

Pharmacokinetics and Pharmacodynamics of Nandrolone Esters in Oil Vehicle: Effects of Ester, Injection Site and Injection Volume¹

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ABSTRACT

We studied healthy men who underwent blood sampling for plasma nandrolone, testosterone and inhibin measurements before and for 32 days after a single i.m. injection of 100 mg of nandrolone ester in arachis oil. Twenty-three men were randomized into groups receiving nandrolone phenylpropionate (group 1, $n = 7$) or nandrolone decanoate (group 2, $n = 6$) injected into the gluteal muscle in 4 ml of arachis oil vehicle or nandrolone decanoate in 1 ml of arachis oil vehicle injected into either the gluteal (group 3, $n = 5$) or deltoid (group 4, $n = 5$) muscles. Plasma nandrolone, testosterone and inhibin concentrations were analyzed by a mixed-effects indirect response model. Plasma nandrolone concentrations were influenced ($P < .001$) by different esters and injection sites, with higher and earlier peaks with the phenylpropionate ester, compared with the decanoate ester. After nandrolone decanoate injection, the highest bioavailability and peak nandrolone levels were ob-

served with the 1-ml gluteal injection. Plasma testosterone concentrations were also influenced ($P < .001$) by the ester and injection site, with the most rapid, but briefest, suppression being due to the phenylpropionate ester, whereas the most sustained suppression was achieved with the 1-ml gluteal injection. Plasma inhibin concentrations were also significantly influenced by injection volume and site, with the lowest nadir occurring after the nandrolone decanoate 1-ml gluteal injection. Thus, the bioavailability and physiological effects of a nandrolone ester in an oil vehicle are greatest when the ester is injected in a small (1 ml vs. 4 ml) volume and into the gluteal vs. deltoid muscle. We conclude that the side-chain ester and the injection site and volume influence the pharmacokinetics and pharmacodynamics of nandrolone esters in an oil vehicle in men.

For decades, administration of androgens such as testosterone and 19-nor-testosterone has been most frequently *via* depot i.m. injections of steroid esters dissolved in a vegetable oil vehicle (Junkman, 1957; Behre *et al.*, 1990). Such i.m. injections provide sustained androgen release into the circulation and have remained the mainstay of androgen replacement therapy for the last few decades (Nieschlag and Behre, 1990), although the basic pharmacological mechanisms are complex and only partially understood (Zuidema *et al.*, 1988). The basic pharmacology of this depot androgen formulation differs among species (van der Vies, 1965) but has been little studied in humans. The current understanding is that the rate-limiting mechanism governing the appearance of active steroid in the bloodstream is the retention of steroid esters from the oil vehicle depot due to oil/water partitioning, with gradual release into the extracellular fluid, where esters are rapidly hydrolyzed to liberate biologically active steroid.

Other physiological and physico-chemical factors that could influence steroid appearance in the bloodstream include the chemistry of the side-chain ester (hydrophobicity, steric hindrance of hydrolysis and solubility), injection factors (depth, site and volume, pH and osmolarity of the solution), exercise and systemic illness. The influence of site and volume of injection on the release kinetics of androgen esters from oil vehicle depots has, however, not been systematically investigated in humans.

This study compared the pharmacokinetics and pharmacodynamics of two currently available esters of nandrolone, the decanoate and phenylpropionate, as well as the influence of i.m. injection sites (gluteal *vs.* deltoid) and injection volumes (4 ml *vs.* 1 ml). In addition to measuring plasma nandrolone to investigate pharmacokinetics, we measured plasma testosterone and inhibin by radioimmunoassay to determine the pharmacodynamic effects of nandrolone-induced inhibition of pituitary gonadotrophin secretion, as reflected in LH-dependent Leydig (testosterone) and FSH-dependent Sertoli (inhibin) cell function in healthy men. We analyzed these data

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using an indirect pharmacodynamic response model, which has demonstrated, for the first time, prominent pharmacological differences between esters differing in only a single carbon in the side-chain, as well as systematic differences attributable to injection site and volume in humans.

Materials and Methods

Experimental design. Twenty-three healthy volunteers were randomly allocated into four groups in two balanced blocks. The first stratum of the study involved comparing two different nandrolone esters while controlling for dose, injection site and volume. In this stratum, volunteers received either nandrolone phenylpropionate (Durabolin; Organon) (group 1) or nandrolone decanoate (Deca-Durabolin; Organon) (group 2), administered as a single deep i.m. injection of 100 mg nandrolone ester into a single injection site (gluteal) in a fixed volume (4 ml of arachis oil vehicle). In the second stratum, a single ester (nandrolone decanoate) with fixed dose (100 mg) and volume (1 ml of arachis oil vehicle) was injected into two different sites, gluteal (group 3) or deltoid (group 4) muscles. This design also allowed comparison between different injection volumes (4 ml *vs.* 1 ml) for a single nandrolone ester with fixed dose and injection site (*i.e.*, group 2 *vs.* group 3).

Subjects. Healthy nonobese men, 18 to 40 years of age, with normal reproductive function, not competing in sports requiring International Olympic Committee-sanctioned urinary drug screening for anabolic steroids, not allergic to peanuts, free from chronic medical illness, not requiring regular prescribed medication and without abnormalities in routine clinical examination and biochemical screening were recruited. Fully informed written consent was obtained from volunteers, and the study had approval from the University of Sydney Human Ethics Committee within National Health and Medical Research Council Guidelines on Human Experimentation, which conform to the Declaration of Helsinki.

Procedures. Blood (5 ml) was sampled before injection (8:00–9:00 A.M.) and at 1, 2, 4, 6, 8 and 10 hr and 1, 2, 3, 4, 7, 9, 11, 14, 16, 18, 21, 23, 25, 28, 30 and 32 days after injection. During the first 10 hr, the participants were in a metabolic ward facility and were allowed to undertake normal daily activities. Subsequently, participants returned for blood sampling on an ambulatory basis while resuming their habitual daily activities.

To calculate absolute bioavailability, two additional subjects received a single i.v. bolus of 1 mg of 19-nor-testosterone, with sampling before injection and at 2, 4, 6, 8, 10, 15, 20, 30, 45, 60, 120, 180, 240, 360, 480, 720, 900, 1440, 1980 and 2880 min. For this study, crystalline nandrolone (Steraloids Inc., Wilton, NH) was dissolved in dehydrated ethanol BP (David Bull Laboratories, P/L, Mulgrave, Australia) at a concentration of 40 g/liter, from which a nandrolone stock solution (2 mg/ml in 15% ethanol) was produced by appropriate dilution with sterile saline. The nandrolone stock was filtered through a 0.2- μ m cellulose acetate filter (Minisart NML; Sartorius GmbH, Gottingen, Germany), and the first 1 ml was discarded. Preliminary experiments showed that >80% of tracer nandrolone was recovered after such filtration. At the start of the study, the stock solution was diluted 10-fold with sterile normal saline in a 20-ml syringe (final ethanol concentration, 1.5%) and 5.0 ml was infused rapidly (within 10 sec) *via* an indwelling cannula in a forearm vein (nominal dose, 1.0 mg), followed by a flush of the syringe and cannula with an additional 5 ml of sterile saline solution. Aliquots of the injection solution were kept for subsequent assay of the exact amount of nandrolone administered. Blood sampling was from another cannula placed in the other arm.

Assays. Plasma samples were stored at -20°C until assay in a single batch per analyte. Steroids were measured by radioimmunoassay after extraction from plasma by a modification of a solid-phase method (Vining, 1980). Serum (200 μ l for the nandrolone assay and 75 μ l for the testosterone assay) was applied to a 5-cm mini-column

of Extrelut (kieselguhr; Merck, Kilsyth, Australia) packed in a Pasteur pipette. Steroids were eluted by four washes of 750 μ l of hexane/ethyl acetate (3:2) at 5-min intervals, the combined eluate was dried and the extract was reconstituted in assay buffer. Extraction efficiency was $97 \pm 5\%$ ($n = 29$) for testosterone and $90 \pm 5\%$ ($n = 25$) for nandrolone; therefore, no corrections were made for extraction losses. Radioactivity was measured with a Wallac 1410 liquid scintillation counter (Wallac Oy, Turku, Finland) and a LKB 1261 Multigamma gamma counter (Wallac Oy), using RIACALC data reduction software on an IBM-compatible computer. All steroid assay reagents were of analytical or higher grade.

