

# Plasma Lipoprotein Profile in the Male Cynomolgus Monkey under Normal, Hypogonadal, and Combined Androgen Blockade Conditions

MATHIAS LEBLANC, MARIE-CLAIRE BÉLANGER, PIERRE JULIEN, ANDRÉ TCHERNOF, CLAUDE LABRIE, ALAIN BÉLANGER, AND FERNAND LABRIE

*Molecular Endocrinology and Oncology Research Center (M.L., A.T., C.L., A.B., F.L.) and Lipid Research Center (M.-C.B., P.J., A.T.), Laval University Medical Center and Laval University, Québec, Canada, G1V 4G2*

**In men, orchiectomy (GDX) produces an atherogenic lipid profile, whereas combined androgen blockade (CAB) induces a favorable lipid pattern. To better understand the opposite effects of GDX and CAB on lipid metabolism, we have compared the changes in plasma lipoproteins, mesenteric fat metabolism, as well as serum and intratissular sex steroid concentrations in intact, GDX, and GDX+FLU [GDX male cynomolgus monkeys treated for 3 months with flutamide (FLU)].**

Serum concentrations of dehydroepiandrosterone, dehydroepiandrosterone sulfate (DHEA-S), and androstenediol remained stable after GDX. Serum androstenedione (–40%), testo (–97%), dihydrotestosterone (–89%), androsterone-glucuronide (–75%), and androstane-3 $\alpha$ ,17 $\beta$ -diol-glucuronide (–80%) levels decreased similarly in both GDX and GDX+FLU animals. Intratissular dihydrotestosterone (–59 to –99%), estradiol (–31 to –53%), and androsterone-glucuronide (–28 to –85%) concentrations also decreased after GDX. GDX induced significant increases in plasma low-density lipoprotein (LDL)

(+78%) and high-density lipoprotein (+34%) cholesterol as well as in LDL-apoB (+58%) and high-density lipoprotein-apoAI (+32%). In the GDX+FLU group, except for the LDL-apoB that showed a tendency to decrease, lipid and apo-protein parameters remained unchanged compared with baseline values measured in intact animals. It is worth noting that these differences in the lipid profile could not be explained by changes in the metabolism of mesenteric adipose tissue.

In summary, in the cynomolgus monkey, GDX and CAB induced opposite effects on the plasma lipoprotein profile. These differences possibly result from differences in the specific activity of the androgens and estrogens derived from adrenal precursors. Such data support the suggestion that androgens and estrogens produced from adrenal precursors in peripheral intracrine tissues could have important, but so-far unsuspected, effects on the homeostasis of lipid and lipoprotein metabolism. (*J Clin Endocrinol Metab* 89: 1849–1857, 2004)

PROSTATE CANCER is the most frequently diagnosed cancer and is the second cause of cancer death in men in North America (1). Based upon the studies of Huggins (2) in 1941, the first gold standard treatment of prostate cancer therapy was limited to blockade of the androgens of testicular origin through orchiectomy (GDX) or high doses of estrogens. Treatment with estrogens, however, resulted in serious cardiovascular complications, and surgical castration is not well accepted by most patients. Our group at Laval University then discovered that medical castration with an LHRH agonist could be achieved without the serious side

effects of previous therapies (3, 4). It was then rapidly established that over 40% of intraprostatic dihydrotestosterone (DHT) was produced from the intracrine transformation of the adrenal precursors dehydroepiandrosterone (DHEA), dehydroepiandrosterone sulfate (DHEA-S), and androstenedione (4-dione) (5). The new gold standard of hormonal therapy of prostate cancer is now aimed at suppressing simultaneously the androgens of gonadal origin with an LHRH agonist while blocking the remaining androgens of adrenal origin, neutralized at the androgen receptor level with a pure antiandrogen (5–10).

In parallel, the studies on the impact of combined androgen blockade (CAB) and intracrinology in prostate cancer therapy have new insights into the controversial role of androgens in lipid metabolism. In fact, increased endogenous serum testosterone (testo) and adrenal C<sub>19</sub> steroids have been associated with a favorable lipid profile, namely reduced plasma triglycerides (Tg), apoB, and low-density lipoprotein cholesterol (LDL-C) and higher levels of high-density lipoprotein cholesterol (HDL-C) (11). On the other hand, it has been reported that the suppression of testicular androgens through surgical castration results in an atherogenic lipid profile (12). In fact, orchiectomy in prostatic carcinoma patients has an adverse effect on the lipid profile by increasing total cholesterol, LDL-C, and LDL-apoB (13). It should be mentioned that LHRH agonist administration in healthy vol-

Abbreviations: ADT-G, Androsterone-glucuronide; CAB, combined androgen blockade; CV, coefficient of variation; DEXA, dual-energy x-ray absorptiometry; DHEA, dehydroepiandrosterone; DHEA-S, dehydroepiandrosterone sulfate; DHT, dihydrotestosterone; 5-diol, androst-5-ene-3 $\beta$ ,17 $\beta$ -diol; 3 $\alpha$ diol-G, androstane-3 $\alpha$ ,17 $\beta$ -diol-glucuronide; 4-dione, androstenedione; E<sub>1</sub>, estrone; E<sub>2</sub>, estradiol; FLU, flutamide; GC-MS, gas chromatography and negative chemical ionization mass spectrometry; GDX, orchiectomy; HDL-C, high-density lipoprotein cholesterol; INT, intact (group); LC-MS, HPLC and mass spectrometry; LDL-C, low-density lipoprotein cholesterol; LOQ, limit of quantification; ns, not significant; LPL, lipoprotein lipase; testo, testosterone; Tg, triglyceride(s); VLDL, very-low-density lipoprotein; WBFM, whole body fat mass; WBLM, whole body lean mass.

JCEM is published monthly by The Endocrine Society (<http://www.endo-society.org>), the foremost professional society serving the endocrine community.

unteers over a relatively short time period results in increased HDL-C, apoAI, and apoB with no other significant changes of the lipid profile (14, 15). On the other hand, CAB in prostatic cancer patients results in an increase in HDL-C with no other significant change to the lipid profile. In fact, Moorjani *et al.* (16) suggested that the opposite effects of GDX and combined CAB on lipid metabolism, especially on the plasma levels of HDL-C, might be attributable to the smaller decrease in estrogens in the later group with no antagonism from androgens. In fact, estrogens might be essential in maintaining HDL-C levels in those men (17).

