic cytokines, adiponectin and leptin, which are normalized during T addback whether T's conversion to E_2 is blocked. The last finding indicates that aromatase activity is not required to transduce T's suppression of these adipocytokines.

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Correspondence: Johannes Veldhuis, MD, Department of Medicine, Endocrine Research Unit, Mayo Clinic, 200 First Street SW, Rochester, Minnesota 55905. E-mail: veldhuis.johannes@mayo.edu.

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