Plasma nandrolone was measured by radioimmunoassay using a rabbit antibody to 19-nor-testosterone 17-hemisuccinate-bovine serum albumin (CER, Marloie, Belgium), tritiated tracer (19-[19- ^3H]nor-testosterone; specific activity, 1.37 TBq/mmol; Amersham, North Ryde, Australia), nandrolone standard (Steraloids), extracts of plasma equivalent to 100 μ l/tube and a dextran-charcoal separation of bound and free steroid. Cross-reactivities (expressed as molar ratios at the ED_{50}) with testosterone (0.04%), dihydrotestosterone (0.7%), androstenedione (0.3%), estradiol (0.02%), nandrolone phenylpropionate (6.3%) and nandrolone decanoate (1.3%) were negligible in relation to circulating levels. The assay detection limit ($B/B_0 = 0.90$) was 8 pg/tube (equivalent to 0.25 nM), and the ED_{50} was 100 pg/tube (equivalent to 3.5 nM). Coefficients of variation at low ($\approx \text{ED}_{80}$), medium ($\approx \text{ED}_{50}$) and high ($\approx \text{ED}_{20}$) levels of the standard curve ($n = 16$ –24 assays) were 11.7, 9.8 and 11.9% (between-assay) and 6.2, 3.5 and 5.3% (within-assay), respectively.

Plasma testosterone was measured by radioimmunoassay using a rabbit antibody to testosterone-3-*O*-carboxymethylloxime-bovine serum albumin (SGT-1, supplied by Dr. B. Caldwell, Yale University), [1,2,6,7,16,17(N)- ^3H]testosterone (specific activity, 5.00–6.66 TBq/mmol; DuPont, North Ryde, Australia), testosterone standard (Steraloids), extracts of plasma equivalent to 12.5 μ l/tube and a dextran-charcoal separation of bound and free steroid. Correction was made for cross-reactivity with nandrolone (20.5%), but cross-reactivities with dihydrotestosterone (21.1%), androstenedione (2.5%), estradiol (0.17%), nandrolone phenylpropionate (0.04%) and nandrolone decanoate ($<0.0002\%$) were negligible in relation to circulating levels. The assay detection limit ($B/B_0 = 0.90$) was 2 pg/tube (equivalent to 0.6 nM), and the ED_{50} was 22 pg/tube (equivalent to 6 nM). Coefficients of variation at low ($\approx \text{ED}_{80}$), medium ($\approx \text{ED}_{50}$) and high ($\approx \text{ED}_{20}$) levels on the standard curve ($n = 8$ –32 assays) were 12.6, 17.1 and 12.9% (between-assay) and 8.3, 3.7 and 6.0% (within-assay), respectively.

Plasma inhibin was measured by a heterologous double-antibody radioimmunoassay established in our laboratory (Handelsman *et al.*, 1990; Crawford and Handelsman, 1994) and validated for humans (McLachlan *et al.*, 1990; Burger, 1992; Dong *et al.*, 1992; Wallace *et al.*, 1993). Reagents, provided by Dr. G. Bialy (Contraceptive Development Branch, National Institute of Child Health and Human Development), were rabbit antiserum to bovine inhibin (rAs-1989), purified 31-kDa inhibin from bovine follicular fluid for iodination (bINH-R-90/1) and bovine inhibin standard (bINH-R-90/1). Duplicate plasma samples (100 μ l) were preincubated overnight with antibody before addition of ^{125}I -labeled inhibin. The standard curve was blanked with castrate human serum to equalize serum protein concentrations in the assay tubes. The detection limit ($B/B_0 = 0.90$) was 19.5 pg/ml, and coefficients of variation at inhibin standard levels of 90, 200 and 390 pg/ml were 3.1, 7.3 and 7.4% (between-assay) and 1.8, 3.0 and 5.0% (within-assay), respectively.

Data analysis. Plasma nandrolone, testosterone and inhibin levels were initially analyzed to evaluate the full time course for each hormone by repeated-measures analysis of variance with BMDP 5V software (Dixon, 1992), testing main effects by the Wald χ^2 test and using suitable linear contrasts to define effects of ester, injection site and volume. Due to the very different time courses of the phenylpropionate and decanoate esters, the three groups (groups 2–4) receiving the decanoate ester were also analyzed separately.

Plasma nandrolone levels were also analyzed by standard pharmacokinetic methods involving polyexponential curve fitting (Gibaldi and Perrier, 1982), with a weighted, nonlinear, least-squares, curve-fitting algorithm, by BMDP 3R software (Dixon, 1992). Standard pharmacokinetic parameters (time of peak, peak concentration, mean residence time, apparent half-times for absorption and clearance, systemic clearance and area under the curve) were derived empirically from the plasma nandrolone concentrations and as mathematical functions of the coefficients of the best-fit curve (Gibaldi and Perrier, 1982). Estimates of absolute bioavailability of nandrolone were calculated from the disappearance curves of plasma nandrolone after i.v. administration, by fitting to a variance-weighted, triexponential curve with corrections for the molecular weight of nandrolone (274.4) and its decanoate (428.7) and phenylpropionate (406.6) esters.

This pharmacokinetic analysis was subsequently extended by building a population pharmacokinetic model using the approach of Mandema *et al.* (1992). The plasma concentration of nandrolone was considered to be the convolution of a monoexponential or biexponential absorption function with a biexponential unit disposition function

$$\text{UDF} = \sum_{i=1}^2 A_i \cdot e^{-\lambda_i \cdot t} \quad (1)$$

The input rate (IR) for the monoexponential absorption model is given by

$$\text{IR} = D \cdot F \cdot k \cdot e^{-k \cdot t} \quad (2)$$

where D is the dose, F is the fraction absorbed (bioavailability) and k is the first-order absorption rate constant. The input rate for the biexponential absorption model is given by

$$\text{IR} = D \cdot F \cdot [P \cdot k_1 \cdot e^{-k_1 \cdot t} + (1 - P) \cdot k_2 \cdot e^{-k_2 \cdot t}] \quad (3)$$

where k_1 and k_2 are first-order absorption rate constants, P is the proportion of the bioavailable dose absorbed by k_1 and $(1 - P)$ is the proportion of the bioavailable dose absorbed by k_2 . The convolution of these input functions with a biexponential disposition function yielded equations 4 and 5, which describe the plasma concentrations over time after i.m. injection for the monoexponential and biexponential absorption models, respectively.

$$Cp(t) = D \cdot F \cdot k \cdot \sum_{i=1}^2 \frac{A_i}{k - \lambda_i} (e^{-\lambda_i \cdot t} - e^{-k \cdot t}) \quad (4)$$

$$Cp(t) = D \cdot F \cdot \sum_{i=1}^2 \left[\frac{k_1 \cdot P \cdot A_i}{k_1 - \lambda_i} (e^{-\lambda_i \cdot t} - e^{-k_1 \cdot t}) + \frac{k_1 \cdot (1 - P) \cdot A_i}{k_2 - \lambda_i} (e^{-\lambda_i \cdot t} - e^{-k_2 \cdot t}) \right] \quad (5)$$

The population pharmacokinetic model did not include the data from the two subjects who received nandrolone i.v. Thus, parameter F was not identifiable and was incorporated into the models as $F \cdot A_1$ and $F \cdot A_2$. The interindividual error on each of the model parameters was modeled using a logarithmic-normal variance model

$$P_i = \theta_{TV} e^{\eta_i}$$

where P_i is the value of the parameter in the individual, θ_{TV} is the typical value of the parameter in the population and η is a random variable with mean 0 and variance ω^2 . The estimates of ω obtained with NONMEM are similar to the coefficient of variation for the parameter, which we report as the population variability, expressed as a percentage. We used a "constant coefficient of variation" model for the variance of the intraindividual residual error. Empirical Bayesian estimates of the individual pharmacokinetic parameters were obtained based on the typical values of the structural model

parameters and on the variances of the interindividual errors. These population pharmacokinetic models were implemented with the computer program NONMEM (Beal and Sheiner, 1992).

We used a GAM (Chambers and Hastie, 1993) to permit identification of linear and nonlinear relationships between the Bayesian estimates of individual model parameters and the volunteer covariates (ester, site and volume of injection, group, age, weight, height, body surface area and lean body mass). The model that resulted in the biggest decrease in the Akaike information criterion was returned by the GAM function as the best model. The structural model incorporating covariates identified by the GAM analysis was then evaluated with NONMEM to develop the final parameter estimates. The final model was examined for parsimony by exclusion of individual covariates and demonstration of a statistically significant increase in the NONMEM objective function and by analysis of the standard errors of the estimated parameters.