The present study compares the effects of GDX and GDX+FLU [GDX combined with 3-month flutamide (FLU) treatment] in male cynomolgus monkeys on changes in body composition, plasma lipoproteins, and mesenteric fat metabolism. Because circulating bioactive androgens, namely testo and DHT, are known markers of gonadal secretion (10), we also determined the serum levels of these steroids, of their conjugated metabolites, and of intratissular steroid levels as an index of the intracellular hormonal milieu.

## Materials and Methods

### Experimental animals

This study was performed at the animal facilities of the Laval University Medical Center (Québec, Canada). Monkeys were selected from the colony of the Molecular Endocrinology and Oncology Research Center. Young adult male cynomolgus monkeys (5–7 yr old), weighing 5.6–8.6 kg, were in good health as verified by a complete veterinary examination, serum biochemistry, and complete blood counts. Animals were housed individually or in pairs in standard stainless steel cages in a room maintained at  $23 \pm 3$  C with a 12-h dark, 12-h light cycle (lights on at 0715 h). Throughout this study, the animals were maintained and handled in accordance with the policies of the Canadian Council on Animal Care and the National Institutes of Health guide for the Care and Use of Laboratory Animals. This protocol has been approved by the Ethical Committee for Animal Protection of the Centre Hospitalier Universitaire de Québec.

### Experimental design

Fifteen adult male cynomolgus monkeys were randomly assigned to three groups of five animals each. The INT (intact) group remained intact, whereas groups GDX and GDX+FLU underwent bilateral GDX under general anesthesia on study d –1 to eliminate steroids of gonadal origin. From study d 1–93, group GDX+FLU received 25 mg/kg FLU orally, twice a day, to block, at the androgen receptor level, the influence of androgens produced locally from adrenal precursors. Fasting morning blood was drawn from the femoral vein twice prior to the beginning of the study as well as at d 33, 61, and 90 to determine serum steroid concentrations, plasma lipids, and lipoproteins as well as complete hematologic and biochemical parameters. All animals were anesthetized on the same study day to determine their body composition by dual-energy x-ray absorptiometry (DEXA). Animals were euthanized on study d 93, using an overdose of sodium pentobarbital. Mesenteric fat was removed during necropsy, for the determination of adipose cell size, lipoprotein lipase (LPL) activity, and adipose tissue lipolytic activity. The prostate, seminal vesicles, liver, lungs, muscle, and skin were snap-frozen in liquid nitrogen to determine their intratissular steroid concentrations.

### Diets

All animals received an identical feeding regimen. Throughout the study period, the monkeys were fed six Certified Hi-Fiber Primate 5K91 cookies (LabDiet, PMI Feeds Inc., Richmond, VA) twice daily at 6-h ( $\pm 1$  h) intervals (Table 1). Animals also received one fruit and one vegetable twice a week (same fruit and same vegetable for all subjects on a given

day). Water was available *ad libitum*. Body weight and food consumption of cookies, fruits, and vegetables were assessed twice weekly before the morning meal.

### Body composition

Monkeys were premedicated with ketamine (10 mg/kg)-glycopyrrolate (0.01 mg/kg) and anesthetized with isoflurane. Whole-body mass, whole-body fat mass (WBFM), and whole-body lean mass (WBLM) were studied using DEXA (QDR 4500 Fan Beam X-Ray Bone Densitometer; Hologic Bedford, MA) and analyzed with the Hologic small-animal software. The technique was validated on six cynomolgus monkeys before the study. Intra- and interassay precision [percent coefficient of variation (CV)] did not exceed 6.5%.

### Serum and intratissular steroid measurements

Adrenal and gonadal steroids, as well as their metabolites, were analyzed at the Molecular Endocrinology and Oncology Research Center. Serum concentrations of DHEA, 4-dione, androst-5-ene-3 $\beta$ ,17 $\beta$ -diol (5-diol), testo, DHT, estrone (E<sub>1</sub>), and estradiol (E<sub>2</sub>) were determined using high-performance gas chromatography and negative chemical ionization mass spectrometry (GC-MS). Intra- and interassay precision (percent CV) did not exceed 5.9% for these assays. DHEA-S, androsterone-glucuronide (ADT-G), and androstane-3 $\alpha$ ,17 $\beta$ -diol-glucuronide (3adiol-G) serum concentrations were determined using HPLC and mass spectrometry (LC-MS) using a PE Sciex (Toronto, Ontario, Canada) API 300 tandem mass spectrometer equipped with a Turbo ionspray source. Intra- and interassay precision (percent CV) did not exceed 6.4% for these assays. Intratissular steroid concentrations were determined for each tissue after pooling equivalent samples from all animals within a group. Intratissular DHT and E<sub>2</sub> concentrations were determined by GC-MS after liquid-liquid and solid-liquid extraction, whereas intratissular ADT-G levels were determined by LC-MS after a solid-liquid extraction. The lower limits of quantification (LOQs) for the assays are indicated in Table 2.

### Plasma lipid determinations

Using fasting blood collected in EDTA at 0 and 3 months, plasma lipoproteins were fractionated by microultracentrifugation to isolate the very-low-density lipoprotein VLDL ( $d < 1.006$ ), LDL ( $d = 1.006–1.063$ ), and HDL ( $d > 1.063$ ) fractions (18). The Tg, cholesterol, and free fatty acid concentrations in lipoprotein fractions and/or total plasma were determined enzymatically using a Technicon (Tarrytown, NY) Auto-Analyzer (Model RA-500), whereas apoB-LDL and apoAI-HDL were measured by automated immunonephelometry (Behring Nephelometer 100 Analyzer, Dade Behring, Germany) using Dade Behring antiapoproteins as previously published (19).

### Adipocyte cell size

Freshly isolated adipocytes were prepared by collagenase digestion (collagenase type I, 860 U/g fat) of 250- to 300-mg tissue samples in

**TABLE 1.** Macronutrient content and major fatty acid components of the certified Hi-Fiber Primate Diet 5K91

Composition	%
Proteins	20.0
Fat (ether extract)	5.0
Cholesterol	Trace
Linoleic acid	1.15
Linolenic acid	0.08
Arachidonic acid	<0.01
Omega-3 fatty acids	0.11
Total saturated fatty acids	1.91
Total monounsaturated fatty acids	1.82
Fiber (crude)	8.5
Carbohydrate	51.2

Metabolizable energy (kcal/g) = 3.06; daily metabolizable energy for a ration of 12 primate cookies, 532 kcal/d.

Krebs-Ringer phosphate-HEPES buffer for 45 min at 37 C. The adipose cell suspension was washed three times with buffer and then examined using an electron microscope connected to a digital camera. Using the Scion Image Software, mean cell diameters were determined from digitalized images based on the measurements of 250 cells per tissue sample.

### Lipolytic activity

Lipolytic capacity was measured *in vitro* with isolated adipose cells in response to 2-h stimulations with isoproterenol (nonselective  $\beta$ -adrenergic agonist) and forskolin (postreceptor  $\beta$ -adrenergic agonist) (20). The glycerol content of the medium was analyzed by bioluminescence using a reduced nicotinamide adenine dinucleotide-linked bacterial luciferase assay and was used as an index of lipolysis.

**TABLE 2.** LOQ for the determination of serum and intratissular concentrations of conjugated and nonconjugated steroids by GC-MS and LC-MS

Steroids	LOQ
Serum steroids	
DHEA (nmol/liter)	1.4
DHEA-S (nmol/liter)	5.1
Androstenedione (nmol/liter)	0.14
Androstenediol (nmol/liter)	0.69
Testosterone (nmol/liter)	0.56
Dihydrotestosterone (nmol/liter)	0.07
3 $\alpha$ diol-G (nmol/liter)	107
ADT-G (nmol/liter)	107
Oestrone sulfate (nmol/liter)	0.13
Oestrone (pmol/liter)	29.6
Oestradiol (pmol/liter)	7.3
Intratissular steroids	
DHT (nmol/kg)	0.68
ADT-G (nmol/kg)	8.6
Oestradiol (pmol/kg)	73.4

**TABLE 3.** Animal characteristics and baseline lipid profile of the male cynomolgus monkeys

	Mean $\pm$ SEM
n	15
Body weight (kg)	7.2 $\pm$ 0.2
Age (years)	6.5 $\pm$ 0.2
Total cholesterol (mmol/liter)	1.91 $\pm$ 0.07
LDL cholesterol (mmol/liter)	0.76 $\pm$ 0.05
HDL cholesterol (mmol/liter)	0.91 $\pm$ 0.04
Triglycerides (mmol/liter)	0.36 $\pm$ 0.05

**TABLE 4.** Body weight, body composition determined by DEXA and total food consumption in INT, GDX, and GDX+FLU cynomolgus monkeys

	INT			GDX			GDX+FLU		
	Baseline	3 months	<i>P</i> value	Baseline	3 months	<i>P</i> value	Baseline	3 months	<i>P</i> value
n	5	5		5	5		5	5	
Weight (kg)	7.5 $\pm$ 0.3	7.8 $\pm$ 0.4	ns	6.8 $\pm$ 0.4	5.6 $\pm$ 0.3 <sup>a</sup>	0.005	7.1 $\pm$ 0.4	5.8 $\pm$ 0.3 <sup>a</sup>	0.001
DEXA (kg)									
WBM	7.3 $\pm$ 0.4	8.2 $\pm$ 0.5	ns	6.6 $\pm$ 0.5	6.1 $\pm$ 0.4	ns	6.8 $\pm$ 0.4	6.3 $\pm$ 0.3 <sup>b</sup>	0.007
WBFM	1.0 $\pm$ 0.2	1.1 $\pm$ 0.1	ns	0.84 $\pm$ 0.05	0.82 $\pm$ 0.08	ns	0.85 $\pm$ 0.09	0.81 $\pm$ 0.10	ns
WBLM	6.0 $\pm$ 0.2	6.8 $\pm$ 0.4 <sup>b</sup>	0.04	5.4 $\pm$ 0.4	5.0 $\pm$ 0.3	ns	5.6 $\pm$ 0.3	5.2 $\pm$ 0.2 <sup>b</sup>	0.04
Total food consumption (kcal-d)		21841 $\pm$ 1668			20292 $\pm$ 2514	ns		19265 $\pm$ 2922	ns

Anthropometric data were collected at baseline and at the end of the experimental period (3 months).

WBM, whole body mass. Data presented are mean values  $\pm$  SEM.

<sup>a</sup> *P* < 0.01 or less.

<sup>b</sup> *P* < 0.05.

### LPL activity

Adipose tissue LPL activity was determined by the method of Taskiran *et al.* (21). In brief, heparin eluates were recovered from 30–50 mg of frozen tissue samples and incubated with an excess of unlabeled and <sup>14</sup>C-labeled triolein in a Tris-albumin buffer emulsified by ultrasound. Excess apo-CII from porcine plasma was used to stimulate LPL activity. The resulting free fatty acids released were then isolated by the Belfage extraction procedure and measured by liquid scintillation counting. Unpasteurized cow's milk was used as an internal LPL activity standard for interassay variation.

### Statistical analyses

Concentrations of plasma lipoproteins and serum steroids were compared between samples collected at baseline and after 3 months of treatment using the Student's two-tailed *t* test. One-way analyses of variance were used to evaluate differences among the groups.

## Results

At baseline, no statistically significant differences in terms of body weight and plasma lipids were detected among the three experimental groups (Table 3). During the following 3 months, the body weight was significantly reduced (–18%, *P* < 0.01) in both the GDX and GDX+FLU groups, whereas the total food intake during the same period was not statistically different among the three groups (Table 4). Body composition determined by DEXA shows that these variations in total body mass are mostly explained by a loss of lean mass, which shows a tendency to decrease in the GDX group (–7.4%) and a statistically significant decrease (–7.1%) in the GDX+FLU group (*P* < 0.05).