Pharmacodynamics. The pharmacodynamic effects of nandrolone on plasma testosterone and inhibin were estimated by nadir concentration, time of nadir, net secretion and duration of suppression. The duration of suppression was defined as the time when plasma testosterone levels were below normal (<10 nM) or inhibin levels were reduced by 50% of base line. The effects of ester, injection site and volume were determined by parametric (testosterone) and nonparametric (inhibin) analysis of variance.

This pharmacodynamic analysis was extended by building a population pharmacodynamic model, again using the approach of Mandema *et al.* (1992). The pharmacodynamic model was based on one of the four basic indirect response models recently proposed by Dayneka *et al.* (1993). Because testosterone and inhibin secretion are both suppressed by nandrolone, model I of Dayneka *et al.* was physiologically the most appropriate to describe the pharmacodynamic effects of nandrolone and is shown in equation 6.

$$\frac{dR}{dt} = k_{in}^0 \cdot \left[1 - \frac{Cp(t)}{Cp(t) + IC_{50}} \right] - k_{out} \cdot R(t) \quad (6)$$

R represents the measured response variable (either testosterone or inhibin concentrations), k_{in}^0 is a zero-order rate constant (the base-line testosterone or inhibin daily input rate), $Cp(t)$ is the plasma concentration of the inhibiting drug (nandrolone) as a function of time and IC_{50} is the drug concentration that results in 50% of maximum inhibition of the production rate. Under steady-state base-line conditions, it is noted that $k_{in}^0 = k_{out} \cdot R_0$, where R_0 is $R(t)$ at $t = 0$, reducing the number of parameters in the model. We have modified equation 6 to allow for incomplete inhibition of testosterone and inhibin synthesis by nandrolone and have included a parameter (γ) to describe the steepness of this relationship

$$\frac{dR}{dt} = k_{out} \cdot \left[R_0 + (R_{min} - R_0) \cdot \left(\frac{Cp(t)^\gamma}{Cp(t)^\gamma + IC_{50}^\gamma} \right) \right] - k_{out} \cdot R(t) \quad (7)$$

where, for either testosterone or inhibin, $R(t)$ is the measured concentration, R_0 is the base-line concentration, R_{min} is the minimum concentration when the input rate is maximally suppressed by nandrolone, k_{out} is the first-order elimination rate constant, $Cp(t)$ is the predicted plasma concentration for nandrolone (based on individual dosing and Bayesian pharmacokinetic parameter estimates) and IC_{50} is the concentration of nandrolone associated with 50% suppression of synthesis. The parameter γ was implemented as $\gamma = 1 + \theta$, to enable a comparison of the full model ($\gamma > 1$) and reduced model ($\gamma = 1$, $\theta = 0$) using the likelihood ratio test.

Alternatively, partial inhibition of the input rate can be modeled with an additional term expressing the fractional inhibition (I_{max}), as shown in equation 8.

$$\frac{dR}{dt} = k_{in}^0 \cdot \left[1 - \frac{I_{max} \cdot Cp(t)}{Cp(t) + IC_{50}} \right] - k_{out} \cdot R(t) \quad (8)$$

We used the parameterization shown in equation 7, because we were interested in estimating the base-line and maximally suppressed concentrations of testosterone and inhibin (and the interindividual variability in these parameters) directly. Based on the parameterization in equation 7, I_{\max} is readily calculated as

$$I_{\max} = 1 - \frac{R_{\min}}{R_0} \quad (9)$$

The interindividual errors in the model parameters (R_0 , R_{\min} , k_{out} , IC_{50} and γ) were assumed to have a logarithmic-normal distribution, and the variance of the residual errors was assumed to be homoscedastic. As described for the pharmacokinetic analysis, a GAM was used to identify significant covariates, and NONMEM was used to develop the final pharmacodynamic model. All data are expressed as mean \pm S.E.M.

Results

Volunteers randomized into the four groups were comparable in anthropometric and hormonal variables (table 1). There were no significant differences between groups in mean dehydroepiandrosterone sulfate, LH, FSH, prolactin, insulin-like growth factor-I, hemoglobin, urea or creatinine concentrations (data not shown), which were normal for all men.

Global statistical analysis. Considering all four groups, global statistical analysis demonstrated significant differences in the time course of plasma nandrolone concentrations (group, $\chi^2 = 84.6$, 3 dF, $P < .001$ by Wald test; group \times time interaction, $\chi^2 = 643$, 66 dF, $P < .001$). These systematic differences were attributable to differences between different nandrolone esters (table 2). Similarly the time course of plasma testosterone concentrations varied significantly by group (group \times time interaction, $\chi^2 = 266$, 66 dF, $P < .001$) due to effects of both ester and injection site (table 2). To adjust for the dominating effect of differences between esters, a global analysis conducted for the three groups receiving nandrolone decanoate (groups 2–4) demonstrated significant effects of injection site on plasma nandrolone levels, as well as effects of injection volume and site on plasma testosterone and inhibin levels (table 3).

Pharmacokinetic analysis. Plasma nandrolone concentrations reached higher and earlier peak concentrations and had a shorter mean residence time after injection of the phenylpropionate ester (group 1; $n = 7$), compared with the other three decanoate ester groups (fig. 1; tables 2 and 4). Analysis of the concentration data obtained from the two subjects who received i.v. nandrolone gave the following values: area under curve/unit dose = 1.3224×10^{-3} days/liter, mean residence time = 25.65 ± 5.22 min, volume of distribution = 11.46 ± 0.30 liters and systemic clearance = 31.52 ± 7.26 liters/hr. From the optimal triexponential curve fit, the following parameters were estimated: $A = 153.3 \pm 2.7$ nM, $\alpha = 0.4132 \pm 0.0030 \text{ min}^{-1}$, $B = 8.8659 \pm 0.4575$ nM, $\beta = 0.0098 \pm 0.0022 \text{ min}^{-1}$, $C = 0.9708 \pm 0.0066$ nM and $\gamma = 0.00043 \pm 0.00045 \text{ min}^{-1}$. Based on the area under the curve estimates for these two subjects, the absolute bioavailability of nandrolone from i.m. injections of esters was significantly higher for nandrolone decanoate injected into gluteal muscle with a 1-ml volume (73%), compared with the other three groups (53–56%).

The final population pharmacokinetic model incorporated ester, site and volume of injection and height as significant covariates. Height was significantly superior to weight, body surface area or lean body mass as a covariate. The type of ester influenced the absorption profile of nandrolone, such that the phenylpropionate ester was best described by a one-compartment absorption model and the decanoate ester was best described by a two-compartment absorption model. This was implemented in the model with parameter P (table 5). The interpretation of this parameter was that, effectively, the total dose of the phenylpropionate ester is administered into the “fast” compartment characterized by the rapid absorption rate constant (k_1), whereas only ~14% of the total dose of the decanoate ester is administered into this compartment, with the remaining ~86% of the total dose being administered into the “slow” compartment characterized by a slower absorption rate constant (k_2). This basic difference in the profiles of the nandrolone concentration data is shown in figure 2. In figure 2, the individual in each of the four groups with the median mean absolute prediction error was selected

TABLE 1
Base-line anthropometric and endocrine variables

Ester	Nandrolone Phenylpropionate	Nandrolone Decanoate	Nandrolone Decanoate	Nandrolone Decanoate	P^e
Injection Site	Gluteal	Gluteal	Gluteal	Deltoid	
Volume (ml)	4	4	1	1	
Dose (mg)	100	100	100	100	
Patients					
Number	7	6	5	5	
Age (yr)	26 ± 2	24 ± 1	24 ± 3	24 ± 2	0.842
Height (cm)	178 ± 2	177 ± 3	173 ± 5	171 ± 3	0.426
Weight (kg)	72.5 ± 3.1	69.7 ± 2.6	66.8 ± 4.4	72.1 ± 2.2	0.580
BSA (m^2) ^a	1.90 ± 0.05	1.86 ± 0.04	1.79 ± 0.08	1.84 ± 0.05	0.616
RBW (% ideal) ^b	101 ± 3	99 ± 4	98 ± 3	108 ± 2	0.165
BMI (kg/m^2) ^c	22.8 ± 0.7	22.4 ± 1.0	22.3 ± 0.6	24.7 ± 0.4	0.152
Total testosterone (nM)	17.6 ± 0.8	20.7 ± 2.5	17.9 ± 2.3	17.1 ± 1.4	0.498
Free testosterone (pM)	323 ± 38	384 ± 62	265 ± 26	298 ± 21	0.290
Estradiol (pM)	130 ± 18	120 ± 11	150 ± 34	83 ± 22	0.251
SHBG (nM) ^d	23.6 ± 2.0	25.8 ± 3.5	34.7 ± 0.7	26.9 ± 5.2	0.121

^a BSA, bodt surface area.
^b RBW, relative body weight.
^c BMI, body mass index.
^d SHBG, sex hormone binding globulin.
^e From analysis of variance.