Table 5 compares the serum concentrations of the steroid hormones in the INT, GDX, and GDX+FLU groups at 3 months compared with their respective baseline values. The values observed in the INT group show that the 3-month experimental period and the diet had no significant effect on the levels of serum steroids. Serum levels of DHEA, DHEA-S, and 5-diol remained unchanged after GDX or treatment of GDX animals with FLU, whereas 4-dione decreased in the GDX and GDX+FLU groups by 47% (*P* < 0.05) and 39% [not significant (ns)], respectively. Serum testo and DHT levels were markedly reduced by about 97 and 89%, respectively, in both the GDX and GDX+FLU groups (*P* < 0.01). ADT-G and 3 $\alpha$ diol-G, the two major metabolites of androgens in the circulation in the monkey and human, reflect the inactivation of locally synthesized androgens in peripheral target tissues



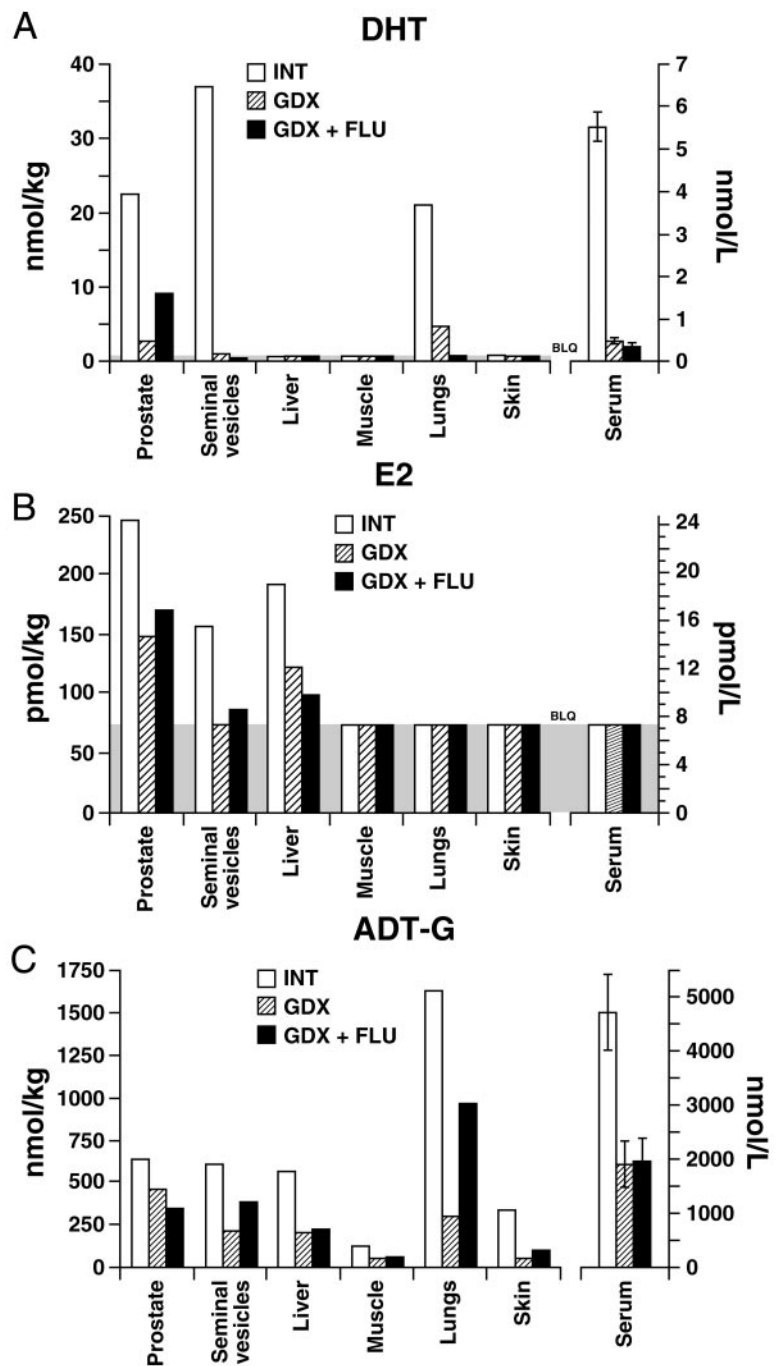


FIG. 1. Intratissular and serum DHT, E<sub>2</sub>, and ADT-G concentrations after 3 months of treatment in INT, GDX, and GDX+FLU cynomolgus monkeys.

for studying hormonal interactions and lipid metabolism (24). In fact, humans and primates are unique in having adrenals that secrete large amounts of DHEA and DHEA-S into the circulation. These inactive steroids act as precursors of active androgens and estrogens in peripheral intracrine tissues, where their action takes place at their site of synthesis with minimal diffusion of the active steroids in the general circulation (25). The rate of transformation of adrenal precursors into active sex steroids in each peripheral target tissues depends upon the local expression level of the various steroidogenic and metabolizing enzymes, thus allowing target tissues to adjust sex steroid intracellular concentrations

to their local requirements (10). The highly sophisticated intracrine system, which is unique to primates, may partly explain discrepancies observed between some animal and human studies on lipid metabolism (26).

The animals originally selected for this study had homogeneous body weight, age, lipid profile, caloric intake, and levels of physical activity. At baseline, plasma total cholesterol, HDL-C, and non-HDL-C levels were slightly lower than those previously reported for the adult macaca fascicularis (27). Such a difference could be explained by the lower fat content of the Hi-Fiber Primate diet used in the present study.

**TABLE 6.** Lipid profiles at baseline and at the end of the 3-month protocol in INT, GDX, and GDX+FLU cynomolgus monkeys

	INT			GDX			GDX+FLU		
	Baseline	3 month	<i>P</i> value	Baseline	3 month	<i>P</i> value	Baseline	3 month	<i>P</i> value
<b>Cholesterol (mmol/liter)</b>									
Total	1.95 ± 0.18	2.36 ± 0.21	ns	1.86 ± 0.19	2.75 ± 0.25 <sup>a</sup>	0.001	1.92 ± 0.08	1.99 ± 0.12	ns
VLDL	0.14 ± 0.04	0.08 ± 0.02	ns	0.09 ± 0.02	0.11 ± 0.05	ns	0.11 ± 0.04	0.18 ± 0.04	ns
LDL	0.87 ± 0.07	1.09 ± 0.09	ns	0.75 ± 0.11	1.26 ± 0.08 <sup>a</sup>	0.001	0.82 ± 0.10	0.85 ± 0.11	ns
HDL	0.94 ± 0.04	1.18 ± 0.15	ns	1.02 ± 0.12	1.37 ± 0.14 <sup>b</sup>	0.02	0.99 ± 0.03	0.94 ± 0.07	ns
<b>Triglycerides (mmol/liter)</b>									
Total	0.48 ± 0.07	0.50 ± 0.04	ns	0.39 ± 0.04	0.40 ± 0.06	ns	0.48 ± 0.05	0.56 ± 0.07	ns
VLDL	0.16 ± 0.03	0.18 ± 0.03	ns	0.14 ± 0.02	0.16 ± 0.04	ns	0.20 ± 0.04	0.23 ± 0.04	ns
LDL	0.16 ± 0.03	0.12 ± 0.03	ns	0.14 ± 0.02	0.10 ± 0.03	ns	0.20 ± 0.04	0.14 ± 0.01	ns
HDL	0.16 ± 0.03	0.21 ± 0.02	ns	0.15 ± 0.01	0.14 ± 0.01	ns	0.17 ± 0.03	0.19 ± 0.02	ns
<b>Free fatty acids (mmol/liter)</b>	0.45 ± 0.10	0.56 ± 0.07	ns	0.64 ± 0.11	0.88 ± 0.17	ns	0.60 ± 0.09	0.87 ± 0.06	ns
<b>Apolipoproteins (g/liter)</b>									
VLDL-apoB	0.07 ± 0.01	0.06 ± 0.02	ns	0.06 ± 0.01	0.09 ± 0.02 <sup>b</sup>	0.04	0.08 ± 0.01	0.08 ± 0.01	ns
LDL-apoB	0.16 ± 0.01	0.18 ± 0.01	ns	0.13 ± 0.03	0.20 ± 0.04 <sup>b</sup>	0.03	0.15 ± 0.02	0.11 ± 0.01	0.09
HDL-apoAI	0.84 ± 0.05	1.04 ± 0.10	ns	0.85 ± 0.07	1.12 ± 0.07 <sup>a</sup>	0.005	0.90 ± 0.03	0.93 ± 0.04	ns
<b>Ratios</b>									
Chol tot/HDL-C	2.07 ± 0.05	2.05 ± 0.10	ns	1.85 ± 0.12	2.01 ± 0.07	ns	1.95 ± 0.09	2.12 ± 0.11	ns
LDL-C/LDL-apoB	5.51 ± 0.31	6.08 ± 0.47	ns	5.75 ± 0.28	6.98 ± 0.42	ns	5.64 ± 0.36	7.94 ± 0.42 <sup>a</sup>	0.001
VLDL-Tg/VLDL-apoB	3.07 ± 1.27	3.21 ± 0.80	ns	2.97 ± 0.75	1.89 ± 0.46	ns	3.13 ± 0.89	2.77 ± 0.25	ns
HDL-C/HDL-apoAI	1.12 ± 0.07	1.12 ± 0.07	ns	1.18 ± 0.06	1.24 ± 0.12	ns	1.10 ± 0.04	1.02 ± 0.06	0.03

Data presented as means ± SEM (n = 5). ns, Not significant; Chol tot, cholesterol total.

<sup>a</sup> *P* < 0.01 or less.

<sup>b</sup> *P* < 0.05.

The present results demonstrate that GDX and CAB have marked effects on body composition despite no significant difference in total food intake over the 3-month study period. WBLM showed a tendency to a decrease in GDX animals and decreased significantly in GDX monkeys treated with FLU. These results are consistent with previous reports showing that WBLM decreases after androgen deprivation in men and increases after testo replacement therapy in patients with hypogonadism (28). On the other hand, androgen deprivation in men causes an increase in body weight and body fat mass, with a redistribution of body fat from the intraabdominal to the sc tissue (28, 29). However, proper control groups were missing in previous reports, and those studies were not controlled for physical activity, diet, or age. As indicated by the WBFBM, which shows a tendency to decrease in both GDX groups, the present data are at variance with previous reports. It may be argued that the observed changes in body composition after 3 months might not reflect the effect of a longer term effect of androgen deprivation. Animals were also fed a low caloric fiber-rich diet, which might account for the lack of increase in adiposity.

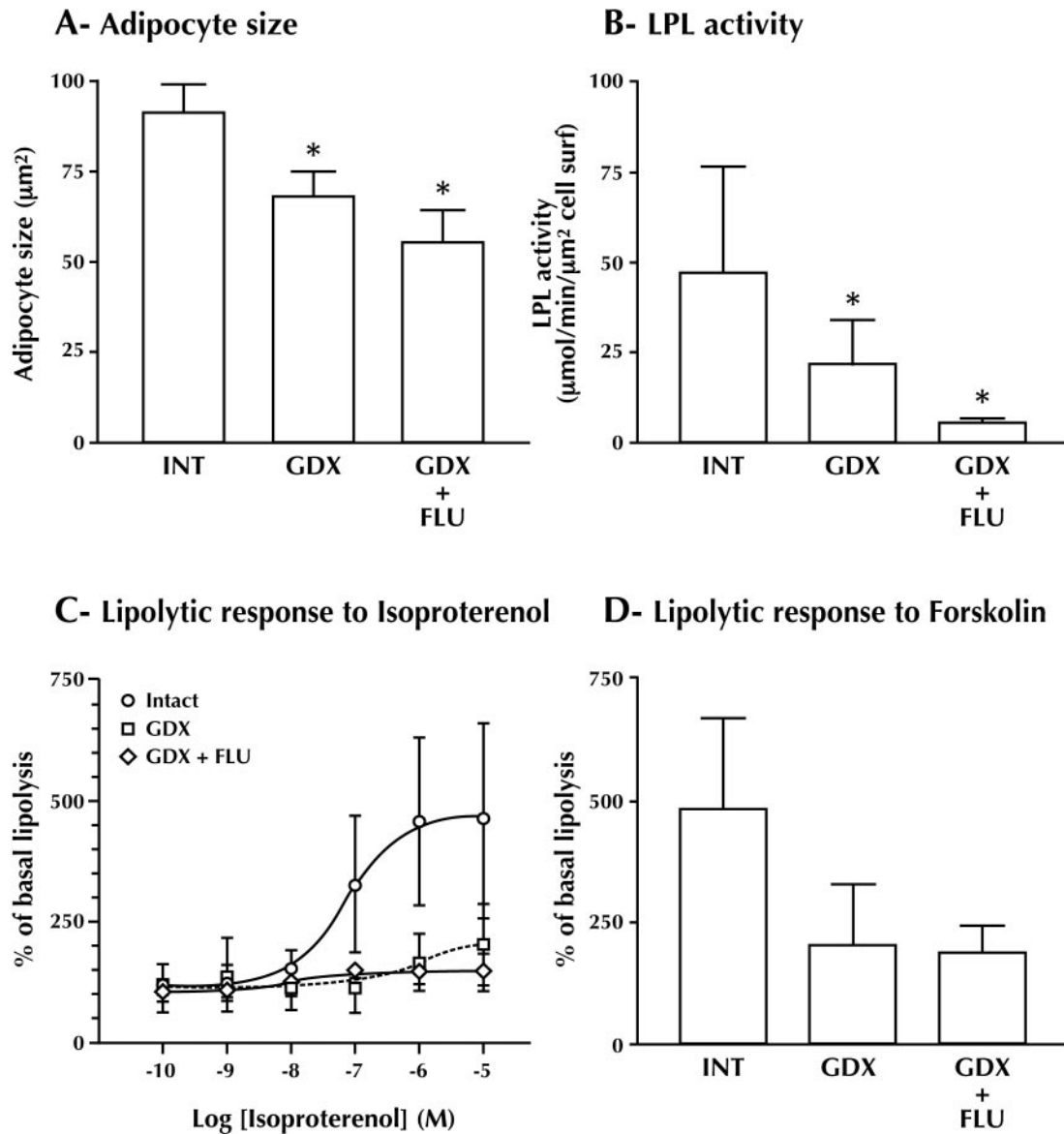
The lack of control data to evaluate the adiposity has been a critical factor put forward to explain some inconsistent findings related to the role of androgens on lipid metabolism (11). In addition, the adipose tissue by itself is an endocrine, paracrine, autocrine, and intracrine tissue that could influence lipid metabolism, the levels of circulating and local steroids, and in excess concentrations could lead to metabolic disorders such as insulin resistance or dyslipidemia (15) (30). The fact that adipose tissue mass was only slightly affected in the present study may have had a favorable impact on the lipid profile of the monkeys.