TABLE 2

Global statistical analysis of plasma nandrolone and testosterone after nandrolone ester injection by time course, ester, injection site and volume

Variable	Nandrolone			Testosterone		
	χ^2	dF	P	χ^2	dF	P
Ester	77.6	1	0	2.8	1	.093
Volume	1.7	1	.188	1.1	1	.291
Site	1.3	1	.261	0.2	1	.663
Time	665	22	0	596	22	<.001
Time \times ester	609	22	0	168	22	<.001
Time \times volume	10.7	22	.979	22.1	22	.455
Time \times site	18.6	22	.671	64.2	22	<.001

TABLE 3

Global statistical analysis of plasma nandrolone, testosterone and inhibin after nandrolone decanoate injection by time course, injection site and volume

Variable	Nandrolone			Testosterone			Inhibin		
	χ^2	dF	P	χ^2	dF	P	χ^2	dF	P
Volume	2.9	1	.090	1.0	1	.315	0.5	1	.482
Site	2.1	1	.148	0.2	1	.681	1.3	1	.262
Time	466	22	0	455	22	0	148	22	<.001
Time \times volume	21.5	22	.489	38.2	22	.017	26.5	22	.001
Time \times site	49.7	22	.001	72.9	22	0	23.8	22	.003

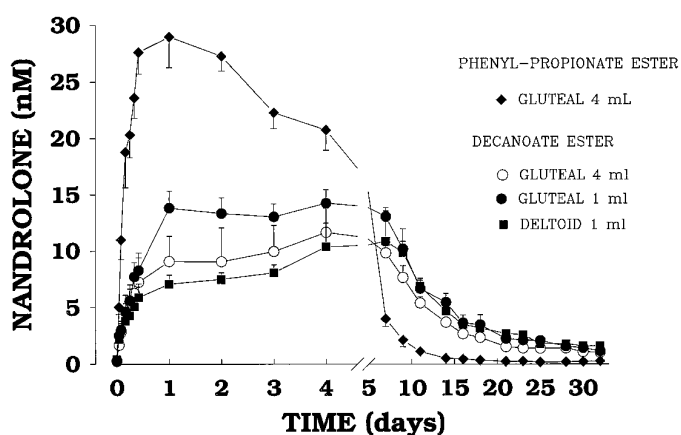


Fig. 1. Time course of plasma nandrolone concentrations in 23 healthy men over 32 days after i.m. injection of 100 mg of nandrolone phenylpropionate in 4 ml of arachis oil vehicle into the gluteal muscle (group 1) (\blacklozenge) or injection of 100 mg of nandrolone decanoate into the gluteal muscle in 4 ml of arachis oil vehicle (group 2) (\circ), into the gluteal muscle in 1 ml of arachis oil vehicle (group 3) (\bullet) or into the deltoid muscle in 1 ml of arachis oil vehicle (group 4) (\blacksquare). Results are expressed as mean and S.E.M., unless the S.E. is smaller than symbol.

to represent the Bayesian predictions based on the individual pharmacokinetic parameters. In addition, the rate of absorption from the fast compartment (k_1) was greater for the deltoid muscle than the gluteal muscle.

A two-compartment disposition function performed significantly better than a one-compartment disposition function. Site and volume of injection were important covariates for the two parameters, with bioavailability as a component ($F \cdot A_1$ and $F \cdot A_2$). $F \cdot A_1$ was greater in the gluteal muscle, compared with the deltoid muscle, and $F \cdot A_2$ was greater with a smaller injection volume (1 ml vs. 4 ml). Although the slow hybrid rate constant λ_2 was difficult to estimate accurately, it describes the very slow terminal elimination phase (fig. 2) and significantly improved the logarithmic-likelihood objective function of the model, as exemplified by the improved fit

of the two-compartment, compared with one-compartment, disposition function.

Pharmacodynamic analysis. Plasma testosterone concentrations were most rapidly and completely suppressed within the first week after injections of the phenylpropionate ester (fig. 3; tables 2 and 6), but this suppression was sustained for the shortest time. The duration of suppression was significantly longest after the gluteal 1-ml injection. Plasma testosterone concentrations returned to base line by day 13 after the phenylpropionate ester but required >20 days to return to base-line levels after the decanoate ester. Among the decanoate ester injections, both injection volume and site significantly influenced plasma testosterone concentrations (tables 3 and 6). Plasma inhibin levels after decanoate ester injections were suppressed to significantly lower nadir levels after 1-ml gluteal injection (fig. 3; table 6). Plasma inhibin was not assayed after nandrolone phenylpropionate (group 1) injection.

The GAM analysis detected no statistically significant covariates for the testosterone pharmacodynamic model (table 7). The inclusion of a parameter to estimate the nadir concentration of testosterone resulting from maximal suppression of testosterone synthesis by nandrolone and of a slope parameter describing the steepness of the relationship between the nandrolone concentration and the testosterone output rate significantly improved the model. Figure 4 shows the predicted testosterone concentrations for the same individuals as shown in figure 2. These predictions were calculated using the Bayesian estimates of the individual's nandrolone pharmacokinetic parameters and testosterone pharmacodynamic parameters.

No statistically significant covariates were detected in the GAM analysis for the inhibin pharmacodynamic model (figs. 5 and 6; table 7). Unlike testosterone, the inclusion of a parameter to estimate the nadir inhibin concentration did not improve the model, although a slope parameter did significantly improve the model fit. Figure 6 shows the pre-

TABLE 4
Pharmacokinetic variables

Variable (mean \pm S.D.)	N-PP ^a	N-D	N-D	N-D	P ^b
Injection site	Gluteal	Gluteal	Gluteal	Deltoid	
Injection volume (ml)	4	4	1	1	
Dose (mg)	100	100	100	100	
Number of patients	7	6	5	5	
Time of peak (days)					
Empirical	1.0 \pm 0.9	5.0 \pm 3.2	2.4 \pm 1.5	3.6 \pm 2.3	.022
Fitted	1.1 \pm 0.1	1.8 \pm 0.3	1.6 \pm 0.2	1.5 \pm 0.2	<.001
Peak concentration (nM)					
Empirical	30.9 \pm 5.8	13.5 \pm 5.0	15.8 \pm 2.8	12.4 \pm 5.5	<.001
Fitted	32.5 \pm 2.5	12.7 \pm 1.1	16.1 \pm 1.0	8.4 \pm 0.5	<.001
Mean residence time (days)					
Empirical	4.5 \pm 0.7	10.1 \pm 1.5	10.0 \pm 1.2	11.4 \pm 1.1	<.001
Fitted	3.1 \pm 0.2	9.5 \pm 0.7	10.6 \pm 0.9	16.9 \pm 1.3	<.001
Half-time (days)					
Absorption	2.4 \pm 0.1	7.0 \pm 0.4	7.7 \pm 0.6	12.0 \pm 0.9	<.001
Clearance	0.25 \pm 0.05	0.42 \pm 0.11	0.34 \pm 0.06	0.25 \pm 0.05	.002
Duration \geq 5 nM (days)	5.1 \pm 2.0	9.4 \pm 1.1	13.3 \pm 2.9	9.0 \pm 5.1	.002
Clearance (liters/day)	1359 \pm 149	1355 \pm 95	1041 \pm 59	1421 \pm 57	<.001
Area under the curve (nmol \cdot day/liter)					
Empirical	155 \pm 21	144 \pm 30	193 \pm 29	162 \pm 34	.065
Fitted	181 \pm 20	172 \pm 12	224 \pm 13	164 \pm 7	<.001

^a Esters are nandrolone decanoate (N-D) and nandrolone phenylpropionate (N-PP).^b From analysis of variance.TABLE 5
Nandrolone population pharmacokinetic model

Parameter	Model ^a	C.V. ^b %
P	1 - 0.863 \cdot ester flag	
k_1 (day ⁻¹)	4.42 + 5.03 \cdot site flag	57
k_2 (day ⁻¹)	0.192	27
$F \cdot A_1$ (liter ⁻¹)	0.000192 - 0.0000319 \cdot site flag	31
λ_1 (day ⁻¹)	0.342 + 0.00297 \cdot [height (cm) - 174]	28
$F \cdot A_2$ (liter ⁻¹)	0.00000197 + 0.00000248 \cdot volume flag	74
λ_2 (day ⁻¹)	0.000388	

^a Ester flag, phenylpropionate = 0, decanoate = 1; site flag, gluteal = 0, deltoid = 1; volume flag, 4 ml = 0, 1 ml = 1.^b Coefficient of variation.

dicted inhibin concentrations for the same individuals as shown in figure 2. Plasma inhibin was not assayed after nandrolone phenylpropionate (group 1) injection. These predictions were calculated using the Bayesian estimates of the individual's nandrolone pharmacokinetic and inhibin pharmacodynamic parameters.