Serum concentrations of testo and DHT are direct and highly reliable markers of testicular secretion. On the other hand, the serum concentrations of the conjugated metabolites of DHT, namely ADT-G and 3 $\alpha$ diol-G, are the most

appropriate markers of the total androgen pool (31). Accordingly, GDX or LHRH agonist treatment in men causes a 90–95% reduction in serum testo concentration, whereas serum levels of 3 $\alpha$ diol-G and ADT-G decrease by only 50–75% (5). Furthermore, the intraprostatic concentration of DHT remains at about 40% of that measured in untreated INT men, thus showing that a large proportion of androgens are produced locally from adrenal precursors without release in the general circulation (32). As observed in the human, the cynomolgus monkey has high serum levels of androgen glucuronides in the circulation (33) and the UGT2B enzymes responsible for the specific conjugation of androgens and their 5 $\alpha$ -reduced metabolites are expressed in various peripheral tissues of the cynomolgus monkey (34).

In the present study, we have observed a 90–97% decrease in circulating levels of the active androgens testo and DHT, whereas their glucuronidated metabolites were reduced by only 75–80% after GDX or CAB. On the other hand, the intratissular concentrations of DHT in various tissues decreased by 59–99%, whereas the levels of ADT-G decreased by only 28–85% after GDX and CAB, thus suggesting that a large proportion of intratissular androgens are produced locally in an intracrine manner from adrenal precursors in the monkey, as described previously in men (5). The intratissular E<sub>2</sub> levels also indicate that the adrenals contribute to over half the estrogen pool in peripheral intracrine tissues such as the prostate, the seminal vesicles, and the liver, even though no estrogens were detectable in the circulation.

This study shows that the elimination of testicular androgens and estrogens by GDX results in a significant increase in total cholesterol. The levels of both cholesterol and apoB also increased in the LDL fraction, whereas the LDL-C/apoB-LDL ratio remained stable. Taken together, these results suggest that the increase in plasma LDL is due to an increase in the number of LDL particles, with no change in its cholesterol composition. Similarly, several studies have



Data presented as means  $\pm$  S.E.M.

FIG. 2. Lipolytic and lipogenic activities in the mesenteric adipose tissue after 3 months of treatments in INT, GDX, and GDX+FLU cynomolgus monkeys. \*,  $P < 0.05$ , experimental *vs.* intact control.

reported that GDX in prostate cancer patients results in an unfavorable lipid profile, namely in increase in total cholesterol, LDL-C, and apoB (12) (13). Based upon these previously reported alterations of the lipoprotein and steroid profiles in men, the increase in the number of LDL particles in the present study could result from a reduction in LDL uptake by the LDL receptor. The LDL receptor is up-regulated by estrogen at the transcriptional level via Sp1 and SREBP in the absence of an estrogen response element in its promoter (35, 36). Croston *et al.* (37) also demonstrated that the androgen receptor antagonizes activation of the LDL receptor by the estrogen receptor.

After GDX, the observed decrease of intratissular, including intrahepatic, estrogen levels could affect the estrogen-driven LDL receptor expression. However, residual androgens in the tissues remain relatively higher than those of estrogens and could, therefore, continue to antagonize the estrogen-dependent LDL receptor transcription. After CAB, total cholesterol, LDL-C, and LDL-apoB remained unchanged compared with INT animals, thus suggesting that CAB, compared with GDX, has an opposite effect on cholesterol and LDL metabolism. Although the intratissular  $E_2$  levels are similar in the GDX and GDX+FLU groups, the action of residual androgens is blocked by the pure anti-an-

drogen FLU in the GDX+FLU group, thus providing a possible explanation for the differences observed. By neutralizing the activity of DHT at the androgen receptor level, FLU might, in fact, favor the residual estrogenic effect in tissues of the GDX+FLU group and thus favor LDL receptor expression induced by estrogens. FLU could also possibly exert an action of its own. Genistein and daidzein, two dietary phytoestrogens known to stimulate the estrogen receptor, have also been shown to decrease apoB secretion in HepG2 cells and to enhance the expression and activity of the LDL receptor, thus contributing to a net decrease in apoB (38). Together, these observations could explain the favorable lipid profile observed in the GDX+FLU animals.

One could argue that alterations in the plasma lipoprotein profile after orchietomy could be the result of an increase in the secretion of hepatic VLDL. Circulating VLDL particles are then catabolized into LDL particles by the action of LPL. However, because the ratio of VLDL-Tg to VLDL-apoB remains stable (Table 6), LPL activity is probably not responsible for the increased levels of plasma LDL. This is further supported by the reduced LPL activity found in the mesenteric adipose tissue in GDX animals. In fact, LPL regulation by androgens in different tissues is still poorly understood (39, 40). Furthermore, direct hepatic secretion of LDL in GDX monkeys could explain the increased levels of plasma LDL, even though this metabolic pathway is not a major contributor to circulating LDL particles in normal animals.

Compared with the INT animals, we believe that mesenteric adipose cells in the GDX and GDX+FLU groups have reached a lower equilibrium state of LPL and lipolytic activities. The similar decrease in both LPL and HSL activities after GDX or treatment of GDX animals with FLU was reflected by unchanged circulating levels of free fatty acids. Again, the low-calorie diet of the present study might account for these observations.

In conclusion, the present data suggest that both androgens and estrogens are important, but differential, regulators of lipid metabolism. The physiological effects of sex steroids on peripheral tissues are mediated through both intracrine and classical endocrine pathways. Circulating concentrations of active sex steroids should, however, be regarded as poor markers of hormonal activity at the estrogen and androgen receptor levels in target tissues. In fact, significant estrogen levels were detectable in tissues even though circulating estrogen levels were below the LOQs in all groups. Furthermore, it should be recalled that exogenous or endogenous testo reaches the androgen receptor usually after conversion into DHT in specific target tissues, whereas the same steroid can also act as an estrogen precursor when transformed by aromatase.

Controversial results from cross-sectional studies on the influence of endogenous androgens and androgen deprivation as well as studies of exogenous androgens in men show that the response of lipid metabolism to sex steroids is not linear and depends on multiple confounding parameters. Based upon the results of our controlled steroid deprivation study performed in the monkey, we suggest that quantity as well as the relative proportions of androgens and estrogens are important factors in the regulation of the plasma lipid profile. Both androgen and estrogen deprivation are prob-

ably responsible for the unfavorable lipid profile in the GDX group. It is also remarkable that after GDX, intratissular androgens could possibly favor their antagonistic effect on the action of estrogens on LDL receptor expression. It is, in fact, known that DHEA is mainly transformed into androgens in the peripheral tissues in the human (31) and rat (41). On the other hand, blocking the androgen receptor with FLU eliminates all residual androgenic effects in peripheral tissues and thus results in a predominant unopposed estrogenic effect and in a substantially different lipid profile.

### Acknowledgments

Received July 17, 2003. Accepted January 6, 2004.

Address all correspondence and requests for reprints to: Pr. Fernand Labrie, Molecular Endocrinology and Oncology Research Center, Laval University Medical Center, 2705 Laurier Boulevard, Québec, Québec, G1V 2G2, Canada. E-mail: fernand.labrie@crchul.ulaval.ca.

This work was supported by the Canadian Institutes of Health Research.

M.L. and M.-C.B. contributed equally to this work and should both be considered first authors.

### References

1. Jemal A, Murray T, Samuels A, Ghafoor A, Ward E, Thun MJ 2003 Cancer statistics, 2003. *CA Cancer J Clin* 53:5–26
2. Huggins C HC 1941 Studies of prostatic cancer. I. Effect of castration, estrogen and androgen injections on serum phosphatases in metastatic carcinoma of the prostate. *Cancer Res* 1:293
3. Faure N, Labrie F, Lemay A, Belanger A, Gourdeau Y, Laroche B, Robert G 1982 Inhibition of serum androgen levels by chronic intranasal and subcutaneous administration of a potent luteinizing hormone-releasing hormone (LHRH) agonist in adult men. *Fertil Steril* 37:416–424
4. Labrie F, Bélanger A, Cusan L, Séguin C, Pelletier G, Kelly PA, Reeves JJ, Lefebvre F-A, Lemay A, Gourdeau Y, Raynaud J-P 1980 Antifertility effects of LHRH agonists in the male. *J Androl* 1:209–228
5. Labrie F, Dupont A, Bélanger A 1985 Complete androgen blockade for the treatment of prostate cancer. In: de Vita VT, Hellman S, Rosenberg SA, eds. *Important advances in oncology*. Philadelphia: J B Lippincott; 193–217
6. Crawford ED, Eisenberger MA, McLeod DG, Spaulding JT, Benson R, Dorr FA, Blumenstein BA, Davis MA, Goodman PJ 1989 A controlled trial of leuprolide with and without flutamide in prostatic carcinoma. *N Engl J Med* 321:419–424
7. Bennett CL, Tosteson TD, Schmitt B, Weinberg PD, Ernstoff MS, Ross SD 1999 Maximum androgen-blockade with medical or surgical castration in advanced prostate cancer: a meta-analysis of nine published randomized controlled trials and 4128 patients using flutamide. *Prostate Cancer Prostatic Dis* 2:4–8
8. Labrie F, Candas B 2000 Androgen blockade in prostate cancer. *Lancet* 356: 341–342
9. Labrie F 2002 Androgen blockade in prostate cancer in 2002: major benefits on survival in localized disease. *Mol Cell Endocrinol* 198:77–87
10. Labrie F, Luu-The V, Labrie C, Simard J 2001 DHEA and its transformation into androgens and estrogens in peripheral target tissues: intracrinology. *Front Neuroendocrinol* 22:185–212
11. Tchernof A, Labrie F, Belanger A, Prud'homme D, Bouchard C, Tremblay A, Nadeau A, Despres JP 1997 Relationships between endogenous steroid hormone, sex hormone-binding globulin and lipoprotein levels in men: contribution of visceral obesity, insulin levels and other metabolic variables. *Atherosclerosis* 133:235–244
12. Xu T, Wang X, Hou S, Zhu J, Zhang X, Huang X 2002 Effect of surgical castration on risk factors for arteriosclerosis of patients with prostate cancer. *Chin Med J (Engl)* 115:1336–1340
13. Moorjani S, Dupont A, Labrie F, Lupien PJ, Gagne C, Brun D, Giguere M, Belanger A, Cusan L 1988 Changes in plasma lipoproteins during various androgen suppression therapies in men with prostatic carcinoma: effects of orchietomy, estrogen, and combination treatment with luteinizing hormone-releasing hormone agonist and flutamide. *J Clin Endocrinol Metab* 66:314–322
14. Goldberg RB, Rabin D, Alexander AN, Doelle GC, Getz GS 1985 Suppression of plasma testosterone leads to an increase in serum total and high density lipoprotein cholesterol and apoproteins A-I and B. *J Clin Endocrinol Metab* 60:203–207
15. Bagatell CJ, Bremner WJ 1995 Androgen and progestagen effects on plasma lipids. *Prog Cardiovasc Dis* 38:255–271
16. Moorjani S, Dupont A, Labrie F, Lupien PJ, Brun D, Gagne C, Giguere M,