Discussion

The present study demonstrates that, in addition to the chemistry of the side-chain ester, both injection site and volume can systematically influence blood nandrolone levels after i.m. injection of nandrolone esters in an oil vehicle formulation. Corresponding to the patterns of blood nandrolone concentrations, pharmacodynamic indices reflecting androgen-induced inhibition of pituitary-testicular function, namely blood testosterone and inhibin concentrations, are also systematically influenced by these factors. Crucially, the mixed-effects pharmacodynamic modeling demonstrated that essentially all of the pharmacodynamic variability in plasma testosterone and inhibin concentrations was accounted for by the variability between esters and the site and volume of injection of the nandrolone injections. The present study extends knowledge of the clinical pharmacokinetics of nandrolone esters, which were reported in two previous studies concerning nandrolone decanoate kinetics in humans

(Belkien *et al.*, 1985; Wijnand *et al.*, 1985); there are no reports of the pharmacokinetics of the phenylpropionate ester.

One feature of this study is the use of the population pharmacokinetic approach to integrating pharmacokinetic and pharmacodynamic data. Whereas the global statistical analysis indicates the significance of some key variables, a structural model allows more physiological interpretation of the findings, especially identifying the relationships between the pharmacokinetic and pharmacodynamic effects. We have used an indirect response model (Dayneka *et al.*, 1993), which allows for more physiologically meaningful models, more realistic interpretation of derived estimates and statistically valid testing for categorical covariables, while more efficiently using all of the experimental data (Jusko and Ko, 1994). The indirect physiological model-based approach also readily allows incorporation, into a general model, of data from different subpopulations where the kinetics and dynamics had differing shapes for the effective dose-response relationships. It is a striking validation of the model-based estimates that the nadir concentrations of testosterone and inhibin correspond very accurately to the known concentrations of these hormones in castrated men (1–3 nM and 0 pg/ml, respectively) (McLachlan *et al.*, 1990; Handelsman, 1994).

An interesting feature of this analysis of testosterone suppression is that the first-order rate constant for the response compartment (k_{out}) does not correspond to the metabolic clearance rate for testosterone. Using either bolus injection or steady-state infusion, the metabolic clearance rate for testosterone in men is \sim 540 liters/m²/day (Gandy, 1977) and the volume of distribution is 10 to 20 liters, which is similar to the 11.5 liters estimated in this study for nandrolone, a molecule almost identical to testosterone. These estimates would indicate a k_{out} value of \sim 50 day⁻¹, whereas our observed estimate for k_{out} was 0.708 day⁻¹; the latter is consistent with a much slower rate (half-time, \sim 1 day). This discrepancy is attributable to the fact that the overall kinet-

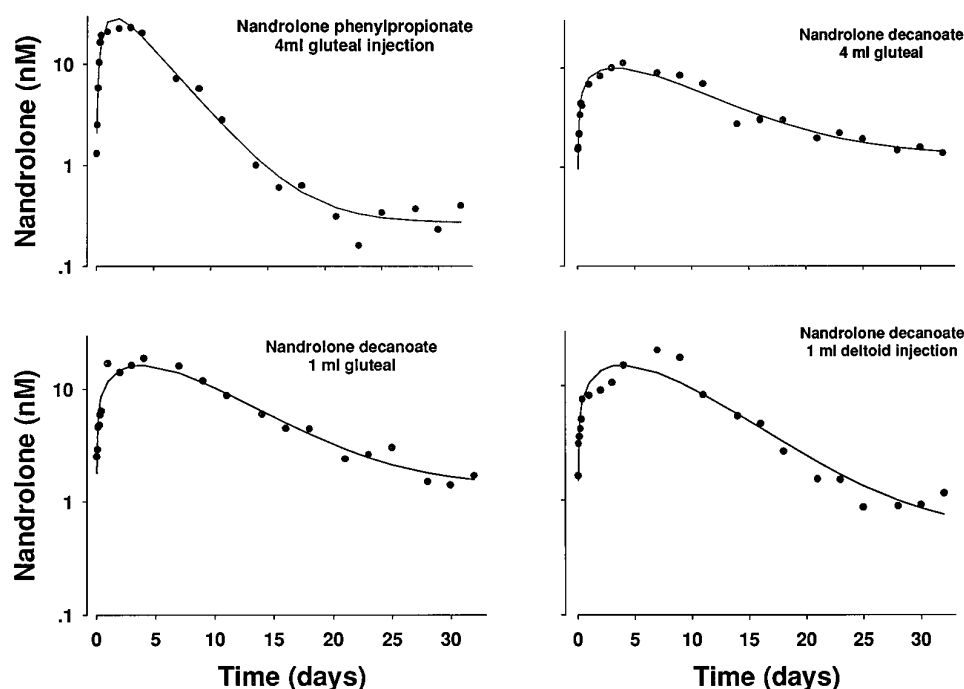


Fig. 2. Observed and model-predicted time course of plasma nandrolone concentrations in four healthy men over 32 days after i.m. injection of 100 mg of nandrolone ester. The four men were selected from each of the treatment groups according to the median predicted error in nandrolone concentrations, so that they were most representative of that group. Note the logarithmic vertical scale. ●, observed data; —, individual Bayesian predictions.

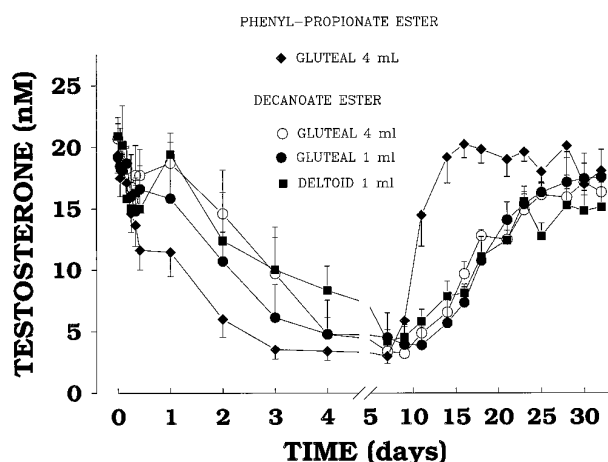


Fig. 3. Time course of plasma testosterone concentrations in 23 healthy men over 32 days after i.m. injection of 100 mg of nandrolone phenylpropionate in 4 ml of arachis oil vehicle into the gluteal muscle (group 1) (♦) or injection of 100 mg of nandrolone decanoate into the gluteal muscle in 4 ml of arachis oil vehicle (group 2) (○), into the gluteal muscle in 1 ml of arachis oil vehicle (group 3) (●) or into the deltoid muscle in 1 ml of arachis oil vehicle (group 4) (■). Results expressed as mean and S.E.M., unless the S.E. is smaller than the symbol.

ics of suppression of testosterone are dominated by the slow negative feedback system, rather than the much faster metabolic clearance of testosterone. This negative feedback is mediated *via* inhibition of pulsatile gonadotropin-releasing hormone secretion from hypothalamic neurons into the pituitary portal system and then pituitary LH secretion from gonadotropes. For example, a highly potent and specific gonadotropin-releasing hormone antagonist that causes immediate cessation of gonadotropin-releasing hormone action leads to castrate testosterone concentrations within 12 hr (Behre *et al.*, 1992), compared with 5 to 10 days in this study. This illustrates the need for physiological insight when interpreting indirect pharmacodynamic models because, in this instance, the relationship between circulating nandrolone

concentration and the input function to the model may itself be indirect. Our paradigm exemplifies a paradigm where the k_{out} parameter may accurately predict the time course of overall behavior of a system without corresponding to the metabolic clearance rate of the drug or the pharmacodynamic endpoint under study.