- Belanger A 1987 Increase in plasma high-density lipoprotein concentration following complete androgen blockage in men with prostatic carcinoma. *Metabolism* 36:244–250
17. Bagatell CJ, Knopp RH, Rivier JE, Bremner WJ 1994 Physiological levels of estradiol stimulate plasma high density lipoprotein2 cholesterol levels in normal men. *J Clin Endocrinol Metab* 78:855–861
  18. Ordovas JM 1998 Fast ultracentrifugation methods for the separation of plasma lipoproteins. *Methods Mol Biol* 110:93–103
  19. Connelly PW, Poapst M, Davignon J, Lussier-Cacan S, Reeder B, Lessard R, Hegele RA, Csima A 1999 Reference values of plasma apolipoproteins A-I and B, and association with nonlipid risk factors in the populations of two Canadian provinces: Quebec and Saskatchewan. Canadian Heart Health Surveys Research Group. *Can J Cardiol* 15:409–418
  20. Kather H, Schroder F, Simon B 1982 Microdetermination of glycerol using bacterial NADH-linked luciferase. *Clin Chim Acta* 120:295–300
  21. Taskinen MR, Nikkila EA 1977 Lipoprotein lipase activity in adipose tissue and in postheparin plasma in human obesity. *Acta Med Scand* 202:399–408
  22. Leblanc M, Labrie C, Belanger A, Candas B, Labrie F 2002 Pharmacokinetics of oral dehydroepiandrosterone (DHEA) in the ovariectomised cynomolgus monkey. *J Steroid Biochem Mol Biol* 81:159–164
  23. Labrie F, Belanger A, Cusan L, Candas B 1997 Physiological changes in dehydroepiandrosterone are not reflected by serum levels of active androgens and estrogens but of their metabolites: intracrinology. *J Clin Endocrinol Metab* 82:2403–2409
  24. Greger NG, Insull Jr W, Probstfield JL, Keenan BS 1990 High-density lipoprotein response to 5- $\alpha$ -dihydrotestosterone and testosterone in Macaca fascicularis: a hormone-responsive primate model for the study of atherosclerosis. *Metabolism* 39:919–924
  25. Labrie F 1991 Intracrinology. *Mol Cell Endocrinol* 78:C113–C118
  26. Weidemann W, Hanke H 2002 Cardiovascular effects of androgens. *Cardiovasc Drug Rev* 20:175–198
  27. Gupta SV, Khosla P 2001 Palmitic and stearic acids similarly affect plasma lipoprotein metabolism in cynomolgus monkeys fed diets with adequate levels of linoleic acid. *J Nutr* 131:2115–2120
  28. Smith MR, Finkelstein JS, McGovern FJ, Zietman AL, Fallon MA, Schoenfeld DA, Kantoff PW 2002 Changes in body composition during androgen deprivation therapy for prostate cancer. *J Clin Endocrinol Metab* 87:599–603
  29. Elbers JM, Asscheman H, Seidell JC, Gooren LJ 1999 Effects of sex steroid hormones on regional fat depots as assessed by magnetic resonance imaging in transsexuals. *Am J Physiol* 276:E317–E325
  30. Mohamed-Ali V, Pinkney JH, Coppack SW 1998 Adipose tissue as an endocrine and paracrine organ. *Int J Obes Relat Metab Disord* 22:1145–1158
  31. Labrie F, Luu-The V, Labrie C, Bélanger A, Simard J, Lin SX, Pelletier G 2003 Endocrine and intracrine sources of androgens in women: inhibition of breast cancer and other roles of androgens and their precursor dehydroepiandrosterone. *Endocr Rev* 24:152–182
  32. Labrie F 1995 Endocrine therapy of prostate cancer: optimal form and timing. *J Clin Endocrinol Metab* 80:1066–1071
  33. Guillemette C, Hum DW, Belanger A 1996 Levels of plasma C19 steroids and 5  $\alpha$ -reduced C19 steroid glucuronides in primates, rodents, and domestic animals. *Am J Physiol* 271:E348–E353
  34. Albert C, Barbier O, Vallee M, Beaudry G, Belanger A, Hum DW 2000 Distribution of uridine diphosphate-glucuronosyltransferase (UGT) expression and activity in cynomolgus monkey tissues: evidence for differential expression of steroid-conjugating UGT enzymes in steroid target tissues. *Endocrinology* 141:2472–2480
  35. Briggs MR, Yokoyama C, Wang X, Brown MS, Goldstein JL 1993 Nuclear protein that binds sterol regulatory element of low density lipoprotein receptor promoter. I. Identification of the protein and delineation of its target nucleotide sequence. *J Biol Chem* 268:14490–14496
  36. Wang X, Briggs MR, Hua X, Yokoyama C, Goldstein JL, Brown MS 1993 Nuclear protein that binds sterol regulatory element of low density lipoprotein receptor promoter. II. Purification and characterization. *J Biol Chem* 268:14497–14504
  37. Croston GE, Milan LB, Marschke KB, Reichman M, Briggs MR 1997 Androgen receptor-mediated antagonism of estrogen-dependent low density lipoprotein receptor transcription in cultured hepatocytes. *Endocrinology* 138:3779–3786
  38. Borradaile NM, de Dreu LE, Wilcox LJ, Edwards JY, Huff MW 2002 Soya phytoestrogens, genistein and daidzein, decrease apolipoprotein B secretion from HepG2 cells through multiple mechanisms. *Biochem J* 366:531–539
  39. Anderson LA, McTernan PG, Harte AL, Barnett AH, Kumar S 2002 The regulation of HSL and LPL expression by DHT and flutamide in human subcutaneous adipose tissue. *Diabetes Obes Metab* 4:209–213
  40. De Pergola G 2000 The adipose tissue metabolism: role of testosterone and dehydroepiandrosterone. *Int J Obes Relat Metab Disord* 24(Suppl 2):S59–S63
  41. Sourla A, Martel C, Labrie C, Labrie F 1998 Almost exclusive androgenic action of dehydroepiandrosterone in the rat mammary gland. *Endocrinology* 139:753–764

JCEM is published monthly by The Endocrine Society (<http://www.endo-society.org>), the foremost professional society serving the endocrine community.