In the present study we have used specific radioimmunoassays to measure the nandrolone, testosterone and inhibin concentrations, with the latter two representing effective markers of endogenous pituitary gonadotropin (LH and FSH, respectively) secretion. This reflects the physiological fact that pituitary LH acts exclusively upon testicular Leydig cells, due to their unique expression of cell surface membrane LH receptors. In healthy men, virtually all circulating testosterone originates from Leydig cells, with an absolute requirement for trophic influence from LH derived from the bloodstream. Similarly, pituitary FSH acts exclusively upon testicular Sertoli cells, which uniquely express FSH receptors on their cell surface membranes, and virtually all circulating immunoreactive inhibin originates from the gonads (Burger, 1992). As a result, blood levels of these two hormones are useful integrated bioassay indicators of endogenous pituitary gonadotropin secretion, as reflected by the testicular hormonal response to ambient blood LH and FSH levels. In the present study, these two pharmacodynamic indices showed physiologically meaningful distinctions between the esters and the effects of injection site and volume.

Variations in side-chain ester chemistry are important in the pharmacokinetics of androgen esters in oil vehicle (Behre *et al.*, 1990). Experimental studies suggest that absorption rates are predicted by the oil/water partition coefficients (or hydrophobicity) and that the oil vehicle is absorbed more slowly than the androgen ester (Tanaka *et al.*, 1974). In humans, the very short propionate (three-carbon aliphatic) ester of testosterone has distinctly shorter duration of action than esters with longer (seven- or eight-carbon) side-chains (Nieschlag *et al.*, 1976; Schulte-Beerbuhl and Nieschlag,

TABLE 6
Pharmacodynamic variables

Variable (mean ± S.D.)	ND-PP ^a	ND-D	ND-D	ND-D	P ^b
Injection site	Gluteal	Gluteal	Gluteal	Deltoid	
Injection volume (ml)	4	4	1	1	
Dose (mg)	100	100	100	100	
Number of patients	7	6	5	5	
Testosterone					
Base-line concentration (nM)	17.6 ± 0.8	20.7 ± 2.5	17.9 ± 2.3	17.1 ± 1.4	.498
Nadir concentration (nM)	2.3 ± 1.4	2.4 ± 1.1	1.7 ± 0.7	3.2 ± 1.3	.295
Time of nadir (days)	5.2 ± 2.1	9.2 ± 2.6	8.4 ± 3.0	7.2 ± 2.0	.060
Net secretion (nM · days)	464 ± 89	348 ± 51	294 ± 65	341 ± 64	.004
Duration of suppressed testosterone (days) ^c	5.8 ± 3.1	11.0 ± 8.2	17.5 ± 5.0	14.9 ± 4.7	.010
Inhibin					
Base-line concentration (μg/liter)	NA ^d	137 ± 29	164 ± 23	189 ± 47	.559
Nadir concentration (μg/liter)	NA	81 ± 55	50 ± 11	79 ± 65	.009
Time of nadir (days)	NA	4.5 ± 2.7	4.0 ± 2.7	3.0 ± 2.2	.291
Net secretion (μg · days/liter)	NA	4052 ± 2735	2429 ± 883	3502 ± 2650	.465
Duration of suppressed inhibin (days) ^c	NA	0 ± 0	6.0 ± 9.7	5.4 ± 10.0	.096

^a ND-PP, nandrolone phenylpropionate; ND-D, nandrolone decanoate.
^b From parametric analysis of variance for testosterone and from nonparametric analysis (Kruskal-Wallis test) for inhibin, due to data skewing reflecting high between-subject variability.
^c Suppressed levels defined as <10 nM for testosterone and <50% base line for inhibin.
^d NA, not assayed.

TABLE 7
Population pharmacodynamic models for testosterone and inhibin

Parameter ^a	Testosterone ^b		Inhibin	
	Population Mean	Population Variability (%)	Population Mean	Population Variability (%)
Base line (R_0)	18.5 ± 0.7	14	153 ± 17	51
Nadir (R_{min})	1.9 ± 0.5		0	
k_{out} (day ⁻¹)	0.708 ± 0.05	87	0.189 ± 0.042	
Nandrolone IC ₅₀ (nM)	2.88 ± 0.51	38	7.49 ± 0.83	
Slope parameter (γ)	2.28 ± 0.22	42	1.98 ± 0.48	

^a For details of parameterization, see equation 7 in "Materials and Methods."
^b Units for baseline and nadir are nM (testosterone) or pg/ml (inhibin).

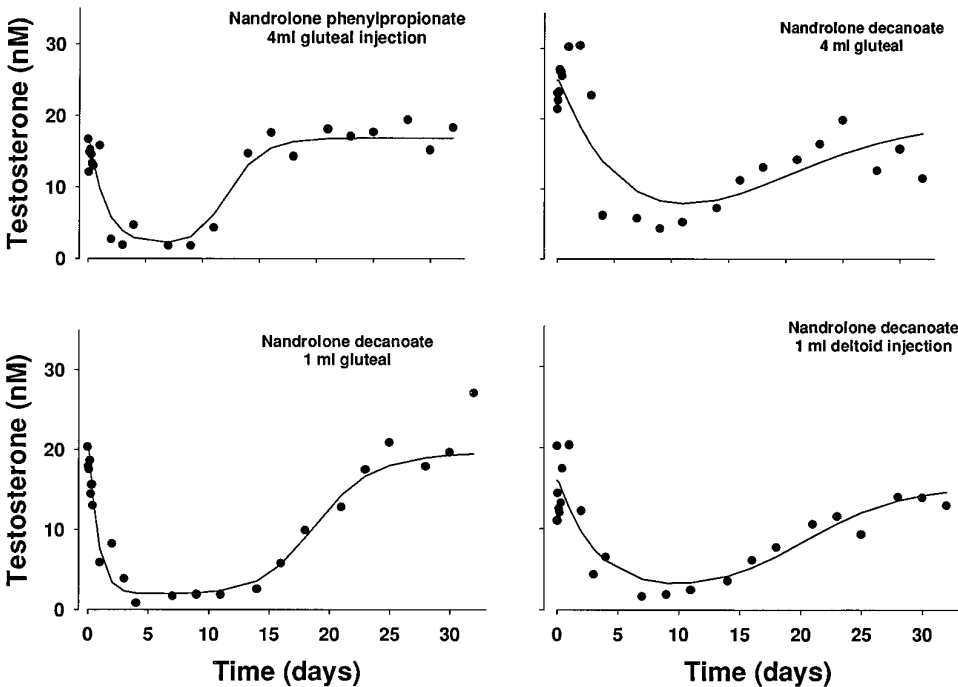


Fig. 4. Observed and model-predicted time course of plasma testosterone concentrations in four healthy men over 32 days after i.m. injection of 100 mg of nandrolone ester. The four men were the same individuals as illustrated in figure 2, who were originally selected from each of the treatment groups according to the median predicted error of nandrolone concentrations, so that they were most representative of that group. ●, observed data; —, individual Bayesian predictions.

1980; Schurmeyer and Nieschlag, 1984; Belkien *et al.*, 1985; Fujioka *et al.*, 1986). More subtle changes in side-chain ester structure have proven ineffective in altering human clinical pharmacokinetics, because substitution of a linear aliphatic side-chain of seven carbons (enanthate) with either a saturated, cyclized, seven-carbon aliphatic chain (cyclohexan-

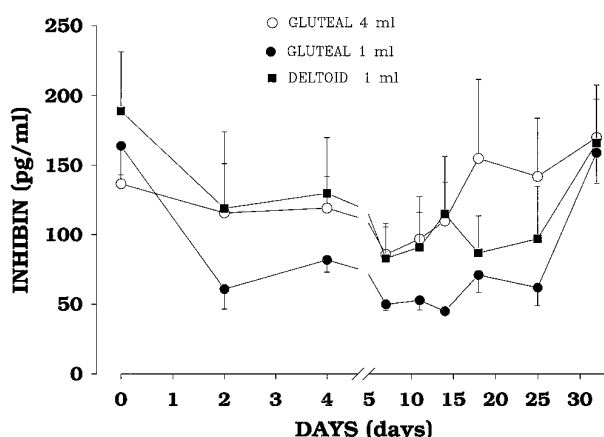


Fig. 5. Time course of plasma inhibin concentrations in 23 healthy men over 32 days after i.m. injection of 100 mg of nandrolone decanoate into the gluteal muscle in 4 ml of arachis oil vehicle (group 2) (○), into the gluteal muscle in 1 ml of arachis oil vehicle (group 3) (●) or into the deltoid muscle in 1 ml of arachis oil vehicle (group 4) (■). Results are expressed as mean and S.E.M. unless the S.E. is smaller than the symbol.

ecarboxylate) (Schurmeyer and Nieschlag, 1984) or a linear, aliphatic, eight-carbon chain (cypionate) (Schulte-Beerbuhl and Nieschlag, 1980) resulted in virtually unchanged kinetics. Wider variation in ester side-chain chemistry to include greater chain length and/or aromatic ring structures is a more effective determinant of ester pharmacokinetics, because nandrolone hexoxyphenylpropionate ester (aromatic ring with 18 carbons) had far better depot properties, with a prolonged and retarded release profile, compared with the decanoate (aliphatic chain with 10 carbons) (Belkien *et al.*, 1985). The present study indicates that a side-chain ester consisting of a 10-carbon aliphatic chain has better depot properties than a nine-carbon chain including an aromatic ring. Because the vehicle (arachis oil) was unchanged during this study and because of the experimental observation that

the oil vehicle influences local reaction to the oil injection (Brown *et al.*, 1944), as well as androgen ester pharmacology (Ballard, 1980; Al-Hindawi *et al.*, 1986), the present conclusions may be extrapolated to other vegetable oil injection vehicles only with caution.

Injection technique, including injection site, volume and concentration, as well as the nature of the vehicle, could theoretically be important for androgen ester release rate. Injection site may be important because of differences in tissue composition (Cockshott *et al.*, 1982) and blood flow (Bederka *et al.*, 1971); indeed, i.m. oil-based injections may more accurately be termed intermuscular (Ballard, 1968) or intralipomatous (Cockshott *et al.*, 1982). The former reflects the tendency of oil vehicle to distribute along intermuscular fascial planes (Ballard, 1968), whereas the latter depends upon the amount of fat at the injection site (including systematic gender differences) (Modderman *et al.*, 1983) together with needle geometry and anatomy of the injection depot. Intralipomatous deposition of injections with a larger vehicle volume may explain the slower release kinetics of nandrolone decanoate in the gluteal region, as well as the differences from the deltoid site, which has a lower fat content. The higher blood flow in the deltoid, compared with the gluteal, muscle (Evans *et al.*, 1975) may also be important. Analogous site-dependent differences in absorption rate and physiological effects have been described for a variety of drugs in aqueous solution (Greenblatt and Koch-Weser, 1976). To our knowledge, there are no previous reports examining the systemic pharmacokinetic and pharmacodynamic effects of injection site and volume for androgen esters in oil vehicle in men.

One possible clinical impact of these observations may lie in recent observations of differences between population groups in the efficacy of regular i.m. injections of testosterone enanthate in an oil vehicle to suppress testicular function for male contraception (World Health Organization Task Force on Methods for the Regulation of Male Fertility, 1990). In

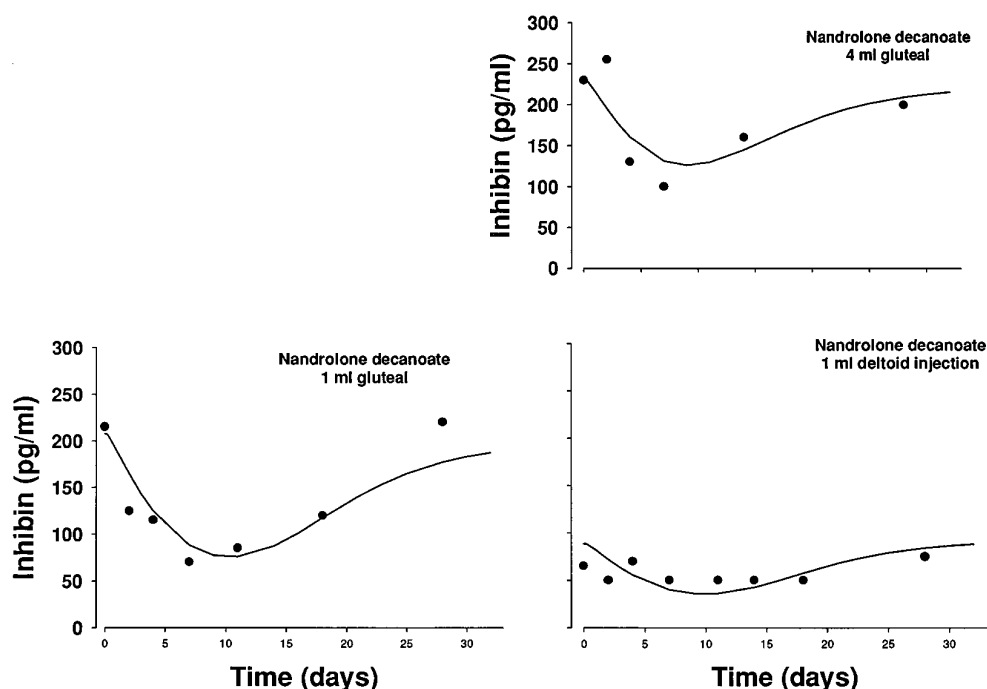


Fig. 6. Observed and model-predicted time course of plasma inhibin concentrations in three healthy men over 32 days after i.m. injection of 100 mg of nandrolone decanoate. No inhibin data were available for the group that received nandrolone phenylpropionate injections. The three men were selected from three of the four treatment groups according to the median predicted error, so that they were most representative of that group. ●, observed data; —, individual Bayesian predictions.

that and related (World Health Organization Task Force on Methods for the Regulation of Male Fertility, 1993) studies, interethnic differences in susceptibility to androgen-induced azoospermia were not due to differences in overall body size or related differences (Handelsman *et al.*, 1995). Evaluation of the possibility of ethnopharmacological differences, however, required a greater understanding of the rate-determining mechanisms of androgen release from androgen ester depots in oil vehicles. The present findings suggest that differences in absorption of androgen esters may contribute to such interethnic differences through possible local mechanical factors (*e.g.*, exercise, compression and muscle and fat mass) at the injection site, and this issue warrants further study. Analogous variations in the pharmacokinetics of steroid esters have been reported among women from different countries using long-acting contraceptive steroids (Garza-Flores, 1994), although no explanation has been advanced. Further analysis of the present observations may facilitate such ethnopharmacological studies, as well as clinical applications of androgen esters in oil vehicle formulations.

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References

- AL-HINDAWI, M. K., JAMES, K. C. AND NICHOLLS, P. J.: Influence of solvent on the availability of testosterone propionate from oily, intramuscular injections in the rat. *J. Pharm. Pharmacol.* **39**: 90–95, 1986.
- BALLARD, B. E.: Biopharmaceutical considerations in subcutaneous and intramuscular drug administration. *J. Pharm. Sci.* **57**: 357–378, 1968.
- BALLARD, B. E.: Prolonged-action pharmaceuticals. In *Remington's Pharmaceutical Sciences*, pp. 1594–1613, Mack Publishing Co., Easton, PA, 1980.
- BEAL, S. L. AND SHEINER, L. B.: NONMEM User's Guide, parts I–VII, University of California Press, San Francisco, 1992.
- BEDERKA, J., TAKEMORI, A. E. AND MILLER, J. W.: Absorption rates of various substances administered intramuscularly. *Eur. J. Pharmacol.* **15**: 132–136, 1971.
- BEHRE, H. M., KLEIN, B., STEINMEYER, E., MCGREGOR, G. P., VOIGT, K. AND NIESCHLAG, E.: Effective suppression of luteinizing hormone and testosterone by single doses of the new gonadotropin-releasing hormone antagonist Cetrorelix (SB-75) in normal men. *J. Clin. Endocrinol. Metab.* **75**: 393–398, 1992.
- BEHRE, H. M., OBERPENNING, F. AND NIESCHLAG, E.: Comparative pharmacokinetics of androgen preparations: Application of computer analysis and simulation. In *Testosterone: Action Deficiency Substitution*, ed. by E. Nieschlag and H. M. Behre, pp. 115–135, Springer-Verlag, Berlin, 1990.
- BELKIEN, L., SCHURMEYER, T., HANO, R., GUNNARSON, P. O. AND NIESCHLAG, E.: Pharmacokinetics of 19-nortestosterone esters in normal men. *J. Steroid Biochem.* **5**: 623–629, 1985.
- BROWN, W., WILDER, V. AND SCHWARTZ, P.: A study of oils used for intramuscular injections. *J. Lab. Clin. Med.* **29**: 259–264, 1944.
- BURGER, H. G.: Inhibin. *Reprod. Med. Rev.* **1**: 1–20, 1992.
- CHAMBERS, J. M. AND HASTIE, T. J., EDS.: *Statistical Models in S*. Chapman and Hall, New York, 1993.
- COCKSHOTT, W. P., THOMPSON, G. T., HOWLETT, L. J. AND SEELEY, E. T.: Intramuscular or intralipomatous injections? *N. Engl. J. Med.* **307**: 356–358, 1982.
- CRAWFORD, B. A. AND HANDELSMAN, D. J.: Failure of recombinant growth hormone or insulin-like growth factor-I to influence gonadotrophin sensitivity of the non-human primate testis *in-vivo*. *Eur. J. Endocrinol.* **131**: 405–412, 1994.
- DAYNEKA, N. L., GARG, V. AND JUSKO, W. J.: Comparison of four basic models of indirect pharmacodynamic responses. *J. Pharmacokin. Biopharm.* **21**: 457–478, 1993.
- DIXON, W.: *BMDP Statistical Software Manual*, University of California Press, Berkeley, CA, 1992.
- DONG, Q., HAWKER, F., MCWILLIAM, D., BANGAH, M., BURGER, H. AND HANDELSMAN, D. J.: Circulating inhibin and testosterone levels in men with critical illness. *Clin. Endocrinol.* **36**: 399–404, 1992.
- EVANS, E. F., PROCTOR, J. D., FRATKIN, M. J., VELANDIA, J. AND WASSERMAN, A. J.: Blood flow in muscle groups and drug absorption. *Clin. Pharmacol. Ther.* **17**: 44–47, 1975.
- FUJIOKA, M., SHINOHARA, Y., BABA, S., IRIE, M. AND INOUE, K.: Pharmacokinetic properties of testosterone propionate in normal men. *J. Clin. Endocrinol. Metab.* **63**: 1361–1364, 1986.
- GANDY, H. M.: Androgens. In *Endocrinology of Pregnancy*, ed. by F. Fuchs and A. Klopper, pp. 123–156, Harper & Row, Hagerstown, MD, 1977.
- GARZA-FLORES, J.: Pharmacokinetics of once-a-month injectable contraceptives. *Contraception* **49**: 347–359, 1994.
- GIBALDI, M. AND PERRIER, D.: *Pharmacokinetics*, Marcel Dekker, New York, 1982.
- GREENBLATT, D. J. AND KOCH-WESER, J.: Intramuscular injection of drugs. *N. Engl. J. Med.* **295**: 542–546, 1976.
- HANDELSMAN, D. J.: Testosterone and other androgens: Physiology, pharmacology and therapeutic use. In *Endocrinology*, ed. by L. J. DeGroot, pp. 2351–2361, W. B. Saunders, Philadelphia, 1994.
- HANDELSMAN, D. J., FARLEY, T. M. M., PEREGUDOV, A., WAITES, G. M. H. AND WORLD HEALTH ORGANIZATION TASK FORCE ON METHODS FOR THE REGULATION OF MALE FERTILITY: Factors in the nonuniform induction of azoospermia by testosterone enanthate in normal men. *Fertil. Steril.* **63**: 125–133, 1995.
- HANDELSMAN, D. J., SPALIVIERO, J. A. AND PHIPPARD, A. F.: Highly vectorial secretion of inhibin by primate Sertoli cells *in vitro*. *J. Clin. Endocrinol. Metab.* **71**: 1235–1238, 1990.
- JUNKMAN, K.: Long-acting steroids in reproduction. *Recent Prog. Horm. Res.* **13**: 380–419, 1957.
- JUSKO, W. J. AND KO, H. C.: Physiologic indirect response models characterise diverse types of pharmacodynamic effects. *Clin. Pharmacol. Ther.* **56**: 406–419, 1994.
- MANDEMA, J. W., VEROTTA, D. AND SHEINER, L. B.: Building population pharmacokinetic-pharmacodynamic models. I. Models for covariate effects. *J. Pharmacokin. Biopharm.* **20**: 511–528, 1992.
- MCLACHLAN, R. I., FINKEL, D. M., BRENNER, W. J. AND SNYDER, P. J.: Serum inhibin concentrations before and during gonadotropin treatment in men with hypogonadotropic hypogonadism: Physiological and clinical implications. *J. Clin. Endocrinol. Metab.* **70**: 1414–1419, 1990.
- MODDERMAN, E. S. M., MERKUS, F. W. H. M., ZUIDEMA, J., HILBERS, H. W. AND WARNDORFF, T.: Sex differences in the absorption of dapsone after intramuscular injection. *Int. J. Leprosy* **51**: 359–365, 1983.
- NIESCHLAG, E. AND BEHRE, H. M.: Pharmacology and clinical uses of testosterone. In *Testosterone: Action Deficiency Substitution*, ed. by E. Nieschlag and H. M. Behre, pp. 92–114, Springer-Verlag, Berlin, 1990.
- NIESCHLAG, E., CUPPER, H. J., WIEGELMAN, W. AND WICKINGS, E. J.: Bioavailability and LH suppressing effect of different testosterone preparations in normal and hypogonadal men. *Horm. Res.* **7**: 138–145, 1976.
- SCHULTE-BEERBUHL, M. AND NIESCHLAG, E.: Comparison of testosterone, dihydrotestosterone, luteinizing hormone, and follicle-stimulating hormone in serum after injection of testosterone enanthate or testosterone cypionate. *Fertil. Steril.* **33**: 201–203, 1980.
- SCHURMEYER, T. AND NIESCHLAG, E.: Comparative pharmacokinetics of testosterone enanthate and testosterone cyclohexanecarboxylate as assessed by serum and salivary testosterone levels. *Int. J. Androl.* **7**: 181–187, 1984.
- TANAKA, T., KOBAYASHI, H., OKAMURA, K., MURANISHI, S. AND SEZAKI, H.: Intramuscular absorption of drugs from oily solutions in the rat. *Chem. Pharm. Bull. (Tokyo)* **22**: 1275–1284, 1974.
- VAN DER VIES, J.: On the mechanism of action of nandrolone phenylpropionate and nandrolone decanoate in rats. *Acta Endocrinol.* **49**: 271–282, 1965.
- VINING, R. F.: Testosterone radioimmunoassay: Rapid extraction technique for steroids based on kieselguhr mini-columns. *Steroids* **36**: 149–159, 1980.
- WALLACE, E. M., GOW, S. M. AND WU, F. C. W.: Comparison between testosterone enanthate-induced azoospermia and oligozoospermia in a male contraceptive study. I. Plasma luteinizing hormone, follicle stimulating hormone, testosterone, estradiol, and inhibin concentrations. *J. Clin. Endocrinol. Metab.* **77**: 290–293, 1993.
- WEINAND, H. P., BOSCH, A. M. G. AND DONKER, C. M.: Pharmacokinetic parameters of nandrolone (19-nortestosterone) after intramuscular administration of nandrolone decanoate (Deca-Durabolin) to healthy volunteers. *Acta Endocrinol. Suppl.* **271**: 19–30, 1985.
- WORLD HEALTH ORGANIZATION TASK FORCE ON METHODS FOR THE REGULATION OF MALE FERTILITY: Contraceptive efficacy of testosterone-induced azoospermia in normal men. *Lancet* **336**: 955–959, 1990.
- WORLD HEALTH ORGANIZATION TASK FORCE ON METHODS FOR THE REGULATION OF MALE FERTILITY: Comparison of two androgens plus depot-medroxyprogesterone acetate for suppression to azoospermia in Indonesian men. *Fertil. Steril.* **60**: 1062–1068, 1993.
- ZUIDEMA, J., PIETERS, F. A. J. M. AND DUCHATEAU, G. S. M. J. E.: Release and absorption rate aspects of intramuscularly injected pharmaceuticals. *Int. J. Pharm.* **47**: 1–12, 1988.

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